PERSONALISING RADIOTHERAPY IN NON-SMALL CELL LUNG CANCER

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy in the Faculty of Biology, Medicine and Health

2018
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School of Health Sciences
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<th>Description</th>
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<tbody>
<tr>
<td>3D-CRT</td>
<td>Three Dimensional Conformal Radiotherapy</td>
</tr>
<tr>
<td>4D-CT</td>
<td>Four Dimensional Computed Tomography</td>
</tr>
<tr>
<td>[$^{18}$F]FDG</td>
<td>[$^{18}$F]Fluoro-2-deoxy-D-glucose</td>
</tr>
<tr>
<td>[$^{18}$F]FLT</td>
<td>3'-deoxy-3'-$[^{18}$F]fluorothymidine</td>
</tr>
<tr>
<td>[$^{18}$F]miso</td>
<td>[$^{18}$F]misonidasole</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
</tr>
<tr>
<td>ALK</td>
<td>Anaplastic lymphoma kinase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>BD</td>
<td>Twice-daily</td>
</tr>
<tr>
<td>CfDNA</td>
<td>cell-free deoxyribonucleic acid</td>
</tr>
<tr>
<td>CBCT</td>
<td>Cone Beam CT</td>
</tr>
<tr>
<td>CHART</td>
<td>Continuous Hyperfractionated Accelerated Radiotherapy</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>CTV</td>
<td>Clinical Target Volume</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>CTC</td>
<td>Circulating Tumour Cell</td>
</tr>
<tr>
<td>CTCAE 4.0</td>
<td>National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0</td>
</tr>
<tr>
<td>CTRT</td>
<td>Chemoradiotherapy</td>
</tr>
<tr>
<td>CTU</td>
<td>Clinical Trials Unit</td>
</tr>
<tr>
<td>cm$^3$</td>
<td>cubic centimetres</td>
</tr>
<tr>
<td>CXR</td>
<td>Chest X-ray</td>
</tr>
<tr>
<td>DCE-MRI</td>
<td>Dynamic Contrast Enhanced Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>DLCO</td>
<td>Gas Transfer Factor for Carbon Monoxide</td>
</tr>
<tr>
<td>DLT</td>
<td>Dose-limiting toxicity</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DW-MRI</td>
<td>Diffusion-weighted magnetic resonance imaging</td>
</tr>
<tr>
<td>EBRT</td>
<td>External Beam Radiotherapy</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>EQD2</td>
<td>Biologically Equivalent Dose in 2 Gy Fractions</td>
</tr>
<tr>
<td>FEV1</td>
<td>Forced Expiratory Volume in 1 second</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
</tr>
<tr>
<td>Gy</td>
<td>Gray (unit of absorbed radiation dose)</td>
</tr>
<tr>
<td>GTV</td>
<td>Gross Tumour Volume</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HU</td>
<td>Hounsfield Unit</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>IMRT</td>
<td>Intensity Modulated Radiotherapy</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>K-RAS</td>
<td>Kirsten rat sarcoma viral oncogene</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left Ventricular Ejection Fraction</td>
</tr>
<tr>
<td>M</td>
<td>Metastases</td>
</tr>
<tr>
<td>MEK</td>
<td>Mitogen Activated Protein Kinase</td>
</tr>
<tr>
<td>MEKi</td>
<td>MEK inhibitor</td>
</tr>
<tr>
<td>MLD</td>
<td>Mean lung dose</td>
</tr>
<tr>
<td>MTD</td>
<td>Maximum tolerated dose</td>
</tr>
<tr>
<td>MRC</td>
<td>Medical Research Council</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>N</td>
<td>Node</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Non-Small Cell Lung Cancer</td>
</tr>
<tr>
<td>OAR</td>
<td>Organ at risk</td>
</tr>
<tr>
<td>OD</td>
<td>Once-daily</td>
</tr>
<tr>
<td>OS</td>
<td>Overall survival</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PET-CT</td>
<td>Positron Emission Tomography-Computed Tomography</td>
</tr>
<tr>
<td>PFS</td>
<td>Progression-free survival</td>
</tr>
<tr>
<td>PFT</td>
<td>Pulmonary Function Test</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>PD-L1</td>
<td>Programmed death ligand 1</td>
</tr>
<tr>
<td>PJP</td>
<td>Pneumocystis jerovecci pneumonia</td>
</tr>
<tr>
<td>PS</td>
<td>Performance status</td>
</tr>
<tr>
<td>PTV</td>
<td>Planning Target Volume</td>
</tr>
<tr>
<td>RADAR</td>
<td>Radiation Damage And Resistance in Patients with Lung Cancer</td>
</tr>
<tr>
<td>RECIST</td>
<td>Response Evaluation Criteria in Solid Tumours</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>RP2D</td>
<td>Recommended Phase 2 Dose</td>
</tr>
<tr>
<td>RT</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>RTOG</td>
<td>Radiation Therapy Oncology Group</td>
</tr>
<tr>
<td>RTTQA</td>
<td>Radiotherapy trials quality assurance</td>
</tr>
<tr>
<td>SABR</td>
<td>Stereotactic ablative body radiotherapy</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>SCF</td>
<td>Supra-clavicular fossa</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SUV</td>
<td>Standardised Uptake Value</td>
</tr>
<tr>
<td>T</td>
<td>Tumour</td>
</tr>
<tr>
<td>TK1</td>
<td>Thymidine kinase 1</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumour Node Metastases</td>
</tr>
<tr>
<td>TMG</td>
<td>Trial Management Group</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper Limit of Normal</td>
</tr>
<tr>
<td>V5 Gy</td>
<td>The volume of normal tissue receiving 5 Gy</td>
</tr>
<tr>
<td>V20 Gy</td>
<td>The volume of normal tissue receiving 20 Gy</td>
</tr>
<tr>
<td>V30 Gy</td>
<td>The volume of normal tissue receiving 30 Gy</td>
</tr>
<tr>
<td>V35 Gy</td>
<td>The volume of normal tissue receiving 35 Gy</td>
</tr>
<tr>
<td>V40 Gy</td>
<td>The volume of normal tissue receiving 40 Gy</td>
</tr>
</tbody>
</table>
Abstract

Personalising radiotherapy in non-small cell lung cancer submitted by Kate Haslett for the degree of Doctor of Philosophy, The University of Manchester, September 2018.

Background

Despite the development of new systemic drugs, targeted agents and technological advances in radiotherapy the outcome remains poor and there has been little improvement in overall survival rates over the last two decades. The clinical trials in this thesis aim to evaluate methods of personalising radical radiotherapy in Non-Small Cell Lung Cancer (NSCLC).

Methods

Different approaches to personalising NSCLC treatment will be evaluated through two separate clinical studies. First, the Isotoxic Intensity Modulated Radiotherapy (IMRT) trial in inoperable stage III NSCLC is a multicentre feasibility study combining a number of intensification strategies; dose escalation, acceleration and hyperfractionation. The dose of radiation was increased until one or more of the organs at risk (OAR) met predefined constraints or the maximum dose of 79.2 Gy was reached. The primary objective was the delivery of isotoxic IMRT to a dose > 60 Gy EQD2. The second study is a single-arm, single-centre, open label phase I trial combining the MEK inhibitor selumetinib (AZD6244) with concomitant thoracic radiotherapy in NSCLC (MEKRT trial). The primary objective was to determine the recommended Phase 2 dose of selumetinib. The exploratory objective was to correlate $^{18}$FFLT PET-CT imaging parameters with response to selumetinib and radiotherapy.

Results

The Isotoxic radiotherapy schedule was found to be a feasible method of dose-escalation. In contrast, the outcome from concomitant radiotherapy and selumetinib was inferior to standard treatment. Based on the uptake of $^{18}$FFLT uptake suggests the MEK inhibitor selumetinib did not effect the uptake of $^{18}$FFLT. There was no association with baseline SUV$_{\text{max}}$ of the primary tumour and survival.

Conclusion

The studies in this thesis have evaluated ways of personalising radiotherapy treatment in non-small cell lung cancer. The isotoxic radiotherapy schedule is currently being tested alongside other dose escalated accelerated schedules in the randomised phase II ADSCaN trial (ISRCTN47674500). The combination of thoracic radiotherapy and selumetinib is not being pursued, but attention is turning to combination with immunotherapy. The future of personalized treatment and better outcomes in NSCLC will require the integration of immunotherapy and thoracic radiotherapy, the integration of targeted agents in the presence of driver mutations, personalised dose escalation and an improvement in the management of co-morbidities so patients can have the best treatment available.
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I would like to acknowledge Pek Koh who was the clinical fellow involved in the dose-escalation phase of the MEKRT trial. I would particularly like to thank Glenda Laviste who was the senior research nurse working on both the trials presented in this thesis. In addition I would like to acknowledge the trial management group for the Isotoxic IMRT trial, the Christie Lung team clinicians for their support and help in identifying suitable trial patients, the trials team including our research nurses and data managers, the radiotherapy planning team, especially Catherine Harris and Phil Whitehurst, and the radiographers. I would also like to thank Damian Mullan, the Consultant Radiologist involved in the MEKRT trial (particularly as he couldn’t say no to this role as he is also my husband!) and for his unfaltering support throughout my time in research.

Finally I would like to thank all our patients and their families who took part in this research, without their support none of this work would have been possible.
Chapter One

Introduction
1 Introduction

1.1 Introduction to Lung cancer

Lung cancer is the leading cause of cancer mortality worldwide. It is the second commonest cancer in the UK after breast cancer (1). In the 1950’s Doll and Hill famously made the link between cigarette smoking and lung cancer, and although the number of people smoking has fallen since a peak in the 1940’s, lung cancer remains a major public health concern (2). Cigarette smoking is still the major risk factor for lung cancer, accounting for over 90% of cases and can also potentiate environmental exposures e.g. asbestos, radon, arsenic and nickel (2, 3). There were 38,381 new cases of lung cancer (20,560 males and 17,821 females) registered in England in 2016 (4). Over the last few years the gap between lung cancer incidence in males and females has continued to narrow (4).

The age-standardised rate of lung cancer has decreased in males from 101.5 per 100,000 males in 2006 to 89.8 in 2016; whilst the opposite has occurred in females with the incidence of lung cancer increasing from 57.9 cases per 100,000 females in 2006 to 65.5 per 100,000 in 2016 (4). Compared to other common cancers, which show continued improvements in survival, less progress has been achieved in lung cancer. The statistics show overall 5 year survival in the UK has only minimally increased from 5% in 1975 to 9.5% in 2016 (1). The outcome from locally advanced disease remains poor, but advanced stage at presentation cannot solely explain the lack of improvement in outcome over the last 30 years, and suggests that a therapeutic plateau has been reached with conventional radiotherapy (RT) approaches (5).

Most patients with lung cancer are symptomatic at presentation, approximately a third with symptoms secondary to the primary tumour (e.g. cough, dyspnoea, chest pain, haemoptysis), a third with systemic symptoms (e.g. weight loss, fatigue) and a third due to metastatic disease (3). In all cases of suspected lung cancer a histological diagnosis should be made, the patient should be staged to define the extent of disease and the patient’s fitness for treatment assessed.

In clinical management the most important pathological distinction is between small-cell lung cancer and non-small-cell lung cancer (NSCLC). Almost 85% of lung cancer patients have non-small cell histology, most commonly squamous carcinoma or adenocarcinoma (2). Standard staging investigations include computed tomography (CT) of chest and abdomen, bronchoscopy and 2-[\textsuperscript{18}F]Fluoro-2-deoxy-D-glucose ([\textsuperscript{18}F]FDG) positron emission tomography-computed tomography (PET-CT). To evaluate the mediastinum endobronchial ultrasound or less commonly mediastinoscopy are utilised. The TNM staging (Tumour, Node, Metastases) is critically important in defining patients who have potentially curative disease, as well as being an important predictor of outcome (1) and the 7th edition which was used in clinical practice for the duration of this research is summarised in Table 1-1 (6) (of note the 8th edition of the lung cancer TNM staging system was introduced in January 2017 and implemented in practice from January 2018 onwards). The introduction of [\textsuperscript{18}F]FDG PET-CT has considerably improved the accuracy of staging in NSCLC
and defining patients suitable for radical treatment. \[^{18}\text{F}]\text{FDG PET-CT images are commonly used in RT planning as they can distinguish between malignant and non-malignant tissue (e.g. tumour and collapsed lung), highlight involved lymph nodes and detect metastatic disease (up to 20% of clinically stage III patients have occult metastases \[^7\]).\n
Table 1-1 7th Lung cancer Tumour Node Metastases (TNM) classification and staging system (6)

<table>
<thead>
<tr>
<th>T/M</th>
<th>Subgroups</th>
<th>( N_0 ) (no lymph nodes)</th>
<th>( N_1 ) (ipsilateral peribronchial ± hilar)</th>
<th>( N_2 ) (ipsilateral mediastinal ± subcarinal)</th>
<th>( N_3 ) (contralateral mediastinal ± hilar, any scalene ± SCF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>(surrounded by lung/visceral pleura, not in main bronchus)</td>
<td>T1a (≤2cm)</td>
<td>stage IA</td>
<td>stage IIA</td>
<td>stage IIIA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T1b (&gt;2cm ≤3cm)</td>
<td>stage IA</td>
<td>stage IIA</td>
<td>stage IIIA</td>
</tr>
<tr>
<td>T2</td>
<td>(involves main bronchus, ≥ 2cm distal to carina, invades visceral pleura)</td>
<td>T2a (&gt;3cm ≤5cm)</td>
<td>stage IB</td>
<td>stage IIA</td>
<td>stage IIIA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T2b (&gt;5cm ≤7cm)</td>
<td>stage IIA</td>
<td>stage IIB</td>
<td>stage IIIA</td>
</tr>
<tr>
<td>T3</td>
<td>(&gt;7cm or invades parietal pleura, pericardium, chest wall (^1), or tumour in main bronchus &lt; 2cm from carina or separate tumour nodule(s) same lobe)</td>
<td>T3 (&gt;7cm)</td>
<td>stage IIB</td>
<td>stage IIIA</td>
<td>stage IIIB</td>
</tr>
<tr>
<td>T4</td>
<td>(invades mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, oesophagus, vertebral body, carina or separate tumour nodule(s) in a different ipsilateral lobe)</td>
<td>Any size</td>
<td>stage IIIA</td>
<td>stage IIIA</td>
<td>stage IIIB</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastases</td>
<td>stage IV</td>
<td>stage IV</td>
<td>stage IV</td>
<td>stage IV</td>
</tr>
<tr>
<td>M1a</td>
<td>(separate tumour nodule(s) in contralateral lobe or malignant pleural/pericardial effusion)</td>
<td>stage IV</td>
<td>stage IV</td>
<td>stage IV</td>
<td>stage IV</td>
</tr>
<tr>
<td>M1b</td>
<td>(distant metastases)</td>
<td>stage IV</td>
<td>stage IV</td>
<td>stage IV</td>
<td>stage IV</td>
</tr>
</tbody>
</table>

Abbreviations: \( T \), Tumour; \( N \), Node; \( M \), Metastases; SCF, supraclavicular fossa,
\(^1\)including superior sulcus tumours

| Chapter 1 - Introduction | 2 |
1.2 Management of Non-small cell lung cancer

1.2.1 Introduction to radiotherapy

Over half of all cancer patients receive RT at some point during their illness (see Table 1-2) (3). RT uses ionizing radiation, most commonly X-rays, but other forms of radiation can be used e.g. electrons, radioactive isotopes, and protons. RT can be delivered using external beam radiotherapy (EBRT), when the radiation source is set at a distance away from the patient, or brachytherapy, when the source is directly inside the patient or tumour. Modern EBRT uses megavoltage X-rays (photons) that are produced by the acceleration of electrons within a vacuum onto a target within the machine (linear accelerator). The beam of X-rays produced can then be modified before it reaches the patient e.g. width, shape, beam flattening and filtration.

The total dose of radiation is prescribed in units of gray (Gy). One Gy created by X-rays is defined as the absorption of one joule of energy by one kilogram (kg) of matter. A single treatment may be given for palliation, but in radical regimens with curative intent the total amount of radiation to be delivered is divided into daily treatments, called fractions. Conventional RT is fractionated to spare normal tissues and allow the desired total dose to be delivered to the tumour. Evidence from standard radical radiation schedules utilised in NSCLC over the past 40 years has confirmed the importance of total dose as a factor in tumour response (8). These schedules often use 1.8–2 Gy fractions per day given 5 days per week for a period of 5-7 weeks e.g. 66 Gy in 33 fractions over 6.5 weeks.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Operable</th>
<th>Medically inoperable</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>Surgery (+/- adjuvant chemotherapy)</td>
<td>Radical radiotherapy (conventional) or stereotactic ablative radiotherapy</td>
</tr>
<tr>
<td>II</td>
<td>Surgery (with adjuvant chemotherapy)</td>
<td>Radical radiotherapy (conventional) or stereotactic ablative radiotherapy</td>
</tr>
</tbody>
</table>
| III   | Surgery (with adjuvant chemotherapy) | Concurrent chemoradiotherapy  
|       |                      | Sequential chemoradiotherapy |
| IV    |          | Palliative chemotherapy or palliative radiotherapy or best supportive care |
1.2.2 Management of early-stage Non-Small Cell Lung Cancer (Stage I and II)

The management of stage I-II lung cancer is aimed at curative intent, provided the patient is fit enough for treatment. The evidence suggests surgery provides the best chance of cure, but only 10-20% of early-stage patients are suitable (3). Lobectomy is the recommended surgical procedure, as more limited resections are associated with increased local recurrence (9). In the UK the average age at presentation is over 70 years of age, with the peak rate of lung cancer cases 2013-2015 in 85-89 year olds (1). Therefore many lung cancer patients have significant co-morbidities, often smoking-related, deeming them medically inoperable. For patients unwilling to accept the risks associated with surgery or those medically unfit, other radical options including stereotactic ablative body radiotherapy (SABR) should be discussed (10). SABR is the recommended treatment for peripheral tumours in stage I NSCLC if the patient is unfit for surgery (11).

1.2.3 Management of locally-advanced Non-Small Cell Lung Cancer (Stage III)

Most patients with locally advanced NSCLC are unsuitable for surgical resection as a consequence of co-morbidities and/or poor performance status. Stage III disease comprises a heterogeneous group of patients with a range of tumour sizes and lymph node involvement (see Table 1-1). The role of surgery is debated in stage III patients, but stage IIIA patients with single station N2 disease may be suitable for surgery combined with adjuvant chemotherapy (see Table 1-2). The non-surgical international standard of care for stage III patients is concurrent platinum-based chemoradiotherapy (CTRT), which is associated with a 5-year survival of 20-30% and a median survival of up to 28 months (12-16). Due to the increased toxicity of concurrent CTRT patient selection is extremely important, and as with surgical candidates, patients with major co-morbidities are unsuitable. In Europe, less than 40% of patients are deemed suitable for this regimen and proceed with concurrent treatment (15, 17, 18). Alternative treatment options include sequential CTRT or RT alone. Local control with standard 3D conformal radiotherapy (3D-CRT) alone remains poor, with reported 2-year loco-regional control rates of 20-44% (19-21). Recent studies have shown that better local control of lung cancer can lead to an improvement in overall survival (20).

Conventional RT either alone or concurrent with chemotherapy appears to have reached a “therapeutic plateau”. However the improvement in 5-year overall survival of 4.5% with concurrent treatment compared to sequential CTRT (20) suggests there is a potential for therapeutic gain by adding drugs to standard radical RT.
1.3 Radiotherapy treatment intensification and personalisation in non-small cell lung cancer

1.3.1 Introduction

The results of a UK-wide survey reported 55 Gy in 20 daily fractions as the commonest fractionation schedule, followed by 66 Gy in 33 daily fractions, the international standard of care (22). High local failure rates after RT have led to the study of various intensification strategies, which include altered fractionation e.g. hyperfractionation (classified as anything < 1.8Gy per fraction), acceleration (reducing overall treatment time), dose escalation with “Isotoxic”/personalised intensity modulated radiotherapy (IMRT) and the addition of molecularly targeted agents to RT. But such combinations often result in increased toxicity, preventing further intensification. However by using increasingly conformal RT regimens, e.g. IMRT and targeted agents with an improved side effect profile, further intensification could be achieved (23).

Furthermore there is significant heterogeneity amongst lung cancer patients, particularly those with stage III disease, highlighting the need for the individualisation of treatment. Treatment strategies to personalise treatment and assess early treatment responses may lead to prompt adaptation of treatment, enhancing the therapeutic ratio for each individual (23).

1.3.2 Dose-escalated radiotherapy

Dose escalation was one of the first strategies adopted to try to improve local control, made possible by improvements in diagnostic imaging and RT techniques over the last 30 years. Martel at al estimated that a dose of 84 Gy with conventional fractionation is needed to achieve a tumour control probability of > 50% at 30 months, establishing a clear dose-response relationship in locally advanced NSCLC (24).

Encouraging results from phase I/II dose escalation studies led to the development of the recent RTOG 0617, which evaluated dose escalation in the context of standard fractionation (2 Gy/day) and concurrent CTRT (16, 25). Unfortunately the study was closed early due to futility, indicating the absence of a survival benefit to high dose radiotherapy (74 Gy in 37 fractions delivered over 7.5 weeks) compared to standard dose (60 Gy in 30 fractions delivered over 6 weeks) (16). The failure of the high dose arm in this trial was multifactorial and likely resulted from a combination of poorer treatment delivery with fewer patients receiving concurrent chemotherapy, reduced compliance with RT, quality assurance issues and a prolonged overall treatment time (26).

1.3.3 Altered fractionation; hyperfractionation and acceleration

Extending the treatment time allows accelerated tumour repopulation, which can potentially be avoided by using hyperfractionation or acceleration. Hyperfractionation is a radiation treatment in which the total dose of radiation delivered is divided into smaller doses and treatments are given more than once a day (typically 2-3 times daily). Acceleration delivers the total dose of radiation over a shorter period of time (fewer days) compared to standard radiation therapy. A meta-
analysis by Mauguen et al evaluated 10 trials, which included 2000 patients, concluded that modifying the radiotherapy schedule by hyperfractionation, acceleration or both resulted in an increase in overall survival (27). Any method of modified RT led to a 12% reduction in the risk of death (p=0.009) and an absolute increase in overall survival in NSCLC. Overall survival was increased by 3.8% at 3 years and 2.5% at 5 years, improving the survival rate from 15.9% to 19.7% at 3 years and from 8.3% to 10.8% at 5 years (27). However modified RT increased the risk of acute severe oesophagitis from 9% to 19% (p<0.001) and, as expected, the most accelerated regimes resulted in the most severe toxicity. Nevertheless at least 90% of patients completed the planned radiotherapy, with compliance in the experimental arms similar to the control arms. See appendix 1 (my published review article) for more information on hyperfractionated and accelerated radiotherapy in non-small cell lung cancer (28).

Despite the fact that continuous hyperfractionated accelerated radiotherapy (CHART) (29, 30), which delivered (54 Gy in 36 fractions of 1.5 Gy three times per day over 12 consecutive days) was shown to be of significant benefit by improving local control and overall survival, this has not become standard UK practice (22). Meanwhile, it should be noted that the control arm of CHART would not be considered current standard of care since chemotherapy was not delivered with RT (either sequentially or concurrently) and a large percentage of patients had stage I-II disease (36%) who would nowadays be considered for a surgical approach, or in some cases SABR.

1.3.4 Personalising radiotherapy treatment using “Isotoxic” radiotherapy

Given the evidence in favour of hyperfractionation and acceleration, this has now been taken a step further with specifically tailored regimes. Isotoxic RT is a novel concept pioneered by the Maastro group, allowing for individualised dose escalation based on pre-defined normal tissue constraints (dose delivered to organs at risk such as lung and spinal cord) and as a consequence the dose will vary from patient to patient (31, 32). A feasibility study by the Maastro group prospectively evaluated 166 NSCLC patients (59% stage III) unsuitable for concurrent CTRT to receive an individualised dose of RT alone or after induction chemotherapy (55% of patients) delivered by using twice-daily fractions of 1.8 Gy using 3D-CRT (31). The median prescribed total tumour dose was 64.8 Gy (range 50.4 – 79.2 Gy) delivered within an accelerated schedule of 1.8 Gy twice daily. Fewer than 10% of patients with stage III received the maximum protocol dose of 79.2 Gy. With a median follow-up of 31.6 months, the median overall survival was 21.0 months (stage IIA 16.2 months, stage II, 17.2 months), with acceptable acute and late radiation toxicity. Despite these stage III patients receiving sequential CTRT or RT alone, the overall survival was similar to that seen with concurrent CTRT at the time of the study, but with less toxicity. The Maastro group conducted a similar study using isotoxic RT in stage III medically inoperable NSCLC patients receiving concurrent CTRT (32). One hundred and thirty seven patients were included in this phase II study, and again results were promising with a median dose of 65 Gy prescribed to large-volume disease, with acceptable toxicity and a median survival of 25 months. It seems likely that dose escalation was limited in the Maastro studies since the patients were treated with 3D-CRT, but the use of IMRT could potentially permit further dose escalation.
1.3.5 Isotoxic treatment using intensity modulated radiotherapy

IMRT modulates the intensity profile of radiation delivered to the patient, allowing improved targeting of the radiation dose with better conformity and a reduction in dose to organs at risk e.g. spinal cord, when compared to 3D-CRT. In the thorax IMRT leads to a reduction in mean lung dose and V20 both strong predictors of RT-induced lung toxicity (33-36). This may lead to better tumour control probability, but with the same normal tissue complication probability (31).

Before prospective data from RTOG 0617 (25), the evidence for the use of IMRT in locally advanced NSCLC came from 3 retrospective publications from the MD Anderson. A large retrospective study at the MD Anderson cancer centre compared the outcome of patients treated using IMRT planned with 4-Dimensional planning CT (4D-CT) (n = 91) versus 3D-CRT with 3D planning CT (3D-CT) (n = 318) (37). 4D-CT creates a video sequence of how the tumour moves during respiration, allowing RT to be delivered to the thorax with increased accuracy. Both the IMRT/4D-CT group and 3D-CRT/3D-CT group received a median dose of 63 Gy (range, 50 – 73 Gy) at a dose of 2 Gy per fraction. Toxicity was reduced in the group treated with IMRT with lower rates of ≥ grade 3 pneumonitis. IMRT was also associated with a significant improvement in median survival (1.4 years for IMRT/4D-CT versus 0.85 years with 3D-CRT/3D-CT), but the impact of stage migration due to the use of [18F]FDG PET-CT for staging should not be underestimated (pre-treatment [18F]FDG PET-CT was used in 82% of IMRT group but only 49% in 3D-CRT group). Another retrospective series from the same institution of 165 patients with inoperable NSCLC treated using IMRT, with or without chemotherapy, was associated with a promising median survival of 21.6 months (89% had stage III-IV disease) and was well tolerated (≥ grade 3 pneumonitis 11% at 6 months and ≥ grade 2 pulmonary fibrosis 7% at 18 months) (38). In these unmatched patient cohorts, during a time period when PET staging was being introduced, this single centre experience was interpreted with a degree of caution but despite the lack of evidence IMRT was adopted worldwide (39). Outcomes from a secondary analysis of RTOG 0617 evaluating 482 patients, 53% treated with 3D-CRT and 47% treated with IMRT, were the same in each group despite the IMRT group having larger planning treatment volumes (higher median PTV 486ml vs. 427ml) and more stage IIIB disease (40). The use of IMRT allows patients with larger tumours to be treated with radical RT, which would have been unachievable with conventional 3D planning.

A planning study by The Christie using IMRT and twice daily fractionation for stage II/III NSCLC showed that this regime had the potential for further individual dose escalation in this group of patients (41). The starting point for dose escalation in this study was 55.8 Gy in 1.8 Gy per fraction delivered twice daily. The number of fractions was then increased until one or more organ at risk tolerance dose was exceeded or a maximum dose of 79.2 Gy (i.e. 44 fraction of 1.8 Gy BD) was reached. IMRT allowed a significant dose increase in comparison to other methods (p<0.0001) while no difference was found between 3D conformal planning and inverse planning (p = 0.06). Inverse planning uses an optimisation program to find the treatment plan which best fits the pre-
defined objectives e.g. dose to the target and the constraints of the organs at risk. This regime was evaluated in a UK multicentre feasibility study of Isotoxic Intensity Modulated Radiotherapy (IMRT) in stage III NSCLC patients not suitable for concurrent CTRT (ClinicalTrials.gov Identifier: NCT01836692) (42), this study will be presented in chapter 2 of this thesis. The Isotoxic IMRT study incorporates acceleration, hyperfractionation, dose escalation and uses state-of-the-art RT techniques, including IMRT and twice-daily cone-beam CT imaging. All these different strategies will be trialled in combination, as one strategy alone is unlikely to result in a significant benefit and an improvement in overall survival. If Isotoxic IMRT proves feasible this regimen will be compared to standard sequential CTRT in the randomised phase II ADSCaN (Accelerated Dose escalated Sequential Chemo-radiotherapy in Non-Small Cell Lung Cancer) trial (ISRCTN47674500) alongside three other dose-escalated regimens currently being evaluated in the UK (discussed in chapter 2 of this thesis).

1.4 Molecularly-targeted agents in combination with radiotherapy

1.4.1 Introduction

Bentzen et al described the radiobiological mechanisms that can be harnessed by adding a targeted drug to RT (43). These include spatial cooperation (RT combats local disease and systemic treatment distant disease), cytotoxic enhancement (cell kill by DNA damage), biological cooperation (in a heterogeneous tumour the drug targets one cell population e.g. hypoxic cells and the RT targets another e.g. more oxygenated cells), temporal modulation (how the drug effects the cell after and during RT – the 5R’s of radiobiology – repair, repopulation, reoxygenation, redistribution, radio sensitivity) and finally normal tissue protection (the drug allows a higher dose of RT to be given because RT toxicity is reduced).

So far the only successful example of adding an epidermal growth factor receptor (EGFR) inhibitor with RT is in squamous cell carcinoma of the head and neck. Cetuximab, a monoclonal antibody that inhibits EGFR, has been shown to improve local control and overall survival, with median survival nearly doubling to 49 months, compared with 29 months for RT alone (44). As expected this combination treatment was associated with increased skin toxicity, with 34% developing a grade 3-4 reaction compared to 18% with RT alone, but overall the treatment was well tolerated. In head and neck patients unsuitable for the gold-standard concurrent CTRT (due to contraindications to cisplatin) cetuximab can be used instead.

Concurrent CTRT is well established, but although research using molecularly targeted drugs in combination with RT in NSCLC has been underway for the last 10 years, none have been of proven benefit and become part of routine clinical practice. Koh et al provide a comprehensive summary of the targeted agents used in combination with RT thus far (45). When trials are developed it is essential that the biological hypothesis for the interaction of the targeted agent and RT is at the forefront of the trial design and reflected in an appropriate clinical endpoint (43, 45). Adding additional drugs may cause increased normal tissue toxicity, with pulmonary toxicity being
of great concern. When combined with CTRT, for both small-cell lung cancer and NSCLC, the novel anti-angiogenic agent bevacizumab led to an unacceptably high incidence of tracheoesophageal fistula formation, resulting in early closure of both trials (46). This highlights the importance of vigilant phase I/II trials robustly designed to detect severe and/or unexpected toxicity.

One of the few targeted drugs currently being evaluated in combination with thoracic radiotherapy is the MEK inhibitor selumetinib (AZD6244) which acts on the mitogen activated protein kinase pathway [ClinicalTrials.gov Identifier:NCT01146756], this study will be presented in chapter 3 of this thesis. The proposed mechanism targeted by selumetinib is temporal modulation, particularly radiosensitivity.

1.4.2 Mitogen activated protein kinase signalling pathway

The Ras/Raf/MEK/ERK signalling cascade has a pivotal role in the regulation of normal cell proliferation by processing various signals from cytokines and extra-cellular growth factors involved in cell division and survival (47). If one of the proteins in the pathway is mutated, it can lead to abnormal cell proliferation and contribute to the development of cancer (48). Mutations to the Kirsten rat sarcoma viral oncogene (KRAS), which subsequently leads to the activation of the Ras pathway, are common and can be found in 15-50% of NSCLC cases (49). In addition to mutations, growth factors and cytokines can lead to aberrant activation of the Ras/Raf/MEK/ERK pathway during a malignant process. As a result this pathway has been extensively researched and is a major focus in the development of novel targeted drugs. MEK is activated downstream by Ras/Raf and is the only known activator of ERK (48) (see Figure 1-1). Therefore as a consequence of MEK inhibition, mitotic signalling should be prevented, leading to decreased tumour proliferation and survival.

![Figure 1-1 Ras/Raf/MEK/ERK pathway](image-url)
1.4.3 Mitogen activated protein kinase inhibitors in clinical trials

PD098059 became the first commercially available MEK inhibitor in 1995, and so far 13 MEK inhibitors have been used in clinical trials (50). Selumetinib is a potent, highly selective, uncompetitive inhibitor of MEK, which has been licensed for development by AstraZeneca Pharmaceuticals from Array BioPharma. Since the start of phase II studies in 2006, selumetinib has now been administered to over 2,000 patients within clinical trials.

Ionising radiation results in rapid activation of the Ras/Raf/MEK/ERK pathway in tumour cells (51). In xenograft models, with or without the addition of radiation, selumetinib resulted in decreased phosphorylation of ERK, with more cells remaining in the G1 phase and fewer cells dividing (51). Cells from the non-small cell cancer cell line A549 were exposed to selumetinib (250nM) or control for 16 hours and irradiated (51). After normalising for cell killing by selumetinib alone, survival curves were produced. Human cancer cell lines, which included A549, were found to be more radiosensitive if exposed to selumetinib before radiation. The mechanism of radiosensitisation by selumetinib is not fully understood. Ionising radiation causes an increase in transforming growth factor alpha (TGF-a) a prosurvival growth factor, and it is thought that selumetinib may partly inhibit this process (52).

1.5 Functional imaging in lung cancer

1.5.1 Introduction

Standard radiological imaging plays a key role in the diagnosis of lung cancer and the assessment of treatment response, but the utility of imaging during treatment and early follow up is poorly established. With the rapid advances in RT technology, the delineation of gross tumour volume (GTV) has become increasingly important. Standard CT or MRI provide accurate structural and anatomical information, but are often unable to distinguish between malignant and non-malignant tissue. Molecular imaging with PET-CT is at the forefront of staging in lung cancer, particularly in NSCLC, combining functional and anatomical imaging. The standard tracer is $^{18}$F-FDG, which exploits the increased glucose metabolism of cancer cells compared with normal tissues. Regional uptake is expressed as a Standardised Uptake Value (SUV). SUV is calculated either pixel-wise creating a parametric image or over a region of interest (ROI). SUV can be calculated at a single time-point or at multiple time-points through a dynamic sequence. SUV is often normalized to the injected dose and patient weight using the following formula:

\[
SUV = \frac{Ci \ (kBq)}{Dose \ (MBq) \times Weight \ (kg)}
\]

(Abbreviations. SUV, standardized uptake value, Ci = curie, radioactivity of the tissues; kBq, kilobequerel; MBq, megabequerel; kg, kilograms)
The prognostic value of the pre-treatment \(^{18}\text{F}\)FDG PET-CT based on baseline SUV’s is uncertain, a meta-analysis suggested primary tumour SUV measurement at baseline had prognostic value (53) whilst other studies have been negative (54, 55). In addition to \(^{18}\text{F}\)FDG other PET tracers targeted at specific molecular cancer pathways are also being investigated, e.g. proliferation (3’-deoxy-3’-[\(^{18}\text{F}\)]fluorothymidine \(^{18}\text{F}\)FLT, L-[methyl-\(^{11}\text{C}\)]-methionine, \([^{1}\text{C}]\)choline chloride), and hypoxia ((\(^{18}\text{F}\))fluoromisonidazole). The main aim is to find tracers to both predict and prognosticate.

Standard radiological imaging cannot evaluate tumour responses to RT within the first few days or weeks, initially because early biological changes precede any objective structural change. \(^{18}\text{F}\)FDG PET-CT has been used to assess early treatment response, showing promise in predicting the outcome to both palliative (56-59) and induction (57, 60-62) chemotherapy, but there is less evidence in radical treatments, including combined modality therapy (23). The main disadvantage of using \(^{18}\text{F}\)FDG PET-CT to assess response is the poor discrimination between inflammation and tumour activity, hence the development of other predictive radiotracers which evaluate tumour proliferation.

It has been proposed that a “biological target volume” could be created in addition to the planned RT volume, potentially allowing targeted dose escalation to the area of tumour with the highest biological activity (63). It has also been suggested that areas of the tumour with persistent high tracer uptake are associated with radioresistance, and a trial is currently underway to assess the feasibility of boosting treatment in these areas [ClinicalTrials.gov Identifier: NCT01024829].

Radiation pneumonitis and radiation-induced fibrosis cause diagnostic uncertainty between true disease progression and pseudo-disease progression, complicating patient management. Other than CXR and standard CT, no other imaging modalities are incorporated into the routine follow up of patients with NSCLC. Functional imaging modalities e.g. dynamic contrast enhanced magnetic resonance imaging (MRI) and diffusion-weighted MRI, require investigation to determine their utility in the assessment of tumour response and in the follow up of thoracic malignancies after RT.

The large heterogeneity amongst lung cancer patients requires new techniques to provide information on the biological and physiological tumour characteristics for both prognostic and predictive purposes. It is hoped that functional imaging could act as an imaging biomarker to define early treatment responses to RT and novel drugs, enable better prognostication, avoid toxicity in futile cases and improve the quality of follow up imaging.

### 1.5.2 The cell cycle and early radiotracers to detect proliferation

Cell proliferation remains an essential component in the pathological classification of tumours. Proliferation is easy to assess on resected tumours, but if imaging became a surrogate biomarker it would enable non-invasive and repeatable assessment over the course of treatment. In addition, it could provide incisive information about the primary tumour and potential metastatic sites.
Cells follow a process of growth and division called the cell cycle. The cell cycle comprises four distinct phases (see Figure 1-2). Cells are either within the active cell cycle or in the resting phase G0 (64, 65). Following an appropriate growth signal, cells proceed from G0 to the first gap phase (G1). During G1 the cell is preparing for the S phase. This phase is particularly important, as the synthesis of nuclear deoxyribonucleic acid (DNA) occurs and following replication the amount of DNA has doubled. Next the cells prepare for mitosis during G2 (second gap phase). During the mitotic (M) phase, the sister chromatids separate and the cell divides into two daughter cells, which can either enter the G0 or G1 phase. The cell cycle is controlled by the cyclin-dependent kinase family (64).

![Figure 1-2 The cell cycle](image)

**Abbreviations:** G1 = first gap phase; S = DNA synthesis; G2 = second gap phase; M = mitosis.

Abnormal and accelerated cell growth is a defining characteristic of cancer. A high cell proliferation rate is usually associated with more aggressive tumours and poorer clinical outcomes (66, 67). In 1960 tritium-labelled thymidine ([³H-methyl] thymidine) was one of the first radiotracers to be used to detect cell proliferation in humans. Repeated intravenous injections of tritium-labelled thymidine was administered to two patients with brain tumours, and from plasma and urine specimens tritium activity was detected by liquid scintillation spectrometry (68). [¹¹C-methyl] thymidine was first synthesised for in vivo imaging in 1972 (69), but due to the rapid catabolism and short half-life of thymidine labelled with ¹¹C it did not become part of routine clinical practice and other radiotracers of DNA synthesis have since been evaluated (65).

### 1.5.3 3’-deoxy-3’-[¹⁸F]Fluorothymidine and the synthesis of thymidine

As synthesis of DNA only occurs in the S phase and not the resting phase, this can be utilised and measured using PET (70). The DNA double helix is comprised of four bases: adenine, cytosine, thymine and guanine. Within DNA the purines (adenine and guanine) pair up with the pyrimidines...
(thymine and cytosine). The exception is ribonucleic acid (RNA), with uracil replacing thymine. There are two main DNA synthetic pathways: de novo synthesis and the ‘salvage’ pathway (71). The salvage pathway is important, as some organs are incapable of de novo synthesis. De novo synthesis produces nucleotides from smaller simpler compounds, compared to the salvage pathway which recycles already-used bases and converts them into nucleotides (71). As the pyrimidine analogue thymidine is present in DNA but not RNA, $[^{18}\text{F}]\text{FLT}$ can be used to evaluate proliferation because cell division is proportional to the rate of DNA synthesis.

Thymidine nucleotides are either synthesized by the de novo pathway undergoing methylation of deoxyuridine monophosphate (dUMP) by thymidylate synthase (TS) or the salvage pathway by phosphorylation of exogenous deoxythymidine (TdR) see Figure 1.3 (65). During the salvage pathway, the enzyme thymidine kinase (TK) catalyses the phosphorylation of TdR to thymidine monophosphate (TMP), an essential precursor of DNA synthesis (65). When $[^{18}\text{F}]\text{FLT}$ enters a cell during S phase, it is phosphorylated by thymidine kinase 1 (TK1) into $[^{18}\text{F}]\text{FLT}$-monophosphate, becoming trapped within the cell, but not the DNA, thus acting as an indirect measurement of proliferation (70, 72). TK1 is found in the cytoplasm and TK2 in mitochondria, see Figure 1-3. The peak concentration of cytosolic TK1 is found during proliferation in the late G1 and S phase of the cell cycle, and is hardly detected in the resting phase (73). Sherley et al showed that during DNA synthesis the concentration of TK1 can be nearly 10 times greater than in the G1 phase (74). Since the synthesis of DNA only occurs in the S phase and the accumulation of $[^{18}\text{F}]\text{FLT}$ is dependent on TK1, this suggests that $[^{18}\text{F}]\text{FLT}$ could be used as a proliferation tracer (65, 70, 75). $[^{18}\text{F}]\text{FLT}$ undergoes glucuronidation in the liver; therefore, measurement of metabolites in blood is needed to completely understand the kinetics of $[^{18}\text{F}]\text{FLT}$ when evaluating tumour proliferation (65).

Figure 1-3 Synthesis of thymidine
Abbreviations: DNA = deoxyribonucleic acid; dUMP = deoxyuridine monophosphate; TDP = thymidine diphosphate; TdR = exogenous deoxythymidine; TK1 = thymidine kinase 1; TK2 = thymidine kinase 2; TMP = thymidine monophosphate; TP = thymidine phosphorylase; TTP = thymidine triphosphate; TS = thymidylate synthase).
1.5.4 3'-deoxy-3'-[18F]Fluorothymidine and Ki-67

Ki-67 is a nuclear protein expressed in proliferating cells but not in G0 cells, thus distinguishing between proliferating and non-proliferating cells (76). MIB-1, a monoclonal antibody against the Ki-67 antigen, is the commonest method used for detection, but a variety of different antibodies can be used. The tumour uptake of [18F]FLT has been shown to correlate to the Ki-67 labelling index (the percentage of tumour cells staining positive for Ki-67), although some clinical studies have not supported this correlation (72, 77-79).

A number of studies have detected a wide range of Ki-67 values in patients with NSCLC (80, 81), highlighting that due to tumour heterogeneity Ki-67 samples from different parts of the tumour may vary significantly (82). A study measuring Ki-67 on the resection specimens of 271 patients with NSCLC found that the correlation with outcome on univariate analysis was significant (P<0.02), the higher the proliferation index the higher the incidence of recurrence and cancer-related death (80). However on multivariate analysis, Ki-67 was eliminated because of confounding effects with the other tests (differentiation, vascular invasion, testing for oncoproteins) suggesting a larger sample size was needed. A similar study looked at the prognostic value of Ki-67 expression in 109 resected tumours in stage I or II NSCLC and found a high Ki-67 labelling index was associated with a poor outcome (p = 0.010) (81).

A meta-analysis of 3983 patients in 37 studies attempted to evaluate whether Ki-67 expression was associated with poor survival in lung cancer patients (76), out of which only 20 studies were included, as the rest provided insufficient data. When the data was subdivided, the stage I-III NSCLC subgroup, which included 1863 patients from 16 studies, showed a significant correlation between Ki-67 positivity and outcome. A more recent meta-analysis evaluating the correlation between [18F]FLT uptake and Ki-67, found a significant correlation for all types of cancer (72), with the combined correlation coefficient for the four lung cancer studies was r = 0.78 (95% CI = 0.56-0.89, p < 0.001).

1.5.5 Clinical studies in lung cancer using 3’-deoxy-3’-[18F]Fluorothymidine for staging

One of the first clinical studies to use [18F]FLT as a radiotracer prospectively evaluated patients with solitary pulmonary nodules. The first of two German studies evaluated 30 patients with suspicious solitary pulmonary nodules, originally discovered on CT, and compared the uptake of [18F]FLT with proliferation using Ki-67 (percentage of MIB-1-stained nuclei) (83). All underwent surgical resection (n=19) or core biopsy (n=11) which confirmed 8 nodules were benign and 22 malignant. Overall the mean [18F]FLT SUV\textsubscript{max} was 4.2. [18F]FLT SUV\textsubscript{max} was significantly increased in the presence of NSCLC, except in a case of carcinoma in situ and a well-differentiated large cell carcinoma with a low proliferation index. In this case series the sensitivity of [18F]FLT PET-CT was 86%. [18F]FLT was not taken up by any of the benign tumours. All malignant lesions were positive.
for Ki-67, which highly correlated with the $^{18}$F FLT SUV$_{\text{max}}$ (correlation coefficient $r = 0.87$ with $P < 0.0001$) (83).

The follow-on study from this group included 47 patients with pulmonary nodules suspicious of primary malignant lung tumours (84). $^{18}$F FLT PET-CT was performed in all 47 patients and $^{18}$F FDG PET-CT in all except 4 patients. Thirty-four patients underwent surgical resection and the remaining 13 had a core biopsy. As in the previous study, $^{18}$F FLT was only taken up by malignant tumours, with 100% specificity, but with two false negatives and an overall sensitivity of 90% for the detection of lung cancer. In comparison, $^{18}$F FDG had 94% sensitivity and 73% specificity. $^{18}$F FLT was however much less accurate for nodal staging with a sensitivity of 53% versus 77% for $^{18}$F FDG, concluding that $^{18}$F FLT PET-CT should not be used for the staging of lung cancer. Yamamoto et al also found in their study of 18 patients with newly diagnosed NSCLC that the sensitivity of $^{18}$F FLT for identifying the primary was 72% compared with 89% for $^{18}$F FDG imaging prior to surgical resection (75). Other clinical studies have shown similar correlations in NSCLC but with lower correlation between $^{18}$F-FDG uptake and Ki-67 (85-87).

1.5.6 Clinical studies evaluating the feasibility of $3'$-deoxy-$3'$-$^{18}$F Fluorothymidine to assess response to radiotherapy

RT in NSCLC is a rapidly advancing field due to improvements in technology, research focusing on the personalization of treatment including the individualisation of RT volume, dose and fractionation. $^{18}$F FLT can potentially assist with this by assessing early response to RT and the molecular effects of RT on tumour metabolism and proliferation, which may not be detectable by CT or $^{18}$F FDG PET-CT. The uptake of $^{18}$F FLT is specific for malignancy, correlates with proliferation, and has the potential for use as a prognostic tool in NSCLC (70).

One of the first pilot studies to assess whether $^{18}$F FLT could be used to image cellular proliferation during treatment was an Australian study, which recruited 5 patients with NSCLC undergoing standard concurrent CTRT (weekly carboplatin/paclitaxel concomitant with radiotherapy 60 Gy in 30 fractions over 6 weeks) (88). $^{18}$F FLT PET-CT was performed at baseline and twice during radiotherapy on either days 2, 8, 15 or 29, to assess early response to treatment on the initial scans, with the potential to observe accelerated repopulation during the later scans. There was uptake of $^{18}$F FLT at baseline by the primary tumour and mediastinal lymph nodes. As expected the $^{18}$F FLT uptake was significantly lower (around 2.2 times) than with staging $^{18}$F FDG. The uptake of $^{18}$F FLT was not uniform within each of the primary tumours. In this small study tumour uptake of $^{18}$F FLT was detected on day 2 (2/2 cases), Day 8 (2/2 cases), day 29 (1/3 cases) but not on day 15. In all 5 cases, the uptake of $^{18}$F FLT in each tumour decreased during the course of treatment and fell markedly in irradiated bone marrow. In one patient, on day 2 after the first 2 Gy fraction of radiotherapy, a flare of $^{18}$F FLT was seen, which then quickly fell. The lymph nodes had reduced in size whereas the primary tumour volume remained unchanged. In one patient, on day 2 after the first 2 Gy fraction of radiotherapy, a flare of $^{18}$F FLT was detected, which then quickly fell. The lymph nodes had reduced in size whereas the primary tumour volume remained unchanged. In two out of the 5 patients no $^{18}$F FLT uptake was identified after 20 Gy, suggesting that tumour cell proliferation had ceased. However the opposite occurred in the final patient when $^{18}$F FLT uptake was
demonstrated after 40 Gy on day 29. The baseline uptake in this case was double that of the others, suggesting it was a highly proliferative tumour. The authors hypothesised that in the presence of ongoing \([^{18}\text{F}]\text{FLT}\) uptake after 40 Gy it may be appropriate to accelerate the remaining radiotherapy treatments to combat accelerated repopulation. They also suggested that the dose of radiotherapy could be boosted to these potentially less responsive areas. Due to the small sample size of 5 patients, a larger study was needed to evaluate whether ongoing proliferation during the latter part of radiotherapy has any correlation with patient outcome, the follow on study from this group is discussed in chapter 3.

Another small study of 5 patients undergoing CTRT for NSCLC, used \([^{18}\text{F}]\text{FLT}\) to assess proliferation, \([^{18}\text{F}]\text{FDG}\) to assess metabolism and \([^{18}\text{F}]\)-misonidazole (\([^{18}\text{F}]\text{miso}\)) to assess hypoxia in the evaluation of tumour responses (89). All 3 scans were performed at baseline and during radiotherapy, at around 46 Gy. RT was associated with a significant decrease in SUV\(_{\text{max}}\) for \([^{18}\text{F}]\text{FLT}\) \((p = 0.0002)\) with an average reduction of 53%, but this was not observed for \([^{18}\text{F}]\text{FDG}\) \((p = 0.12)\) or \([^{18}\text{F}]\text{miso}\) \((p = 0.79)\).

Furthermore a study from our centre has supported these findings in NSCLC. The aim of this study was to assess whether \(^{18}\text{F}-\text{FLT}\) changes occur early in response to RT without concurrent chemotherapy and whether such changes exceed test-retest variability (90). Patients underwent one or two (optional) baseline \([^{18}\text{F}]\text{FLT}\) PET-CT scans, with the second assessing reproducibility, followed by a further scan during their second week of RT to assess response. Most of the 16 patients included had been treated with primary chemotherapy \((n=13)\) and were undergoing sequential CTRT, with the remaining 3 patients receiving radical RT alone. In total there were 35 \([^{18}\text{F}]\text{FLT}\) PET-CT scans available for evaluation. After 5-11 RT fractions, the primary tumour SUV\(_{\text{mean}}\) decreased significantly by 25% \((p = 0.0001)\) without any significant change in tumour volume. This percentage decrease was less than that seen in the previous studies evaluating concurrent CTRT (88, 89). However involved nodal volume decreased by 31% \((p = 0.02)\) with a larger SUV\(_{\text{mean}}\) reduction of 40% \((p<0.0001)\). Similar changes were seen for SUV\(_{\text{max}}\). The data also confirmed that decreases in tumour \([^{18}\text{F}]\text{FLT}\) uptake exceeded test-retest variability evaluated in 7 patients. These results highlight the potential for \([^{18}\text{F}]\text{FLT}\) PET-CT to be used as an imaging biomarker in the assessment of early response to radiation alone, or in combination with a novel molecular agent, but due to the paucity of data future studies are needed.

1.5.7 3'-deoxy-3'-[\(^{18}\text{F}\)Fluorothymidine as a biomarker to assess tumour response to targeted therapies in Non-Small Cell Lung Cancer

As many of the novel drugs, used alone or in combination with RT, are cytostatic agents the early effects of the drug may not be detectable by standard radiological imaging. Some studies have used functional imaging such as PET to evaluate those physiological changes, which occur before any visible structural change (91). Sohn et al evaluated the use of gefitinib in 31 lifetime non-smokers with advanced adenocarcinoma of the lung using \([^{18}\text{F}]\text{FLT}\) (92). Gefitinib is an inhibitor of EGFR with a response rate of 9-19% seen in previously treated advanced NSCLC. This study
compared baseline [\(^{18}\)F]FLT PET-CT with a repeat scan after 7 days of treatment and response evaluation with CT after 6 weeks of gefitinib. Responders had a significant decrease in tumour uptake of [\(^{18}\)F]FLT after 7 days of treatment (SUV\(_{\text{max}}\) = 3.2 ± 1.0 vs. 2.0 ± 0.7; \(P < 0.001\)). However in the non-responders there was no significant change in tumour uptake of [\(^{18}\)F]FLT. There was no significant difference in the change in SUV\(_{\text{max}}\) between those with stable versus progressive disease. The [\(^{18}\)F]FLT “responders” who showed a significant decrease in tumour uptake of [\(^{18}\)F]FLT on day 7 had a median time-to-progression of 7.9 months compared with 1.2 months for the [\(^{18}\)F]FLT “non-responders”. However the median overall survival between each group was not statistically significant, which may be due to the small study size and the additional treatment given after progression on gefitinib. The authors also suggested that the presence of tumour necrosis might cause an underestimation tumour uptake of [\(^{18}\)F]FLT. The study shows that the responders can be easily defined, but the inability to distinguish those with stable versus progressive disease raises some uncertainty about its clinical value.

A laboratory study used [\(^{18}\)F]FLT and [\(^{18}\)F]FDG to assess early response to another EGFR inhibitor erlotinib (93). Cell lines which expressed mutations making them sensitive to erlotinib, were compared to resistant controls. After 24 hours of exposure to erlotinib the cells in culture were arrested in the G1 phase of the cell cycle, with a decrease in the number of cells in the S phase, which soon led to significant cell death by apoptosis. No changes were seen in the resistant cell population. A nude mouse tumour xenograft model was then transplanted subcutaneously with the same cell lines. Erlotinib was given orally and imaging was performed with [\(^{18}\)F]FLT PET-CT and [\(^{18}\)F]FDG PET-CT. In EGFR-sensitive tumours the uptake of [\(^{18}\)F]FLT was dramatically reduced after 48 hours (mean reduction 38.8%, \(p = 0.04\)). In contrast the uptake of [\(^{18}\)F]FDG decreased slightly and was not statistically significant (\(p = 0.13\)). However Scheffler et al showed that [\(^{18}\)F]FLT PET-CT did not demonstrate any advantages over [\(^{18}\)F]FDG PET-CT in the prediction of outcome in 40 metastatic patients receiving first-line erlotinib for NSCLC (94). Prior to treatment the median SUV\(_{\text{max}}\) was 6.6 for [\(^{18}\)F]FDG and 3.0 for [\(^{18}\)F]FLT, a comparable ratio to that demonstrated in other trials (79, 95). A lower tumour uptake for [\(^{18}\)F]FDG (SUV\(_{\text{max}}\) < 6.6) was associated with a longer median overall survival (16.3 months (7.1 – 25.4 months)) compared to those with a higher tumour uptake (SUV\(_{\text{max}}\) > 6.6) significantly with a decreased survival (3.1 months (0.6 – 5.5 months)), \(P < 0.001\) (94). A lower tumour uptake of [\(^{18}\)F]FLT (SUV\(_{\text{max}}\) < 3.0) was associated with a longer median overall survival (10.3 months (0-23.3 months)) compared to those with a higher tumour uptake (3.4 months (0-8.1 months)), \(p = 0.027\). Both [\(^{18}\)F]FLT and [\(^{18}\)F]FDG were found to be prognostic on univariate analysis, but only [\(^{18}\)F]FDG was an independent prognostic factor in multivariate analysis, suggesting a lack of tumour specificity of [\(^{18}\)F]FLT (94).
1.6 Rationale and objectives

Despite the development of new systemic drugs, targeted agents and technological advances in radiotherapy the outcome from lung cancer remains poor and there has been little improvement in overall survival rates. These studies aim to evaluate methods of personalising radical radiotherapy in non-small cell lung cancer (NSCLC). This will be achieved by evaluating new drugs in combination with radical radiotherapy, using novel imaging approaches to assess tumour response during radiotherapy treatment and assessing the feasibility of a new “Isotoxic” individualised radiotherapy protocol.

Thesis objectives:

Chapter 2) Isotoxic Intensity Modulated Radiotherapy (IMRT) in stage III non-small cell lung cancer (NSCLC) – A feasibility study

- Primary objective
  - to assess the feasibility of delivering isotoxic IMRT to a dose of > 60 Gy equivalent dose in 2Gy fractions (EQD2)

- Secondary objectives
  - assessing the suitability of the study population for isotoxic IMRT
  - acceptability of isotoxic IMRT amongst patients
  - estimation of patients with acute grade 3 or more non-haematological toxicity
  - estimation of local control and overall survival
  - the development of a robust QA process for lung IMRT

Chapter 3) Phase I trial evaluating MEK inhibitor selumetinib with concomitant thoracic radiotherapy in non-small-cell lung cancer

- Primary objective
  - to determine the recommended phase II dose (RP2D) of selumetinib in combination with thoracic RT

- Secondary objectives
  - collect data on the safety profile and dose delivery
  - overall response rate, by response evaluation criteria in solid tumours (RECIST) (16) and local control by Green criteria (17), of selumetinib in combination with thoracic RT.

Chapter 4) Evaluation of $^{18}$FFLT uptake in patients with NSCLC treated with the MEK inhibitor Selumetinib concomitant with radical thoracic radiotherapy (within the Phase 1 MEKRT trial)

- evaluation of $^{18}$FFLT as a biomarker for response to MEKi and RT
- assess correlation between $^{18}$FFLT PET-CT parameters and survival.
### 1.7 References – Chapter One


91. Stroobants S, Goeminne J, Seegers M, Dimitrijevic S, Dupont P, Nuys J, et al. 18FDG-Positron emission tomography for the early prediction of response in advanced soft tissue


Hyperfractionated and accelerated radiotherapy in non-small cell lung cancer

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ABSTRACT
Radical radiotherapy plays a major role in the treatment of non-small cell lung cancer (NSCLC) due to the fact that many patients are medically or surgically inoperable. Advances in technology and radiotherapy delivery allow targeted treatment of the disease, whilst minimizing the dose to organs at risk. This in turn creates an opportunity for dose escalation and the prospect of tailoring radiotherapy treatment to each patient. This is especially important in patients deemed unsuitable for chemotherapy or surgery, where there is a need to increase the therapeutic gain from radical radiotherapy alone. Recent research into fractionation schedules, with hyperfractionated and accelerated radiotherapy regimes has been promising. How to combine these new fractionated schedules with dose escalation and chemotherapy remains open to debate and there is local, national and international variation in management with a lack of overall consensus. An overview of the current literature on hyperfractionated and accelerated radiotherapy in NSCLC is provided.

KEYWORDS
Accelerated radiotherapy; hyperfractionated radiotherapy; non-small cell lung cancer (NSCLC)

Introduction
Lung cancer is a major public health concern worldwide. Progress in improving 5-year survival is lagging behind comparable survival rates in other common cancers. Population-based lung cancer registry data analysis shows only a minimal increase in survival from 7.1-6% between 1995-1999 to 8.1-8% between 2005-2007 (1).

The majority of patients with locally advanced non-small cell lung cancer (NSCLC) are not suitable for surgical resection, often due to pre-existing co-morbidities and poor performance status. The international standard of care is concurrent chemo-radiotherapy which is associated with a 5-year survival of 20-30% and a median survival of 17-28 months (2-6). Due to the potential toxicity of concurrent chemo-radiotherapy patient selection is important. Patients with a good performance status, without major co-morbidities and assuming an acceptable radiation dose to normal tissues are eligible for this intensive treatment (7,8). Alternative treatment options are sequential chemo-radiotherapy or radiotherapy alone. Radiotherapy alone is associated with a 5-year survival of less than 5% due to local, regional and distant relapse. Local control with standard 3D conformal radiotherapy remains poor, with reported two years loco-regional control rates of 20-44% (9-11).

However, recent studies have shown that better local control of lung cancer can lead to an improvement in overall survival (10), prompting interest in altering radiotherapy delivery regimes. High dose stereotactic ablative body radiotherapy typically delivering >100 Gy biologically effective dose (BED) in 3-8 fractions is associated with very high in-field local control rates, but such doses cannot be delivered safely to locally advanced tumours due to the proximity of organs at risk such as the proximal bronchial tree, heart and spinal cord. A gap between radiation fractions allows recovery of damage in normal tissues and may also increase the sensitivity of the tumour cells to radiation by processes such as reoxygenation (12). If the individual fraction size is reduced and the fractions delivered closer together (e.g., twice daily), it may be possible to increase...
the dose without detriment to normal tissues.

One of the strategies to improve local control is dose escalation. Evidence gathered from the standard radiation schedules utilised in NSCLC over the past 40 years have confirmed the importance of total dose as a factor in tumour response (13). These schedules often use a single treatment of 1.8-2.0 Gy fractions per day over 5 days per week for a period of 5-7 weeks.

The RTOG 0617 study has evaluated dose escalation in the context of standard fractionation (2 Gy/day) and concurrent chemo-radiotherapy (5). Unfortunately the study was closed early due to futility indicating the absence of a survival benefit to high dose radiotherapy (74 Gy in 37 fractions delivered over 7.5 weeks) compared to standard dose (60 Gy in 30 fractions delivered over 6 weeks) (5).

An alternative approach to increasing the biological tumour dose in NSCLC is to develop new fractionation regimes, most commonly by hyperfractionation or acceleration. Hyperfractionation is a radiation treatment in which the total dose of radiation delivered is divided into smaller doses and treatments are given more than once a day (typically 2-3 a day). Acceleration means radiation treatment in which the total dose of radiation is given over a shorter period of time (fewer days) compared to standard radiation therapy. A recent meta-analysis by Mauguen and co-workers, evaluated ten trials including 2,000 patients and concluded that modifying the radiotherapy schedule by hyperfractionation, acceleration or both resulted in an increase in overall survival (14). The use of modified radiotherapy led to a 12% reduction in the risk of death (P=0.009). The absolute increase in overall survival in the NSCLC patients was by 3.8% at three years and 2.5% at five years, improving the survival rate from 15.9% to 19.7% at three years and from 8.3% to 10.8% at five years (14). Modified radiotherapy increased the risk of acute severe oesophagitis from 9% to 19% (P<0.001), and as expected the most accelerated regimes were associated with the most severe toxicity. However, at least 90% of patients completed the planned radiotherapy, with compliance in the experimental arms similar to the control arms. A summary of both hyperfractionation and acceleration is presented below.

### Hyperfractionation

Early clinical trials evaluating hyperfractionation in the late 1980’s and early 1990’s investigated the benefit of adding chemotherapy to radiotherapy. The RTOG 8808-ECOG 4588 randomised 458 patients to two months of induction chemotherapy with cisplatin and vinblastine, followed by conventional radiotherapy (60 Gy in 2 Gy per fraction), or radiotherapy alone, with either the same radiotherapy regime or a hyperfractionated regime of 1.2 Gy per fraction delivered twice daily to a total dose of 69.6 Gy (15,16). This study showed that patients receiving induction chemotherapy did best, with a median survival of 13.2 months and a 5-year overall survival of 8% (P=0.04). Although the twice-daily radiation arm performed slightly better compared with the conventional radiation arm, the difference was not statistically significant (median survival 12 vs. 11.4 months, 5-year overall survival 6% vs. 5%).

The trials evaluating hyperfractionated radiotherapy are summarised in Table 1. One of these pivotal trials in demonstrating the advantage of concurrent over sequential chemo-radiotherapy was the RTOG 9410 study (17). It also addressed the important question of overall treatment time in the management of stage III NSCLC. This 3-arm study randomised patients to sequential chemo-radiotherapy with cisplatin/vinblastine followed by radiotherapy (60 Gy in 30 fractions of 2 Gy over six weeks) beginning on day 50 (arm 1); concurrent chemo-radiotherapy with combination cisplatin/vinblastine and the same radiotherapy beginning on day 1 (arm 2); vs. concurrent chemo-radiotherapy using combination cisplatin/etoposide with hyperfractionated radiotherapy beginning on day 1 (69.6 Gy in 58 fractions of 1.2 Gy twice daily, over six weeks) (arm 3).

Table 1. Description of included trials using hyperfractionation radiotherapy schedule in non-small cell lung cancer.

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. patients randomised</th>
<th>Inclusion period</th>
<th>RT dose/no. of fractions</th>
<th>Duration (weeks)</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTOG 8808-ECOG 4588 (15,16)</td>
<td>326</td>
<td>1989-1992</td>
<td>Control arm: 60 Gy/30, Experimental arm: 69.6 Gy/38</td>
<td>2 Gy OD, 1.2 Gy BID</td>
<td>6</td>
</tr>
<tr>
<td>RTOG 9410 (17)</td>
<td>610</td>
<td>1994-1998</td>
<td>Study 1: 63 Gy/34, Study 2: 63 Gy/34</td>
<td>1.8 Gy x 25, 2.0 Gy x 9 OD, 1.8 Gy x 25, 2.0 Gy x 9 OD</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Study 3: 69.6 Gy/58</td>
<td>1.2 Gy BID</td>
<td>6</td>
</tr>
</tbody>
</table>

Abbreviations: RT, radiotherapy; BID, RT given twice a day; ECOG, Eastern Cooperative Oncology Group; No, number; OD, RT given once a day; RTOG, Radiation Therapy Oncology Group.
Phase II data suggested that the hyperfractionated regimen in arm 3 would be superior (17). However survival in the RTOG 9410 study was actually higher for patients treated with the concurrent regimen with once-daily radiotherapy (arm 2) compared with the concurrent regimen using twice-daily radiotherapy (arm 3) (P=0.046) (17). Median survival times were 14.6%, 17% and 15.6%, with five years survival of 10%, 16% and 13% for arms 1-3, respectively (P=0.046). This trial highlighted that dose escalation by a hyperfractionation regimen delivered over a standard overall treatment time does not improve survival. In addition the results supported the use of concurrent chemo-radiotherapy with conventional fractionation, which has since become the gold standard treatment in good performance status stage III patients (3).

**Accelerated hyperfractionation**

**Three fractions per day regime**

Treatment using continuous hyperfractionated accelerated radiotherapy (CHART) was shown to be of significant benefit by improving local control and overall survival (18, 19). The randomised trial recruited 563 patients, PS 0-1, medically inoperable, and compared CHART (54 Gy in 36 fractions of 1.5 Gy 3 times per day over 12 consecutive days) to conventionally fractionated radiotherapy (60 Gy in 30 once daily fractions of 2 Gy over six weeks). As anticipated the main toxicity during treatment was dysphagia, which was more severe in the CHART patients, with 19% experiencing severe dysphagia, compared with 3% in the conventional group. Overall there was a 24% reduction in the relative risk of death in the CHART arm and the overall survival rates were significantly higher: 30% vs. 21% at two years and 12% vs. 7% at five years respectively for the CHART and conventional radiotherapy arm (P=0.004) (18, 19). On subgroup analysis, CHART demonstrated an even greater improvement for squamous cell carcinomas, with an overall survival at three years of 21% compared with 11% for the conventional regime (P=0.0007). This evidence suggests that reducing overall treatment time in an effort to reduce tumour repopulation plays a key role in tumour control and treatment of NSCLC. Meanwhile, it should be noted that (i) the control arm of CHART would not be considered standard current standard of care as chemotherapy is not delivered with radiotherapy (either sequentially or concurrently) and (ii) a large percentage of patients had stage I-II disease (36%) who would nowadays be considered for a surgical approach or in some cases stereotactic ablative body radiotherapy. Despite the overall benefit seen with hyperfractionated accelerated radiotherapy in the CHART trial, this has not become standard practice. Recently published data gathered from a survey of UK clinical oncologists (20), revealed 55 Gy in 20 daily fractions as the commonest fractionation schedule for NSCLC in the UK, followed by 66 Gy in 33 daily fractions. Only 14/50 centres offered CHART despite the National Institute for Health and Clinical Excellence (NICE) recommending CHART as highly cost-effective (21). It is widely recognised that the schedule is demanding for patients and requires flexible and ad hoc radiotherapy department staffing willing to work extended day. If patients are unable to travel this treatment often necessitates a 12-day inpatient stay.

Between 1991 and 1994, Fu et al. conducted a phase I/II trial evaluating hyperfractionated accelerated radiation therapy (HART) which was published as a comparative cohort study. HART was delivered by 1.1 Gy per fraction, three fractions per day at intervals of four hours with five treatment days per week (22). The clinical disease was irradiated to 74.3 Gy delivered in 66-69 fractions over 33 days (not corrected for lung density), and the subclinical disease to 50.0 Gy delivered in 44-46 fractions over 33 days. There were 60 patients in the HART group and their survival and local control results were compared to those of 50 patients treated by conventional fractionated irradiation during the same period. Survival and local control were improved in the HART group. Three-year survival was 28% vs. 6% (P<0.001). Three-year local control was 29% vs. 5% (P=0.008). Median survival for HART was 22.6 months compared with 14.0 months for standard radiotherapy patients (P<0.05).

The evolving evidence in favour of concurrent chemo-radiotherapy led to the premature closure of a number of clinical trials evaluating accelerated and hyperfractionated regimen. The trials which evaluated both these fractionation schedules as the primary treatment modality are summarised in Table 2. The ECOG 2,597 trial was closed in June 2001 when 141 patients had been recruited, reaching 42% of the overall target (25). This trial randomly assigned stage III NSCLC patients to induction chemotherapy followed by standard thoracic radiotherapy (64 Gy, 2 Gy once daily over 6.5 weeks), vs. induction chemotherapy followed by HART (57.6 Gy, 1.5 Gy in three daily fractions over 2.5 weeks, with weekend breaks). Although not statistically significant there was an improvement in survival with HART (20.3 vs. 14.9 months; P=0.28).

The CHART schedule was logistically difficult for radiotherapy departments to implement due to the additional weekend and evening treatments. This led to the CHARTWEL-trial evaluating hyper-fractionated accelerated radiotherapy which omitted weekend treatments (24). The CHARTWEL-trial compared 60 Gy in 1.5 Gy fractions, delivered 3 times per day, on the 5 weekdays, over an average of 17 days vs. conventional
### Table 2. Description of included trials using acceleration or hyperfractionation radiotherapy schedules in non-small cell lung cancer.

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. patients randomised</th>
<th>Inclusion period</th>
<th>RT dose/no. of fractions</th>
<th>Dose per fraction</th>
<th>Duration (weeks)</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ball 1999 (23)</td>
<td>204</td>
<td>1989-1995</td>
<td>Control arm: 60 Gy/30</td>
<td>2 Gy OD</td>
<td>6</td>
<td>+/- concurrent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experimental arm: 60 Gy/30</td>
<td>2 Gy BID</td>
<td>3</td>
<td>+/- concurrent</td>
</tr>
<tr>
<td>CHART (18,19)</td>
<td>563</td>
<td>1990-1995</td>
<td>Control arm: 60 Gy/30</td>
<td>2 Gy OD</td>
<td>6</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experimental arm: 54 Gy/36</td>
<td>1.5 Gy TID</td>
<td>1.5</td>
<td>None</td>
</tr>
<tr>
<td>Fu 1997 (22)</td>
<td>69</td>
<td>1991-1994</td>
<td>Control arm: 60-64 Gy/22-34</td>
<td>1.8-2.0 Gy OD</td>
<td>7</td>
<td>Adjuvant or none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experimental arm: 74.3 Gy/66-69</td>
<td>1.1 Gy TID</td>
<td>6.5</td>
<td>Adjuvant or none</td>
</tr>
<tr>
<td>CHARTWEL-trial (ARO 97-1) (24)</td>
<td>406</td>
<td>1997-2005</td>
<td>Control arm: 66 Gy/33</td>
<td>2 Gy OD</td>
<td>6.5</td>
<td>Induction or none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experimental arm: 60 Gy/40</td>
<td>1.5 Gy TID</td>
<td>2.5</td>
<td>Induction or none</td>
</tr>
<tr>
<td>ECOG 2597 (25)</td>
<td>119</td>
<td>1998-2001</td>
<td>Control arm: 64 Gy/32</td>
<td>2 Gy OD</td>
<td>6.5</td>
<td>Induction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experimental arm: 57.6 Gy/36</td>
<td>1.6 Gy TID</td>
<td>2.5</td>
<td>Induction</td>
</tr>
<tr>
<td>Nyman 2009 (26)</td>
<td>152</td>
<td>2002-2005</td>
<td>Control arm: 60 Gy/30</td>
<td>2 Gy OD</td>
<td>6</td>
<td>Induction &amp; concurrent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control arm: 60 Gy/30</td>
<td>2 Gy OD</td>
<td>6</td>
<td>Induction &amp; concurrent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experimental arm: 64.6 Gy/38</td>
<td>1.7 Gy BID</td>
<td>4.5</td>
<td>Induction &amp; concurrent</td>
</tr>
<tr>
<td>Van Baardwijk 2012 (27)</td>
<td>137</td>
<td>2006-2009</td>
<td>Total dose 51-69 Gy</td>
<td>1.5 Gy BID</td>
<td>3</td>
<td>Concurrent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Study dose: phase 1 45 Gy/30</td>
<td>2 Gy OD for</td>
<td>3-4</td>
<td>Concurrent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Study dose: phase 2 iso-toxic</td>
<td>remainder</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CHART, Continuous Hyperfractionated Accelerated Radiation Therapy; CHARTWEL, CHART Week-End Lung, ECOG, Eastern Cooperative Oncology Group; No, Number; RT, Radiotherapy; OD, RT given once a day; RTOG, Radiation Therapy Oncology Group; BII, RT given twice a day; TID, RT given three times a day.

### Treatment of 66 Gy in 33 fractions delivered once daily over 45 days

The study found no significant difference between the two arms, with two years survival rates of 32% in the conventional arm and 31% in the CHARTWEL arm (P=0.43). However, this study confirmed the importance of a time factor in this disease as the lower total dose in the CHARTWEL arm was compensated by the shorter overall treatment time.

Another strategy is to dose escalate CHART. Continuous hyperfractionated accelerated radiotherapy escalated dose (CHART-ED) was a multi-centre phase I feasibility study which completed recruitment in September 2012. It compared dose-escalated CHART, adding twice daily fractions after completion of 54 Gy in 36 fractions over 12 days (28). Patients were treated on day 15 in group 1 (total dose 57.6 Gy in 38 fractions), days 15-16 in group 2 (total dose 61.2 Gy in 40 fractions) and days 15-17 in group 3 (total dose 64.8 Gy in 42 fractions). The incidence and grade of potentially dose-limiting toxicities will be assessed to determine whether dose escalation of around 6-10 Gy using this approach is safe, and the data is currently awaited.

### Two fractions per day regime

An Australian study by Ball et al. used a 2x2 factorial design to evaluate shortening of the overall treatment time and the addition of carboplatin in patients with inoperable NSCLC (23). The trial randomised 204 patients between conventional radiotherapy (60 Gy in 30 fractions, once daily over six weeks) or accelerated radiotherapy (60 Gy in 30 fractions, twice daily, over three weeks) with or without concurrent carboplatin chemotherapy. Oesophageal toxicity was significantly higher in the three week radiotherapy arms and no significant survival difference between the groups was found.

Between June 2002 and May 2005 152 patients with stage III NSCLC, PS 0-1 were randomised in a Swedish 3-arm
(A, B and C) phase II study by Nyman et al. (26). All arms started with two cycles of induction chemotherapy (carboplatin/paclitaxel), a third cycle was given concomitant with the start of accelerated radiotherapy in arm A (64.6 Gy in 1.7 Gy twice-daily fractions over 4.5 weeks), while in the remaining arms (B and C) conventional radiotherapy (60 Gy in 2 Gy daily fractions over 6 weeks) was combined with daily or weekly chemotherapy. Toxicity for all arms was similar and manageable with 12% grades 3-4 esophagitis, 1% grades 3-4 pneumonitis (all arms combined). Median survival was 17.8 (14.4–23.7) months (17.7, 17.7 and 20.6 months for A, B and C respectively). The 1-, 3- and 5-year overall survival was 63%, 31% and 24%. This study demonstrated that similar survival results could be achieved by intensifying treatment with either accelerated fractionated radiotherapy or concomitant chemo-radiotherapy.

Between 1995 and 2003 the German Lung Cancer Co-operative Group (GLC50) evaluated the role of accelerated hyperfractionated chemo-radiotherapy regimes in the pre-operative setting (29). The trials which included this fractionation schedule in the neoadjuvant setting are summarised in Table 3. 558 patients with stage IIIA-IIIB NSCLC were randomised between pre-operative chemo-radiotherapy and chemotherapy alone. In the control arm three cycles of cisplatin and etoposide chemotherapy were delivered followed by surgical resection, then adjuvant radiotherapy at 1.8 Gy daily fractions, the total dose dependent on surgical resection margins (54 Gy for negative margins, 64.4 Gy for positive margins). In the experimental arm the same induction chemotherapy was delivered, but followed by concurrent chemo-radiotherapy 45 Gy at 1.5 Gy twice daily fractions with carboplatin and vindesine, prior to surgical resection. If the margins were negative no further radiotherapy was given. But in the presence of positive margins, additional radiotherapy of 24 Gy at 1.5 Gy twice daily fractions was delivered. Pneumonectomies were performed in 35% of the patients in each group, with an increase in treatment-associated mortality seen in the experimental arm. Overall a similar number of patients underwent surgery, with a slightly higher complete resection rate in the experimental arm of 37% compared with 32% in the control arm. However there was no difference in progression-free survival, the primary endpoint of this trial (29).

Pötgen et al. also evaluated neo-adjuvant accelerated hyperfractionated chemo-radiotherapy. In an observational study, 239 patients with stage III NSCLC were treated with neoadjuvant radiochemotherapy using either accelerated hyperfractionation (45 Gy in 1.5 Gy twice-daily fractions over three weeks) or conventional fractionation (46 Gy in 2 Gy once daily fractions over 4.5 weeks) prior to thoracotomy (30). The crude pathological complete response (pCR) rates of 37% and 24% were seen in the accelerated hyperfractionated group and conventional fractionated group respectively, with a significant relationship between pCR rates and the BED suggesting an improvement in local effectiveness of accelerated hyperfractionation in lung cancer.

This accelerated regimen was further evaluated in a prospective trial by the same group in stage III NSCLC patients not deemed resectable, mainly stage IIIb (31). After three cycles of induction chemotherapy (cisplatin/paclitaxel) concurrent chemoradiotherapy was delivered (accelerated hyperfractionated, 45 Gy in 1.5 Gy twice daily fractions over three weeks, with cisplatin/vinorelbine). Once 45 Gy was reached, a multidisciplinary
panel decision was made regarding operability. Inoperable patients received definitive radiotherapy (total dose 65 or 71 Gy, depending on the mean lung dose) with additional concurrent chemotherapy (cisplatin/vinorribine). The majority (21 of 28 patients) received 71 Gy. Oesophagitis Grade 3+ was observed in 18% and pneumonitis Grade 3+ in 4% of the patients. At three years, the loco-regional control rate was 52% (95% CI, 29.7-75%). In an exploratory analysis, those patients receiving 71 Gy had a loco-regional rate at two and three years of 74% (95% CI: 51.2-96.3%) and 63% (95% CI: 36.1-90.4%), while in those patients receiving the lower total dose (65 Gy), loco-regional control at two and three years was 18% (95% CI: 0.4-9.2%; P=0.001, Wilcoxon test), respectively. Overall survival at three years was 31% (95% CI: 12.50%) for all patients. This study led to the EUSPAT trial, a phase III multicentre study that compared induction chemotherapy followed by definitive concurrent chemo-radiotherapy to trimodality treatment (induction chemotherapy followed by concurrent chemo-radiotherapy followed by surgery). The study recently closed and results are awaited.

Given the evidence in favour of hyperfractionation and acceleration, this has been taken a step further with specifically tailored regimes. The MAASTRO group have pioneered the concept of "isoevic" radiotherapy allowing for individualised dose escalation in stage I-II patients based on dose delivered to organs at risk (such as lung and spinal cord), using hyperfractionated accelerated radiotherapy (32). In the first MAASTRO study 166 NSCLC patients (59% stage III) not suitable for concurrent chemo-radiotherapy received an individualised dose of radiotherapy alone or after induction chemotherapy (55% of patients). Using 3D conformal therapy, the total dose delivered was between 50.4-79.2 Gy (delivered within an accelerated schedule of 1.5 Gy twice daily). With a median follow-up of 31.6 months, the median overall survival was 21.0 months—95% CI, 15.8 to 26.2 months, (stage IIA 16.2 months—95% CI, 7.6 to 24.8 months; stage IIIA, 17.2 months—95% CI, 8.4 to 26.0 months) with a 2-year overall survival of 45.0%. Only eight patients (4.8%) developed acute grade 3 dysphagia. Less than 10% of patients with stage III received the maximum dose as per protocol of 79.2 Gy.

A further MAASTRO study, evaluated the same strategy in the concurrent setting (27), only in stage III NSCLC patients. One hundred and thirty seven patients were included in this phase II study and treated with 3D conformal radiotherapy. The individually prescribed dose was based on mean lung dose of 19 Gy, spinal cord dose of 54 Gy, brachial plexus dose of 66 Gy and central mediastinal structure dose of 74 Gy. A total dose between 51 and 69 Gy was delivered in 1.5 Gy twice daily up to 45 Gy, followed by 2 Gy once daily and radiotherapy was started at the 2nd or 3rd course of chemotherapy. The median dose was 65.016.0 Gy delivered in 35±5.7 days. With a median follow-up of 30.9 months, the median overall survival was 25.0 months (95% CI: 19.8-30.3 months) and 2-year overall survival 52.4%. Thirty five patients (25.5%) developed G3+ dysphagia.

It should be noted that patients in the two MAASTRO group studies were treated with 3D CRT, probably limiting individualised dose escalation. The use of Intensity Modulated Radiotherapy (IMRT) could potentially allow for further dose escalation. IMRT modulates the intensity profile of radiation delivered to the patient, permitting improved targeting of the radiation dose, and in the thorax leads to a reduction in dose to organs at risk. This could therefore lead to increased tumour control probability yet with the same normal tissue complication probability (33). A planning study by The Christie using IMRT and twice daily fractionation for stage II/III NSCLC showed that this had potential to allow a further individual dose escalation in this group of patients (34). The starting point for dose escalation in this study was 55.8 Gy in 1.8 Gy per fraction delivered twice daily. The number of fractions was then increased until one or more organ at risk (OAR) tolerance dose was exceeded or a maximum dose of 79.2 Gy (i.e., 44 fraction of 1.8 Gy BD) was reached. IMRT allowed a significant dose increase in comparison to other methods (P=0.0001) while no difference was found between 3D conformal planning and inverse planning (P=0.06).

This regime will be assessed in a UK feasibility multicentre study of isoevic hyperfractionated accelerated radiotherapy in stage III NSCLC patients not suitable for concurrent chemo-radiotherapy (ClinicalTrials.gov Identifier: NCT01836692). If isoevic IMRT is proven to be feasible this regimen will be compared to standard sequential chemo-radiotherapy in a national phase II "pick-the-winner" trial alongside three other dose-escalated regimens currently being evaluated in the UK.

The use of concurrent chemo-radiotherapy with accelerated hyperfractionated schedules is compromised by high rates of acute mucosal toxicity which can be challenging for both patient and clinicians, however these side effects are usually transient and resolve within a few weeks of completion of radiotherapy. The Bortfeld group have raised the interesting issue that the optimal fractionation schedule (hyperfractionated vs. hyperfractionated) may depend on the OAR doses (35). For larger tumours, their model which minimizes maximum BED within a serial organ suggests hyperfractionation. Thus, accelerated hyperfractionation may eventually turn out as an ideal alternative to pure dose-escalation in locally advanced NSCLC and should deserve further evaluation within properly designed randomised trials.
Conclusions

There is significant evidence that prolonging the overall treatment time can allow cancer stem cells to repopulate, and thus be detrimental to disease outcome (36). CHART has shown improved survival over standard radiotherapy, in patients with inoperable stage I-II NSCLC. Selected patients (with ECOG performance status ≤2 who do not fit the criteria for sequential or concurrent chemotherapy or patients who prefer radiotherapy only) may be considered for CHART (7,8).

Within the field of thoracic oncology evidence is emerging to suggest that an accelerated hyperfractionated radiotherapy schedule may be superior to conventional treatment. We believe that such treatment should be closely combined with other strategies in order to improve local control and survival. Dose escalation and individualised radiation doses facilitated by the use of IMRT should be combined in order to increase local control and survival. This is an exciting time for thoracic radiotherapy with these developments leading towards the goal of personalised treatment.

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References


Chapter Two

Isotoxic Intensity Modulated Radiotherapy in stage III non-small cell lung cancer – A feasibility study
2 Isotoxic Intensity Modulated Radiotherapy in stage III non-small cell lung cancer – A feasibility study

2.1 Introduction

The current 5-year survival of stage III NSCLC with standard treatment is approximately 20-30% at best (1, 2) highlighting the urgency to improve outcomes. Concurrent chemoradiotherapy (CTRT), chemotherapy and radiotherapy (RT) given at the same time, is the standard of care in stage III NSCLC, with median survival rates of approximately 28 months. However, the majority of patients are not suitable for this treatment due to poor performance status (PS) and comorbidities (3). In a national survey of concurrent CTRT practice within the UK, it was estimated less than 30% of patients with stage III NSCLC were suitable for concurrent treatment (4). The alternative, sequential CTRT (chemotherapy given prior to radiotherapy), has inferior local control and survival rates (1). Local control with current RT doses delivered by standard three-dimensional conformal RT (3D-CRT) is poor, with local progression-free survival rates of about 30%. However, recent data has shown that improved local control in lung cancer can lead to improvement in survival (1).

We are now accelerating into an era of personalized medicine. However, to date, fixed doses of radiation are still delivered to patients, which do not account for volume of disease, stage of disease (IIIA vs. IIIB) or anatomical location within the thorax. Stage III NSCLC is a heterogeneous disease and there is a need to move away from ‘one-size-fits-all’ treatment approach. A strategy to improve local control is to escalate the dose of radiation delivered to the tumour. Martel et al (5) demonstrated a clear dose–response relationship in NSCLC, with 84 Gy using conventional fractionation required to achieve 50% probability of tumour control at 3 years.

However, a recent Radiation Therapy Oncology Group (RTOG) phase III study that randomised patients between 60 Gy in 30 daily fractions and 74 Gy in 37 daily fractions failed to demonstrate a survival advantage for the high-dose arm, indicating that dose escalation using conventional fractionation is not the way forward. Failure of the high-dose arm was likely multifactorial and in addition to prolonged overall treatment time may have resulted from a combination of poorer treatment delivery with fewer patients receiving concurrent chemotherapy, reduced compliance to RT, quality assurance issues and unreported treatment toxicity (6). Following the publication of RTOG 0617, 60 Gy delivered in 2 Gy per fraction is now considered standard treatment in patients with stage III NSCLC (7). Accelerated hyperfractionation has been studied in an attempt to reduce the overall treatment time and counteract repopulation in lung cancer. Modified fractionation (acceleration, hyperfractionation or both) has been shown to improve overall survival as
compared with conventional fractionation in NSCLC resulting in an absolute benefit of 3% at 5 years (8).

Intensity modulated radiotherapy (IMRT) modulates the intensity profile of the beam and increases conformity to the target. This technique reduces dose to organs at risk (OAR). As a result the dose delivered to the tumour can be escalated while keeping the dose to normal tissues within tolerance (9). Although IMRT is becoming standard for the treatment of lung cancer in large international academic centres, at the time our study opened, implementation of IMRT in the UK was not widespread.

The MAASTRO group has pioneered the concept of ‘isotoxic RT’ using individualised tailored dose escalation (10). They have shown with hyperfractionated, accelerated 3D-CRT that increasing the radiation dose to pre-specified normal tissue dose constraints could lead to increased tumour control probability with the same normal tissue complication probability (10, 11). In the MAASTRO group the mean dose delivered to patients with stage III disease was 61.2 Gy but less than 10% of patients received the maximum dose of 79.2 Gy in 44 fractions twice a day (10).

Given these promising results and the need to intensify treatment in the sequential CTRT setting we decided to set up a study investigating the feasibility of delivering isotoxic IMRT using a hyperfractionated accelerated schedule in stage III NSCLC patients.

2.1.1 Study contributions chapter 2

In this study I was involved in the day-to-day running of the trial at the Christie: recruiting patients, planning the radiotherapy treatment, reviewing patients on treatment and during follow up and completing the case report forms (CRF). I reviewed the serious adverse events (SAE) with the Chief Investigator, presented trial updates at the trial management group (TMG), interpreted and collated the data provided by the Clinical Trials Unit (CTU) and using the raw data created survival curves. I wrote the BMJ protocol paper (first author), and presented the data in a short oral presentation at the 18th World Conference on Lung Cancer in Japan in October 2017.

Trial management group contributions: Prof Corinne Faivre-Finn of the Christie NHS Foundation Trust and University of Manchester is the Chief Investigator of the Isotoxic IMRT trial, Principal Investigators who recruited patients at the other sites (Dr Matthew Hatton of Sheffield Teaching Hospitals NHS Foundation, Dr Gerard Hanna of Belfast Health & Social Care NHS Trust, Dr Kevin Franks of Leeds Teaching Hospitals NHS Trust, Dr Susan Harden of Cambridge University Hospitals NHS Foundation Trust, Dr Fiona McDonald of The Royal Marsden NHS Foundation Trust), radiotherapy quality assurance (RTTQA) by Nicki Groom,
and statisticians Linda Ashcroft and David Ryder collated the data from the completed CRF’s. Dr Paula McCloskey a clinical research fellow was involved in developing the Isotoxic IMRT atlas with Prof Corinne Faivre-Finn and Dr Ben Taylor Consultant Radiologist.

2.2 Methods

2.2.1 Study design and participants

The Isotoxic IMRT study was a prospective, multicentre, non-randomised feasibility study with early stopping rules, details of the trial design have been published (12). Patients were treated with individualised doses of radiation based on prespecified normal tissue doses (spinal cord, brachial plexus, lung tissue, heart and great vessels/proximal bronchial tree, Table 2-1) up to maximum of 79.2 Gy in 44 fractions. The study flow diagram is shown in Figure 2-1.
Patients were enrolled from 7 UK centres: Addenbrookes Hospital (Cambridge), Beatson Cancer Centre (Glasgow), The Christie NHS Foundation Trust (Manchester), Northern Ireland Cancer Centre (Belfast), the Royal Marsden (London), St James's Hospital (Leeds) and Weston Park Hospital (Sheffield). Eligible patients were aged 18 years or older; had inoperable stage III NSCLC (T3 N1-3, any T4, any N2-3) confirmed by histology/cytology and on PET scanning, +/- mediastinoscopy / thoracoscopy; Eastern Cooperative Oncology Group performance status 0–2 (PS 2 allowed if due to disease-related symptoms not co-morbidities); not suitable for concurrent CTRT; prior treatment with at least two cycles of...
platinum-based induction chemotherapy, ability to start RT within 5 weeks of the last cycle of chemotherapy.

Mandatory investigations prior to trial registration included: a contrast-enhanced CT scan of the thorax and upper abdomen (within 4 weeks prior to registration), contrast-enhanced CT (or MRI) brain scan (within 4 weeks prior to registration), fluorodeoxyglucose-positron emission tomography (FDG-PET) CT within 4 weeks prior to registration and lung function tests. Participants gave written informed consent and the study was done according to the Declaration of Helsinki and Good Clinical Practice Guidelines. The trial was reviewed in the UK by the National Research Ethics Service Committee, which granted ethics approval for the study on 08/08/2013. The protocol was also approved by the institutional review board at each study centre.

A radiotherapy planning scan using free breathing 4D-CT with intravenous contrast was mandatory to account for tumour motion (patients with a medical contraindication to contrast were not included in the study). Patients were planned and treated in the supine position, immobilised using either a chest board and fixed arm position above the head or a 5-point fixation shell for upper lobe tumours.

The gross tumour volume (GTV) was contoured depending on local practice to include either 1) the combined GTV exhale (defined on the maximum exhale 4D-CT dataset) and GTV inhale (defined on the maximum inhale 4D-CT dataset) 2) combined GTV from all phases of the 4D-CT dataset or 3) GTV as defined on the maximum intensity projection dataset (MIP). The GTV is defined as identifiable tumour and involved lymph nodes from cross-sectional imaging, using CT to define nodal involvement if nodes ≥ 1cm in short axis or PET positive lymph nodes (SUV > 3 if information on uptake of blood pool is not available). As induction chemotherapy was mandated, the GTV will include the post-chemotherapy tumour volume and the pre-chemotherapy lymph node volume.

The clinical target volume (CTV) comprised the GTV with a 5mm margin of radiologically normal tissue in all directions. In case of a complete remission of a lymph node, the whole anatomical area as defined by Chapet et al (13) will be included. Manual editing of the CTV was permitted to reduce the dose to the spinal cord e.g. when disease is adjacent to a structure such as a vertebra but is not though to invade the structure. Elective nodal irradiation was not permitted. The planning target volume (PTV) comprises the CTV with a 0.9cm margin superiorly and inferiorly and 0.7cm margin laterally. Editing of the PTV was not permitted. The RT planning guidelines and quality assurance (QA) document were provided as a reference to contour organs at risk (OAR). Treatment planning and optimisation of inverse planned IMRT was undertaken by an experienced dosimetrist / physicist in lung planning. The use of volumetric modulated arc therapy (VMAT) / Rapid Arc /
tomotherapy / fixed-beam IMRT was allowed in this study. The OAR tolerance doses were specified to a volume of 1cc, with the exception of a mean heart dose and mean lung dose (MLD) (see Table 2-1). At least 95% of the PTV should have received 90% (ideally 95%) of the prescribed dose and the mean dose to the CTV should be 100%. Hotspots should not exceed 107% of the prescribed dose within a 1 cc volume. The dose of radiation was increased until one or more of the OAR tolerances or the maximum dose of 79.2 Gy was reached. Radiotherapy was delivered twice-daily (a minimum 6-hour interval between fractions) on consecutive weekdays in 1.8 Gy per fraction over a maximum of 44 fractions.

The trial was subject to a RT Quality Assurance (QA) programme tailored to the technical requirements of lung IMRT. The QA programme for the study was coordinated by the National Cancer Research Institute (NCRI) Radiotherapy Trials QA (RTTQA) Group. The details of the programme can be found at the RTTQA website, http://www.rttrialsqa.org.uk. Prior to recruitment each centre delineated a benchmark case. The first patients’ RT plan from each centre was reviewed prospectively before a second patient was recruited. All RT plans were reviewed retrospectively by the RTTQA group to ensure adherence to the trial protocol.

Data was collected at each trial visit regarding any Serious Adverse Events (SAEs, as defined by Good Clinical Practice). All SAEs causally related to the RT treatment were reported to the Manchester Academic Health Science Centre Trials Coordination Unit and followed until they resolved or stabilised. Acute and late radiation toxicities continue to be recorded at each follow-up visit (according to the Common Terminology Criteria for Adverse Events (CTCAE) V.4.0 grading system).

Patients were followed-up for 2 years post-treatment (4 monthly in years 1 and 2). A late toxicity assessment was performed at each visit. CT scans were performed every 4 months for the first 2 years.

<table>
<thead>
<tr>
<th>Table 2-1 Pre-specified normal tissue doses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organ at risk</strong></td>
</tr>
<tr>
<td>Brachial plexus</td>
</tr>
</tbody>
</table>
| Heart | Max dose = EQD2 ≤ 76Gy  
Mean dose ≤ 46Gy |
| Lung | Mean lung dose (lung – GTV) ≤ 20Gy |
| Mediastinal envelope¹ | Max dose = EQD2 ≤ 76Gy |
| Spinal canal PRV | Max dose = EQD2 ≤ 50Gy |

¹ Comprising = heart, proximal bronchial tree, trachea, oesophagus and edited manually to include the blood vessels in the upper mediastinum. 
Abbreviations: PRV, planning organ at risk volume; EQD2, Equivalent dose in 2Gy fractions; GTV; gross tumour volume. 
2.2.2 Outcomes and statistics

The primary objective was the delivery of isotoxic IMRT to a dose of > 60 Gy equivalent dose in 2Gy fractions (EQD2). The secondary objectives included the suitability of the study population for isotoxic IMRT, acceptability of isotoxic IMRT amongst patients, estimation of recruitment rates, estimation of patients with acute grade 3 or more non-haematological toxicity, estimation of local control, estimation of overall survival and the development of a robust QA process for lung IMRT.

This feasibility study was two-stage (with early stopping rules) using the design of Bryant and Day with 85% power and 15% significance level for both completion (an acceptable rate of 90% and unacceptable rate of 70% of patients receiving > 60 Gy EQD2) and acute radiation pneumonitis rates an acceptable rate of grade 3 or more acute radiation pneumonitis of 8.5% and unacceptable rate of 22.5%) (14).

After the first 11 out of 35 patients were enrolled (stage I of the study) if less than 7 of 11 patients were planned to a dose > 60 Gy EQD2 or more than 3 of 11 patients had experienced grade 3 or more acute radiation pneumonitis the trial would be stopped. If three patients experienced grade 3 or more acute radiation pneumonitis before enrolment of the last patient in stage I, recruitment to stage II would only start when the last patient enrolled in stage I had reached 3 months follow-up. A negative study would be concluded if less than 27 of 35 patients were planned to a dose > 60 Gy EQD2 or more than 6 of 35 patients experienced grade 3 or more acute radiation pneumonitis.

2.3 Results

Between June 2014 and March 2016, 37 patients were enrolled from 7 UK centres, the baseline characteristics are shown in Table 2-2. Initial recruitment target was 35 patients in total, but two patients were replaced.
### Table 2-2 Patient characteristics

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>On trial (n = 35)</th>
<th>Off trial (n = 2) (unable to dose escalate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient 1</td>
<td>Patient 2</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
<td>18 (51%)</td>
<td>17 (49%)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>69.9</td>
<td>52 years</td>
</tr>
<tr>
<td>Mean (years)</td>
<td>46 – 86</td>
<td>48 years</td>
</tr>
<tr>
<td>Range (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOG PS</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>5 (14.2%)</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>27 (77.1%)</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3 (8.6%)</td>
<td>1</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td>IIb</td>
</tr>
<tr>
<td>IIIa</td>
<td>22 (62.9%)</td>
<td>IIla</td>
</tr>
<tr>
<td>IIIb</td>
<td>13 (37.1%)</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>Squamous</td>
<td>16 (45.7%)</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>14 (40%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Lung function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1 (litres)</td>
<td>(median/range)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.8 (0.7 – 3.4)</td>
<td></td>
</tr>
<tr>
<td>DLCO (% predicted)</td>
<td>66.8 (26.2 – 102)</td>
<td></td>
</tr>
<tr>
<td>GTV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (cm3)</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>Range (cm3)</td>
<td>8.2 – 260.7</td>
<td></td>
</tr>
<tr>
<td>PTV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (cm3)</td>
<td>330</td>
<td></td>
</tr>
<tr>
<td>Range (cm3)</td>
<td>146 – 807</td>
<td></td>
</tr>
</tbody>
</table>

Out of 37 patients, 2 (5.4%) failed to achieve a planned dose of EQD2 >60Gy due to large tumour size and inability to meet OAR constraints. Thus 35 out of 37 patients achieved an EQD2 > 60Gy and received treatment within the trial, see Table 2-2. Both patients who failed to achieve the planned dose received 55 Gy in 20 fractions once daily over 4 weeks. The median prescribed tumour dose for the 35 patients treated with dose>60 Gy EQD2 was 77.4Gy (61.2 – 79.2Gy) with the maximum dose of 79.2Gy delivered to 14 (37.8%) patients. In addition to the prescribed tumour dose, the doses delivered to the normal tissues are summarised in Table 2-3. All patients completed RT as scheduled, except in one patient for whom treatment was stopped due to disease progression after 8 fractions (4 days). See Figure 2-2 for a case example showing radiotherapy planning images displaying selected OAR’s and target volumes.
Figure 2-2 Case example of a 65 year old man with T2 N3 M0 adenocarcinoma of the left lung stage III B, radiotherapy dose of 7020 cGy in 39 fractions given. 1a – c) motion adapted gross tumour volume outlined in green, clinical target volume in purple, planning target volume in light blue, mediastinal envelope in red, brachial plexus in brown.
| Table 2-3 Prescribed tumour doses and normal tissue dosimetry in 35 patients treated with dose > 60 Gy EQD2 |

<table>
<thead>
<tr>
<th></th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PTV</strong></td>
<td></td>
</tr>
<tr>
<td>Prescribed dose (Gy)</td>
<td>77.4 Gy (61.2 – 79.2)</td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td></td>
</tr>
<tr>
<td>V5Gy (Lung – PTV)</td>
<td>63.2% (29.2 – 91.5)</td>
</tr>
<tr>
<td>V20Gy (Lung – PTV)</td>
<td>26.6% (14 – 41.4)</td>
</tr>
<tr>
<td>MLD (Lung – GTV)</td>
<td>18.5 Gy (6.8 – 20.0)</td>
</tr>
<tr>
<td><strong>Oesophagus</strong></td>
<td></td>
</tr>
<tr>
<td>V35Gy</td>
<td>28.6% (0 – 69)</td>
</tr>
<tr>
<td>V50Gy</td>
<td>21.3% (0 – 62.5)</td>
</tr>
<tr>
<td>V60Gy</td>
<td>17.4% (0 – 53.5)</td>
</tr>
<tr>
<td>Mean</td>
<td>21.5 Gy (8.0 – 44.4)</td>
</tr>
<tr>
<td>Max 1cc</td>
<td>74.1 Gy (21.2 – 78.6)</td>
</tr>
<tr>
<td><strong>Heart</strong></td>
<td></td>
</tr>
<tr>
<td>V5Gy</td>
<td>47.6% (8.2 – 100)</td>
</tr>
<tr>
<td>V30Gy</td>
<td>23.6% (0.1 – 93.1)</td>
</tr>
<tr>
<td>V40Gy</td>
<td>18.2% (3.4 – 63.5)</td>
</tr>
<tr>
<td>V50Gy</td>
<td>11.2% (0.9 – 34.7)</td>
</tr>
<tr>
<td>Mean</td>
<td>17.0% (1.4 – 79.1)</td>
</tr>
<tr>
<td>Max 1cc</td>
<td>77.6% (21.8 – 79.1)</td>
</tr>
<tr>
<td><strong>Other OAR’s</strong></td>
<td></td>
</tr>
<tr>
<td>Max 1cc Brachial plexus</td>
<td>4.51 Gy (0.6 – 72.4)</td>
</tr>
<tr>
<td>Max 1cc Mediastinal envelope</td>
<td>78.2 Gy (39.2 – 79.2)</td>
</tr>
<tr>
<td>Max 1cc Proximal Tree</td>
<td>77.2 Gy (20.1 – 79.0)</td>
</tr>
<tr>
<td>Max 1cc Trachea</td>
<td>65.6 Gy (1.12 – 79.2)</td>
</tr>
<tr>
<td>Max 1cc Spinal Canal</td>
<td>43.7 Gy (14.3 – 57.7)</td>
</tr>
<tr>
<td>Max 1cc Spinal Canal + 0.5 cm</td>
<td>49.4 Gy (14.9 – 59.1)</td>
</tr>
</tbody>
</table>

**Abbreviations:** PTV, Planning Target Volume; Gy, Gray; V5Gy/V20Gy/V35Gy/V50Gy/V60Gy the volume receiving more than 5 Gy; V20Gy/V35Gy/V50Gy/V60Gy respectively; MLD, mean lung dose; cc, cubic centimetre; cm, centimetre

### 2.3.1 Toxicity

The most common grade 3 acute adverse events included: dysphagia 1 (2.9%), dypsnoea 2 (5.7%), lung infection 3 (5.7%) radiation oesophagitis 2 (5.7%) and trachea-oesophageal fistula 1 (2.9%) (see Table 2-4). There were two G5 events: the first from acute radiation pneumonitis and the second from a bronchopulmonary haemorrhage, which were probable treatment related deaths. Both patients received the maximum dose of 79.2 Gy in 44 fractions and had similar volumes of disease (PTV 311cm$^3$ and 344cm$^3$). The first patient who died after 4 months from acute radiation
pneumonitis had a V20 of 23.5%, and MLD of 19.5 Gy. The second patient who died from a bronchopulmonary haemorrhage after 18.6 months had a V20 of 27.1% and a MLD of 15 Gy. Overall the G3 late toxicities included: fatigue 1 (2.9%), dyspnoea 3 (8.6%) and 1 (2.9%) case of late G4 lung infection (see Table 2-4).

Table 2-4 Toxicity

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Acute</th>
<th></th>
<th>Late</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchopulmonary haemorrhage</td>
<td>0</td>
<td>0</td>
<td>1 (2.9)</td>
<td>1 (2.9%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>31 (88.6%)</td>
<td>0</td>
<td>0</td>
<td>14 (40%)</td>
<td>1 (2.9%)</td>
<td>0</td>
</tr>
<tr>
<td>Weight loss</td>
<td>15 (42.9%)</td>
<td>0</td>
<td>0</td>
<td>8 (22.9%)</td>
<td>1 (2.9%)</td>
<td>0</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>20 (57.1%)</td>
<td>1 (2.9)</td>
<td>0</td>
<td>4 (11.4%)</td>
<td>1 (2.9%)</td>
<td>0</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>22 (62.9%)</td>
<td>2 (5.7)</td>
<td>0</td>
<td>15 (42.9%)</td>
<td>3 (8.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Radiation oesophagitis</td>
<td>23 (65.7%)</td>
<td>2 (5.7)</td>
<td>0</td>
<td>1 (2.86%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Radiation dermatitis</td>
<td>12 (34.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Radiation pneumonitis</td>
<td>5 (14.3%)</td>
<td>0</td>
<td>1 (2.9)</td>
<td>4 (11.4%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>5 (14.3%)</td>
<td>0</td>
<td>0</td>
<td>8 (22.9%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oesophageal stenosis</td>
<td>1 (2.9%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tracheo-oesophageal fistula</td>
<td>0</td>
<td>1 (2.9%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

2.3.2 Survival and local control

At the time of analysis the median follow-up was 25.4 (8.0 – 44.2) months for 11 out of 35 survivors. The 2-year overall survival (see Figure 2-3) was 33.6% with 95% CI (17.9, 50.1) and progression-free survival was 23.9% with 95% CI (11.3, 39.1) see Figure 2-3. The median survival was 18.1 months with 95% CI (13.9, 30.6).
2.3.3 Quality assurance

Treatment plans for each study patient were assessed within one week of the start of treatment. For the treatment planning benchmark case 2 out of the 7 centres (28.6%) were able to achieve the maximum dose/number of fractions (79.2Gy/44#). Dose escalation was limited in all cases by the dose to 1cc of the mediastinal envelope.

34 on trial treatment plans were reviewed, and QA reports sent to each centre. For all 34 plans the OAR dose constraints were met. Out of the 34 treatment plans two failed to meet one of the PTV criteria (the PTV D (Total Volume -1cc) ≥85%), but were within 2% of it. There were some deviations from the protocol for organ at risk outlining which was highlighted to appropriate centre.
2.4 Discussion

This study combines a number of strategies to intensify radiotherapy: personalised dose escalation, acceleration, and hyperfractionation, facilitated by the use of IMRT. The primary endpoint was met, as it was feasible to intensify treatment using Isotoxic IMRT with 35 out of 37 patients (95%) receiving dose escalated radiotherapy. The 1 year overall survival in Isotoxic IMRT of 75% and PFS at 1 year of 59% compares favourably to historical survival reported in sequential CTRT trials (1) and to other recently published studies including the standard arm of RTOG 0617 (PFS 49.2% at 1 year) and experimental arm of the Pacific study (PFS of 55.9% at 1 year).

Despite technological advances, a conventional radiotherapy fractionation of 60 Gy in 30 fractions and chemotherapy devised over 30 years ago, remains standard of care in stage III inoperable NSCLC (6, 7, 9, 15, 16). It is important that the potential survival benefit of dose intensification is balanced with the risk of treatment-related toxicity. RTOG 0617 which compared 60 Gy in 30 daily fractions to 74 Gy in 37 daily fractions concurrently with chemotherapy, showed a detrimental effect on survival in the high-dose arm. As an alternative to using conventional fractionation in NSCLC, Isotoxic IMRT along with other trials including IDEAL-CRT (isotoxic dose-escalated concurrent chemoradiotherapy) CHART-ED (continuous hyperfractionated accelerated radiotherapy without chemotherapy) and PLANET (dose escalation to 84Gy with concurrent chemotherapy) have investigated dose escalation schedules (17-19).

In our study despite the intensified regime used, the rate of grade 3-5 pneumonitis (3%) was comparable to the standard arm of RTOG 0617 and other intensified schedules (see Table 2-5). The incidence of grade ≥ 3 oesophagitis (6%) was the same as the IDEAL-CRT study (17), and less than RTOG 0617 (7), CHART-ED (18), and PLANET (19) (see Table 2-5). This can be explained in part by the fact that the chemotherapy was delivered sequentially in the Isotoxic IMRT study.
Table 2-5 Summary of the incidence of radiation pneumonitis and radiation oesophagitis from selected radiotherapy dose escalation clinical trials

<table>
<thead>
<tr>
<th></th>
<th>Isotoxic IMRT</th>
<th>IDEAL-CRT (17)</th>
<th>CHART-ED (18)</th>
<th>RTOG 0617 (7)</th>
<th>PLANET (19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation pneumonitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3–5</td>
<td>3%</td>
<td>4%</td>
<td>0</td>
<td>4%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>G3</td>
<td>0</td>
<td>4%</td>
<td>0</td>
<td>4%</td>
<td>0</td>
</tr>
<tr>
<td>G4</td>
<td>0</td>
<td>0</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>0</td>
</tr>
<tr>
<td>G5</td>
<td>3%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Radiation oesophagitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3-5</td>
<td>6%</td>
<td>6%</td>
<td>18%</td>
<td>7%</td>
<td>15%</td>
</tr>
<tr>
<td>G3</td>
<td>6%</td>
<td>6%</td>
<td>18%</td>
<td>7%</td>
<td>15%</td>
</tr>
<tr>
<td>G4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Results thus far suggest that dose escalation in thoracic radiotherapy might be limited by pneumonitis and oesophagitis (19), but other toxicities such as bronchopulmonary haemorrhage and the development of fistulas may be rarer, but related to severe morbidity and mortality (16, 20). A phase I trial delivering hypofractionated radiotherapy using IMRT up to doses of 85.5 Gy in 25 fractions, reported no cases of grade 3 radiation pneumonitis or oesophagitis, but was prematurely terminated following 5 treatment related deaths which included 3 from fatal haemoptysis (20). It is well documented that centrally located tumours are associated with a high risk of grade 5 haemoptysis (16, 20, 21). This phase 1 trial protocol did not include any OAR constraints for the proximal bronchial tree but demonstrated a dose-response relationship, with higher doses to the proximal bronchial tree resulting in severe late toxicity. In our study the maximum dose permitted to the mediastinal envelope was EQD2 ≤ 76 Gy. We report two treatment related deaths, one from acute pneumonitis and the other from a late bronchopulmonary haemorrhage, both recorded as probable treatment related deaths. One patient had a non-fatal G3 tracheoesophageal fistula. A treatment related death rate of 5.7% is comparable to other dose escalation studies (4.8% IDEAL-CRT (17)) and less than the 74Gy high dose arm of RTOG 0617 (7.5%)(7).

One of the strengths of the study is the radiotherapy quality assurance programme, which is crucial to the robustness of multicentre studies such as Isotoxic IMRT. The higher median prescribed tumour dose of 77.4 Gy (61.2 – 79.2 Gy) in Isotoxic IMRT compared to the median dose within the MAASTRO group of 65 Gy (51 – 69 Gy), can be explained by the mandatory use of IMRT. IMRT increases the accuracy of radiation delivery, improves dose conformity to the target whilst sparing the surrounding normal tissues. Before prospective data from RTOG 0617, the evidence for the use of IMRT in locally advanced NSCLC came from three retrospective publications from the MD
Anderson. In these unmatched patient cohorts, during a time period when PET staging was being introduced, this single centre experience was interpreted with a degree of caution but despite the lack of evidence lung IMRT was adopted worldwide in a secondary analysis of RTOG 0617, despite the IMRT group having larger planning treatment volumes and more stage IIIIB disease, survival outcomes were the same as those treated in the 3D-CRT group (22). In addition to toxicity data, quality of life (QOL) data was collected prospectively in RTOG 0617 using FACT Trial Outcome Index (FACT-TOI), showed that QOL was better in IMRT group despite some unfavourable prognostic factors (e.g. more stage 3B and larger volume of disease) (23). Furthermore the incidence of grade 3 radiation related toxicities of pneumonitis, cardiovascular and oesophagitis were less in the IMRT group (3.5%, 4.8%, 13.2%) compared to 3D-CRT (7.9%, 8.3%, 15.4%). The lung V20Gy did not differ between the two groups but the lung V5Gy was higher in the IMRT group. However all heart doses were significantly less in those receiving IMRT compared with 3D-CRT (V40 6.8 vs. 11.4 respectively).

An advantage of IMRT over 3D-CRT is that the dose to the heart can be reduced. Over the years dose constraints to the heart have been poorly defined and radiotherapy related cardiac toxicity is often underreported. More recently the evidence shows heart dose is significantly and independently associated with overall survival (7, 24-26) and cardiac events (27). The correlation between a higher mean heart dose and higher cardiac events was established by Dess et al evaluating 125 patients within 4 prospective radiotherapy trials (28). In RTOG 0617 the heart dose was higher in the high dose arm and on multivariate analysis of the survival data the higher heart V5Gy and V30Gy was associated with poorer survival (22). Historically the heart is contoured as a single organ, an imaging data mining study in our centre has shown the base of the heart, which received the highest doses of radiation, was associated with poorer survival and indicated patients receiving > 8.5Gy to the identified region had poorer survival (25).

In addition to state of the art image-guided intensity modulated radiotherapy, which allowed dose escalation up to OAR constraints, further strengths of this study include the multicentre setting, and the delivery of radiotherapy overseen by a robust QA programme. On the contrary, the main limitations are that of a single arm study, the recruitment of a heterogeneous group of stage III patients and potential implications of the imaging approach. A PET-CT was performed at baseline, however a standard contrast enhanced CT post chemotherapy was used as an assessment of disease response, and to guide radiotherapy planning. In comparison to PET-CT, a CT scan may underestimate the burden of disease, and therefore patients with undiagnosed disease progression or occult metastatic disease could have been included in the trial and negatively impacted upon survival. In the future this could be addressed by repeating the PET-CT for radiotherapy planning, as is the case in other dose escalation trials (29). In the case of stage III NSCLC the burden of, anatomical location (the location of tumour proximity to critical structures e.g. heart varies widely due to the large volume of the lungs), histological subtype, tumour and host genomics differ widely, which may influence the outcomes from trials (15).
The heterogeneity of stage III disease is one of the reasons why a “one size fits all” radiotherapy approach is failing to improve patient outcomes. In addition to personalised dose escalation, other methods include boosting subvolumes of the tumour based on FDG-PET, as used in Artforce/PET boost trial (29) and RTOG 1106/ACRIN 667 (ClinicalTrials.gov: NCT01507428). Both evaluate whether redistributing the dose based on FDG-PET can improve outcome and deliver treatment in a dose-individualised way. Other strategies of treatment intensification include combining radical radiotherapy with novel drugs e.g. immunotherapy, using hypofractionated stereotactic body radiotherapy to the primary site and conventionally fractionated radiotherapy to central mediastinal lymph nodes (ClinicalTrials.gov: NCT01933568), adaptive techniques, targeting hypoxia and using radiomics (using quantitative imaging parameters to predict clinical outcomes) characteristics to stratify patients. The future may involve the use of genomic signatures to predict the radioresistant tumours that may benefit from additional dose-escalation and also identify those whom have a high risk of relapse and additional toxicity outweighs the benefit. Currently there are no dose escalation approaches that take into account molecular or genomic tumour characteristics, or individual patient characteristics and current trials focusing on personalisation are lacking in patient numbers.

In conclusion there is an unmet need to intensify treatment in stage 3 NSCLC patients who are unsuitable for concurrent CTRT as outcomes are poor and a larger proportion of patients will be ineligible for this treatment option. We have demonstrated that Isotoxic IMRT is a well tolerated method of treatment intensification with promising outcome. This regime is currently being tested in a UK phase II randomized controlled trial (ADSCaN- ISRCTN47674500) alongside three other dose-escalated and accelerated sequential chemo-radiotherapy schedules.
2.5 References – Chapter Two


10. van Baardwijk A, Reymen B, Wanders S, Borger J, Ollers M, Dingemans AM, et al. Mature results of a phase II trial on individualised accelerated radiotherapy based on


Chapter 2

Isotoxic radiotherapy


2.6 Chapter Two Appendix

**BMJ Open**

Protocol for the isotoxic intensity modulated radiotherapy (IMRT) in stage III non-small cell lung cancer (NSCLC): a feasibility study

Kate Haslett,1 Kevin Franks,2 Gerard G Hanna,3 Susan Harden,4 Matthew Hatton,5 Stephen Harrow,6 Fiona McDonald,7 Linda Ashcroft,6 Sally Falk,6 Nicki Groom,9 Catherine Harris,10 Paula McCloskey,11 Philip Whitehust,10 Neil Bayman,12 Corinne Fairve-Finn1,13

**ABSTRACT**

Introduction: The majority of stage III patients with non-small cell lung cancer (NSCLC) are unsuitable for concurrent chemoradiotherapy, the non-surgical gold standard of care. As the alternative treatment options of sequential chemoradiotherapy and radiotherapy alone are associated with high local failure rates, various intensification strategies have been employed. There is evidence to suggest that altered fractionation using hyperfractionation, accelerated, dose escalation, and individualisation may be of benefit. The MAASTRO group have pioneered the concept of 'isotoxic' radiotherapy allowing for individualised dose escalation using hyperfractionated accelerated radiotherapy based on predefined normal tissue constraints. This study aims to evaluate whether delivering isotoxic radiotherapy using intensity modulated radiotherapy (IMRT) is achievable.

Methods and Analysis: Isotoxic IMRT is a multicentre feasibility study. From June 2014, a total of 35 patients from 7 UK centres, with proven histological or cytological diagnosis of resectable NSCLC unsuitable for concurrent chemoradiotherapy will be recruited. A minimum of 2 cycles of induction chemotherapy is mandated before starting isotoxic radiotherapy. The dose of radiation will be increased until one or more of the organs at risk tolerance or the maximum dose of 79.2 Gy is reached. The primary and point is feasibility, with accrual rates, local control and overall survival our secondary and points. Patients will be followed up for 5 years.

Ethics and dissemination: The study has received ethical approval (REC reference 13/NW/0490) from the National Research Ethics Service (NRES) Committee North-West—Greater Manchester South. The trial is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP). The trial results will be published in a peer-reviewed journal and presented internationally.

Trial registration number: NCT01836562; P2E-results

**INTRODUCTION**

Lung cancer is the leading cause of cancer mortality worldwide with approximately 40,000 new cases diagnosed annually in the UK. Of these cases, 34,000 will present with non-small cell lung cancer (NSCLC) and one-third (~12,000) of patients with NSCLC will present with locally advanced (stage III) disease. The 5-year survival from lung cancer in the UK has changed little (from 3% to 8%) over the past 50 years, with progress lagging significantly behind other common cancers.

The 5-year survival of stage III NSCLC with current standard treatment is approximately 10-15% highlighting a real urgency to improve outcomes for these patients.

Radiotherapy (RT), alone or combined with chemotherapy, plays a major therapeutic role in the treatment of stage III NSCLC. Despite this, most patients still progress locally and at sites of distant spread. Concurrent chemoradiotherapy (chemotherapy and RT given at the same time; CTRT) is the standard of care in stage III NSCLC with median survival rates of approximately 21 months. However, the majority of patients are not suitable for this treatment based on poor performance status (PS) and comorbidities. In a recent UK national survey of CTRT practice, the majority of clinical oncologists estimated that <50% of patients with stage III NSCLC were suitable for concurrent CTRT. The
alternative treatment offered to patients who are not suitable for concurrent CRT is sequential CRT (chemotherapy given prior to RT), but local control and survival rates are inferior compared with concurrent CRT. As the majority of patients with stage III NSCLC cannot be treated concurrently, strategies to improve outcome in this large group of patients treated sequentially is vital.

Local control with current RT doses delivered with standard three-dimensional conformal RT (3D-CRT) is poor with local progression-free survival rates of about 50%. However, recent data has shown that improved local control in lung cancer can lead to improvement in survival. A meta-analysis of concurrent versus sequential CRT in locally advanced NSCLC based on individual patient data demonstrated that although concurrent treatment decreases locoregional progression (HR=0.73; p=0.01); its effect is not different from that of sequential treatment on distant progression (HR=1.06; p=0.59). The decrease in locoregional progression translated into a significant survival benefit in favour of concurrent CRT (HR, 0.84; 95% CI 0.74 to 0.95; p=0.004), with an absolute benefit of 5.7% at 3 years and 4.5% at 5 years.

We are now accelerating into an era of personalized medicine for the systemic treatment of NSCLC. However, to date, fixed doses of radiation are still delivered to patients not taking into account volume of disease, stage of disease (IIA vs IIIB) or anatomical location within the thorax. Stage III NSCLC is a heterogeneous disease and there is a need to move away from a ‘one-size-fits-all’ approach to more personalized radiation treatments. The concept of isotoxic RT was recently introduced allowing the radiation dose prescription to the tumour to be tailored based on normal tissue constraints.

A strategy to improve local control is to escalate the dose of radiation delivered to the tumour. Manci et al demonstrated a clear dose-response relationship in NSCLC, with 84 Gy using conventional fractionation required to achieve 50% probability of tumour control at 3 years. Subsequently we have learned from the stereotactic body RT studies in NSCLC that biologically effective dose (BED) in excess of 100 Gy are necessary to achieve >90% local control rates.

However, a recent Radiation Therapy Oncology Group (RTOG) phase III study that randomised patients between 50 Gy in 30 daily fractions and 74 Gy in 37 daily fractions has failed to demonstrate a survival advantage for the high-dose arm indicating that dose escalation using a conventional fractionation resulting in increased overall treatment time is not the way forward in this disease. The failure of the high-dose arm was likely multifactorial and in addition to prolonged overall treatment time may have resulted from a combination of poorer treatment delivery with less patients receiving concurrent chemotherapy, reduced compliance to RT, QA issues and unreported treatment toxicity. Following the presentation of RTOG 0617 a dose of 60 Gy biologically equivalent dose in 2 Gy fractions (EQD2) is considered to be standard in patients with stage III NSCLC.

Accelerated hyperfractionation has been studied in an attempt to reduce the overall treatment time and counteract repopulation in lung cancer. In the national continuous hyperfractionated accelerated radiotherapy (CHART) study there was a staggering 24% reduction in the relative risk of death, which is equivalent to an absolute improvement in 2-year survival of 6% with hyperfractionated accelerated RT (54 Gy in 36 fractions over 12 days) compared with conventional RT (60 Gy in 30 fractions over 6 weeks). Despite CHART showing this significant benefit, it has not become standard practice in the UK. First, a large percentage of patients included had stage I-II disease (50%) who would now be considered for surgery or stereotactic body radiotherapy (SABR), and second, the control arm would not be considered standard care since chemotherapy was not delivered with RT (either sequentially or concurrently). Subsequently an individual patient data meta-analysis of 2009 patients from 10 trials, demonstrated that modified fractionation (acceleration, hyperfractionation or both) improves overall survival as compared with conventional fractionation in NSCLC resulting in an absolute benefit of 3% at 5 years.

Intensity modulated radiotherapy (IMRT) modulates the intensity profile of the radiation delivered to the patient allowing improved targeting of the radiation dose. This technique allows a decrease in the mean lung dose (MLD), V20 (percentage volume of total lung receiving ≥20 Gy), and maximal spinal cord dose. As a result the dose delivered to the tumour can be escalated while keeping the dose to the normal tissue within tolerance. Although IMRT is becoming standard for the treatment of lung cancer in large international academic centres, in the UK implementation of IMRT is currently poor. In September 2010 Cancer Research UK reported that UK RT practice is ‘lagging behind’ with only 7% of patients receiving IMRT compared with 20% in Europe. The MAASTRO group have pioneered the concept of ‘isotoxic RT’ allowing for individualised dose escalation using hyperfractionated accelerated RT based on predefined MLD and spinal cord dose in stage I-III patients. They have shown with 3D-CRT delivered two times a day over 4 weeks that increasing the radiation dose to prespecified normal tissue dose constraints could lead to increased tumour control probability with the same normal tissue complication probability. In the stage III group of patients the mean dose delivered was 61.2 Gy (ie, 72.2 Gy BED10 and 60.2 Gy EQD2), range 50.4-79.2 Gy (ie, 59.5 Gy BED10 and 49.6 Gy EQD2 – 85.5 Gy BED10 and 77.9 Gy EQD2) and <10% of patients received the maximum dose as per protocol of 72.2 Gy in 30 fractions two times a day with 3D-CRT (ie, 95.5 Gy BED10, 79.4 Gy EQD2). The survival rates of stage III patients in this study, all of whom were treated with sequential CRT, were comparable to the results expected with concurrent CRT with acceptable acute and late toxicity. It is important to note that IMRT was not used in the MAASTRO study. Our in-house planning
study has confirmed the use of IMRT could allow further individual dose escalation in this group of patients, which may ultimately translate into improved survival.14

METHODS AND ANALYSIS

The Isotoxic IMRT study is a non-blinded multicentre feasibility study. The study is sponsored by The Christie NHS Foundation Trust and coordinated by the Manchester Academic Health Science Centre Trial Coordination Unit (MAHSC-CTU) based at The Christie NHS Foundation Trust. Data management is undertaken by the MAHSC-CTU. The trial is registered on the clinicaltrials.gov database (NCT01856602) and jointly funded by Cancer Research UK’s Clinical Trials Awards and Advisory Committee (CTAAC) and the British Lung Foundation (BLF). The study is included in the National Institute for Health Research (NIHR) Clinical Research Network portfolio (ID: 14937). The trial is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP).

The primary research question is to evaluate the feasibility of delivering isotoxic RT using IMRT and hyperfractionated accelerated RT in patients with stage III NSCLC unsuitable for concurrent CRT.

The secondary research questions are:
1. Estimate the feasibility of delivering lung isotoxic IMRT
2. Estimate the proportion of patients with acute grade 3+ non-haematological toxicity
3. Estimate late toxicity
4. Estimate local control and overall survival
5. Develop a robust QA process for lung IMRT

Setting
In total, 35 patients with a histological or cytological proven diagnosis of NSCLC will be recruited from seven UK centres Addenbrookes (Cambridge), Beatson (Glasgow), The Christie NHS Foundation Trust (Manchester), Royal Marsden (London), Northern Ireland Cancer Centre (Belfast), Weston Park (Sheffield), St James’s Hospital (Leeds). The study started recruitment in June 2014.

Patients with stage III NSCLC, PS 0-2, not suitable for concurrent CRT, will be treated with individualised doses of radiation based on prespecified normal tissue doses (spinal cord, brachial plexus, lung tissue, heart and great vessels/proximal bronchial tree table 1). The study flow diagram is shown in figure 1.

Participant screening and selection

Patients have been deemed inoperable by the lung multidisciplinary team (MDT) and suitable for sequential CRT. Eligible patients are invited to participate and provided with a patient information sheet (see online supplementary appendix 1). Patients are only recruited into the Isotoxic IMRT study once. Mandatory investigations prior to registration included: a Contrast CT scan of the thorax and upper abdomen (within 4 weeks prior to registration), Contrast-enhanced CT (or MRI) brain scan (within 4 weeks prior to registration if patients have not had imaging of the brain prior to starting induction chemotherapy), fluorodeoxyglucose-positron emission tomography (FDG-PET) CT within 4 weeks prior to registration if patients have not had a PET-CT prior to starting induction chemotherapy and lung function tests.

Inclusion criteria

- Histologically or cytologically confirmed NSCLC
- Inoperable stage III disease (T3N1-3, any T4, any N2-3) confirmed by PET scanning, mediastinoscopy or bronchoscopy
- Patients treated with at least two cycles of platinum-based induction chemotherapy and able to start RT within 5 weeks of the last cycle of chemotherapy
- Tumour judged inoperable by a lung MDT
- Age ≥18, no upper age limit
- PS—Eastern Cooperative Oncology Group (ECOG) scale 0-2. Patients with PS 2 whose general condition is explained by disease can be included at the discretion of the local investigator. Patients with PS 2 as a result of comorbid conditions will be excluded
- Patient considered suitable for radical RT
- Tumour that can be encompassed within a radical RT treatment volume (MLD expected to be ≤20 Gy).

Exclusion criteria

- Patients suitable for standard concurrent CRT
- Patients only suitable for radical RT alone

Informed consent

Eligibility to participate is confirmed by a clinician prior to consent being taken. Patients are given at least 24 h to consider the patient information sheet and time to ask questions prior to written informed consent being taken by a trial doctor. The consent form can be viewed in online supplementary appendix 2.

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Table 1 Prespecified normal tissue doses (specified to a volume of 1 cc)

<table>
<thead>
<tr>
<th>Organ at risk</th>
<th>Prespecified normal tissue doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial plexus</td>
<td>Maximum dose: EQD2 ≤ 56 Gy</td>
</tr>
<tr>
<td>Heart</td>
<td>Maximum dose: EQD2 ≤ 76 Gy</td>
</tr>
<tr>
<td>Mean dose ≤ 46 Gy</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>Maximum lung dose (lung–GTV) ≤ 50 Gy</td>
</tr>
<tr>
<td>Mediastinal nodes</td>
<td>Maximum dose: EQD2 ≤ 56 Gy</td>
</tr>
<tr>
<td>Spinal cord PRV</td>
<td>Maximum dose: EQD2 ≤ 50 Gy</td>
</tr>
</tbody>
</table>

*Including heart, proximal bronchial tree, thyroid, oesophagus and the blood vessels in the upper mediastinum. EQD2, equivalent dose in 2 Gy fractions; GTV, gross tumour volume; PRV, planning organ at risk volume.
Stage III NSCLC
- Unsuitable for concurrent CTRT
- Inoperable stage II* (T3N1-3, any T4, any N2-3)
- ECOG 0-2*, no upper age limit

≥ 2 cycles platinum based induction chemo

Start RT within 3 weeks of last chemo

Individualised doses of radiation based on pre-specified normal tissue doses
No toxic ED IMRT delivered over a max. 4.5 weeks
Max dose 79.2 Gy

Investigations
- ECG
- CT chest/abdo
  If not done pre-chemo
  - PET
  - PET-CT
  - MR/CT head

Mandatory for RT planning
- contrast 4DCT
- IMRT
- bidaily CBCT

Figure 1 Trial schema. BD, two times a day; CBCT, Cone beam computer tomography; CTRT, chemoradiotherapy; ECOG, Eastern Cooperative Oncology Group; IMRT, intensity modulated radiotherapy; NSCLC, non-small cell lung cancer; PET, positron emission tomography; PTFs, pulmonary function tests; RT, radiotherapy.

Registration
Registration into this study takes place after completion of chemotherapy. Once a patient is deemed eligible for the study and has consented to participate, the MAHSC-CTU will allocate a patient identification number by telephone and confirm enrolment by email.

Standard care
In patients with inoperable stage III NSCLC who are unsuitable for concurrent CTRT, sequential CTRT is the standard of care. While on study patients will not co-enrol in other clinical trials offering therapeutic intervention. Patients can withdraw from the trial at any time without any effect on clinical care.

RT intervention and planning
4D-CT scanning is mandatory to account for tumour motion during the breathing cycle. The use of innumeros coronal cuts is mandated (patients with a medical contraindication should not be included in the study). The whole thorax (cricoid to L2) should be covered to allow dose volume histograms to be calculated for the lung, heart, spinal cord, brachial plexus, great vessels, proximal bronchial tree and the oesophagus. RT should be started within 3 weeks of 4D-CT planning scan date.

- The motion adapted gross tumour volume (GTV) is defined as idenfiable tumour and involved lymph nodes.
- The clinical target volume (CTV) will encompass regions at risk of microscopic extension.
- The CTV comprises the GTV with a 5 mm margin of radiologically normal tissue in all directions.

Dosimetry/dose specifications
Treatment planning will be with inverse planned IMRT. This optimisation must be performed by an experienced dosimetrist/physicist in lung planning.

The use of volumetric modulated arc therapy (VMAT)/RapidArc/immotherapy/fixed-beam IMRT is allowed in this study. If fixed-beam IMRT is used, it is suggested that five or more fields should be used to avoid creating hotspots in normal tissue and ensure optimal dose distribution. The use of these techniques facilitates the mandatory PTV coverage of D92 ≥95% (ideally 95%) of the prescribed dose and a 1 cc maximum <107%.

Patients are treated with individualized doses of radiation based on prespecified normal tissue constraints (spinal cord, brachial plexus, lung tissue, heart and great vessels/proximal bronchial tree). RT is delivered two times a day over a maximum period of 45 weeks using IMRT and the dose of radiation is increased until one or more of the OAR tolerance or the maximum dose of 70.2 Gy is reached.

Follow-up
Patients are followed up for 5 years post-treatment (4 monthly in years 1 and 2, 6 monthly from years 2–5). A late toxicity assessment will be performed at each visit.

Statistical considerations
This feasibility study will be stage-two (with early stopping rules) using the design of Bryant and Day with 85% power and 12% significance level for both completion and acute radiation pneumonitis rates. Using the design of Bryant and Day55 to combine an acceptable rate of 90% and unacceptable rate of 70% of patients receiving >60 Gy EQD2 and an acceptable rate of grade 3+ acute radiation pneumonitis of 8.5% and unacceptable rate of 22.5%, 35 patients will be recruited from seven centres over 1 year.

Stage I: Enrol 11 patients to stage II.
1. Less than 7 of 11 patients can be planned to a dose >60 Gy EQD2
2. More than 3 of 11 patients experience grade 3+ acute radiation pneumonitis before enrolment of the last patient in stage I, recruitment to stage II would only start when the last patient enrolled in stage I has reached 3 months followup. This would be done to ensure the absence of acute radiation pneumonitis in the fourth patient treated before proceeding to stage II.

Stage II: Enrol a further 24 patients, totalling 35, conclude a negative study if:
1. Less than 27 of 35 patients can be planned to a dose >60 Gy EQD2
2. More than 6 of 35 patients experience grade 3+ acute radiation pneumonitis.

There is no planned interim analysis. The study will stop after stage I if the conditions aforementioned are met.

Primary analysis
Total radiation dose, proportion of patients receiving >60 Gy EQD2 to those receiving ≤60 Gy EQD2 and the reason why isotoxic IMRT ≤60 Gy EQD2 will be reported.

Secondary analysis
Percentage of patients who are deemed suitable to receive isotoxic IMRT, withdrawal rates, recruitment rates, incidence of toxicity, incidence of serious adverse events (SAEs), estimation of local control and estimation of overall survival (calculated from date of registration) will be reported.

Changes to the protocol after the start of the trial
The trial details documented here are consistent with Isotoxic IMRT study protocol V5.0 (dated: 8 December 2013). There have not been any significant changes to the protocol after the start of the study.

End of the trial
The study will close 5 years after the last patient completes RT treatment. The chief investigator and/or the trial management group (TMG) have the right at any time to terminate the study for clinical or administrative reasons. The end of the study will be reported to the REC and Regulatory Authority (where applicable) within the required timeframes.

QA programme
The trial is subject to a RT QA programme which is tailored to the technical requirements of lung IMRT. This programme will facilitate an audit of UK lung IMRT practice and dosimetric accuracy of delivery, as well as providing a standardised framework for implementation of lung IMRT in participating centres. The QA programme for the study is coordinated by the National Cancer Research Institute (NCRI) Radiotherapy Trials QA (RTTQA) Group. The details of the programme can be found at the RTTQA website, http://www.rettqa.org.uk.

As part of the pretrial QA, sites were asked to complete the following:

- RTTQA facility questionnaire.
- Outline a benchmark case, delineating the OAR on the CT data set provided according to the study specific isotoxic IMRT atlas.
- Plan a benchmark case according to the trial protocol on the provided CT (OAR’s, GTV, CTV and PTV).
- The first patient from each centre is reviewed before that centre recruits a second patient. If there are any problems with the first patient a prospective case review is mandated for the second patient.
- All RT plans are subject to review (retrospectively) by the Mount Vernon NCRI RTTQA Group to ensure adherence to the trials RT planning and delivery protocol.
- Finally, a site visit and complex treatment dosimetry check is carried out to review participating centres.
4D-CT and treatment verification processes and the RTTQA lung IMRT dosimetry audit.

End of the trial
The trial will end once 30 patients have been recruited and all patients have completed 6 months follow-up or have died, whichever is sooner.

ETHICS AND DISSEMINATION
Safety reporting
Data is collected at each trial visit regarding any SAEs (as defined by GCP). All SAEs causally related to the RT treatment are reported to the MAHSC-CTU and followed until they resolve or stabilise. Acute and late radiation toxicities continue to be recorded at each follow-up visit (according to the CTCAE V4.0 grading system).

Trial monitoring and oversight
Formal on site data monitoring activities are performed as part of the Isotoxic IMRT study.

As this is a feasibility study data is not reviewed by an independent data monitoring committee, however, individual patient and treatment experiences are discussed at the regular TMC meetings. The TMC coordinates and manages the trial’s day-to-day activities. The TMC is comprised of health professionals, a patient representative and members of the direct study team, including the principal investigators from each participating site.

Dissemination
Data from all centres will be analysed together and published promptly. Individual participants may not publish data concerning their patients that are directly relevant to questions posed by the trial until the TMC has published its report. The TMC will form the basis of the Writing Committee and advise on the nature of publications. The trial will be publicised at regional and national conferences. The final results will be presented at scientific meetings and published in a peer-reviewed journal (authorship will be according to the journal’s guidelines). In addition a lay summary of the results will be produced for interested parties for example, Cancer Research UK and BLF.

Trial status
The first patient was registered in June 2014 and recruitment continues to stage II of the study. The study completed recruitment in March 2016. The Isotoxic IMRT trial along with three other phase I/II trials of dose-escalated sequential CIRT, will be compared with a UK standard sequential CIRT regime (55 Gy in 29 fractions) using state-of-the-art RT in a randomised phase II trial. As it would be impossible to test all schedules in phase III study it was decided to proceed with a combined randomised phase II screening “pick the winner” approach to select one schedule for further testing in a randomised phase III study.

Acknowledgements
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Contributions
CF received funding for the study. CF, NF, NB, KY, GGH, SAH, MHT, SST, FM, LA and SP initiated the study design and CH, PW and SP helped with implementation. CF is the grant holder. IA provided statistical expertise in clinical trial design. All the authors contributed to refinement of the study protocol and approved the final manuscript.

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Competing interests None declared.

Ethics approval NRES Committee North West—Greater Manchester South (REC ref. 12/W0064/09).

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES


Chapter Three

Phase I trial evaluating MEK inhibitor selumetinib with concomitant thoracic radiotherapy in non-small-cell lung cancer
3 Phase I trial evaluating MEK inhibitor selumetinib with concomitant thoracic radiotherapy in non-small-cell lung cancer

3.1 Introduction

Radiotherapy (RT) plays a major role in the treatment of non-small cell lung cancer (NSCLC). Two thirds of patients present with locally advanced (stage III) or metastatic disease (stage IV). Currently there is no curative therapy for stage IV NSCLC and radical treatment it is not standard outside of a clinical trial. Standard treatment is with palliative intent to optimize symptom control, improve quality of life, and prolong survival. For patients without an epidermal growth factor receptor (EGFR) sensitising mutation or anaplastic lymphoma kinase (ALK) gene rearrangement, combination chemotherapy is recommended (1). Response rates to doublet chemotherapy using a platinum agent in combination with another chemotherapy drug (gemcitabine, vinorelbine, paclitaxel, docetaxel or pemetrexed) are in the region of 20-30% with a median survival of 10 – 12 months (2, 3). For those with sensitizing EGFR mutations, tyrosine kinase inhibitors such as erlotinib, gefitinib and afatinib are used, and in presence of ALK or c-ros oncogene (ROS1) arrangement crizotinib is given (1). In patients who have stable disease or had a response to first-line pemetrexed regimens, maintenance therapy with pemetrexed is an effective and tolerated treatment option (4). However in locally advanced stage III disease, chemotherapy and radiotherapy is given with radical intent. The gold standard for inoperable stage III NSCLC is concurrent chemoradiotherapy (chemotherapy and RT given at the same time; CTRT) with median survival rates of approximately 28 months (5). A meta-analysis of concurrent versus sequential CTRT in locally advanced NSCLC demonstrated a significant survival benefit in favour of concurrent CTRT, with an absolute benefit of 4.5% at 5 years (6). However, in the UK and across Europe, less than half of patients are suitable for concurrent treatment with chemotherapy, the remainder unsuitable due to poor performance status and co-morbidities (7-9). There is therefore an unmet need to improve patient outcome from sequential CTRT.

It is imperative that new effective treatments are found to combine with radiotherapy; one such option is to use molecularly targeted drugs with radiation as an alternative to chemotherapy (10). For an experimental drug-radiotherapy combination to be successful it should be based upon established radiobiology, molecular biology and pharmacology principles (11). There is now a greater understanding of the molecular mechanisms that may drive malignancy in NSCLC and targeted drugs have been developed to block these pathways. However despite numerous clinical trials investigating targeted agents in combination with RT (10), none to date have become part of routine clinical practice in lung cancer (12). Furthermore adding drugs to thoracic RT may increase normal tissue toxicity.
with pulmonary toxicity being one of the main concerns. For example the addition of the anti-angiogenic agent bevacizumab to paclitaxel-carboplatin chemotherapy was found to improve response rates (35% versus 15%) and median survival (12.3 months versus 10.3 months) in NSCLC (13). However when combined with chemo-radiotherapy, for both small-cell lung cancer and NSCLC, adding bevacizumab led to an unacceptably high incidence of tracheoesophageal fistula formation, resulting in early closure of both trials (14). This highlights the importance of vigilant phase I/II trials robustly designed to detect severe and/or unexpected toxicity in order to safely combine targeted agents with radiotherapy.

One family of targeted agents that has been under investigation in recent years are MEK inhibitors. PD098059 became the first commercially available MEK inhibitor in 1995, and so far 13 MEK inhibitors have been used in clinical trials (15). Selumetinib (also known as AZD6244 and ARRY-142886)) a selective, uncompetitive inhibitor of MEK (licensed for development by AstraZeneca Pharmaceuticals) has been used in combination with chemotherapy, but this is the first study that combines this agent with thoracic radiotherapy. The mitogen activated protein kinase pathway comprising the Ras/Raf/MEK/ERK signaling cascade has a key role in the regulation of normal cell proliferation. The action of selumetinib as a MEK inhibitor, enhanced radiosensitivity seen in preclinical studies and enhanced cytotoxic effects associated with KRAS mutant xenografts provided the basis for the development of this phase 1 trial as a strategy to improve outcome from sequential CTRT.

### 3.1.1 Study contributions chapter 3

I was involved in the day-to-day running of the expanded cohort of the MEKRT trial: identifying and recruiting patients, planning the radiotherapy treatment, attending the 3'-deoxy-3'-[18F]Fluorothymidine ([18F]FLT) positron emission tomography-computed tomography (PET-CT) scans, reviewing patients on treatment and during follow up, completion of CRF’s, interpreting and collating the data from the study report issued by the CTU, using the raw data to generate survival curves, submitting abstracts, and presenting the data in an oral presentation at the 18th World Conference on Lung Cancer in Japan in October 2017.

Trial management group contributions: Prof Corinne Faivre-Finn is the Chief Investigator of the MEKRT trial with Prof Fiona Blackhall as a co-investigator and collaborator. Dr Pek Koh was the research fellow who ran the dose escalation cohort of the MEKRT trial. The trial statisticians Linda Ashcroft and David Ryder collated the data. Professor Malcolm Ranson chaired the safety review committee which decided upon the dose of selumetinib administered in the expanded cohort.
3.2 Patients and methods

3.2.1 Patients

Patients were eligible if they were ≥ 18 years of age, with histological or cytological confirmation of NSCLC, either inoperable stage III or stage IV with dominant chest symptoms, and previously untreated by RT or investigational agents. Prior chemotherapy was permitted provided the interval of day 8 of the last cycle of chemotherapy and day 1 of selumetinib dosing was ≥ 2 weeks. Thoracic disease needed to be encompassable within a radical RT treatment volume. Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1, Medical Research Council (MRC) dyspnoea score < 3 and a life expectancy of > 3 months were further requirements. Additional inclusion criteria were: forced expiratory volume in one second (FEV1) and gas transfer factor for carbon monoxide (DLCO) both > 40% of predicted, left ventricular ejection fraction (LVEF) > 50% on baseline echocardiogram, glomerular filtration rate (GFR) > 50ml/min, white cell count > 3 x 10^9/l, neutrophil count 1.5 x 10^9/l, haemoglobin 10.0g/dl and platelet count > 100 x 10^9/l, aspartate aminotransferase (AST) / alanine aminotransferase (ALT) < 2.5 x upper limit of normal (ULN) and bilirubin < 1.5 x ULN. In the expanded cohort undergoing [18F]FLT PET-CT scans, in order to limit the impact of partial volume effect, it was mandated that the diameter of the residual tumour was ≥ 2cm for inclusion in the study.

Exclusion criteria included: mixed non-small cell and small cell tumours, presence of clinically significant fluid accumulations in the third spaces which could not be adequately drained prior to study inclusion, history of interstitial pneumonitis (any diffuse alveolar damage, pneumonitis, bronchiolitis and pulmonary fibrosis), unstable diabetes, hypertension defined as a systolic blood pressure ≥160 or diastolic blood pressure ≥ 100 (antihypertensive medication was permitted to achieve the parameters), cardiac event (myocardial infarction, unstable angina, congestive cardiac failure New York Heart Association > class II, within previous 1 year, major surgery within 4 weeks, and known brain metastases (brain imaging was not mandatory if asymptomatic).

Screening and baseline assessments were included in the pre-treatment evaluation on all patients within 21 days prior to trial entry (e.g. history, examination, electrocardiogram (ECG), echocardiogram, ophthalmic evaluation), CT (computed tomography) scan and pulmonary function tests within 28 days and baseline bloods within 24 hours of starting selumetinib.

3.2.2 Study design and treatments

This was a single-arm, single-centre, open-label phase I trial of concurrent selumetinib with thoracic RT. Recruitment to a dose-finding stage using a Fibonacci 3+3 design (maximum number = 18) to evaluate safety and tolerability of selumetinib was followed by recruitment of
an expanded cohort (n = 15). Oral selumetinib was administered as a single agent twice daily commencing 7 days prior to RT, then in combination with thoracic RT for 6 – 6.5 weeks (60 – 66 Gy in 30 – 33 fractions), the drug was then stopped on the final day of RT (Figure 3-1).

The recommended phase 2 dose of selumetinib as a monotherapy is 75mg twice daily. The aim of the study was to determine the recommended phase 2 dose of selumetinib in combination with standard dose thoracic RT. Using the modified Fibonacci scheme each cohort contained 3 to 6 patients. The initial dose of selumetinib administered was 50mg twice daily, with the intention to escalate to a maximum dose of 75mg twice daily or de-escalate to a dose of 75mg once daily see Table 3-1. All patients were asked to donate surplus tumour tissue from initial diagnostic biopsy and blood samples for the exploratory biomarker study. Blood samples were taken before, during and after RT.

<table>
<thead>
<tr>
<th>Group</th>
<th>Explanation</th>
<th>Dose level</th>
<th>RT Dose (Gy) in 2Gy per fraction</th>
<th>Selumetinib dose schedule</th>
<th>Minimum number of evaluable patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>De-escalation</td>
<td>0</td>
<td>60 - 66</td>
<td>75mg od</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>Starting</td>
<td>1</td>
<td>60 - 66</td>
<td>50mg</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>Escalation</td>
<td>2</td>
<td>60 - 66</td>
<td>75mg bd</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>Expanded Cohort</td>
<td>RP2D</td>
<td>60 - 66</td>
<td>RP2D</td>
<td>15</td>
</tr>
</tbody>
</table>

Abbreviations: bd, twice daily; od, once daily; RP2D, recommended phase 2 dose; RT, radiotherapy.

Participants gave written informed consent and the study was conducted according to the Declaration of Helsinki and Good Clinical Practice Guidelines. The trial was reviewed by the research ethics committee, which granted ethics approval for the study on 31/12/2009. Patients gave consent for surplus tumour tissue taken at diagnosis to be analysed in the...
study. Bloods for biomarkers including evaluation of Kirsten rat sarcoma viral oncogene (KRAS) status were taken throughout the study following the schedule outlined in Table 3-2.

<table>
<thead>
<tr>
<th>Time-point (day)</th>
<th>Screen</th>
<th>Selumetinib</th>
<th>Selumetinib with RT</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue: KRAS, pMAPK</td>
<td>X</td>
<td>-7 to 1</td>
<td>Day 1, 8, 15, 22, 29, 36</td>
<td></td>
</tr>
<tr>
<td>Cf DNA: KRAS mutation</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Blood: M30, M65, nDNA, angiogenesis multiplex, OPN</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Abbreviations: cfDNA, cell-free deoxyribonucleic acid; KRAS, Kirsten rat sarcoma viral oncogene; pMAPK, phosphorylated mitogen-activated protein kinase; nDNA, nuclear deoxyribonucleic acid, OPN, osteopontin.

3.2.3 Thoracic radiotherapy

The first fraction of RT was delivered 7 days after the first dose of selumetinib. The minimum interval between the last chemotherapy administered and the first day of RT was 14 days. The total dose of RT 60-66Gy was delivered in 30-33 fractions, 2 Gy per fraction, over approximately 40-45 days. The gross tumour volume (GTV) was defined as residual tumour (minimum of 2cm in the expanded cohort) and involved lymph nodes (nodal involvement on CT defined as pre-chemotherapy nodes > 1cm in short axis). The clinical target volume (CTV) was defined as the GTV plus a 0.5cm margin in all directions. The CTV to planning target volume (PTV) expansion followed standard departmental protocols accounting for the use of 3D or 4DCT (e.g. 3D-CT 1.3 cm margin superiorly and inferiorly, and 1.0 cm margin laterally, at the 95% isodose, for 4D-CT 0.9cm margin superiorly and inferiorly, and 0.7cm margin laterally). Prophylactic nodal irradiation was not permitted. The dose was specified at the international commission on radiation units (ICRU) reference point and fully corrected for heterogeneity. The dose distribution within the PTV should ideally be within ± 5% of the prescribed dose, and no more than ± 7% of the prescribed dose.

The normal tissue constraints (for a standard dose of 2 Gy per fraction): maximum dose to spinal cord ≤ 48 Gy, the percentage of lung minus PTV receiving more than 20 Gy would not exceed 35% (V20=35%, based on dose-volume histograms), the mean lung dose was also recorded (mean dose to lung minus GTV) and the heart could receive the total dose to < 30% of its volume. For > 50% of cardiac volume, dose < 50% of the total dose was recommended. Cone beam or orthogonal images were obtained on days 1 to 3 (or 2 to 4) and weekly thereafter. Additional cone beam imaging was at the discretion of the Principle Investigator, if during treatment any discrepancies were noted on the radiotherapy planning CT scan.
3.2.4 Objectives and outcome measures

The primary objective was to determine the recommended phase II dose (RP2D) of selumetinib in combination with thoracic RT. The secondary objectives were to collect data on the safety profile, dose delivery, and overall response rate, by response evaluation criteria in solid tumours (RECIST) (16) and local control by Green criteria (17), of selumetinib in combination with thoracic RT. Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan-Meier method. Exploratory objectives included the analysis of [$^{18}$F]FLT PET-CT imaging parameters to assess treatment response or toxicity (will be addressed in chapter 4).

Dose-limiting toxicity (DLT) was assessed during treatment until 12 weeks after completion of thoracic RT and graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTCAE 4.0). Over the assessment period toxicity, that if observed would be defined as DLT related to the combination of selumetinib and radiotherapy, included grade ≥ 3 toxicity from dyspnoea, oesophagitis or pneumonitis that persisted for > 7 days and any of the following toxicity: neutropenia with fever grade ≥ 3, thrombocytopenia with bleeding grade ≥ 3, toxicity resulting in administration of ≤ 80% of the planned course of selumetinib and toxicity leading to interruption of RT for > 7 days. All adverse events were reviewed by an investigator to assess if they were attributable to selumetinib in combination with RT. The protocol stated the recommended phase two dose of selumetinib will be the dose level at which < 2/6 patients experience dose limiting toxicity (DLT). The protocol outlined specific guidelines for dose modifications and interruptions, a summary is outlined in Table 3-3. For any toxicity grade ≤ 3, treatment with RT could continue at the discretion of the Principal Investigator (PI). Selumetinib was discontinued if the dose interruption was ≥ 2 weeks.

Table 3-3 Dose modifications for Selumetinib

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-haematological</td>
<td>Continue same dose</td>
<td>Continue same dose (except if elevated AST/ALT selumetinib withheld)</td>
<td>Omit until grade ≤ 1, or returned to baseline. Resume same/reduced dose at discretion of PI</td>
<td>Omit until grade ≤ 1, or returned to baseline. Reduce dose/discontinue at discretion of PI</td>
</tr>
<tr>
<td>Haematological</td>
<td>Continue same dose</td>
<td>Continue same dose (except if neutropenia/thrombocytopenia selumetinib withheld)</td>
<td>Omit until grade ≤ 2, or returned to baseline. Resume same/reduced dose at discretion of PI</td>
<td>Omit until toxicity grade ≤ 1, or returned to baseline. Reduce dose/discontinue at discretion of PI</td>
</tr>
</tbody>
</table>

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase
3.3 Results

Between June 2010 and February 2015, 21 patients were enrolled (6 to the dose finding stage and 15 to the expanded cohort). The baseline demographics and disease characteristics are shown in Table 3-4. The majority of patients had stage III disease and were performance status 1 at trial entry.

Table 3-4 Patient characteristics

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>n=21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Male</td>
<td>12 (57%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Median (years)</td>
<td>62</td>
</tr>
<tr>
<td>Range (years)</td>
<td>50 - 73</td>
</tr>
<tr>
<td>ECOG PS</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7 (33%)</td>
</tr>
<tr>
<td>1</td>
<td>14 (67%)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>5 (24%)</td>
</tr>
<tr>
<td>IIb</td>
<td>11 (52%)</td>
</tr>
<tr>
<td>IV</td>
<td>5 (24%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Other (mixed, undifferentiated, other)</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Lung function</td>
<td>(median/range)</td>
</tr>
<tr>
<td>FEV1 (litres)</td>
<td>2.3 (0.9 – 5)</td>
</tr>
<tr>
<td>DLCO (% predicted)</td>
<td>65 (33 – 99)</td>
</tr>
<tr>
<td>GTV</td>
<td></td>
</tr>
<tr>
<td>Median (cm³)</td>
<td>31</td>
</tr>
<tr>
<td>Range (cm³)</td>
<td>1 – 224</td>
</tr>
<tr>
<td>PTV</td>
<td></td>
</tr>
<tr>
<td>Median (cm³)</td>
<td>360</td>
</tr>
<tr>
<td>Range (cm³)</td>
<td>241 – 785</td>
</tr>
</tbody>
</table>

Abbreviations: Eastern Cooperative Oncology Group; PS, performance status; FEV1, forced expiratory volume in 1 second, DLCO, diffusing capacity of the lung for carbon monoxide; GTV, gross tumour volume; PTV, planning target volume.

3.3.1 Dose finding cohort

A total of six consecutive patients with inoperable stage III (n=3) or stage IV (n=3) NSCLC were given selumetinib 50 mg twice daily (dose level 1) with concomitant thoracic RT. Baseline characteristics for both the dose finding cohort and expanded cohort are presented in Table 3-4. Two patients were considered to have DLT’s. One patient was admitted to hospital with grade 3 diarrhoea and prolongation of hospitalisation by grade 2 fatigue and grade 2 radiation oesophagitis. Diarrhoea is an expected toxicity with selumetinib but due to the duration of this serious adverse event it was classified as a treatment related toxicity.
second patient developed a pulmonary embolism during week 3 of RT. Pulmonary embolisms are not uncommonly found in cancer patients, often diagnosed on routine interval CT scans. In this case most likely caused by underlying disease but selumetinib cannot be ruled out as a contributing factor and it was decided by the safety review committee that this should be counted as a dose limiting toxicity but not attributable to the trial treatment. In other studies using selumetinib there have been no evidence of increased thromboembolic events.

In the dose escalation cohort there were only a few interruptions to the delivery of selumetinib due to, hypotension (1 day), acneiform rash (3 days), social reasons (1 day) and the serious adverse event described when the patient was admitted with diarrhoea and fatigue (2 days).

In the first 6 patients receiving 50mg twice daily no enhanced RT related toxicity was seen. The original protocol permitted dose escalation to dose level 2 if < 2/6 patients experienced a dose limiting toxicity. As these criteria were not met, dose escalation was not considered. Technically this suggests that the dose of 50mg twice daily would not be a tolerated dose and the dose could be de-escalated to 75mg once daily. However after extensive discussion by the safety review committee due to the small number of very heterogeneous patients in the dose-escalation study it was concluded that further examination of the 50mg twice daily dose was required rather than de-escalating the dose. The dose finding part of the trial was closed and recruitment to the expanded cohort of 15 patients (having additional [18F]FLT PET-CT scans) with the recommended dose of 50mg twice daily was opened. (Of note I was not involved in the dose-escalation study or the safety review committee).

3.3.2 Results - all patients
All 21 patients completed thoracic radiotherapy as planned 12 (57%) received 66 Gy in 33 fractions, 1 received (5%) 64 Gy in 32 fractions and 8 (38%) received 60 Gy in 30 fractions, over a mean duration of 44 days (40 – 48). The dose of radiation received by the normal tissues is summarised in Table 3-5, the V20 Gy in all patients was ≤ 35%. The median MLD of 17.8 Gy, although up to 24.1 Gy was permitted.
Table 3-5 Normal tissue dosimetry

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>V5 Gy (Lung – PTV)</td>
<td>65.7% (36.9 – 81.3)</td>
</tr>
<tr>
<td>V20 Gy (Lung – PTV)</td>
<td>32.2% (17.6 – 35)</td>
</tr>
<tr>
<td>MLD (Lung – GTV)</td>
<td>17.8 Gy (10.5 – 24.1)</td>
</tr>
<tr>
<td>Oesophagus</td>
<td></td>
</tr>
<tr>
<td>V35 Gy</td>
<td>41.9% (16.1 – 73.5)</td>
</tr>
<tr>
<td>Maximum dose</td>
<td>66.2 Gy (60.2 – 68.5)</td>
</tr>
<tr>
<td>Length oesophagus &gt; 40Gy</td>
<td>10.8cm (6.9 – 18.0)</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
</tr>
<tr>
<td>V30 Gy</td>
<td>27.9% (1.3 – 45.5)</td>
</tr>
<tr>
<td>V40 Gy</td>
<td>18.8% (0.6 – 24.1)</td>
</tr>
<tr>
<td>Spinal cord</td>
<td></td>
</tr>
<tr>
<td>Maximum dose</td>
<td>44.2 Gy (35.6 – 47.6)</td>
</tr>
</tbody>
</table>

Abbreviations: V5, volume receiving ≥ 5Gy; V20, volume receiving ≥ 20Gy; MLD, Mean Lung Dose; V35, volume receiving ≥ 35Gy; V30, volume receiving ≥ 30Gy; V40, volume receiving ≥ 40Gy

Due to the heterogeneity seen in stage III and IV NSCLC there was a wide range of GTV and PTV volumes (see Table 3-4). All 21 patients received induction standard of care chemotherapy, 19 (90%) received 4 cycles and 2 (10%) 3 cycles. The most commonly used regime was carboplatin and gemcitabine (n = 12, 57%) followed by cisplatin and pemetrexed (n = 4, 19%), cisplatin and gemcitabine (n = 3, 14%) and carboplatin and pemetrexed (n = 2, 10%). No radiotherapy treatments were concluded early thus all patients received the initial planned dose. Compliance to selumetinib was > 80%. The quality control of grade 1-2 adverse events is not as robust as that of grade 3-4 adverse events (see Table 3-6 and Table 3-7). The commonest grade 3-4 adverse event was lymphopaenia. Out of the 21 patients, 3 patients had confirmed pneumocystis jeroenec pneumonia (PJP, previously known as pneumocystis carinii pneumonia) and an additional 3 patients were treated empirically for PJP. One patient died within 25 days of completing treatment (selumetinib and radiotherapy) from PJP following a myocardial infarction. In addition to the high incidence of confirmed and suspected PJP, and due to the increased incidence of post-treatment changes observed in the lungs on CT imaging, I suspect the patients in the MEKRT trial may have had increased lung toxicity beyond that expected with RT alone.
Response assessment by RECIST three months following completion of treatment showed 1 patient had a complete response, 3 patients had a partial response, 7 patients had stable disease, 8 patients had progressive disease and 2 patients were deceased. Applying the Green criteria (residual radiographic abnormality assessed by chest CT at 3 and 6 months after completion of thoracic RT, which then remains stable for an additional 6 months or more) (17), 3 out of 8 alive patients had controlled disease at 1 year. Out of the 19 deaths, 18 were reported as lung cancer deaths and 1 cardiovascular death. The main cause of first relapse was disease progression from distant metastases (9/21, 43%), then locoregional progression (6/21, 29%) and one patient had both distant metastases and locoregional progression (1/21, 5%). Two patients were alive (2/21, 10%) at the time of analysis and there was no information on locoregional progression for three patients (3/21, 14%). Out of the three patients with missing data on cause of first relapse, two of these patients deaths were attributed to lung cancer and the other death from a cardiovascular event.
There were 2 survivors (24 & 26 months) at the time of analysis who were both alive with disease. The 1-year survival was 38% for stage III disease vs. 44% for stage IV 20%, 2-year survival was 24% for stage III 31% vs 0 % for stage IV, see Figure 3-2. The 1-year progression-free survival (PFS) was 23.8% and 2 year PFS was 9.5%. The median overall survival was 9.7 months (95% confidence interval (C.I) 6 – 18.) and median PFS was 6.9 months (95% CI 3-11).

Figure 3-2 Overall and progression-free survival 1a) overall survival. 1b) Progression-free survival
Unfortunately no archival biopsies were suitable for KRAS testing. KRAS testing on circulating free DNA by polymerase chain reaction (PCR) revealed no KRAS mutants. Unfortunately all the biomarker samples from this trial were due to be analysed on the day of the devastating fire which damaged the Paterson research institute beyond repair. It remains uncertain as to whether the samples are in a condition to be analysed in the future, but unfortunately no data is currently available.

The commercial sponsor was not involved in the interpretation or presentation of the MEKRT trial results, but passed comment and approved the oral abstract that was submitted to the 18th World Conference on Lung Cancer in Japan in October 2017.

### 3.4 Discussion

This is the first phase I trial assessing selumetinib in combination with thoracic radiotherapy in patients with lung cancer. The combination of thoracic radiotherapy and selumetinib was feasible, with all patients completing radical radiotherapy. The survival reported in this heterogeneous unselected small group of patients was inferior to that expected but this cohort is small, heterogeneous and included metastatic patients.

It is not ideal to compare concurrent chemoradiotherapy studies with sequential chemoradiotherapy studies, but there are no contemporary studies using sequential chemoradiotherapy in the era of modern RT, which can be used for comparison. In our study radiation induced toxicity was greater than the standard radiotherapy dose arm of the RTOG 0617 with and without cetuximab but comparable that reported in the dose escalation study IDEAL-CRT, toxicity data summarised in Table 3-8.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Radiation pneumonitis (%)</th>
<th>Radiation oesophagitis (%)</th>
<th>Lymphocyte count decreased (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G 1</td>
<td>G 2</td>
<td>G 3</td>
</tr>
<tr>
<td>MEKRT</td>
<td>71</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>RTOG 0617 *(5)</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>No cetuximab</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>IDEAL-CRT *(18)</td>
<td>27</td>
<td>27</td>
<td>4</td>
</tr>
</tbody>
</table>

Abbreviations: standard radiotherapy arm of RTOG 0617, concurrent chemoradiotherapy 60 Gy, RT without cetuximab, RT with cetuximab.

As the majority of patients are unsuitable for concurrent chemoradiotherapy, it is imperative that new effective treatments are found to combine with radiotherapy. One option is to use
molecularly targeted drugs with radiation as an alternative to chemotherapy (10). Bentzen et al described the radiobiological mechanisms that can be harnessed by adding a targeted drug to RT (19). These include spatial cooperation (RT combats local disease and systemic treatment distant disease), cytotoxic enhancement (cell kill by DNA damage), biological cooperation (in a heterogeneous tumour the drug targets one cell population e.g. hypoxic cells and the RT targets another e.g. more oxygenated cells), temporal modulation (how the drug effects the cell after and during RT – the 5R’s of radiobiology – repair, repopulation, reoxygenation, redistribution, radio sensitivity) and finally normal tissue protection (the drug allows a higher dose of RT to be given because RT toxicity is reduced).

The MEK inhibitors are a family of targeted drugs which have been combined with chemotherapy and for the first time with thoracic radiotherapy in this study. The mitogen activated protein kinase pathway comprising the Ras/Raf/MEK/ERK signaling cascade has a key role in the regulation of normal cell proliferation. Ionising radiation results in rapid activation of the Ras/Raf/MEK/ERK pathway in tumour cells (20). In xenograft models, with or without the addition of radiation, selumetinib resulted in decreased phosphorylation of ERK, with more cells remaining in the G1 phase and less cells dividing (20). Ionising radiation causes an increase in transforming growth factor alpha (TGF-a) a prosurvival growth factor, and it is thought that selumetinib may partly inhibit this process (21). The mechanism of radiosensitisation by selumetinib is not fully understood (22). In addition, mutations to the oncogene KRAS, which can activate the RAS pathway, are found in 15-50% of NSCLC cases (23). In KRAS mutant xenografts, selumetinib led to increased growth inhibition when used synergistically with cytotoxics (docetaxel, temozolamide) and targeted agents (gefitinib) compared to monotherapy (24, 25).

However what is striking is the incidence of high grade lymphopaenia in our study, grade 3 (81 %) and grade 4 (9.5%) compared to RTOG 0617 which reported the same grade 3 (13%) and grade 4 (8%) in both standard radiotherapy arms (with and without cetuximab), suggesting cetuximab did not affect the lymphocyte count (5). Furthermore the incidence of ≥ grade 3 lymphopaenia of 90.4% in our study was significantly higher than that reported in the study IDEAL-CRT of 11%. Despite the significant and maintained decrease in lymphocytes seen in our study, this has not been shown in any other clinical trials using selumetinib; suggesting that selumetinib in combination with thoracic RT in the sequential chemo-radiotherapy setting may increase the risk of lymphopaenia. It is likely that the reduction in lymphocytes may have contributed to the development of the confirmed and clinically suspected cases of PJP. In the non-HIV population PJP is associated with significantly higher mortality rates (34 – 50%) compared to those with HIV (6-7%) (26-28). Given the intensive follow-up of patients on this phase I trial patients were identified and treated quickly with antibiotics and there were no deaths directly as result of PJP infection,
A number of different molecularly targeted agents have been studied in combination with radiotherapy in lung cancer patients summarised in the review by Koh et al (10) including EGFR inhibitors ((29-32), proteasome inhibitors (33) and mTOR inhibitors (34, 35). Pneumonitis is a known side effect of some of the drugs trialled e.g. mTOR inhibitors even when the drugs are used alone (36) and thus pose a greater risk when used in combination with RT. However despite the risk of increased pulmonary toxicity generally the combinations were tolerable but so far none have led to a change in clinical practice (10).

Pulmonary toxicity is one of the major concerns when combining targeted agents and radiotherapy. Given that reports of cetuximab-associated pulmonary toxicity are rare, it looked promising from the initial studies that the EGFR monoclonal antibody cetuximab could be combined with radiotherapy without increasing side effects except for the expected cetuximab-related skin reactions (29, 38). Cetuximab was further evaluated within the large randomised two-by-two factorial phase III study RTOG 0617 in NSCLC (5). RTOG 0617 compared high-dose (74 Gy in 2 Gy per fraction) versus standard-dose conformal radiation (60 Gy in 2 Gy per fraction) with concurrent and consolidation chemotherapy (carboplatin/paclitaxel) administered with or without cetuximab (5). The overall survival in the high-dose arm was significantly worse than the standard arm (20.3 months versus 28.7 months) and the study closed early due to futility (5). The compliance to cetuximab was the same in both treatment arms, but the severe toxic effects were greater (86%) in the cetuximab group versus the no cetuximab group (70%) and there were double the treatment related deaths in the cetuximab group (n = 10) (5). The addition of cetuximab provided no benefit in overall survival for these patients (5), thus supporting the need to explore alternative combinations. The rapidly developing field of immunotherapy has introduced new agents which have the potential to be combined with thoracic RT. Immunootherapy with immune checkpoint inhibitors has become a ‘game changer’ and a standard of care in the treatment of stage 4 NSCLC (39). However until recently evidence supporting the use of checkpoint inhibitors for earlier stage NSCLC was lacking. The recently published PACIFIC study compared the anti–programmed death ligand 1 (anti-PD-L1) antibody durvalumab delivered as consolidation therapy (after concurrent chemo-radiotherapy with placebo in patients with stage 3 NSCLC who did not have disease progression after two or more cycles of platinum-based CTRT (32). Progression-free survival was significantly longer with durvalumab than with placebo. Durvalumab has been recently approved by FDA as consolidation treatment after concurrent chemo-radiotherapy and is leading to a change in standard of care in stage 3 NSCLC (40). Preclinical data suggests the optimal scheduling of RT and anti-PD-1 is concomitant (41). There are more than 30 registered studies on ClinicalTrials.gov that combine immunotherapy with RT for lung cancer, but there is little current data on the safety of combined treatment. There are no prospective clinical trials combining thoracic RT and immunotherapy concurrently that have been published to date.
The main limitation of this trial is the small numbers and the heterogeneity of the patient group in terms of stage and disease volume. In addition there was no stratification based on KRAS testing and the lack of sensitivity to selumetinib may be in part due to the absence of KRAS mutants. There was no archival tumour tissue suitable for KRAS testing. The limited tumour tissue available may be due to small tumour biopsies being taken which undergo multiple testing at diagnosis leaving little tissue remaining. Obtaining adequate tumour tissue without performing another biopsy is a wider problem faced with current phase I trials. Pre-clinical studies did suggest enhanced efficacy in NSCLC with activation of the Ras/Raf/MEK/ERK pathway due to the presence of KRAS mutation [42 & 43]. The poor survival could also be due to patient selection, as those included had locally advanced disease unsuitable for concurrent CTRT or metastatic disease.

Unfortunately the clinical outcome was poor, however as is the case with most small single arm radiotherapy trials, outcome data should be interpreted with caution and such studies are mainly hypothesis generating. In the future there is a need to use more efficient design and recruit patients at multiple sites. Conventional early phase clinical trials are typically designed to evaluate one radiotherapy-drug combination at a time. However, platform or umbrella trials provide an opportunity to study multiple targeted therapies in the same disease area in a more efficient and scientifically rich manner (11).

**Conclusion**

Conclusions drawn from a small heterogeneous number of patients such as ours should be made with caution. However our results suggest that given the inferior outcome and the toxicity this combination should not be pursued in a subsequent phase II trial.
3.5 References – Chapter three


Chapter Four

Evaluation of 3’-deoxy-3’-[\(^{18}\text{F}\)]fluorothymidine uptake in patients with non-small cell lung cancer treated with the MEK inhibitor selumetinib concomitant with radical thoracic radiotherapy (within the Phase 1 MEKRT trial)
4 Evaluation of 3'-deoxy-3'-[18F]fluorothymidine uptake in patients with non-small cell lung cancer treated with the MEK inhibitor selumetinib concomitantly with radical thoracic radiotherapy (within the Phase 1 MEKRT trial)

4.1 Introduction

Standard radiological imaging plays a key role in the diagnosis of lung cancer and the assessment of treatment response, but the use of imaging during treatment and early follow-up is poorly established. Molecular imaging with positron emission tomography-computed tomography (PET-CT) combines functional tumour imaging and anatomical imaging and is now standard practice for staging in lung cancer, particularly in NSCLC. Standard imaging cannot evaluate tumour responses to radiotherapy (RT) within the first few days or weeks of treatment, because early biological changes precede any objective structural change. Functional imaging has the potential to act as an imaging biomarker to define early treatment responses to RT and novel drugs, enable better prognostication, avoid toxicity in futile cases and improve the quality of follow-up imaging. 2-[18F]Fluoro-2-deoxy-D-glucose ([18F]FDG) positron emission tomography-computed tomography (PET-CT) has been used to assess early treatment response, showing promise in predicting the outcome to both palliative (1-4) and induction (2, 5-7) chemotherapy, but there is less evidence in radical treatments, including combined modality therapy (8). One of the disadvantages of using [18F]FDG PET-CT to assess tumour response is its poor discrimination between inflamed tissues and tumour.

The radiotracer 3'-deoxy-3'-[18F]fluorothymidine ([18F]FLT) used with PET is thought to act as a non-invasive marker of tumour proliferation. When [18F]FLT enters a cell during S-phase, it is phosphorylated by thymidine kinase 1 (TK1) into [18F]FLT-monophosphate, becoming trapped within the cell, but not the DNA. Since the synthesis of DNA only occurs in the S-phase and the accumulation of [18F]FLT is dependent on TK1 (TK1 is upregulated 10-to-20 fold during the S phase (9)), this suggests that [18F]FLT could be used as a surrogate marker for the S-phase fraction and proliferation rate (9-13). As the pyrimidine analogue thymidine is present in DNA but not RNA, [18F]FLT can be used to evaluate proliferation because cell division is proportional to the rate of DNA synthesis. At baseline, tumour [18F]FLT uptake, as quantified by standard uptake values (SUV’s), has been shown in a meta-analysis to correlate with the histological proliferation marker Ki-67 in three cancer types including lung cancer (12).
A study published by the Australian group Everitt et al evaluating $[^{18}F]$FDG and $[^{18}F]$FLT in patients undergoing concurrent chemoradiotherapy for NSCLC, showed an agreement between the staging assessments on both baseline scans, but differences in the tumour uptake in weeks two and four, suggesting that chemo-radiotherapy affects tumour cell proliferation more than cell metabolism (14). A study in our centre, the substudy of RADAR (Radiation Damage And Resistance in lung cancer), evaluated $[^{18}F]$FLT PET-CT scans from 16 patients with NSCLC treated with radical radiotherapy. $[^{18}F]$FLT PET-CT scans were performed twice at baseline for repeatability assessment and during RT to assess response. Our results were similar to the Everitt et al pilot study (15) findings that in patients with NSCLC, RT induced an early, significant decrease in lesion $[^{18}F]$FLT uptake which exceeded test-retest variability (16).

Thus, $[^{18}F]$FLT has potential as a sensitive early biomarker of response to targeted treatments such as tyrosine kinase inhibitors (17, 18) and the MEK inhibitor (MEKi) selumetinib (also known as AZD6244 and ARRY-142886) (19). The first pilot study using $[^{18}F]$FLT PET-CT to assess the effect of selumetinib evaluated four patients with metastatic disease (two patients had metastatic melanoma and two had metastatic colorectal cancer) performed $[^{18}F]$FLT PET-CT at baseline and after two weeks of treatment with selumetinib (19). The one patient who demonstrated a decrease in $[^{18}F]$FLT uptake had stable disease (reduction in tumour size by 12% using RECIST 1.1), in contrast the patient who had an increase in $[^{18}F]$FLT uptake had stable disease but then subsequently progressed, whilst the other two patients with progressive disease had no significant changed in $[^{18}F]$FLT uptake (19).

In our study we evaluate the use of $[^{18}F]$FLT as a biomarker of response in twelve patients from a phase 1 trial (MEKRT trial) using the MEK inhibitor (MEKi) selumetinib in combination with a radical dose of thoracic radiotherapy for patients with NSCLC. This is the first study to combine selumetinib with thoracic radiotherapy.

4.1.1 Study contributions chapter 3

I was involved in the day-to-day running of the MEKRT trial. I attended the $[^{18}F]$FLT PET-CT scans of patients in the expanded cohort, I either administered the radiotracer or collected the blood samples during the scan. I outlined the regions of interest, following the RADAR protocol the regions of interest were then reviewed by a Consultant Radiologist Dr Damian Mullan. The data (SUV data and volume) from the $[^{18}F]$FLT PET-CT scans was generated by Dr Marie-Claude Asselin. I analysed this data using graph pad prism version 7.0 software to create my own graphs and perform the statistical tests as outlined in this chapter.
4.2 Materials and methods

The clinical details of the MEKRT trial are described in detail in Chapter 3. In summary, eligible patients had confirmed NSCLC, either inoperable stage III or stage IV with dominant chest symptoms, and previously untreated by RT or investigational agents. Prior chemotherapy was permitted and the thoracic disease needed to be encompassable within a radical RT treatment volume. It was mandated that in the expanded cohort of patients undergoing $^{18}$FFLT PET-CT the diameter of the residual primary tumour had to exceed 2cm in order to reduce the impact of partial voluming.

4.2.1 Study design and treatments

The MEKRT trial was a single-arm, single-centre, open-label phase I trial of concurrent selumetinib with thoracic RT. Oral selumetinib was administered as a single agent twice daily commencing 7 days prior to RT, then in combination with thoracic RT for 6 – 6.5 weeks (60 – 66Gy in 30 – 33 fractions), the drug was then stopped on the final day of RT (see Chapter 3, Figure 3-1).

To evaluate safety and tolerability of selumetinib, patients were recruited to a dose-finding stage before recruitment of an expanded cohort (n = 15). In the expanded cohort, patients underwent $^{18}$FFLT PET-CT scan at baseline (scan 1), after 7 days of selumetinib alone (scan 2) and during RT combined with selumetinib (scan 3) (see Figure 4-1).

![Figure 4-1 $^{18}$FFLT PET-CT schedule in the MEKRT trial](image)

Figure 4-1 $^{18}$FFLT PET-CT schedule in the MEKRT trial
4.2.2 Objectives

The primary objective of the MEKRT trial was to determine the recommended phase II dose (RP2D) of selumetinib in combination with thoracic RT. The secondary objectives were to collect data on the safety profile, dose delivery, and overall response rate (RECIST criteria (20) and local control by GREEN criteria (21)) of selumetinib in combination with thoracic RT. In the expanded cohort the role of $^{18}$FFLT in measuring tumour proliferation before treatment, during the first week of treatment with single agent MEK inhibitor and during combined treatment (thoracic radiotherapy and MEK inhibitor) was evaluated. In addition $^{18}$FFLT imaging parameters were correlated with response to the MEK inhibitor and radiotherapy.

4.2.3 PET data acquisition

All PET scans were performed on the Siemens Biograph TruePoint 6 TrueV 3D PET-CT scanner at the Wolfson Molecular Imaging Centre in Manchester, UK. $^{18}$FFLT was synthesized in-house or externally supplied (PETNET Solutions Inc., Nottingham, UK or the Wolfson Brain Imaging Centre, University of Cambridge, UK). The target dose of $^{18}$FFLT, 330 MBq for men and 280 MBq for women, was administered as a 30-sec bolus per scan. 3D dynamic data were acquired over the 60-min duration of each scan, in a single bed position with free breathing throughout. Following the PET scan a non-contrast-enhanced CT scan was acquired, to enable corrections for scatter and attenuation and allow tissue localisation. PET data acquired 0-60 min (dynamic scan) and 45-60 min (single frame) post-injection were reconstructed using the filtered back projection (FBP) or ordered subset expectation maximization (OSEM) algorithm, respectively, in order to provide images with voxel dimensions (256 x 256 x 109 matrix, corresponding to a voxel size of 2.67 x 2.67 x 2.00 mm$^3$) (16). A 3D 4-mm Gaussian filter was used to smooth the reconstructed PET images.

The plasma input function was derived from the time-course of radioactivity in the aorta determined from the $^{18}$FFLT images combined with discrete venous samples, alleviating the need for arterial lines often required for quantitative PET studies. The input function was corrected for the partition of radioactivity between erythrocytes and plasma as well as for the radiolabelled metabolite fluoro-glucuronide in plasma, determined by reverse-phase high-performance liquid chromatography.

The maximum (max) and mean standard uptake values (SUV’s) were calculated from the radioactivity concentration (Ci) within the regions of interest during the 45-60 min summed $^{18}$FFLT images, once normalized to the dose injected and the patients weight using the following formula:

\[
SUV = \frac{Ci (kBq/ml)}{Dose (MBq) \times Weight (kg)}
\]
4.2.4 Image analysis
Using the image analysis software Analyze (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN, USA), a clinical oncologist (KH) manually outlined the evaluable primary tumour and involved lymph nodes on all transaxial slices to create volumes of interest (VOI’s) on each co-registered scan. The VOI’s were then reviewed by an experienced oncological radiologist (Dr Damian Mullan). The whole lesion was delineated irrespective of $[^{18}\text{F}]$FLT avidity, thus the ROI was based on the CT image. Standardised window settings were used during delineation (lung windows; level -500HU, width 1,500 HU, soft tissues; level 0 HU, width 400 HU). A fixed visualisation window between 0 and 4 was used for the smoothed SUV maps. In accordance with the protocol delineation in the preceding RADAR trial, enlarged mediastinal nodes or nodes of normal size showing increased $[^{18}\text{F}]$FLT uptake were delineated (16). Normal proliferating bone marrow signal could affect nearby tumour voxels of interest, so a margin was left between the delineated VOI’s, which were close to bone structures. Movement can also affect the quality of PET-CT co-registration, and thus can be compromised by patient movement. This was assessed, and manual adjustments made if required. For the nodal lesions, an averaged nodal value was calculated per patient to avoid any influence from the variability in nodal volume. For example for a patient with 3 nodes:

$$\text{Nodes (averaged)} = \frac{(s_1 \times v_1) + (s_{ii} \times v_{ii}) + (s_{iii} \times v_{iii})}{(v_1 + v_{ii} + v_{iii})}$$

(2)

where $s_i$ is the individual node SUV and $v_i$ its volume with $i$ varying between 1 and the number of evaluable nodes for each patient. The max value across the nodes was used for the SUV$_{\text{max}}$ of the averaged nodes and the total volume of the nodes as its volume. In order to limit the impact of partial volume effects, any lesions < 2cm$^3$ at baseline were not evaluated.

4.2.5 Statistical analysis
For each evaluable VOI, three parameters were extracted: SUV$_{\text{max}}$, SUV$_{\text{mean}}$ and volume in cubic centimetres (cm$^3$). Descriptive summary statistics for each parameter, including the mean and standard deviation (SD) where appropriate, were calculated. The change in the parameters and volume for the primary tumour and the nodes (averaged) between each of the three scans was calculated and assessed using the Wilcoxon signed-rank test (two-sided). Potential correlations amongst baseline parameters, between baseline SUVs and the time interval since induction chemotherapy and between SUV$_{\text{max}}$ ratios and the RT dose at time of scanning, were evaluated using Spearman’s rho coefficient test (two-sided).
4.2.6 Analysis of outcome data

Median times for overall survival were calculated using the Kaplan-Meier method. Potential associations of outcome end points with $[^{18}F]$FLT PET parameters were investigated using linear regression. Statistical tests were performed using graph pad prism version 7.0 and p-values below 0.05 were considered significant. No corrections for multiple comparisons were applied.

4.3 Results

Between June 2010 and February 2015, 21 patients were enrolled to the MEKRT study (6 to the dose finding stage and 15 to the expanded cohort). The expanded cohort of 15 patients opened with the recommended dose of 50mg twice daily. Due to technical difficulties with the PET-CT scanner and $[^{18}F]$FLT production, there was a delay in commencing the $[^{18}F]$FLT PET-CT scans in the expanded cohort until May 2013. Out of 15 patients recruited to the expanded cohort, the last 12 underwent $[^{18}F]$FLT PET-CT imaging. The clinical characteristics of the 12 patients are summarised in Table 4–1 (for further information on the clinical study, see chapter 3). The response assessment by RECIST 1.1 (20) conducted three months following completion of treatment showed that 1 patient had had a partial response, 7 patients had stable disease, 2 patients had progressive disease and 2 patients had died.

Table 4–1 Clinical characteristics of patients in the expanded cohort of the MEKRT trial undergoing $[^{18}F]$FLT PET-CT scans

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender/Age (y)</th>
<th>Histology</th>
<th>Stage</th>
<th>Induction chemotherapy</th>
<th>MEK inhibitor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RT&lt;sup&gt;b&lt;/sup&gt; Gy</th>
<th>RECIST 1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M / 60</td>
<td>Squamous</td>
<td>IIIB</td>
<td>Carboplatin Gemcitabine</td>
<td>42 / 6</td>
<td>n/a&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Deceased</td>
</tr>
<tr>
<td>2</td>
<td>M / 62</td>
<td>Squamous</td>
<td>IIA</td>
<td>Cisplatin Gemcitabine</td>
<td>34 / 6</td>
<td>8</td>
<td>Deceased</td>
</tr>
<tr>
<td>3</td>
<td>F / 61 Adeno-carcinoma</td>
<td>IIIB</td>
<td>Cisplatin&lt;sup&gt;*&lt;/sup&gt; Carboplatin Pemetrexed</td>
<td>23 / 6</td>
<td>26</td>
<td>PD</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M / 60</td>
<td>Squamous</td>
<td>IIA</td>
<td>Cisplatin Gemcitabine</td>
<td>30 / 6</td>
<td>20</td>
<td>PR</td>
</tr>
<tr>
<td>5</td>
<td>F / 69</td>
<td>Adeno-carcinoma</td>
<td>IIIB</td>
<td>Carboplatin Gemcitabine</td>
<td>22 / 6</td>
<td>10</td>
<td>PR</td>
</tr>
<tr>
<td>6</td>
<td>M / 58</td>
<td>Adeno-carcinoma</td>
<td>IV</td>
<td>Carboplatin Pemetrexed</td>
<td>28 / 6</td>
<td>16</td>
<td>PD</td>
</tr>
<tr>
<td>7</td>
<td>M / 64</td>
<td>Adeno-carcinoma</td>
<td>IIIB</td>
<td>Carboplatin Gemcitabine</td>
<td>20 / 6</td>
<td>16</td>
<td>PD</td>
</tr>
<tr>
<td>8</td>
<td>F / 59</td>
<td>Squamous</td>
<td>IIIB</td>
<td>Carboplatin Gemcitabine</td>
<td>15 / 6</td>
<td>10</td>
<td>PD</td>
</tr>
<tr>
<td>9</td>
<td>F / 67</td>
<td>Squamous</td>
<td>IIIB</td>
<td>Carboplatin Gemcitabine</td>
<td>15 / 6</td>
<td>12</td>
<td>SD</td>
</tr>
<tr>
<td>10</td>
<td>M / 62</td>
<td>Adeno-carcinoma</td>
<td>IV</td>
<td>Carboplatin Pemetrexed</td>
<td>28 / 6</td>
<td>6</td>
<td>PD</td>
</tr>
<tr>
<td>11</td>
<td>M / 67</td>
<td>Squamous</td>
<td>IIIB</td>
<td>Carboplatin Gemcitabine</td>
<td>43 / 4</td>
<td>10</td>
<td>SD</td>
</tr>
<tr>
<td>12</td>
<td>M / 68</td>
<td>NSCLC (not specified)</td>
<td>IIIB</td>
<td>Carboplatin Gemcitabine</td>
<td>20 / 6</td>
<td>10</td>
<td>PD</td>
</tr>
</tbody>
</table>

<sup>1</sup>Before scan 1; <sup>2</sup>At time of scan 2; <sup>3</sup>At time of scan 3; <sup>4</sup>Scan 3 not acquired; <sup>a</sup>drug switch

Abbreviations. ID, patient identification; M, male; F, female; PD, progressive disease; PR, partial response; SD, stable disease.
Of the 12 patients in the expanded cohort, 9 underwent all 3 scans. The other 3 patients had a baseline scan and one out of the two on-treatment scans. Information on the timing of $[^{18}\text{F}]$FLT PET-CT scans is summarised in Table 4-2.

Table 4-2 $[^{18}\text{F}]$FLT PET-CT scans and patient details

<table>
<thead>
<tr>
<th>Patient</th>
<th>Scan dates (days to RT start)</th>
<th>Condition</th>
<th>Weight (kg)</th>
<th>$[^{18}\text{F}]$FLT Dose (MBq)</th>
<th>Deviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21/05/2013 (-8) 28/05/2013 (-1) n/a</td>
<td>Baseline MEKi n/a</td>
<td>80.6 83.0 n/a</td>
<td>331.9 337.0 n/a</td>
<td>Scan 3 omitted as patient was admitted to hospital</td>
</tr>
<tr>
<td>2</td>
<td>17/06/2013 (-8) 24/06/2013 (-1) 28/06/2013 (3)</td>
<td>Baseline MEKi MEKi+RT</td>
<td>84.2 83.8 84.6</td>
<td>330.1 321.8 324.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>23/08/2013 (-12) 03/09/2013 (-1) 24/09/2013 (20)</td>
<td>Baseline MEKi MEKi+RT $^2$</td>
<td>59.4 59.4 59.6</td>
<td>277.1 276.8 278.2</td>
<td>Scan 3 delayed by 1 week due to $[^{18}\text{F}]$FLT production issue</td>
</tr>
<tr>
<td>4</td>
<td>05/09/2013 (-13) 01/10/2013 (13)</td>
<td>Baseline n/a $^3$ MEKi+RT</td>
<td>57.2 n/a 60.6</td>
<td>334.0 n/a 331.8</td>
<td>PET-CT scanner not operational, scan 2 omitted</td>
</tr>
<tr>
<td>5</td>
<td>08/10/2013 (-15) 22/10/2013 (-1) 29/10/2013 (6)</td>
<td>Baseline MEKi MEKi+RT</td>
<td>61.4 61.0 61.4</td>
<td>283.3 284.6 292.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>03/12/2013 (-8) 10/12/2013 (-1) 20/12/2013 (9)</td>
<td>Baseline MEKi $^4$ MEKi+RT $^4$</td>
<td>72.1 73.0 72.8</td>
<td>332.9 333.5 331.6</td>
<td>In error patient took half the prescribed dose of MEKi</td>
</tr>
<tr>
<td>7</td>
<td>07/04/2014 (-9) 15/04/2014 (-1) 25/04/2014 (9)</td>
<td>Baseline MEKi MEKi+RT</td>
<td>94.0 93.2 94.2</td>
<td>328.0 329.9 336.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>02/12/2014 (-8) 09/12/2014 (-1) 17/12/2014 (7)</td>
<td>Baseline MEKi MEKi+RT</td>
<td>70.8 71.4 72.0</td>
<td>280.7 219.2 283.9</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>02/12/2014 (-8) 09/12/2014 (-1) 16/12/2014 (6)</td>
<td>Baseline MEKi MEKi+RT</td>
<td>66.4 67.8 69.4</td>
<td>280.7 245.2 205.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>08/12/2014 (-9) 19/12/2014 (2)</td>
<td>Baseline n/a $^5$ MEKi+RT</td>
<td>78.2 n/a 79.6</td>
<td>332.6 n/a 326.4</td>
<td>$[^{18}\text{F}]$FLT dose too low for injection, scan 2 omitted</td>
</tr>
<tr>
<td>11</td>
<td>09/01/2015 (-5) 13/01/2015 (-1) 20/01/2015 (6)</td>
<td>Baseline $^6$ MEKi MEKi+RT</td>
<td>98.2 98.4 99.0</td>
<td>334.5 333.5 319.6</td>
<td>Due to bank holiday, shorter time interval between starting MEKi and baseline</td>
</tr>
<tr>
<td>12</td>
<td>10/02/2015 (-8) 17/02/2015 (-1) 24/02/2015 (6)</td>
<td>Baseline MEKi MEKi+RT</td>
<td>80.0 78.8 79.4</td>
<td>323.6 335.3 323.0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: n/a: not applicable; MEKi: MEK inhibitor selumetinib
Overall, 8 out of 12 patients had both primary and lymph node metastases, 2 patients had a single primary and 2 patients had only lymph node metastases evaluable (see Table 4-3). In total, 31 baseline lesions (primary tumours and lymph node metastases) were evaluable. See Figure 4-2 for a representative case which includes images from the RT planning scan and [\(^{18}\)F]FLT PET-CT study from patient 2.

### Table 4-3 Regions of interests manually delineated on lesions

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Primary(^1)</th>
<th>Nodes</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LUL</td>
<td>Central nodal mass</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>RUL</td>
<td>Hilar Subcarinal</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>Paratracheal Subcarinal</td>
<td>No primary tumour</td>
</tr>
<tr>
<td>4</td>
<td>LUL</td>
<td>Prevascular Paratracheal</td>
<td>Diffuse [(^{18})F]FLT uptake in consolidated lung Nodal volume too small to evaluate on scan 3*</td>
</tr>
<tr>
<td>5</td>
<td>RML</td>
<td>None</td>
<td>Moved at 45min during scan 1</td>
</tr>
<tr>
<td>6</td>
<td>RUL</td>
<td>Paratracheal Hilar (contralateral)</td>
<td>Contralateral hilar node outside RT field</td>
</tr>
<tr>
<td>7</td>
<td>LUL</td>
<td>Aorto-pulmonary Paratracheal Subcarinal</td>
<td>Primary volume too small to evaluate on scans 2 and 3</td>
</tr>
<tr>
<td>8</td>
<td>RML</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>LUL</td>
<td>Aorto-pulmonary Paratracheal</td>
<td>Moved during scan 3</td>
</tr>
<tr>
<td>10</td>
<td>RUL</td>
<td>Aorto-pulmonary Subcarinal</td>
<td>[(^{18})F]FLT uptake in consolidated lung</td>
</tr>
<tr>
<td>11</td>
<td>None</td>
<td>Aorto-pulmonary Subcarinal</td>
<td>No primary tumour</td>
</tr>
<tr>
<td>12</td>
<td>LUL</td>
<td>Aorto-pulmonary Prevascular</td>
<td>Moved at start of scan 1</td>
</tr>
</tbody>
</table>

**Abbreviations:** L/R: left/right; LL/ML/UL: lower/middle/upper lobe
Figure 4-2. Case example of patient in MEKRT trial. Patient 2, a 63-year-old man with stage T3 N2 M0 squamous cell carcinoma in right lung. Images 1a) Baseline RT planning scan and 1b) repeat RT planning scan required during RT, showing new lobar lung collapse, which is also seen, on the PET-CT scans (2a, 3a, 4a). In images 1a) and 1b), the green line corresponds to motion-adapted gross tumour volume and the yellow line indicates the 95% isodose line (6270 cGy). The baseline scan is 2(a+b), the MEK inhibitor alone is scan 3(a+b), combined MEKi and RT is scan 4(a+b). Images 2-4 a) CT scan, b) PET[^18F]FLT SUV map (45-60min), with ROI’s outlined & numbered for 1) primary tumour (white), 2) hilar node (blue), and 3) subcarinal node (yellow). There is a dramatic reduction in bone marrow uptake following radiotherapy, which was also seen in the RADAR study (16).
The primary tumour volumes were slightly larger, with a mean volume of 33 cm$^3$ ± 28 compared to the mean nodal volumes of 11 cm$^3$ ± 15 at baseline (see Figure 4-3). The lesion uptake of $[^{18}\text{F}]$FLT at baseline varied widely between patients, the SUV$_{\text{mean}}$ of the primary tumour was 3.7 ± 1.7 with similar variability in the nodes SUV$_{\text{mean}}$ 2.45 ± 0.93. In 5 out of 8 patients, the primary tumour uptake was greater than the nodes, whereas in 2 out of 8 patients nodal uptake was greater than the primary tumour, and in 1 out of 8 patients it was similar (see Figure 4-3). Baseline volume, SUV$_{\text{max}}$ and SUV$_{\text{mean}}$ for all the evaluable lesions are shown in the Figure A.1.
Figure 4-3 Baseline $[^{18}\text{F}]$FLT PET-CT parameters of the primary tumour and nodes. Figure 1a) $SUV_{\text{max}}$, 1b) $SUV_{\text{mean}}$, and 1c) volume of primary tumours (circles) and metastatic nodal lesions (squares) on the baseline (scan 1). As expected (see Figure A.2), there was a significant correlation between baseline $SUV_{\text{max}}$ and $SUV_{\text{mean}}$ for the primary (rho 0.95, p<0.0001) and for the node (averaged) (rho 0.75, p=0.0174). There was no significant correlation between the primary $SUV_{\text{max}}$ (rho 0.62, p=0.06) or $SUV_{\text{mean}}$ (rho 0.69, p=0.03) and volume. There was also no correlation for individual nodes between $SUV_{\text{max}}$ (rho=0.3, p=0.18) or $SUV_{\text{mean}}$ (rho=0.02, p=0.94) and volume.
All 12 patients received induction chemotherapy (as outlined in Table 4-1) prior to entering the study and all patients received standard platinum doublet chemotherapy with cisplatin or carboplatin combined with either gemcitabine or pemetrexed. There was a trend between the time from last administration of gemcitabine chemotherapy and the baseline SUV$_{\text{max}}$ of the nodes (averaged) (rho 0.76, p=0.058) and no significant correlation for the primary (rho 0.42, p=0.3). There were too few patients to perform the correlation analysis in the pemetrexed group (n=3) separately, but when added to the gemcitabine group there was no correlation (days from chemotherapy for primary tumour, rho 0.44, p=0.2, and for the nodes rho 0.32, p=0.4). There were no correlations between SUV$_{\text{mean}}$ and the time from the last administered induction chemotherapy for either the primary or the nodes (averaged) for gemcitabine or both drugs combined. These results suggest that the type of chemotherapy drug given, and the duration from the last administered chemotherapy to the baseline scan, did not impact upon baseline lesion uptake SUV$_{\text{max}}$ or SUV$_{\text{mean}}$ of the primary or nodes (averaged) (see Figure 4-4).
Figure 4-4 Days from last administered chemotherapy to the baseline [18F]FLT PET-CT (scan 1), and the parameters of SUV\textsubscript{max} (figure 1a) and SUV\textsubscript{mean} (figure 1b), for the primary (circle) and nodes (squares). The different chemotherapy regimes are also identified, gemcitabine (closed symbol) and pemetrexed (open symbol). At baseline there was no dependence of SUV\textsubscript{max} or SUV\textsubscript{mean} for the primary tumour or nodes (averaged) on time (in days) from last administered chemotherapy (one platinum (cisplatin or carboplatin) with either gemcitabine (closed symbols) or pemetrexed (open symbols)) to baseline [18F]FLT PET-CT (scan 1).
Figure 4-5. Relative change (response) in 1) SUV\textsubscript{max}, 2) SUV\textsubscript{mean}, and 3) volume of a) primary tumour and b) nodes (averaged) over the 3 response time points: MEK inhibitor alone (scan 2 relative to scan 1), combined MEK inhibitor and radiotherapy (scan 3 relative to scan 1) and radiotherapy (scan 3 relative to scan 2). The dotted line represents the 1.96 x standard deviation of repeatability scans from the RADAR study (16) at our centre (repeatability coefficient RC = 29.9\% for SUV\textsubscript{max}, 29.2\% for SUV\textsubscript{mean}, and 37\% for volume for all lesions).

The individual parameter values for the three $^{18}$FFLT PET scans are displayed in Figure A.3 and the relative change of individual patients to the MEK inhibitor and to radiotherapy is plotted in Figure 4-5 for the different parameters (SUV\textsubscript{max}, SUV\textsubscript{mean}, and volume). A Wilcoxon signed-rank test was performed on all the individual values (see Table 4-4). The SUV\textsubscript{max} and SUV\textsubscript{mean} responses for the nodes (average) were significant for RT and combined MEKi and
RT, but not for MEKi alone (see Table 4-4), this suggests that the MEKi did not modify the nodal $[^{18}\text{F}]$FLT uptake. The relative decreases in the $[^{18}\text{F}]$FLT uptake of the primary tumour were not significant in this study, albeit in small numbers. However the reduction in volume of the primary tumour following the combined treatment (MEKi + RT) reached significance (p=0.05) in comparison to the reduction in nodal volume which was significant in response to RT (p=0.003).

Table 4-4. Lesion $[^{18}\text{F}]$FLT uptake at 45-60 min and volume (mean ± standard deviation, SD) at baseline, after MEK inhibition (MEKi) and during concurrent RT (MEKi+RT) and associated percentage changes (responses) for the primary and nodes (averaged).

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Values</th>
<th>Responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline$^2$</td>
<td>MEKi</td>
</tr>
<tr>
<td>SUV$_{\text{max}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>7.2 ±3.3 (n=9)</td>
<td>6.6 ±3.3 (n=7)</td>
</tr>
<tr>
<td>Nodes (average)</td>
<td>6.6 ±3.5 (n=10)</td>
<td>5.9 ±3.1 (n=8)</td>
</tr>
<tr>
<td>SUV$_{\text{mean}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>3.7 ±1.7</td>
<td>3.6 ±1.6</td>
</tr>
<tr>
<td>Nodes (average)</td>
<td>3.0 ±1.2</td>
<td>2.4 ±1.0</td>
</tr>
<tr>
<td>Volume (cm$^3$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>33 ±28</td>
<td>34 ±35</td>
</tr>
<tr>
<td>Nodes (total)</td>
<td>20 ±22</td>
<td>19 ±20</td>
</tr>
</tbody>
</table>

$^1$ Significant changes are highlighted in bold, $^2$ Patient 7 with no treatment scans excluded, $^3$ Wilcoxon signed-ranked test $^4$ Patient 1 with the largest node was not scanned during RT
There was a significant negative dose-response relationship for SUV$_{\text{max}}$ during RT relative to baseline SUV$_{\text{max}}$ for all lesions (rho=-0.48, p=0.03) or the nodes (averaged) alone (rho=-0.865, p=0.0041). An increase in the dose of radiation was associated with a lower ratio, suggesting an increasing response (lower uptake of $[^{18}\text{F}]\text{FLT}$) as the dose of radiation increases (see Figure 4-6). However there was no significant dose-response relationship for the primary tumour alone (rho=-0.39, p=0.34).

In the evaluation of survival versus the different parameters, there was a trend between overall survival and baseline SUV$_{\text{max}}$ of the primary tumour, suggesting higher SUV is associated with poorer overall survival (see Figure 4-7 & 4-8). This is not significant but the small patient numbers will have had an impact, as only 10 out of 12 patients had a primary tumour (rho=-0.55, p=0.10) and 10 out of 12 patients had evaluable lymph nodes (rho=-0.43, p=0.22). There were no correlations between SUV$_{\text{mean}}$ and the primary (rho=-0.02 p=0.97) nor the nodes (rho=-0.14, p=0.71). There was a trend between overall survival and the total evaluable nodal volume (rho=-0.57, p=0.08) whereas the association with the primary volume was weaker (rho=-0.32, p=0.37). However, this study was not powered for a survival analysis.
Figure 4-7 Baseline parameters at scan 1 versus overall survival, 1a) $SUV_{\text{max}}$ of the primary tumour versus overall survival 1b) $SUV_{\text{max}}$ of the nodes (averaged) versus overall. 2a) $SUV_{\text{mean}}$ of the primary tumour versus overall survival 2b) $SUV_{\text{mean}}$ of the nodes (averaged) versus overall. 3a) Volume of the primary tumour versus overall survival 3b) Volume of the nodes (averaged) versus overall survival. Baseline tumour [18F]FLT uptake or lesion volume are not predictive of overall survival. Stronger associations were found for the primary tumour $SUV_{\text{max}}$ and total evaluable nodal volume. Note that 2 patients have only a primary and 2 other patients have only nodes (highlighted by the dotted line).
Figure 4-8 Percentage change in parameters (compared to baseline) versus overall survival, 1a) Percentage change in SUV$_{\text{max}}$ of the primary tumour versus overall survival 1b) Percentage change in SUV$_{\text{max}}$ of the nodes (averaged) versus overall survival. 2a) Percentage change in SUV$_{\text{mean}}$ of the primary tumour versus overall survival 2b) Percentage change in SUV$_{\text{mean}}$ of the nodes (averaged) versus overall survival. 3a) Percentage change in volume of the primary tumour versus overall survival 3b) Percentage change in volume of the nodes (averaged) versus overall survival. Response by percentage change in [18F]FLT uptake or lesion volume are not predictive of overall survival.
4.4 Discussion and future directions

4.4.1 Discussion

This study evaluated 12 patients within the expanded cohort of the MEKRT trial who underwent longitudinal [\(^{18}\)F]FLT PET-CT imaging whilst undergoing treatment with a radical dose of thoracic radiotherapy combined with the MEKi selumetinib. Patients were followed up for at least two years after treatment.

In this study the decreases in the [\(^{18}\)F]FLT uptake of the nodes (average) were significant in response to RT and combined treatment (MEKi and RT), but not for MEKi alone suggesting the MEKi did not modify the [\(^{18}\)F]FLT uptake in the nodes. A similar trend was seen for the primary tumour, but the smaller reductions were not significant. The suggestion that the MEKi did not change tumour uptake of [\(^{18}\)F]FLT are in keeping with the results from the clinical study (discussed in chapter 3), which concluded that there was no benefit to outcome from adding the MEK inhibitor to radical thoracic radiotherapy. Although this study is limited by a small sample size, there was at best a trend between primary tumour SUV\(_{\text{max}}\) at baseline and overall survival.

The repeatability data from the RADAR study in our centre suggested that individual changes of the primary tumour SUV\(_{\text{mean}}\) exceeding 17% and SUV\(_{\text{max}}\) exceeding 25% are likely to be significant, and changes in the nodes exceeding 35% for SUV\(_{\text{mean}}\) and 34% for SUV\(_{\text{max}}\) are likely to be significant(16). There was a significant decrease in the [\(^{18}\)F]FLT uptake of the primary tumour in only 1 patient compared to 3 from of a similar number of patients in the RADAR study (16). Unlike in the RADAR study where no change in the primary tumour volume was detected with RT alone, the decrease in [\(^{18}\)F]FLT uptake may have been confounded by a reduction in the primary tumour volume with the addition of selumetinib to RT in our study.

A further confounding factor to consider is the impact of thymidylate synthase inhibitors on [\(^{18}\)F]FLT uptake. The flare effect seen with thymidylate synthase inhibitors, including pemetrexed and gemcitabine, is usually within hours of administration of the drug and has a delayed effect on bone marrow rather than tumour lesions (22, 23). Given the interval between the last administered chemotherapy and the baseline [\(^{18}\)F]FLT PET-CT is more than 15 days, the effect on baseline tumour proliferation status was expected to be negligible, as the results demonstrate.

4.4.2 Dose-response between uptake of [\(^{18}\)F]FLT and dose of radiation

There was a significant negative dose-response relationship for SUV\(_{\text{max}}\) during RT relative to baseline SUV\(_{\text{max}}\) for all lesions and for the nodes (averaged) alone. This suggests an
increasing response (lower uptake of \([^{18}\text{F}]\text{FLT}\)) as the dose of radiation increases. These findings are in keeping with the pilot study by Everitt et al (15), as there was a reduction in \([^{18}\text{F}]\text{FLT}\) tumour uptake on eight on-treatment scans, but in contrast to the RADAR study which showed no dose-response relationship between SUV\(_{\text{max}}\) response and dose of RT for both the primary and nodes (16).

4.4.3 Correlation of \([^{18}\text{F}]\text{FLT}\) tumour uptake with overall survival

A number of studies in the literature suggest that an early decrease in \([^{18}\text{F}]\text{FLT}\) tumour uptake during treatment represents a reduction in cellular proliferation, indicating a good treatment response and is associated with a longer survival (24-27). However more recent data from Everitt et al (28) suggests the opposite to this and to their own pilot study (15). In their study, 60 patients with NSCLC treated with concurrent chemoradiotherapy underwent \([^{18}\text{F}]\text{FLT}\) PET-CT and \([^{18}\text{F}]\text{FDG}\) PET-CT scans at baseline and during weeks 2 and 4 of treatment (28). They scored the metabolic response by using the European Organisation for Research and Treatment of Cancer (EORTC) response criteria. Metabolic responses were calculated using the mean SUV\(_{\text{max}}\) for up to 3 lesions: the primary tumour; and the first 2 nodal stations closest to the primary gross tumour volume. Tumour response was characterized as either a complete metabolic response, partial metabolic response, stable metabolic response or progressive metabolic response (29). The association between the \([^{18}\text{F}]\text{FLT}\) week 2 response and PFS and OS was the strongest. Patients who had a stable \([^{18}\text{F}]\text{FLT}\) uptake response compared with baseline had better outcomes than those with a partial or complete reduction in \([^{18}\text{F}]\text{FLT}\) uptake or disease progression (28). Everitt et al speculate that this unexpected difference with “responders” associated with a poorer outcome may be due to tumour cells undergoing cell cycle arrest at the G1/S checkpoint, allowing time for repair of tumour DNA damage and protecting tumours from radiation therapy (28).

4.4.4 Limitations

In our study, on-treatment scans were performed within 7 days of starting MEKi to assess the effect of the drug and between fractions 3-10 whilst on combined treatment with radical radiotherapy. Ideally, this should be a narrower window to decrease the impact of intrinsic radiosensitivity, minimise the confounding effect of volumetric changes and potentially radiotherapy related inflammatory change on tumour radiotracer uptake. In comparison to \([^{18}\text{F}]\text{FDG}\), \([^{18}\text{F}]\text{FLT}\) is highly tumour-specific and is not taken up by macrophages or other inflammatory cells (as demonstrated in Figure 4-2, the collapsed and consolidated lung does not take up \([^{18}\text{F}]\text{FLT}\)). In addition the uptake of \([^{18}\text{F}]\text{FLT}\) is significantly lower than \([^{18}\text{F}]\text{FDG}\), by approximately half, so it is much less sensitive.

Although data was collected on individual nodes, the results have been pooled together for data interpretation which is a limitation of the analysis. It would be of value to analyse
individual lesion data in this diverse cohort of patients and explore the heterogeneity that exists in NSCLC particularly in stage III disease. This data may help in predicting response to radiotherapy and if a robust tracer was established could support dose-painting radiotherapy, which targets radioresistant areas of a tumour defined by functional imaging. [$^{18}$F]FLT does not seem to be of value in assessing response to the MEKI combined with thoracic radiotherapy, and it is possible that other tracers e.g. [$^{18}$F]FDG may be more suitable.

A further limitation of our study is the difficulty of evaluating outcomes from small, single-centre imaging studies. Due to technical reasons, some patients missed a scan on treatment. This reduced the strength of the conclusions that could be drawn from this study.

### 4.4.5 Future considerations

Despite its limitations, this study demonstrates how such investigations can be useful by generating hypotheses with the potential to be tested in collaborative multicentre studies. This, however, would require the standardisation of methodology which can be challenging across different centres using different imaging equipment.

It is crucial that any imaging biomarkers used as surrogates for early tumour response are evaluated in the context of long-term patient outcomes. There is conflicting data in the literature as to the direction and magnitude of [$^{18}$F]FLT uptake that indicates a “response” and whether it has prognostic value. Additionally, there are limitations of [$^{18}$F]FLT; mainly low sensitivity and the potential for drug interactions e.g. chemotherapy drugs effecting enzyme pathways particularly TK1 activity (22, 26). However [$^{18}$F]FLT does highlight heterogeneity in tumours and may guide the best site for a targeted biopsy (10).

Response assessment using [$^{18}$F]FDG PET-CT has been shown to be better than standard CT for predicting survival after chemoradiotherapy (30). During fractionated courses of radiotherapy, metabolic responses are detected more rapidly on PET-CT versus CT and interim [$^{18}$F]FDG PET scans have become the imaging modality of choice if adaptive radiotherapy is planned (31, 32). In future studies using an established and widely available tracer such as [$^{18}$F]FDG might yield better results than using experimental tracers such as [$^{18}$F]FLT.

### 4.4.6 Conclusion

Further work in larger patient cohorts is needed as thus far the evidence does not suggest that [$^{18}$F]FLT is a robust enough radiotracer to use for assessing early clinical response to treatment.
Figure A1: $SUV_{\text{max}}$ uptake across all 3 scans for all lesions. Individual nodes and primary response for 1a) scan 1, 1b) scan 2 and 1c) scan 3. Displayed to show the average node calculation amongst the other nodes.
Figure A2: Parameters ($SUV_{max}$ and $SUV_{mean}$) and volume at baseline (scan 1). 1a) $SUV_{max}$ of the primary tumour versus $SUV_{mean}$ 1b) $SUV_{max}$ of the nodes (averaged) versus $SUV_{mean}$ 2a) Volume of the primary tumour versus $SUV_{max}$ 2b) Volume of each individual node versus $SUV_{max}$. 3a) Volume of the primary tumour versus $SUV_{mean}$ 3b) Volume of each individual node versus $SUV_{mean}$. Due to the limited spatial resolution of the PET scanner (partial volume effects), tumour $^{18}$FFLT uptake depends on tumour volume. As a consequence of blurring, the tumour uptake is lower than in reality and the effect is greater the smaller the tumour is.
Figure A3: Parameters 1a) $SUV_{\max}$ 1b) $SUV_{\text{mean}}$ 1c) volume changes of the primary tumour for each individual patient for all 3 scans. Patient 3 and 11 do not have a primary tumour.
4.6 References – Chapter Four


28. Everitt S, Ball D, Hicks RJ, Callahan J, Plumridge N, Trinh J, et al. Prospective Study of Serial Imaging Comparing Fluorodeoxyglucose Positron Emission Tomography (PET) and Fluorothymidine PET During Radical Chemoradiation for Non-Small Cell Lung


Chapter Five

Discussion and future directions
5 Discussion and future directions

5.1 The unmet need in stage III NSCLC

As the current 5-year survival of stage III NSCLC with standard treatment remains poor, approximately 30% at best with concurrent chemoradiotherapy, (4, 5), there is an urgent need to improve patient outcomes. As the majority of patients are unsuitable for this treatment due to poor performance status and comorbidities (6) there is an unmet need in stage III NSCLC to develop alternative methods of treatment intensification. Little progress has been made over the last two decades, for example dose escalation using conventional fractionation and the addition of new chemotherapy drugs to concurrent treatment both failed to improve outcome. Neither study took into account histology or driver mutations as part of the treatment algorithm. The only real recent advance is in the field of immunotherapy, the use of durvalumab after chemoradiotherapy in stage III NSCLC was associated with a significantly longer progression-free survival than placebo (8).

The overall aim of this thesis is to evaluate methods of personalising radical radiotherapy in non-small cell lung cancer (NSCLC). This involves assessing the feasibility of delivering an individualised (isotoxic) dose escalated radiotherapy schedule using intensely modulated radiotherapy (IMRT), evaluating a novel drug selumetinib in combination with radical thoracic radiotherapy (RT), and a novel imaging biomarker 3'-deoxy-3'-[\(^{18}\)F]Fluorothymidine (\([^{18}\)F]FLT). The main strength of this thesis is the presentation of original work, with respect to these individual subsections, and each will be discussed in turn.

5.2 Methods of personalising radiotherapy in NSCLC

5.2.1 Dose-escalated radiotherapy

The Isotoxic radiotherapy schedule (discussed in chapter 2) was found to be a feasible method of dose-escalation. It is now being tested alongside other dose escalated accelerated schedules in the randomised phase II ADSCaN trial (ISRCTN47674500) which compares experimental arms against the UK standard sequential chemoradiotherapy regimen of 55 Gy in 20 fractions for patients with NSCLC (see Figure 5-1).
Figure 5-1 Randomised Phase II study of Accelerated Dose escalated Sequential Chemoradiotherapy in Non-Small Cell Lung Cancer (ADSCaN)

The hypothesis is that sequential chemoradiotherapy using accelerated and dose escalated radiotherapy will intensify loco-regional treatment, and will improve local control and overall survival in comparison to using conventional fractionation. In routine practice as the majority of patients receive sequential chemoradiotherapy rather than concurrent treatment, particularly in the UK, it is important to turn attention to this patient group. Over the last 2 decades all efforts have focused on concurrent chemoradiotherapy, and until ADSCaN there has been an unmet need for a clinical trial in sequential chemoradiotherapy. The main objective of the randomised phase II ADSCaN trial is to select a single schedule to take forward for phase III testing using a ‘pick the winner’ design. A limitation of each experimental schedule included in the ADSCaN trial was it was first tested as a single arm study. The advantage of the ADSCaN study is the scope for testing 4 experimental arms simultaneously against standard treatment. The heterogeneity between patients with stage III NSCLC, (volume of disease, anatomical location, histology, tumour and host genomics) is a limitation of this patient group which may significantly impact on outcome (9). In the ADSCaN trial, prognostic factors that reflect the differing case mix (e.g. histological type, epidermal growth factor receptor (EGFR) mutation status, radiotherapy technique (3D conformal RT vs. IMRT), stage (IIIA vs. IIIB), gross tumour volume, albumin and age), will be taken into account in the primary analysis model, to fully adjust for case-mix. The reflection of case-mix in all multi-arm trials is important, but particularly important in a heterogeneous population to allow a true estimation of treatment effect. The question as to which sequential radiotherapy schedule is superior has been extensively debated over the last decade. It is expected that the phase III...
randomised control trial that will follow on from ADSCaN will guide future practice in this
group of patients.

5.2.2 Molecularly targeted drugs combined with radiotherapy

Adding molecularly targeted drugs to radiotherapy is another method of personalising
treatment, and is an alternative for those unsuitable for concurrent treatment with
chemotherapy. Although the MEK inhibitor selumetinib has been trialed in over 2,000
patients, and used in combination with chemotherapy, the MEKRT trial (discussed in chapter
3) is the first study in the world to use selumetinib concurrently with radical thoracic RT. The
main limitation of the MEKRT trial is the small number of patients, and also the heterogeneity
of the stage III patient group. Unfortunately the clinical outcome was poor, the results
suggested this combination should not be pursued in a subsequent phase II trial. However as
is the case with most small single arm radiotherapy trials, outcome data should be interpreted
with caution and such studies are mainly hypothesis generating. In the future there is a need
to use more efficient design and recruit patients at multiple sites. Conventional early phase
clinical trials are typically designed to evaluate one radiotherapy-drug combination at a time.
However, platform or umbrella trials provide an opportunity to study multiple targeted
therapies in the same disease area in a more efficient and scientifically rich manner (10). The
integration of a calibration control arm is incorporated into this multi-arm platform study to
ensure the safety and toxicity data in the combination arms are interpretable within the
context of the current study, given the use of modern radiotherapy techniques (11).

Such multi-institution phase I trial involving a number of UK centres is in set-up and will test
multiple DNA damage response inhibitors (DDRi) given in combination with fixed dose
curative intent radiotherapy in patients with stage IIIB/III NSCLC (12). In addition to the
standard radiotherapy dose of 60 – 66 Gy in 30 – 33 fractions, the dose delivered to the
organs at risk will also be standardised so any additional tissue toxicity can be attributed to
the novel drug rather than a differences in the dose of radiotherapy (12). This novel umbrella
study will be set up using a Bayesian design to optimise accrual and incorporate valuable
toxicity data. The primary outcome measure for each arm will be the recommended phase 2
dose of the drug to take forward to subsequent trials. The study plans to incorporate “proof of
mechanism” biomarkers and the adaptive design will allow any future biomarkers to be
incorporated (12).

Another limitation of the MEKRT study is that out of the 21 patients none had KRAS
mutations. The mitogen activated protein kinase pathway comprising the
RAS/RAF/MEK/ERK signaling cascade has a key role in the regulation of normal cell
proliferation. Mutations to the oncogene K-RAS, which can activate the RAS pathway, can be
found in 15-50% of NSCLC cases (13). In K-RAS mutant xenografts selumetinib led to
increased growth inhibition when used synergistically with cytotoxics (docetaxel, temozolamide) and targeted agents (gefitinib) compared to monotherapy (14, 15). When used in combination with ionizing radiation, preclinical models using NSCLC cell lines have demonstrated enhanced radiosensitivity, the mechanism of which is not fully understood (16). As there is some preclinical evidence which suggests selumetinib may effect growth inhibition to a greater extent in K-RAS mutants, the absence of K-RAS mutants in this study may have contributed to the poor outcome.

Over the last 10 years there has been increasing interest into molecular and genomic profiling leading to the stratification of patients according to mutation status. However there are very few studies currently recruiting in the UK combining a molecularly targeted drug combined with radiotherapy. Furthermore to date only two drugs are licensed to be delivered with radiotherapy, namely cetuximab in cancers of the head and neck and temozolomide in glioblastoma multiforme (10). Cetuximab preferentially radiosensitises tumour over normal tissues by effecting the EGFR pathway involved in regulating intrinsic radioresistance (17, 18) and showed improvements in overall survival and 3-year local control in patients with advanced-stage head and neck cancer (17, 19). The radiosensitiser temozolomide is not only of benefit concurrently in glioblastoma multiforme but also as an adjuvant systemic treatment following radiotherapy (20). In contrast there are a number of biomarker-driven studies evaluating targeted therapies and immunotherapies in patients with advanced NSCLC. For example in TRACERx (Tracking Non-small cell lung cancer evolution through therapy) (NCT01888601) which evaluates evolutionary genomic landscape between primary and metastatic sites and intratumour heterogeneity over time in NSCLC, 2 sub-studies are set-up to study the personalised delivery of anti-cancer drugs. In the first study, DARWIN I (NCT02183883) targeted drugs (e.g. trastuzumab emtansine, alectinib) are prescribed according to the mutation status of patients. In DARWIN II (NCT02183883) those without an actionable mutation, but programmed death-ligand 1 (PD-L1) positive status, will receive an upfront monoclonal antibody targeting anti-PDL1 (MPDL3280A), and in those who are PD-L1 negative targeted treatment (MPDL3280A) will follow after chemotherapy. These studies include the analysis of exploratory biomarkers to investigate a number of markers that may predict the response to treatment and influence future trial design. In the future radiotherapy could be incorporated within the trial design considering alternative options to chemotherapy or in addition to chemotherapy.

It is not only important to stratify patients appropriately to receive the best drug combination with RT, but also important that a durable response is not at the expense of toxicity. Treating lung cancer with novel agents, especially if used in combination with radiotherapy, necessitates increased vigilance for pulmonary toxicity as pneumonitis can be fatal. In addition to pneumonitis, another serious complication of treatment is the development of pneumocystis jiroveci pneumonia (PJP) in the presence of lymphopaenia. PJP is an
opportunistic fungal infection, previously known as pneumocystis carinii pneumonia, which causes respiratory symptoms in humans. The majority of cases occur in immunocompromised patients. PJP has been widely studied in patients with Human Immunodeficiency Virus (HIV) and in the presence of a low CD4 lymphocyte count is recognised as an Acquired Immune Deficiency Syndrome (AIDS) defining illness. PJP also occurs in patients with cancer-related immunosuppression, with or without the concomitant use of steroids. There is a significantly higher mortality rate from PJP in non-HIV patients compared with those with HIV (21, 22). This is the first clinical trial using selumetinib to report severe and prolonged lymphopaenia, and thus the thoracic RT is likely to have had a confounding effect. Over the last few years there has been an increasing awareness amongst clinicians in our institution of PJP in the lung cancer population.

Following the interim results of the MEKRT study, I was involved in a quality improvement project reviewing all confirmed cases of PJP in patients in our centre who had been treated for lung cancer between 2013 – 2017. The outcome of our analysis has resulted in the implementation of PJP guidelines which outline the recommendations for the use of PJP prophylaxis. As a result, it was thus necessary for this PhD research to provoke guidance on PJP. I wrote guidance on PJP which was included in the ADSCaN trial protocol. It is important to raise awareness of PJP in lung cancer patients and the clinical trial community, particularly trials using chemoradiotherapy, should be encouraged to report incidence of lymphopaenia and PJP. It is critical that all clinicians remain vigilant to the early signs of PJP and use PJP prophylaxis when appropriate in order to prevent unnecessary deaths.

5.2.3 Biomarkers evaluating early response to treatment

Given the risk of toxicity with intensified treatments, biomarkers evaluating early response to treatment may help prevent toxicity from futile treatments. In the MEKRT study, 3'-deoxy-3'-[18F]Fluorothymidine ([18F]FLT) was evaluated as a proliferation tracer and a measure of early response to cancer treatment. However there is conflicting evidence in the literature, as [18F]FLT responses in both directions have been shown to be prognostic. In addition there are a number of limitations, compared to 2-[18F]Fluoro-2-deoxy-D-glucose ([18F]FDG) there is significantly lower uptake of [18F]FLT (approximately half that of [18F]FDG. [18F]FLT has a higher specificity than [18F]FDG but a lower sensitivity. [18F]FLT strongly correlates with histopathological proliferation markers (23) giving the potential to use this as a non-invasive method for tumour grading. As it highlights heterogeneity in tumours, it may guide to the best site for a targeted biopsy, rather than replacing a biopsy, and at present is potentially most useful in brain tumours (23). As an alternative to imaging biomarkers, another example of a non-invasive biomarker, is the use of circulating free DNA or circulating tumour cells. This “liquid biopsy” has the potential to be easily repeated to assess tumour evolution and has the potential to be used as a predictive biomarker. Technology in this field has rapidly developed.
over the last few years and it will be interesting to see which methods of analysis become validated and whether "liquid biopsies" can facilitate real-time decision making in the clinic.

5.3 Conclusion and future directions

This PhD highlights the poor outcome from NSCLC suggesting the need to intensify our treatments. However given the heterogeneity of disease in patients with NSCLC, especially those with stage III disease, this emphasises the need to personalise treatment to the individual in order to improve outcome.

During my time in research I really enjoyed gaining experience in clinical trials involving a novel drug, functional imaging and dose escalated radiotherapy. I am particularly interested in clinical trial design and hope to use these skills in my future work as a Consultant in Clinical Oncology. The future of personalised treatment and better outcomes in NSCLC will require the integration of immunotherapy and RT (both in the sequential and concurrent CTRT setting), the integration of targeted agents in patients with driver mutations (e.g. Erlotinib Hydrochloride or Crizotinib and Chemoradiation Therapy in Treating Patients with Stage III NSCLC, NCT01822496), personalized dose escalation (e.g. ADSCaN trial (ISRCTN47674500)), a better understanding of RT-induced cardiac toxicity and an improvement in the management of co-morbidities so patients can have the best treatment available,
5.4 References – Chapter five


