Improving Outcomes for the Lung Cancer Patient with Impaired Lung Function

MD(Res)

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Declaration of Authenticity

The work presented in this thesis is my own, except where stated.

I received statistical support for the analyses in chapters 2, 5 and 6 from the Royal Marsden Hospital (RMH) lung unit statistician, Ms. Ranga Gunapala. The sample size calculation in chapter 3 was supported by the RMH statistics department.

For the translational work in chapter 4, technical support was provided by histopathologists within the Breast Cancer Now (BCN) department of The Institute of Cancer Research. The NanoString assay was ran by the BCN NanoString Facility.

Chapter 6 presents the results of a study that was open to recruitment prior to the commencement of this MD(Res). My contributions are declared within the chapter.

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Dr David Walder

Abstract

Lung function impairment is common amongst patients with lung cancers. Identifying the patients that are most likely to benefit from anti-cancers therapies, without suffering significant toxicities, is central to improving their outcomes.

Radiotherapy

Method: A retrospective review with survival and regression analyses to establish associated factors related to pneumonitis, relapse and survival from 208 SBRT treatments.

Results: Median overall survival 2.6 years (2.3 – 3.3). Mediastinal staging associated with risk of pneumonitis (OR: 11.79 (2.66 – 52.26) $p = 0.001$). No association between lung function parameters and risk of pneumonitis. Low TLCO associated with worse overall survival (HR: 0.98 (0.97 – 0.99) $p = 0.010$). Increased tumour size associated with shorter time to relapse (HR: 1.05 (1.02 – 1.08) $p = 0.001$)

Conclusion: Low TLCO is a poor prognostic marker for patients undergoing SBRT for early stage NSCLC

Radiotherapy/Immunotherapy Combination

Part A

Method: A phase 1b/II study to assess the safety of adjuvant nivolumab (240mg every 2 weeks) commencing within 24 hours of the final fraction of SBRT.

Results: After a minimum of 3 months follow up of the first 5 recruited patients, no episodes of grade 3 pneumonitis were observed. Based on this, the trial has been expanded to include recruitment of patients of ECOG performance status 2.

Conclusion: Early data suggests unacceptable lung toxicity is not seen with adjuvant nivolumab following SBRT

Part B

Method: NanoString analysis of RNA from macrodissected NSCLC biopsies including the tumour microenvironment.

Results: Successful immunogenomic profiling from 12 degraded NSCLC biopsies. Tendency for lower expression of MICB and IFN in biopsies from patients who went on to respond to combination radiotherapy/immunotherapy treatment.

Conclusion: The immune makeup of the tumour microenvironment may help to predict responses to combination immunotherapy and radiotherapy treatment regimens

Chemotherapy

Method: A retrospective analysis of 52 patients to establish the patient factors associated with tolerability and outcome from second-line docetaxel for NSCLC.

Results: FEV_1 was the factor most associated with overall survival (HR: 0.96 (0.93 - 0.99 $p = 0.009$). Patients with an FEV_1 less than 50% predicted had significantly worse survival (HR: 0.15 (0.04 – 0.57) $p = 0.005$) and were also more likely to discontinue treatment due to toxicity ($p = 0.023$).

Conclusion: An FEV_1 less than 50% predicted is a poor prognostic marker in patients being considered for docetaxel chemotherapy for advanced NSCLC.

Symptom Control

Method: An open label, randomised, controlled trial comparing the effect of adding optimal inhaled therapies to best supportive care alone in 64 patients with co-existing untreated COPD and lung cancer.

Results: Inhaled therapies led to an increase in the proportion of patients achieving a minimum 2-point improvement in VAS breathlessness after 4 weeks. Response rate in those receiving inhaled therapies was 53% (35 -71) compared to 26% (12 - 45) in the group that received BSC alone ($p = 0.027$).

Conclusion: Spirometry performed in the lung cancer clinic can identify undiagnosed COPD and treating this with inhaled therapies improves breathlessness.

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Publications During Period of Study

In Submission: Impact of Inhaled Therapies for Breathlessness in Patients with Undiagnosed COPD and Co-Existing Thoracic Cancers: A Randomised-Controlled Trial. **Walder D**, Punwani R, Kumar R, Nimako K, Minchom A, Gunapala R, Chua F, Bhosle J, Popat S, O'Brien MER. The Clinical Respiratory Journal. April 2020

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Poster Presentations During Period of Study

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Walder DP, Bhosle J, Ratoff J. Is the chest x-ray sensitive enough to diagnose lung cancer in symptomatic patients? BTOG 2017

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List of Abbreviations

6MWD	6-Minute Walk Distance
AE(s)	Adverse Events
ARSAC	Administration of Radioactive Substances Advisory Committee
BCN	Breast Cancer Now
BED	Biological Effective Dose
BMS	Bristol-Myers Squibb
BSC	Best Supportive Care
BTS	British Thoracic Society
CCI	Charlson Co-morbidity Index
CCR	Committee for Clinical Research
cGAS	Cyclic GMP-AMP Synthase
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CTC(s)	Circulating Tumour Cells
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T Lymphocyte-associated Antigen 4
DAMPs	Damage-Associated Molecular Patterns
DC	Dendritic Cells
DCB	Durable Clinical Benefit
DFS	Disease Free Survival
DR	Distant Relapse
EBUS	Endobronchial Ultrasound
ECOG	Eastern Conference Oncology Group
ESMO	European Society for Medical Oncology
FDG-PET	Fluorodeoxyglucose Positron Emission Tomography
FDR	False Discovery Rate
FEV_1	Forced Expiratory Volume in 1 Second
FEV_1%pred	FEV_1 as a Percentage of Predicted Normal
FFPE	Formalin-Fixed Paraffin-Embedded
FNA	Fine Needle Aspiration
FVC	Forced Vital Capacity
G-CSF	Granulocyte-Colony Stimulating Factor
GOLD	Global Initiative for Obstructive Lung Disease
Gy	Gray
HR	Hazard Ratio
HRA	Health Research Authority
HRQoL	Health Related Quality of Life
IDMC	Independent Data Monitoring Committee
IFN-1	Type-I Interferon
IHC	Immunohistochemistry
IMP	Investigational Medical Product
IQR	Interquartile Range
ISG	IFN-I stimulating genes
KCO	Carbon Monoxide Coefficient
LFT(s)	Liver Function Tests
LLP	Liverpool Lung Project
LLR	Loco-regional Relapse
LR	Local Relapse
MHC	Major Histocompatibility Complex
MRC	Medical Research Council

mRNA	Messenger RNA
NGS	Next Generation Sequencing
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NKG2D	Natural Killer Group 2, Member D
NLST	National Lung Screening Trial
NSCLC	Non-Small Cell Lung Cancer
OR	Odds Ratio
OS	Overall Survival
PACS	Picture Archiving and Communication System
PD-1	Programmed Death-1
PD-L1	Programmed Death-1 Ligand
PEFR	Peak Expiratory Flow Rate
PFS	Progression Free Survival
PFT(s)	Pulmonary Function Tests
pGGO(s)	Pure Ground Glass Opacities
PIS	Patient Information Sheet
PPI	Patient and Public Involvement
PS	Performance Status
QC	Quality Control
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
RDI	Relative Dose Intensity
REC	Research and Ethics Committee
RECIST	Response Evaluation Criteria in Solid Tumours
RIN	RNA Integrity Number
RM-CTU	Royal Marsden Clinical Trials Unit
RMH	Royal Marsden Hospital
RNA-Seq	RNA-Sequencing
RR	Relative Risk
RTOG	Radiation Therapy Oncology Group
SAE(s)	Severe Adverse Events
SBRT	Stereotactic Body Radiotherapy
SCLC	Small Cell Lung Cancer
SD	Standard Deviation
SE	Standard Error
SGRQ	St. George's Respiratory Questionnaire
SUV_{max}	Maximum Standardized Uptake Value
TCR	T Cell Receptor
TFT(s)	Thyroid Function Tests
TIL(s)	Tumour Infiltrating Lymphocytes
TLCO	Carbon Monoxide Transfer Factor
TLCO%pred	TLCO as a Percentage of Predicted Normal
TMB	Tumour Mutational Burden
TPS	Tumour Proportion Score
TSH	Thyroid Stimulating Hormone
UICC	Union for International Cancer Control
V/Q	Ventilation-Perfusion
VA	Alveolar Volume
VAS	Visual Analogue Scale
VEGF	Vascular Endothelial Growth Factor
VO_2max	Peak Oxygen Consumption

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Overview of Thesis

In this thesis I will explore the impact that impaired lung function has on various lung cancer treatment modalities. Lung cancer patients with impaired lung function have poor outcomes due to a combination of unsuitability for curative treatment options, intolerance to treatments and the occurrence of more severe side effects. In this series of studies, I will highlight the importance of lung function when making decisions regarding the risks and benefits of anti-cancer treatments and provide evidence to help deliver better treatment strategies for the lung cancer patient with impaired lung function. The aims and hypotheses of each study are presented below.

Part 1: Radiotherapy (Chapter 2)

Risk factors for Pneumonitis and Survival following Stereotactic Body Radiotherapy in Early Stage NSCLC

- **Aim:** To explore the patient and tumour factors that affect the toxicity and prognosis following stereotactic body radiotherapy (SBRT) for early stage NSCLC
- **Hypotheses:**
    - The rate of pneumonitis (≥ grade 2) following SBRT for NSCLC is not influenced by lung function parameters
    - Expression of programmed death ligand-1 (PD-L1) on biopsy samples is a negative prognostic marker for patients treated with SBRT alone for NSCLC

Part 2. Combining Radiotherapy and Immunotherapy (Chapters 3 & 4)

Part 2A: Stereotactic Body Radiotherapy with Immunotherapy: Tolerability and Lung Effects – The STILE Trial (Chapter 3)

- **Aim:** To assess the lung toxicity of nivolumab after stereotactic body radiotherapy (SBRT) for early stage NSCLC
- **Hypothesis:**
    - Nivolumab following SBRT for stage I-II NSCLC (T3 (≤5cm) N0M0 is a safe treatment and rates of pneumonitis at grade 3 or higher within 6 months of the final fraction of SBRT occur at a rate that does not exceed 20%

Part 2B: Immunogenomics of Patients with NSCLC and Impact on Response to Combination Radiotherapy and Immunotherapy (Chapter 4)

- **Aim:** To assess the ability to extract RNA and then perform NanoString immunogenomic profiling from archival fixed-formalin paraffin embedded (FFPE) NSCLC biopsy samples from patients receiving combination radiotherapy and immunotherapy treatment
- **Hypothesis:**
    - The immune profile of the tumour microenvironment can predict response to combination radiotherapy and immunotherapy for advanced NSCLC

Part 3: Chemotherapy (Chapter 5)

Predicting Toxicity and Outcome from Docetaxel in NSCLC

- **Aim:** Understand the factors that affect the tolerability and outcomes for patients receiving docetaxel for NSCLC
- **Hypothesis:**
    - Patients with poorer lung function parameters are less tolerant of second-line docetaxel for NSCLC and have worse survival outcomes

Part 4. Symptom Control (Chapter 6)

Optimal Management of Breathlessness in Co-Existing Undiagnosed COPD and Lung Cancer – The ADOPT Study

- **Aim:** To understand the effects that the addition of inhaled therapies has on breathlessness over best supportive care alone in patients with lung cancer and untreated COPD
- **Hypothesis:**
    - The addition of appropriate inhaled therapies to best supportive care will improve breathlessness for patients with co-existing COPD and lung cancer

Chapter 1: **Background on the Management of Non-Small Cell Lung Cancer and the Relationship with Lung Function**

1.1 Introduction

Lung cancer is the leading cause of cancer-related death worldwide. Non-small cell lung cancer (NSCLC), which includes adenocarcinoma, squamous cell carcinoma and large cell carcinoma accounts for approximately 80-85% of all lung cancers.[49] Small cell lung cancer (SCLC), a more aggressive and widely metastatic form, is pathologically and clinically very different from NSCLC and accounts for the remaining 15%.[40] While most patients with SCLC initially respond to cytotoxic chemotherapy, tumours very frequently recur, and mortality is high. For this reason, SCLC are very rarely surgically resected and follow different treatment algorithms to NSCLC. This thesis will explore the significance of lung function on multiple treatment modalities, focussing only on NSCLC, and aims to provide evidence towards the development of better management strategies for these lung cancer patients with impaired lung function.

Impairment in lung function parameters are seen across nearly all chronic respiratory pathologies. Both restrictive and obstructive patterns of lung function impairment on spirometric testing are associated with an increased risk of developing lung cancer. Obstructive spirometry is characterised by low forced expiratory volume in 1 second (FEV_1) with relatively preserved forced vital capacity (FVC), which leads to a reduced FEV_1/FVC ratio of less than 0.7. Obstructive spirometry is seen in patients with chronic obstructive pulmonary disease (COPD), emphysema and asthma. Although tobacco smoke is a common aetiological factor for both COPD and lung cancer, the presence of airways obstruction is a well-established additional risk factor for the development of lung cancer.[65, 117] This effect is seen even after controlling for smoking status, with the presence of COPD leading to between a 2 and 5 fold increased risk of developing lung cancer.[90] Restrictive lung disease is characterised by spirometry findings of a low FEV_1 and a low FVC with a preserved FEV_1/FVC ratio of greater than 0.7. A restrictive spirometry pattern (or test result) is found in patients with multiple conditions, including

interstitial lung disease, pleural disease, obesity and cardiovascular disease and is only weakly associated with smoking.[66] Despite this weak association, restrictive spirometry is also associated with a higher incidence of lung cancer compared to those with normal lung function.[89] Chronic inflammation of the lung parenchyma, a common pathological process related to several restrictive lung diseases such as interstitial lung disease and pneumonitis, is a postulated mechanism behind the development of lung cancer.[73]

Lung function impairment is therefore a common finding in patients diagnosed with lung cancer. COPD co-exists in 50-70% of lung cancer cases[117] and may be underdiagnosed. A large retrospective study of hospitalised patients with lung cancer, found a new diagnosis of COPD in 7.1% of the patients.[120] More severe impairment in lung function leads to reduced physiological reserve and a decline in exercise tolerance. Two meta-analyses have found COPD to be a negative prognostic marker in NSCLC, although there was significant heterogeneity between the studies in each analysis.[23] For surgically resected patients, the largest decrease in survival is seen in patients with more severe COPD.[13] The degree of lung function impairment is therefore an important factor when considering treatment options for a patient with lung cancer in both early and advanced stage disease.

1.2 Lung Cancer Screening

Lung cancer screening programs using low dose CT scans have been shown to increase the proportion of cancers diagnosed at an earlier stage. The US based National Lung Screening Trial (NLST) was the first trial to show that CT screening of high-risk individuals could reduce lung cancer mortality. In this landmark screening trial of 53,454 high-risk individuals, lung cancer mortality with annual low dose CT was reduced by 20% compared to annual plain chest x-ray. Mortality from any cause was also improved in the CT arm with a relative risk reduction of 6.7%.[76] In both arms of this trial the proportion of stage IA and IB tumours was highest in screen detected cancers. A recent update with extended follow-up has confirmed that the reduction in lung cancer mortality is as much as 19%.[75] Following this trial the US have implemented annual CT screening for patients that meet the NLST criteria of being

current or former smokers (within 15 years of quit date) between the ages of 55 and 74 and with a minimum of 30 pack years smoking history. In a subgroup analysis from the NLST, which included 18,475 participants with baseline pre-bronchodilator spirometry, the presence of COPD was associated with a two-fold increase in lung cancer incidence, a more favourable stage shift and no apparent overdiagnosis.[116]

In 2020, the Dutch-Belgian NELSON trial also reported that low dose CT scans with intervals of 1, 2 and 2.5 years reduced lung cancer mortality by 24% compared to standard of care.[25] The smoking history criteria in the NELSON study was current or former smokers (maximum of 10 years since quit date) who had smoked >15 cigarettes/day for 25 years or >10 cigarettes/day for 30 years. This study, which had fewer participants than the NLST was not able to show a significant reduction in all-cause mortality. A key finding from the NELSON study was the lung cancer stage shift achieved with CT screening compared to standard of care. In the CT arm, 30.5% and 9.9% of cancers were diagnosed in stage IA and IB respectively. In comparison, the proportions in the standard of care arm were 6.9% (IA) and 6.6% (IB).[25] The NELSON trial had several advantages over the NLST including using a volumetric nodule follow-up protocol which reduced the number of subsequent scans and invasive diagnostic procedures of non-malignant nodules. The NELSON trial was initially only focused on male participants, but the protocol was amended during the trial to include females, and in the final analysis 16.4% of participants were women. In the subgroup analysis of female participants, the rate ratio for lung cancer death was 0.67 (95% CI, 0.38 to 1.14) at 10 years of follow-up.

Following the publication of the NELSON study there seems little doubt that there will be widespread implementation of CT lung cancer screening programs across Europe. This is likely to cause an increase in the number and proportion of early stage NSCLCs being diagnosed, who will be candidates for radical treatment. Regular lung function testing was not included in the protocols of either the NELSON study or the NLST. However, in the Danish Lung Cancer Screening Trial, spirometry was performed at each screening round. Although overall, this smaller study failed to show a difference in lung cancer mortality with CT screening, for patients with extensive smoking history

(≥ 35 pack years) and COPD, the risk of death due to lung cancer was two to six times higher than in the other subgroups.[114]

The UK Lung Cancer Screening trial aimed to determine the best recruitment and screening strategies for implementation of a lung cancer screening program in the UK. In this trial, high risk individuals were identified using the Liverpool Lung Project (LLP) risk prediction model version 2, and recruited those with a 5-year lung cancer risk of ≥ 5%.[34] The LLP risk prediction model incorporates age, gender, family history of lung cancer, smoking duration, personal history of other cancers and non-malignant respiratory diseases including COPD, and occupational exposure to asbestos.[15] Similarly, the SUMMIT study, a pilot study for the introduction of lung cancer screening in the UK, incorporates a 'lung health check' at baseline, which includes spirometry. The eligibility requirement for patients to enter low-dose CT screening is a 6-year lung cancer risk of ≥ 1.3%, as assessed by the PLCOm2012 risk prediction model.[52] As with the LLP model, the PLCOm2012 includes the presence of COPD within its algorithm.[52, 110] Hence, if lung cancer screening is to be introduced nationally in the UK, it seems likely that patients with impaired lung function will form a significant proportion of the eligible population.

1.3 Management of Early Stage NSCLC

1.3.1 Surgical Management

Lung cancers are commonly diagnosed at an advanced stage. A national database survey found that stage I cancers made up only 24.8% of all diagnosed NSCLC.[72] The reporters found evidence of a decrease in the proportion of stage I cancers being diagnosed from the year 2002 onwards. Stage I cancers represented 27.5% in 2000 but only 24.8% in 2002, with a corresponding increase in stage IV disease from 35.4% to 38.8%.[72] The decrease was attributed to the introduction of widespread use of fluorodeoxyglucose positron emission tomography (FDG-PET) with radiological upstaging of some tumours. The gold standard treatment for stage I to IIIA disease remains lobectomy with hilar and mediastinal lymph node dissection or sampling. A minimally invasive thoracoscopic lobectomy is an alternative approach to open lobectomy with less perisurgical morbidity.[70] Sublobar resections, wedge resections

and segmental resections are frequently used when there is borderline lung function, a necessity to preserve healthy lung tissue and in patients with poor cardiorespiratory reserve. However these more limited procedures correspond with higher rates of local and distant recurrence.[43]

1.3.2 Surgical Ineligibility

Lacking fitness for surgery is the primary reason for not offering surgery to patients who have resectable tumours. The major areas for concern regarding fitness for surgery are pulmonary function, cardiovascular risk and nutritional and performance status. While poorer performance status and co-morbidities are more common in older patients, there is no strong evidence that increasing age on its own, including patients over 80 years of age, should be the sole reason for declining a patient for lobectomy or sub-lobar resections.[82, 104] However the perioperative morbidity is likely to be higher in older patients.[111] Survival following surgery in older patients undergoing pneumonectomy is poor and therefore age should be a factor when considering surgery in such cases.

The British Thoracic Society (BTS) recommend a tripartite risk assessment model when assessing a patient's suitability for surgery that incorporates operative mortality, perioperative cardiovascular events and post-operative dyspnoea.[60] Evaluation of lung function parameters provides important information when attempting to predict operative mortality and post-operative dyspnoea. The once long held consensus was that the optimum cut-off for predicted post-operative FEV_1 was 40%. However, there is no strong evidence that such a cut-off is independently predictive of perioperative mortality and FEV_1 in this respect may only be a surrogate for performance status.[60] FEV_1 and carbon monoxide transfer factor (TLCO) measure different aspects of lung function and are poorly correlated with each other. TLCO is likely to be the most accurate lung function predictor of perioperative mortality. Post-operative dyspnoea and quality of life measures are also poorly correlated with predicted postoperative lung function parameters. Predicted post-operative FEV_1 and TLCO are performed using segment counting but this method may overestimate the degree of decline following surgery.[12] This phenomenon is particularly seen when the resected lobe is

disproportionately emphysematous or when the tumour is causing compression of the pulmonary artery and therefore leading to a ventilation-perfusion (V/Q) mismatch. In cases with anticipated V/Q mismatch, consideration should be given to performing split lung function testing with either ventilation or perfusion scintigraphy. The shuttle walk test with a minimum target of 400 metres and formal cardiopulmonary exercise testing (CPET) with a cut-off peak oxygen consumption (VO_2 max) of 15 ml/kg/min can help to predict post-operative dyspnoea in high risk groups. However, with all potential surgical candidates a frank, personalised discussion of the risks of complications and post-operative dyspnoea is necessary so that the patient can make an informed decision. The BTS algorithm for risk assessment of postoperative dyspnoea is shown in

Figure 1.1. Impaired lung function remains the major objective factor why patients with resectable tumours are not offered gold-standard surgery.

Around 20% of patients with resectable tumours do not receive definitive surgery[93] due to either declining surgery or surgical inoperability. Historically, patients deemed unsuitable for surgery were offered external beam radiotherapy, which has an inferior 5 year local control rates of between 40 and 70%.[54, 96] Stereotactic body radiotherapy (SBRT) that delivers focused, hypofractionated high dose radiotherapy is now the treatment of choice for inoperable early stage NSCLC where there is no evidence of nodal involvement on FDG-PET imaging or from endobronchial ultrasound (EBUS) sampling.

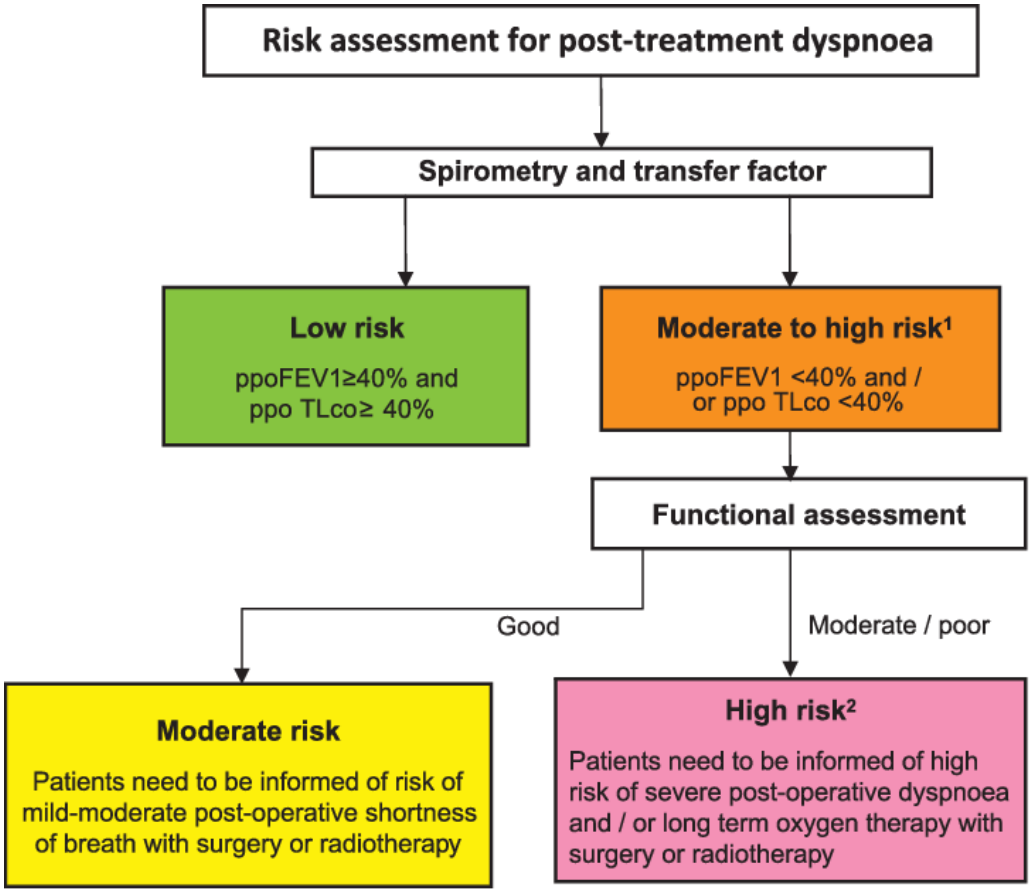

Figure 1.1: BTS algorithm for risk assessment for post-treatment dyspnoea.[60] FEV1: forced expiratory volume in 1 s; LVRS: lung volume reduction surgery; ppo: projected postoperative; TLCO: lung carbon monoxide transfer factor

1.3.3 Stereotactic Body Radiotherapy (SBRT)

SBRT achieves superior tumour cell death and survival outcomes compared to conventional radiotherapy by delivering higher biologic-equivalent doses. SBRT can be delivered in up to 10 fractions with doses of between 5 and 20 Gray (Gy) per fraction. In comparison, conventional treatment is delivered in 15 to 30 fractions of between 2 and 3 Gy. The higher dose per fraction is delivered to a smaller volume of lung, thus there is greater sparing of normal tissues with SBRT compared to conventional radiotherapy. This allows a greater number of patients with poor lung function to be treated radically than could be achieved with conventional radiotherapy. In a randomised study of 101 patients with stage I NSCLC, the rate of freedom from local treatment failure was significantly higher with SBRT compared to standard radiotherapy (hazard ratio 0·32, 95% CI 0·13 – 0·77, p =0·0077).[3] Overall survival rates of between 40% and 80% at 3 years have been achieved with SBRT.[105]

For operable patients, phase III trials comparing surgery against SBRT have proved difficult to recruit to. Despite this, a systemic review has shown equivalent local control and overall survival at one year between surgery and SBRT for stage I NSCLC.[119] A subsequent meta-analysis in 2014 showed unadjusted overall survival rate to be higher for the surgically resected patients at 1, 3 and 5 years (92.5%, 77.9% and 66.1%) compared to SBRT (83.4%, 56.6% and 41.2%). However, when adjusted for age and patient eligibility for surgery there was no significant difference in overall survival between the treatments.[121]

1.3.4 Prognostic Factors in SBRT

Increasing age and male sex have been shown to be negative prognostic factors for overall survival following SBRT.[48] There is inconsistent evidence for patient co-morbidities to affect overall survival following SBRT. Holmes et al. did not find that the burden of comorbid disease, as measured by the Charlson Co-Morbidity Index (CCI) added extra prognostic information over age alone.[48] The CCI was developed to predict 10 year mortality in any patient admitted to a medical service. It includes scores

for twenty medical conditions, which are weighted based on their presence or not, but no account is made for the severity of the co-morbid disease. In one study, age adjusted CCI was shown to be the most effective measure of co-morbid disease to predict overall survival following SBRT.[30]

Increased tumour size has also consistently been shown to be a negative prognostic factor following SBRT.[24] A Japanese study of 71 patients did not identify any independent factors to be associated with overall survival but a lower biological effective dose of delivered radiotherapy (BED) and higher median maximum standardized uptake value (SUV_{max}) on FDG-PET were negatively associated with disease-specific survival.[1] A different group found that tumour size was prognostic with regards to local control, disease progression and overall survival.[68] In the multivariate analysis, female sex was again associated with improved overall survival. Similarly, a Nordic meta-analysis has shown that increasing tumour size is an important negative predictor of local disease control.[6] In this meta-analysis, central or pleural proximity of the tumour and increased target definition were also associated with local failure.

1.3.5 Pneumonitis following SBRT

Radiation pneumonitis is one of the major toxicities associated with SBRT and limits the maximum radiation dose that can be delivered safely.[101] Symptomatic radiation pneumonitis presents with new cough, shortness of breath and fever. Clinically it may be difficult to differentiate from lung infections or exacerbations of underlying airways disease. SBRT can cause an early acute radiation pneumonitis with clinical symptoms developing at approximately 3 to 6 months after treatment.[115] CT changes of radiation pneumonitis usually occur in two stages. Early acute radiation changes occur within the first 6 months, although it would be rare for there to be any changes seen until at least 3 months from treatment. Fibrotic changes occur more than 6 months after treatment.

SBRT is generally a well-tolerated procedure with rates of pneumonitis consistently less than 10%. In a large multi-centre study of 483 patients, the rate of pneumonitis at grade 2 (see Table 1.1) or above was only 7% and there was no correlation between pre-treatment pulmonary function tests (PFTs) and rates of pneumonitis.[46] Central, perihilar lesions are more likely to be associated with severe, including lethal pulmonary toxicities such as pneumonia and major bleeding.[112] Methods to predict the risk of severe radiation pneumonitis following SBRT are not well established.

SBRT appears to have no significant impact on pulmonary function. An analysis of the phase II North American study, RTOG 0236 found that poor baseline PFTs did not predict pulmonary toxicity and that SBRT caused minimal deterioration in FEV_1 or TLCO. At 2 years, the mean decline in FEV_1 as a percentage of predicted normal (FEV_1%pred) and TLCO as a percentage of predicted normal (TLCO%pred) were 5.8% and 6.3% respectively.[107]

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Pneumonitis	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self-care ADL; oxygen indicated	Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death

Table 1.1 Common Terminology Criteria for Adverse Events (CTCAE) for pneumonitis definitions[113]

1.3.6 Adjuvant Therapy Following Radical Treatments

Following surgery, adjuvant chemotherapy is currently offered as standard treatment for patients with completely resected stage II or III NSCLC. The Lung Adjuvant Cisplatin Evaluation (LACE) Collaborative group published a meta-analysis of five adjuvant cisplatin-based trials that demonstrated a 5.3% improvement in survival at 5 years with adjuvant chemotherapy. The evidence for stage IB disease was not significant and there was no benefit for adjuvant chemotherapy in those with stage IA disease.[87] The prospective CALGB study suggested that patients with stage IB disease, whose tumours were ≥ 4cm diameter benefited from adjuvant chemotherapy also.[45] Adjuvant chemotherapy is therefore currently offered to stage II and stage III disease and to stage IB disease with tumours ≥ 4cm in maximum dimension.

Although SBRT can treat lesions up to a maximum diameter of 5cm, there is currently no recommended adjuvant treatment for patients post SBRT. The STEREO trial, a feasibility study assessing the ability to deliver platinum doublet chemotherapy post SBRT was closed early due to poor recruitment.[88] Delivery of chemotherapy to the more frail patients that undergo SBRT would be unlike treating the surgically resected population, given that the toxicity from chemotherapy in frail patients in general could outweigh any potential benefit. In this population overall survival and cancer-specific survival also appears to be inferior with SBRT than with wedge resection.[86] This may be partly due to the lack of pathological nodal staging for the majority of patients that undergo SBRT and an underestimation of local metastatic spread.

1.4 Immune Regulation in NSCLC

Cancer is an inflammatory disease in which the innate and adaptive immune systems play a central role in the regulation of cancer growth. Escape from immune destruction is a key feature that allows cancer cells to survive and proliferate. In order to do this, tumour cells acquire the ability to promote immunosuppressive mechanisms to avoid recognition and destruction.

The programmed death-1 (PD-1) receptor is expressed on several immune cells including CD4 and CD8 lymphocytes, B lymphocytes, natural killer (NK) cells and T regulatory cells (Tregs).[55] One of the ligands for PD-1, PD-L1 is expressed on T and B lymphocytes, dendritic cells and macrophages.[109] The PD-L1 ligand is upregulated in a range of solid tumours including in around 25-60% of NSCLC.[56] Overexpression of PD-L1 in resected NSCLC is associated with poorer outcomes.[83] PD-L1 interaction with PD-1 on T cells leads to downregulation of T cell effector function. A second ligand, PD-L2 is expressed less extensively and predominantly limited to macrophages and dendritic cells.[94] The increased interaction between PD-1 and its ligands leads to a downregulation of T cell activation and proliferation and therefore reduced T-cell mediated anti-tumour response.

In the last decade, immunotherapies have revolutionised systemic cancer treatment options across a range of tumour types. Immunotherapies attempt to disinhibit the immune system by modulating regulatory checkpoints.[32] In advanced NSCLC, blockade of a number of these checkpoints have been shown to elicit anti-tumour responses that can be significantly more durable than those seen with cytotoxic chemotherapy. The PD-1 receptor, PD-L1 ligand and the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) receptor are all checkpoints that can be successfully blocked in the treatment of advanced NSCLC. Downregulation of the immune inhibitory pathway can be achieved by the PD-1 inhibitors Nivolumab and Pembrolizumab or by blocking the PD-L1 and PD-L2 ligands with Atezolizumab, Avelumab and Durvalumab.[69] Interaction between CTLA-4 and CD-80 and CD-86 on antigen presenting cells also leads to a downregulation in the immune response. The agents Ipilimumab and Tremelimumab can inhibit CTLA-4 and therefore downregulate T cell inhibition.[69] In early stage and locally advanced NSCLC, there is evolving evidence for the use of checkpoint inhibitors as adjuvant and neo-adjuvant treatment options.

1.5 Nivolumab

Nivolumab is a genetically engineered, fully human immunoglobulin G4 (IgG4) monoclonal antibody that is specific for human PD-1.[9, 85] Nivolumab binds to PD-1 with high affinity and therefore blocks interaction with its ligands (PD-L1 and PD-L2) and hence has the potential to activate T cell anti-tumour responses.[10] PD-L1 expression on tumour cells is a biomarker that predicts likelihood of response to PD-1/PD-L1 inhibition.[8]

There is substantial data on the efficacy and safety of monotherapy with nivolumab in advanced NSCLC from Phase I (CA209-003)[41], II (CA209063/CheckMate 063)[92] and III (CA209017[11], CA209057[8]) studies. CA209003 showed an objective response rate (ORR) of 24.3% with 3mg/kg nivolumab and 20.3% with 10mg/kg administered every 2 weeks (q2w). The estimated median duration of response for NSCLC patients was 74 weeks with 3mg/kg nivolumab and 83.1 weeks with 10mg/kg.[41]

Nivolumab was FDA approved in 2015 to treat metastatic squamous NSCLC based on the results of the data from the phase II study, CheckMate 063 and the phase III study CheckMate 017.[11, 92] CheckMate 017 randomly assigned 272 squamous cell NSCLC patients to receive nivolumab at a dose of 3mg/kg every two weeks or docetaxel (75mg/kg) every 3 weeks in the second line treatment setting. The objective response rate was significantly higher in the nivolumab arm (20% vs. 9% p=0.008). Although the median progression free survival (PFS) improvement was only modest (3.5 months v 2.8 months), those patients that did respond tended to have a prolonged response and hence PFS rate at 1 year was 21% in the nivolumab group compared to only 6% in the docetaxel group. The median overall survival (OS) was 9.2 months with nivolumab versus 6 months with docetaxel. The OS rate at 1- and 2- years was 42% and 23%, respectively in the nivolumab arm and 24% and 8% respectively in the docetaxel arm.[5, 11] In a retrospective analysis of PD-L1 expression on pre-treatment biopsy samples, PD-L1 did not predict response or prognosis.[11]

The CheckMate 057 second line study randomised 582 patients with non-squamous NSCLC post platinum doublet chemotherapy to either nivolumab (3mg/kg q2w) or docetaxel.[8] The confirmed objective response rate was 19% with nivolumab and 12% with docetaxel (p=0.02). Median PFS did not favour nivolumab although PFS rate at 1 year was higher with nivolumab (19 % vs. 8%). This again points to the enduring response of responders. Median OS was 12.2 months with nivolumab versus 9.5 months with docetaxel. The 1- and 2- year OS rates were 51% and 29% respectively for nivolumab and 29% and 16% respectively for docetaxel.[5] In contrast to the squamous patients, higher levels of PD-L1 were associated with a greater magnitude of benefit in this patient population.[5, 8] In September 2016, the FDA licence for nivolumab was changed to a flat dose of 240mg every 2 weeks.[50] Flat dosing offers several advantages over body weight normalized dosing, including reduced potential for dosing errors and shortened dose preparation time. After further pharmacokinetic studies have shown a similar time-averaged steady state exposure and toxicity profile, nivolumab is now licenced at a dose of 480 mg every 4 weeks.[62]

Nivolumab has a more tolerable safety profile compared to chemotherapy. Pooled analyses from CheckMates 017[11] and 057[8] with a minimum of 2 years follow-up, showed that treatment-related adverse events (AEs) were reported in fewer nivolumab-treated patients versus docetaxel-treated patients (any grade: 68% vs. 88%; grade 3–4: 10% vs. 55%) and less frequently led to discontinuation (any grade: 6% vs. 13%; grade 3–4: 4% vs. 7%).[5] The overall incidence of grade 3-4 immune-mediated pneumonitis observed with nivolumab in clinical trials is low.[5, 51] Immune-related adverse events can occur at any time during treatment and for several months after the final dose. However, the median time to the appearance of pneumonitis is 3.5 months (range 0.0-19.6).[51] Treatment-related AEs with a potential immunologic cause, which may require management through immune-modulating medications such as corticosteroids, were uncommon and they rarely led to discontinuation.[5] Serious adverse events were also less frequent with nivolumab compared to docetaxel (7% vs. 20% of patients had events of any grade; 5% vs. 18% had events of grade 3 or 4).[8]

As oncologist experience with checkpoint inhibitors has grown, a greater comfort in the use of these agents has developed. While the toxicity from immunotherapies is generally less than seen with cytotoxic chemotherapy, an awareness of the more varied and unpredictable side effects is necessary.

1.6 Combining Radiotherapy & Immunotherapy

The development and progression of cancer is a result of the balance between tumour cells and the immune system. The immunoediting of the tumour occurs in three phases described as elimination, equilibrium and escape.[71] The innate immune system detects tissue changes during neoplastic transformation, which leads to cytotoxic cell death by macrophages. Cytotoxic action leads to release of tumour-associated antigens which are loaded into the major histocompatibility complex on dendritic cells and recognised by CD4 and CD8 T cells.[35] The activation of tumour-specific T cells leads to the elimination of further tumour cells.[98] However, where elimination is incomplete surviving tumour cells generate mutations due to genetic instability and escape destruction. Eventual resistance to immune rejection ultimately leads to tumour progression. Despite the loss of control over cell proliferation, the immune system continues to play a role in slowing tumour progression and in doing so generates further neoantigens that can be recognised by T cells.[100]

Cell death has recently been established as an efficient process to transfer tumour antigens to dendritic cells. Dendritic cells (DC) process the antigens into peptides that are loaded into the major histocompatibility complex (MHC) class 1 and 2 molecules and these are subsequently recognised by CD8 and CD4 T cells.[35] Three key molecular signals have been established that are necessary to achieve an immunological cell death.[38] The currently known steps are cell surface translocation of calreticulin, extracellular release of high-mobility group protein B1 and release of ATP.[2, 42, 80, 81]

Historically, high dose radiotherapy was felt to be immunosuppressive characterised by reduced blood counts. SBRT benefits from a more targeted treatment volume and

subsequently less irradiated bone marrow and blood volume. Radiotherapy is known to promote a range of immune modulatory responses. Immunogenic cell death following radiotherapy triggers the release of several endogenous damage-associated molecular patterns (DAMPs). DAMPs contribute to the immune priming of dendritic cells and therefore improved tumour antigen presentation.[4]

Ionising radiation leads to the release of proinflammatory cytokines including interleukin 1β, tumour necrosis α and type 1 and 2 interferons.[35] Exposure to ionizing radiation results in a high rate of tumour cell destruction and subsequent release of tumour neoantigens, which prime the adaptive immune system for immunological cell death and hence holds the potential to act as an *in situ* vaccine. Furthermore, tumour cells that receive sublethal doses of radiation undergo phenotypic changes such as increased expression of MHC class 1 molecules that enhances their susceptibility to immunological cell death.[39] Radiotherapy, by causing release of tumour neoantigens and pro-inflammatory sequelae could potentially engage both the innate and adaptive arms of the immune system. The generation of tumour-specific T cells provides immune memory and may explain radiotherapy's ability to improve final outcomes for irradiated patients.[36]

The introduction of immune checkpoint inhibitors to the oncologist's arsenal has helped to improve outcomes for patients with advanced NSCLC. However, durable responses are only seen in a minority of patients receiving immunotherapy and only in the most immunogenic tumours. Current biomarkers to predict response to such agents, including expression of PD-L1 and tumour mutational burden (TMB), help to predict response but better biomarkers are needed. For example, PD-L1 expression is not mandatory for seeing a response to PD-1/PD-L1 inhibitors and conversely, a lack of response or even overt progression can be observed in those with high PD-L1 expression.[14]

The potential for radiotherapy to be immune priming makes it an attractive treatment option to combine with immunotherapies to augment the natural anti-tumour immune

response. This has been suggested historically by the rare phenomenon of tumour shrinkage being observed outside of the radiotherapy field, the so called abscopal effect. There is substantial preclinical evidence to support this, including in animal models. In one study using a mouse model of lung carcinoma, the administration of Flt3-ligand, a DC growth factor, following radiotherapy led to the induction of anti-tumour T cells that were able to inhibit metastases. The effect of combining radiotherapy and the DC growth factor was greater than with either therapy alone.[16] In another preclinical study using mouse models of mammary and colorectal cancer, the use of an antibody that targets CTLA-4 was not effective as a single agent in the relatively poorly immunogenic tumours that mimic the clinical setting. However, when combined with radiotherapy, anti-CTLA-4 could inhibit early lung metastases and elicit a response in bulky tumours outside of the irradiated field.[26, 29] Similarly, in a mouse model of glioblastoma multiforme, SBRT when combined with blockade of PD-1 produced a pronounced treatment effect. Median survival for the combination therapy was 53 days, compared to 25 days in the control group and 27 days and 28 days for monotherapy with either anti-PD-1 therapy or SBRT, respectively. Immunological data from cadaveric samples at 21 days showed increased tumour infiltration by cytotoxic T cells and decreased regulatory T cells (Tregs) in the combination treatment group compared to either single modality arm.[118]

For patients undergoing SBRT for NSCLC, immunotherapy, with its more favourable toxicity profile compared to chemotherapy may provide a more tolerable and effective adjuvant treatment option. Furthermore, the potential for radiotherapy to act as an *in-situ* vaccine to prime the adaptive immune system makes immunotherapies such as nivolumab an attractive option to target micrometastatic disease, believed to be responsible for tumour relapse.

1.7 Staging and Prognostic Factors in Advanced NSCLC

The TNM classification for NSCLC, which is regularly updated by the Union for International Cancer Control (UICC) provides a guide for possible treatment options and expected prognosis. This classification system grades tumours by their tumour

size (T), nodal involvement (N) and metastatic spread (M). The composites of these domains stage the cancer and through statistical analysis of the large international database provides prognostic information. The most recent, 8th edition of UICC staging reviewed cases from 1999 to 2010, from 35 sources and 16 countries.[61] In the current classification, cancer stages can range from stage IA1 to IVB with worse prognoses at each gradation. However, as the stage only reflects the anatomical distribution of disease it can be difficult to accurately estimate prognosis on an individual basis, where multiple tumour and patient factors are likely to have a large influence on prognosis. There is also wide geographical variation in outcomes and with the recent updates in cancer treatments, UICC staging may provide outdated figures.[28] Nevertheless tumour stage forms a large component of estimating a patient's prognosis. Data from the 7th edition of the classification showed that patients with a clinical stage 1A had a 5 year survival rate of 50% compared to only 2% for stage IV.[44]

Within this classification each component affects prognosis with larger tumours (T stage) having a worse prognosis. Similarly increasing nodal staging has an impact on prognosis and can be responsible for limiting options to a palliative paradigm of treatment. Within advanced stage disease, the extent of disease also affects prognosis. The most recent classification reflects this with an acknowledgement that patients with only a single site of extrathoracic metastasis have a better prognosis than those with multiple. The median overall survival for patients with a single extrathoracic metastasis is 11.4 months compared to 6.3 months with multiple extrathoracic metastases, regardless of the number of organs involved.[61] Along with tumour staging, more recent data have identified that histological subtype affects prognosis in patients with resectable disease, with squamous cell histology being favourable over adenocarcinoma.[17] However, in advanced stage disease the opposite is true with patients with adenocarcinoma having a better prognosis.[47]

The majority of the data regarding specific prognostic markers in advanced NSCLC are patient characteristics prior to first line treatment. The most reproducible prognostic marker in advanced disease is patient performance status (PS), as measured by Eastern Conference Oncology Group (ECOG) scale or Karnofsky scale.

Patients with an ECOG PS of 2 consistently do worse than those with an ECOG PS of 0-1.[7, 22] There is however evidence that patients of ECOG PS 2 do benefit from first line treatment.[99] Other patient factors that confer a poorer prognosis include increasing age, recent weight loss[106] and male sex[74].

Despite evidence of clinical efficacy, there is much less knowledge about the patient factors that are predictive and prognostic for second line chemotherapy. This provides a challenge for clinicians deciding on which patients to recommend for second line cytotoxic agents. A review of the individual data of 1197 patients from nine second line treatment studies in NSCLC studies was performed by Di Maio et al.[31] Their analysis found performance status, gender, histology, stage, use of platinum-based first line and best response to first chemotherapy were independent prognostic factors. There was a heterogeneity in the chemotherapy agents that were used in these trials including docetaxel in several of the arms. In this analysis, age was not an independent prognostic marker. All variables that were considered in this analysis were found to be independently prognostic however more detailed patient factors including co-morbidities or lung function tests were not included. Smaller, single centre studies are in agreement that performance status and response to first line therapy are prognostic factors in second line chemotherapy for NSCLC, while co-morbidities including hypertension and diabetes mellitus were not significant factors.[53, 58]

1.8 Chemotherapy in Advanced NSCLC

1.8.1 First-line chemotherapy

Until the recent development of checkpoint inhibitors, chemotherapy was the mainstay of treatment for advanced NSCLC in patients that lacked a targetable driver mutation. Chemotherapy continues to have a critical role in the treatment of patients with a performance status of 2 and in those who are ineligible for or have progressed on immunotherapy. A large meta-analysis indicated that first-line platinum-doublet chemotherapy can improve survival, reduce the burden of disease symptoms and improve quality of life.[18] The hazard ratio for platinum-doublet was 0.73 over

supportive care alone, which equated to an absolute improvement in 1-year survival rate from 20% to 30%. While previously, in general, histological subtypes within NSCLC had little bearing on outcomes, a landmark study in 2008 by Scagliotti et al. demonstrated that the combination of cisplatin and pemetrexed chemotherapy in advanced disease was more active than previous regimens in patients with non-squamous histology and this could improve 12 and 24 month survival rates to 43.5% and 18.9% respectively.[97] Together, these studies provide compelling evidence for the use of platinum-doublet chemotherapy in all patients without significant comorbidities and of reasonable PS (ECOG 0-2). However, with a median PFS of only around 4-8 months with platinum-doublet chemotherapy, many patients will be candidates for second line chemotherapy on progression.

1.8.2 Docetaxel

Docetaxel's primary mechanism of action is by stabilisation of microtubules with subsequent disruption to cell division. Due to this indiscriminate mechanism of action, docetaxel has a wide spectrum of side effects including mucositis, diarrhoea, nausea, hair loss and myalgias.[33] The effect on the bone marrow causing myelosuppression leads to the dose-limiting toxicity of neutropaenia.

Docetaxel is recommended by the European Society for Medical Oncology (ESMO) as a second-line chemotherapy treatment option in advanced NSCLC. This recommendation is supported largely by two phase 3 trials. The multicentre Tax 317 study enrolled 204 patients who had progressed on or after at least 1 prior platinum-based chemotherapy regimen.[103] The study recruited patients with performance status 0-2 and were randomised to docetaxel 100 mg/m^2 or docetaxel 75mg/m^2 every 3 weeks or best supportive care. The protocol was subsequently amended to remove the higher dose of docetaxel due to unexpected high adverse events in this arm. The medium time to progression in the docetaxel 75 mg/m^2 arm was 12.3 weeks compared to 7 weeks with best supportive care (p = 0.004). The median overall survival in the docetaxel 75 mg/m^2 group was 7.2 months versus 4.7 months with best supportive care. The major additional toxicity associated with docetaxel compared to best

supportive care was grade 3/4 neutropaenia (grade 3: <1 – 0.5 x 10^9/L, grade 4: < 0.5 x10^9/L)[102], which occurred in 67% of patients receiving 75mg/m^2. There was only one case of febrile neutropaenia at the lower dose and this was non-fatal.

These findings were supported by the US-led Tax 320 study, which enrolled 373 patients and compared docetaxel at either 75 or 100 mg/m^2 every 3 weeks against investigator's choice of vinorelbine 30mg/m^2/week or ifosfamide 2mg/m^2 for 3 days.[37] Patients who had received a prior taxane regimen (usually paclitaxel) remained eligible. Partial response rates were higher in both docetaxel arms compared to the control arms and the median response duration with docetaxel 75 mg/m^2 was 9 months. The median time to progression was equivalent in each group at approximately 8 weeks but the 26-week progression free survival rate was higher with docetaxel 100 mg/m^2 (19% p = 0.013) and docetaxel 75 mg/m^2 (17% p = 0.031) compared to the control arm (8%). Median overall survival was also equitable across all three groups at approximately 5.5 months but with docetaxel 75 mg/m^2 having a higher 1-year survival rate (32%) than either docetaxel 100 mg/m^2 (21%) or the control arm (19%).

1.8.3 Nintedanib

Tumour development, progression and metastasis requires angiogenesis. The tumour microenvironment requires large amount of nutrients and oxygen to supply the proliferating cells. Angiogenesis is a regulated process that is controlled by a balance of proangiogenic and antiangiogenic mechanisms. The proliferating cells dysregulate the normal angiogenic process and lead to increased secretion of proangiogenic molecules. This in turn leads to the development of multiple, tortuous, highly-permeable blood vessels which are necessary for tumour growth and spread.[79] The vascular endothelial growth factor (VEGF) is one of the most important factors in promoting angiogenesis. Blockade of VEGF's interaction with its receptor is therefore an attractive target for anti-cancer therapy. Indeed, the anti-VEGF monoclonal antibody bevacizumab, when given in addition to first-line platinum-doublet chemotherapy for advanced NSCLC, improves overall survival by 3 months compared

to chemotherapy alone.[95] However, resistance inevitably develops to bevacizumab due to the redundancy of pathways involved in angiogenesis.[21] Nintedanib is an oral angiokinase inhibitor that can potentially inhibit the angiogenic pathways mediated by VEGF, platelet-derived growth factor and fibroblast growth factor receptors.[67]

The LUME-lung 1 study was a randomised phase III trial of second-line docetaxel plus nintedanib versus docetaxel with placebo in advanced NSCLC. A total of 1,314 patients were randomised with stratification for histology, performance status, prior bevacizumab therapy and the presence of brain metastasis (only those with stable brain metastases were eligible for the study).[91] The addition of nintedanib significantly increased the progression free survival regardless of age, gender, ethnicity or performance status. The median PFS in the nintedanib group was 3.4 months (95% CI, 2.9 to 3.9 months) versus 2.7 months (95% CI 2.6 to 2.8 months) in the placebo arm. However, OS was only significantly increased in patients with adenocarcinoma histology subtype. For patients with adenocarcinoma, nintedanib when given with docetaxel increased overall survival to 12.6 months (95% CI, 10.6 to 15.1 months) compared to 10.3 months (95% CI, 8.6 to 12.2 months) when docetaxel was given with placebo. Increased side effects in the nintedanib group included an increased rate of diarrhoea, nausea and liver function test (LFT) derangement. There was no significant difference in rates of bleeding or hypertensive events, a concern given its mechanism of action. Following this trial, the National Institute for Health and Care Excellence (NICE) recommend nintedanib as a treatment option with docetaxel for second and subsequent line chemotherapy in advanced NSCLC of adenocarcinoma histology.

1.8.4 Febrile Neutropenia

The development of fever while neutropaenic, so called febrile neutropaenia, is a common but serious complication of patients receiving myelosuppressive treatments. The risk of this potentially life-threatening event is directly related to the severity and duration of neutropaenia.[19, 108] A model-based meta-analysis identified a linear dose-response relationship between docetaxel dose and grade 3/4 neutropaenia incidence,

with a 5% increase in the odds of neutropaenia per mg/m^2.[108] Known risk factors for the development of febrile neutropaenia in patients receiving chemotherapy are older age, the type of cancer, the type and the number of myelosuppressive agents used and co-morbidities.[63] Other factors identified for the risk of chemotherapy induced neutropaenia include patient performance status, nutritional status, chemotherapy dose-intensity and low baseline blood counts.[63] In a population based study, the rate of early mortality for patients experiencing episodes of febrile neutropaenia was significantly higher than their matched controls.[64] Of note, the highest mortality was seen in lung cancer patients. Rates of hospitalisation for lung cancer patients were also highest for lung cancer patients and lowest for breast cancer.[64] In the multivariate analysis, patients with lung cancer had the greatest hazard for overall mortality compared with patients who had breast cancer (hazard ratio (HR) 5.26, 95%CI, 4.42-6.26). This multivariate analysis included variables of age, sex, comorbidities including COPD, and other selected risk factors (history of infection, history of hospitalisation, history of surgery, receipt of radiotherapy). In this analysis, the use of granulocyte-colony stimulating factor (G-CSF) decreased the hazard of mortality by 45%.

Episodes of febrile neutropaenia require hospitalisation for intravenous, broad spectrum antibiotics and supportive care with a significant impact on patient quality of life. The mean length of hospital stay for a patient with febrile neutropaenia is between 3 and 15 days, which is a significant morbidity for patients with an already poor prognosis.[64] In addition to this, the adjusted mortality for patients that experience an episode of febrile neutropaenia is at least 15% higher than matched patients without febrile neutropaenia.[64]

1.8.5 Granulocyte-Colony Stimulating Factor (G-CSF)

Neutrophils are the primary cells from within the innate immunity responsible for the clearance of bacterial pathogens. G-CSF is the major regulator of neutrophilic granulocytes.[59] G-CSF production is induced in states of inflammation and particularly infection by inflammatory mediators including interleukin-1β, tumour necrosis factor-alpha and lipopolysaccharide.[27] The receptor for G-CSF is found on myeloid precursor

cells and on maturing neutrophils where it acts to promote their stability.[78] High levels of G-CSF drive proliferation of neutrophils and are responsible for stimulating the immune response to bacterial infection. Along with promoting the production of neutrophils, G-CSF also shortens the transition time of developing granulocytes through the bone marrow compartments leading to increased number of circulating neutrophils.[84] This response is necessary for the increased and prolonged neutrophil circulating levels during episodes of infection.[84]

Recombinant G-CSF is known to reduce the severity and duration of neutropaenia in patients receiving myelosuppressive therapy. In the original study with patients being treated for SCLC, G-CSF reduced the percentage of patients that had an episode of febrile neutropaenia to 40%, compared to 77% in the placebo control group ($p < 0.001$).[20] Length of hospital admission, duration of intravenous antibiotics and rates of confirmed infections were also reduced by 50% in those receiving G-CSF. The median duration of grade 4 neutropaenia (neutrophil count of less than $0.5x10^9$/L) in the G-CSF arm was one day compared to six days in the placebo group. This was despite allowing G-CSF prescription for patients in the placebo arm who suffered an episode of febrile neutropaenia, in all subsequent treatment cycles. The most common significant side effect from G-CSF treatment was mild to moderate medullary bone pain, seen in 20% of patients.

A large meta-analysis of randomised controlled trials comparing the use of G-CSF and placebo in chemotherapy for solid organ cancers and malignant lymphoma found a relative risk (RR) reduction for infection-related mortality of 45% with G-CSF (RR 0.55, 95% CI 0.33 to 0.90, $p = 0.018$).[57] There was also a relative risk reduction of febrile neutropaenia of 46% with the use of G-CSF compared to placebo (RR 0.54, 95% CI 0.43 to 0.67, $p < 0.01$).[57] Average relative dose intensity (RDI), a marker of treatment tolerability that incorporates treatment delays and discontinuations, was also significantly higher in patients who received G-CSF.[57]

In England and Wales, NICE are responsible for providing guidelines on the use of health technologies based on the evidence supporting their efficacy, safety and cost-effectiveness. Current NICE guidelines recommend the use of G-CSF as primary prophylaxis to prevent febrile neutropaenia in adult chemotherapy only if it is an integral part of the regimen or to maintain dose intensity. In practice this equates to chemotherapy regimens with an expected minimum rate of febrile neutropaenia of 20% or for patients receiving chemotherapy as part of a radical paradigm. As per NICE guidelines, primary prophylaxis with G-CSF is not recommended for patients with advanced stage NSCLC receiving docetaxel as a second or subsequent line of therapy.[77]

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Chapter 2: **Risk factors for Pneumonitis and Survival following Stereotactic Body Radiotherapy in Early Stage NSCLC**

First, I explored potential patient factors that may influence the rates of lung toxicity and the effectiveness of SBRT. In real-world practice, I attempted to clarify the significance that impaired lung function has on the toxicity and prognosis from SBRT and if significant, which lung function parameter is most predictive. To provide insights for future work, I also investigated the relative tumour immunogenicity, as characterised by the expression of PD-L1, in early stage NSCLC biopsy samples. In an exploratory analysis I investigated what impact the degree of pre-treatment PD-L1 expression on tumour cells had on subsequent response to SBRT monotherapy.

2.1 Aim

To explore the patient and tumour factors that affect the toxicity and prognosis following SBRT for early stage NSCLC

2.2 Objectives

- Assess the patterns of relapse, disease free survival and overall survival for patients receiving SBRT for early stage NSCLC
- Perform univariate and multivariate regression analyses to identify patient and tumour factors that are associated with symptomatic radiation pneumonitis, rate of relapse and overall survival
- Assess the rate of invasive mediastinal lymph node staging prior to SBRT

2.3 Hypotheses

- The rate of pneumonitis (≥ grade 2) following Stereotactic Body Radiotherapy (SBRT) for NSCLC is not influenced by lung function parameters
- Expression of PD-L1 on biopsy samples is a negative prognostic marker for patients treated with SBRT alone for NSCLC

2.4 Study Design

2.4.1 Overall Plan

I performed a retrospective data collection and statistical analysis on all patients that had been treated with SBRT for stage I/II NSCLC at the Royal Marsden Hospital (RMH). A list of all patients was identified through the RMH radiotherapy department. I completed a thorough notes review for each episode of SBRT to establish baseline characteristics and the most up-to-date PFT results prior to treatment. It was local policy for all patients to have PFTs within three months of the planned start date for SBRT. Each diagnostic CT and post treatment scan were reviewed in picture archiving and communication system (PACS). I generated tumour measurements myself, which correlated with CT reports where available. The inclusion and exclusion criteria for this analysis were as follows:

Inclusion Criteria

- NSCLC - Histologically proven or presumed based on radiological criteria
- T1-3 (≤5cm) N0 M0 stage by CT ± PET
- SBRT delivered with doses of 50 to 55 Gy in 5 fractions or 54 Gy in 3 fractions for peripheral disease or 60 Gy in 8 fractions or 55 Gy in 10 fractions for central disease
- 1st fraction of SBRT administered between 1/1/09 and 1/1/17

Exclusion Criteria

- Unobtainable patient follow-up data

2.4.2 Tumour Measurements & Data Collection

Tumour size was measured as the longest two-dimensional diameter on the latest diagnostic CT after assessment in 3 planes. Nearly all patients also underwent an FDG-PET as part of their workup before SBRT. However, as this does not include a diagnostic quality CT scan, tumour measurements were not taken from PET scans, despite frequently being closer to the start of treatment. Similarly, the lesion type was

categorised from the most recent diagnostic CT. Lesions with no visible component on mediastinal window were classified as pure ground glass opacities (pGGOs). Lesions that comprised some ground glass component were deemed part solid.

Tumours were designated as being central or peripherally located as per the Radiation Therapy Oncology Group (RTOG) definition. Central lesions were inside the 'no-fly zone' of a 2cm radius of main airways and proximal bronchial tree. This is defined as 2cm from the bifurcation of the second order bronchus e.g. where the right upper lobe bronchus splits. (Figure 2.1)

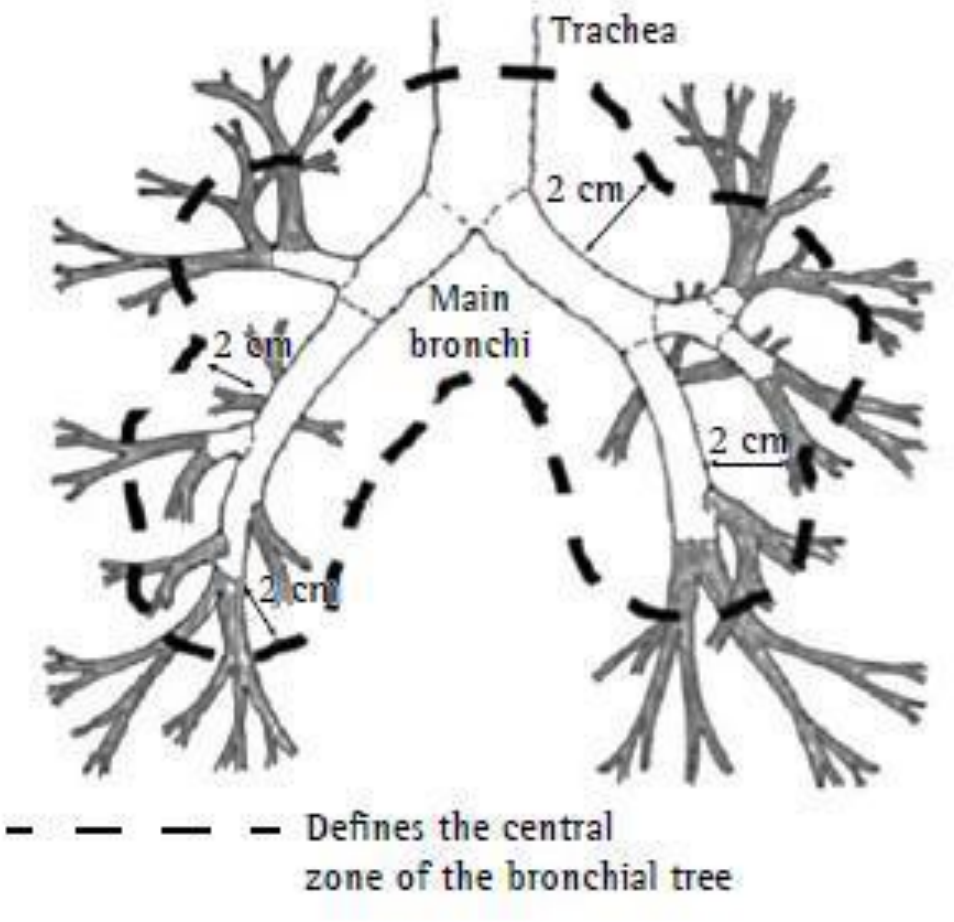


Figure 2.1 Definition of central zone: region within a radius of 2 cm around the proximal bronchial tree (within the dashed line). Adapted from the Radiation Therapy Oncology Group

Past medical history and calculation of Charlson Co-morbidity Index (CCI) was made through a thorough notes review. A result for tumour expression of PD-L1 was available for a minority of those with histologically confirmed NSCLC. The pre-treatment biopsy was used in all cases. These results were available due to reflex PD-L1 testing of all NSCLC biopsies in some laboratories regardless of cancer staging. PD-L1 expression was performed by standard immunohistochemistry methods and presented as percentage of tumour cells expressing PD-L1, known as the tumour proportion score (TPS).

Pneumonitis was assessed by a combination of notes reviews and a review of follow up scans at 3 and 6 months. A thorough interrogation of all investigations was performed in cases where patients were admitted into hospital within the first 6 months following SBRT completion.

Relapse was determined as per MDT decisions where appropriate or in any case with histological confirmation of NSCLC. Patterns of relapse were defined as follows:

- **Local relapse (LR):** relapse within the planned treatment volume or involved lobe
- **Regional relapse:** relapse within the regional lymph node stations
- **Loco-regional relapse (LRR):** any relapse that is local, regional or both local and regional
- **Distant relapse (DR):** relapse outside of LRR

2.4.3 Outcome Measures & Statistical Analysis

<u>Overall Survival</u>

Overall survival (OS) was calculated from the date of the first fraction of SBRT until the date of death; patients that were alive or lost to follow-up were censored at the date of last visit. The median OS and 95% confidence interval were determined using the Kaplan-Meier method.

Disease-Free Survival (DFS)

Disease Free Survival (DFS) was calculated from the date of SBRT treatment until the date of relapse or death, whichever occurred first, otherwise they were censored at their last follow-up date. The median DFS and 95% confidence interval were determined using the Kaplan-Meier method.

Time to Relapse

Time to relapse was calculated from the date of SBRT treatment until the date of relapse, otherwise they were censored at their last follow-up date. The median time to relapse and 95% confidence interval were determined using the Kaplan-Meier method. Any survival differences between groups were assessed using the log-rank test.

I used logistic regression to assess factors that may affect rates of pneumonitis (grade <2 vs. grade ≥2). I used Cox regression to assess factors that may affect overall survival and rate of relapse. Univariate analysis was performed with each factor first, and only those with a p-value < 0.2 were tested in the multivariate model. Factors with a p-value < 0.05 in the multivariate analysis were considered significant.

In instances of synchronous primaries or where a patient received SBRT on multiple occasions all survival curves and regression analyses were performed using the first episode of SBRT. Baseline characteristics and episodes of pneumonitis are described for the entire population.

2.5 Results

There were 208 SBRT treatments in 187 patients for early stage NSCLC across both sites of the Royal Marsden Hospital between 1/1/09 and 1/1/17. The median age of patients treated with SBRT was 78 years (interquartile range (IQR) 72 – 83). The baseline characteristics for all patients treated with SBRT are displayed in

Table **2.1**. There were 11 (5.3%) centrally located treated tumours. A histological diagnosis was made prior to treatment in 142 (68.3%) cases. Solid lesions were the most common lesion type treated (76.4%), with part-solid lesions making up 19.2% and pGGOs 4.3%.

PD-L1 results were available in 29 (20.4%) of histologically diagnosed tumours. There was no evidence of PD-L1 expression on tumour cells in 17 (58.6%) of tested samples and only 3 (10.3%) showing high expression as defined by TPS ≥ 50%.

Mediastinal staging was performed in eight cases in total, of which six were obtained via EBUS fine needle aspiration (FNA) and the remaining two by mediastinoscopy. There were 66 tumours that were either greater than 30 mm in diameter or centrally located. Pre-treatment mediastinal staging was obtained in five (7.6%) (x2 mediastinoscopy, x3 EBUS) of these high-risk cases.

Age: Median (IQR)	78 (72 – 83)
Gender	
Female	96 (51.3)
Male	91 (48.7)
Ethnicity	
White	165 (88.2)
Black	3 (1.6)
Asian	12 (6.4)
Other	3 (1.6)
Not disclosed	4 (2.1)
Tumour size: Median (IQR)	23 (17 – 31)
Tumour location	
Central	11 (5.3)
Peripheral	197 (94.7)
Histology (%)	
Adenocarcinoma	89 (42.8)
Squamous	46 (22.1)
Large Cell Neuroendocrine	1 (0.5)
Carcinoid	1 (0.5)
NSCLC: Not Otherwise Stated (NOS)	5 (2.4)
Radiological Diagnosis	66 (31.7)
Lesion Type	
Solid	159 (76.4)
Part Solid	40 (19.2)
Pure Ground Glass	9 (4.3)
Smoking status	
Never	12 (6.4)
Former	133 (71.1)
Current	41 (21.9)
Unknown	1 (0.5)
COPD	
No	59 (31.7)
Yes	127 (68.3)
Charlson Co-morbidity Index - Median (range)	7 (4 – 13)
FEV_1 (L) mean +/- SD (n =182)	1.52 +/- 0.61
FEV_1 % predicted mean +/- SD	67.6 +/- 25.4
TLCO mean +/- SD (n =160)	4.04 +/- 1.54
TLCO (% predicted) mean +/- SD	53.4 +/- 17.7
PD-L1 status (n = 29)	
<1%	17 (58.6)
1-49%	9 (31.0)
>50%	3 (10.3)
Mediastinal Staging	
No	200 (96.2)
EBUS	6 (2.9)
Mediastinoscopy	2 (1.0)

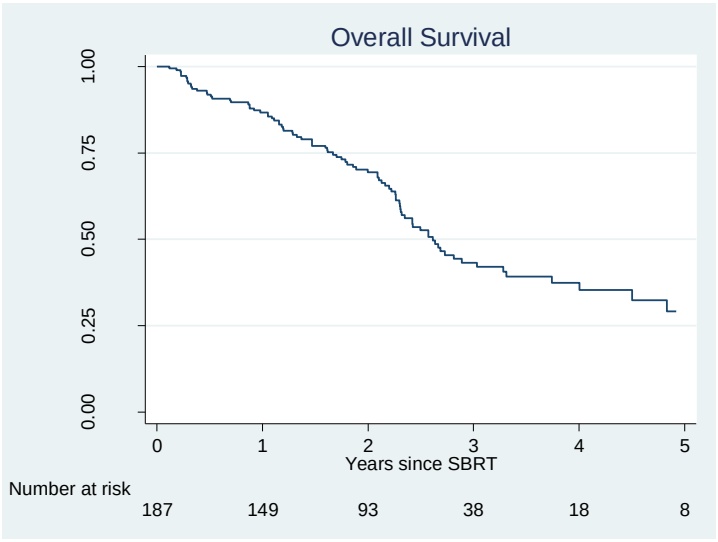
Table 2.1: Patient and tumour baseline characteristics for all SBRT treatments. Number of patients (percentage) unless otherwise stated. EBUS = Endobronchial Ultrasound

For the 187 patients included in the analysis, the median OS from the date of first fraction of SBRT was 2.6 years (95% CI 2.3 – 3.3). Using the reverse Kaplan-Meier method, the median follow-up was 2.7 years (95% CI 2.5 – 3.2) (Figure 2.2A). There were 47 episodes of disease relapse of which 14 (29.7%) were local, 6 (12.8%) were loco-regional and 27 (57.4%) were distal relapses. The overall local control rate with SBRT was 92.5%. The median disease-free survival (DFS) was 2.4 years (95% CI 2.0 - 2.7) (Figure 2.2B). The median time to relapse was not reached but the relapse rate at 2 years was 25% (95% CI 18 – 33) (Figure 2.2C).

There were 20 episodes of symptomatic pneumonitis (grade ≥ 2). Of these, there were 2 cases of grade 5 pneumonitis where the pneumonitis contributed to the death of the patient. A grade 5 pneumonitis occurred in less than 1% of SBRT treatments. The results of the survival analyses are displayed in Table 2.2.

Overall Survival (OS)	
Median (95% CI)	2.6 years (2.3 to 3.3)
1-year OS rate (95% CI)	87% (81% to 91%)
2-year OS rate (95% CI)	69% (62% to 76%)
5-year OS rate (95% CI)	29% (19% to 41%)
Disease Free Survival (DFS)	
Median (95% CI)	2.4 years (2.0 to 2.7)
1-year DFS rate (95% CI)	78% (71% to 83%)
2-year DFS rate (95% CI)	59% (51% to 66%)
5-year DFS rate (95% CI)	25% (16% to 35%)
Relapse Rate (RR)	
1-year RR (95% CI)	13% (9% to 19%)
2-year RR (95% CI)	25% (18% to 33%)
Location of relapse n = 47	
Local	14 (29.7)
Regional	6 (112.8)
Distal	27 (57.4)
Pneumonitis n = 207 (%)	
No pneumonitis	140 (69.4)
Grade 1	47 (22.7)
Grade 2	15 (7.2)
Grade 3	3 (1.4)
Grade 4	0 (0.0)
Grade 5	2 (0.9)

Table 2.2: Results of survival analyses for overall survival (OS), disease free survival (DFS) and relapse rare (RR). Descriptions of patterns of relapse and severity of pneumonitis observed.



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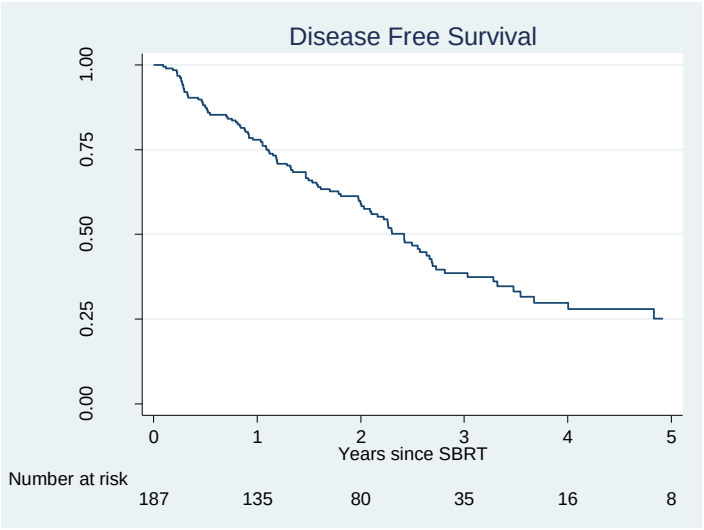

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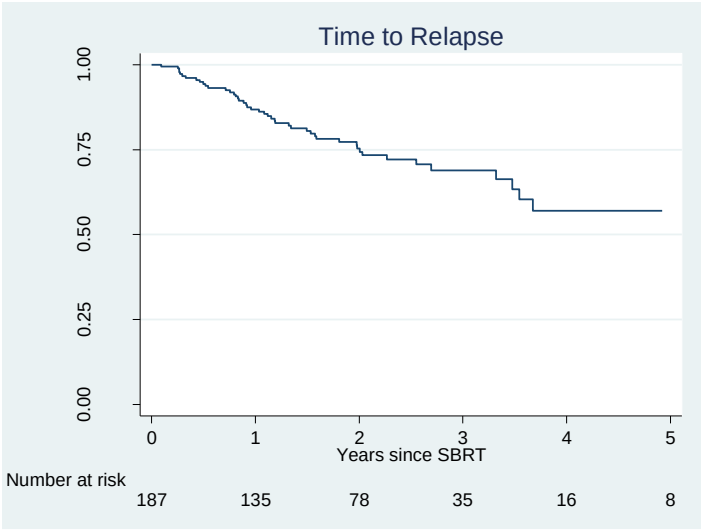

C

Figure 2.2: Kaplan-Meier survival curves for overall survival (A), disease-free survival (B) and time to relapse (C)

The association between patient and tumour factors on rate of pneumonitis is shown in Table 2.3 . In the univariate analysis, increasing age, patients with tumours with a PD-L1 expression of greater than 1% and having had mediastinal staging performed were associated with increased risk of suffering a grade 2 or more radiation pneumonitis. There was no association between potential lung function parameters, observed FEV_1 as a percentage of predicted normal (FEV_1%pred) or TLCO as percentage of predicted normal (TLCO%pred) with rates of symptomatic pneumonitis. Due to PD-L1 expression results only being available for 29 patients, PD-L1 status was not taken forward into the multivariate models. In the multivariate model, the only significant factor associated with risk of symptomatic pneumonitis was having had mediastinal staging (OR 11.79 (95% CI 2.66 – 52.26). (Table 2.3)

In the univariate analysis, CCI, increasing age, male sex, increasing tumour size, solid lesions and low TLCO%pred were all associated with poorer overall survival. In this analysis part-solid and pGGOs were combined as there were no deaths in the pGGO category. All variables with a *p*-value less than 0.2 were entered into the multivariate model in a forward stepwise manner. Significant factors in the multivariate model for overall survival were tumour size, low TLCO%pred, age and solid lesions. Lesion type was the most significant factor contributing to overall survival. If the patient's age, TLCO%pred and tumour size are assumed to remain constant then the increased hazard of death increases by 208% for being a solid lesion rather than a part-solid or a pure ground glass opacity. (Table 2.4)

Tumour size was the only factor significantly associated with the rate of relapse. In the multivariate model, for each millimetre increase in tumour size, the rate of relapse increases by 5% ($p = 0.003$) (Table 2.5)

				Univariate Analysis		Multivariate Analysis	
Variable	**N**	**No or Grade 1 only Pneumonitis**	**Pneumonitis Grade ≥2**	**Odds Ratio (95% CI)**	***p*-value**	**Hazard Ratio (95% CI)**	***p*-value**
Age	187	169 (90.4%)	18 (9.6%)	1.05 (0.98 – 1.12)	**0.136**	-	-
Gender							
Female	96	88 (91.7%)	8 (8.3%)	1.00 (Reference)			
Male	91	81 (89.0%)	10 (11.0%)	1.36 (0.51 – 3.61)	0.539	-	-
Tumour size	186	168 (90.3%)	18 (9.7%)	1.02 (0.97 – 1.08)	0.428	-	-
Tumour location							
Central	11	9 (81.8%)	2 (18.2%)	1.00 (Reference)			
Peripheral	176	160 (90.9%)	16 (9.1%)	0.45 (0.09 – 2.26)	0.333	-	-
Smoking status							
Current	41	40 (97.6%)	1 (2.4%)	1.00 (Reference)			
Never/Former	146	129 (88.4%)	17 (11.6%)	5.27 (0.68 – 40.85)	**0.112**	-	-
COPD							
No	59	54 (91.5%)	5 (8.5%)	1.00 (Reference)			
Yes	127	114 (89.8%)	13 (10.2%)	1.23 (0.42 – 3.63)	0.706	-	-
FEV1 (% predicted)	182	165 (90.7%)	17 (9.3%)	0.99 (0.98 – 1.02)	0.660	-	-
TLCO (% predicted)	160	144 (90.0%)	16 (10.0%)	0.98 (0.95 – 1.01)	0.212	-	-
PD-L1 status							
<1%	17	16 (94.1%)	1 (5.9%)	1.00 (Reference)			
>=1%	12	8 (66.7%)	4 (33.3%)	8.00 (0.76 – 83.88)	**0.083**	-	**-**
Mediastinal Staging							
No	179	165 (92.2%)	14 (7.8%)	1 (Reference)		1	
Yes	8	4	4	11.79 (2.66 – 52.26)	**0.001***	11.79 (2.66 – 52.26	**0.001***

Table 2.3: Pneumonitis - Univariate and Multivariate Regression Analyses of factors associated with ≥ grade 2 pneumonitis. All factors with a $p < 0.2$ (bold) were taken forward to the multivariate model. * denotes $p < 0.05$

					Univariate Analysis		Multivariate Analysis	
		N	Deaths (event)	Median survival (95% CI) (years)	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Age		187	89	2.6 (2.3 – 3.3)	1.04 (1.01 – 1.07)	**0.010***	1.05 (1.01 – 1.09)	**0.023***
Gender	**Female**	96	36	3.3 (2.6 - NE)	1			
	Male	91	53	2.3 (1.9 - 2.7)	1.77 (1.16 – 2.71)	**0.008***	-	-
Tumour size		186	89	2.6 (2.3 – 3.3)	1.05 (1.02 – 1.07)	**<0.001***	1.03 (1.01 – 1.06)	**0.010***
Tumour location	**Central**	11	6	1.9 (0.3 - NE)	1			
	Peripheral	176	83	2.6 (2.3 - 3.3)	0.70 (0.30 – 1.60)	0.396	-	-
Smoking status	**Never**	13	4	3.3 (2.9 - NE)	1			
	Former	134	66	2.6 (2.3 – 4.0)	1.58 (0.57 – 4.36)	0.374	-	-
	Current	40	19	2.6 (1.7 – NE)	2.23 (0.76 – 6.58)	0.146	-	-
	Overall					0.243	-	-
Lesion type	**Solid**	145	76	2.4 (2.2 - 3.0)	1		1	
	Part-Solid/ pGGO	42	13	2.9 (2.6 – NE)	0.49 (0.27 – 0.88)	**0.017***	0.48 (0.24 – 0.93)	**0.031***
FEV1 (% predicted)		182	87	2.6 (2.3 – 3.3)	1.00 (0.99 – 1.01)	0.566	-	-
TLCO (% predicted)		160	76	2.5 (2.3 – 3.0)	0.98 (0.97 – 0.99)	**0.016***	0.98 (0.97 – 0.99)	**0.010***
PD-L1 status	**<1%**	17	5	3.3 (2.3 – NE)	1			
	>=1%	12	5	2.3 (1.2 – NE)	2.78 (0.73 – 10.61)	**0.133**	-	-
Charlson index		186	89	2.6 (2.3 – 3.3)	1.23 (1.10 – 1.38)	**<0.001***	-	-
Histology	**Pathology**	128	67	2.6 (2.3 – 3.0)	1			
	Radiology	59	22	4.8 (2.1 – NE)	0.82 (0.51 – 1.33)	0.426	-	-
Mediastinal Staging	**No**	179	84	2.6 (2.3 – 3.3)	1			
	Yes	8	5	2.3 (1.2 - NE)	1.52 (0.61 – 3.76)	0.368	-	-

Table 2.4: Overall Survival - Univariate and Multivariate Regression Analyses of factors associated with overall survival. All factors with a $p < 0.2$ (bold) were taken forward to the multivariate model. * denotes $p < 0.05$

					Univariate Analysis		Multivariate Analysis	
		N	Relapse (event)	Time to Relapse (95% CI) (years)	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Age		187	46	NE (3.5 – NE)	0.99 (0.96 – 1.02)	0.560	-	-
Gender	**Female**	96	22	NE (3.7 - NE)	1			
	Male	91	24	NE (2.7 - NE)	1.28 (0.72 – 2.28)	0.409	-	-
Tumour size		186	45	NE (3.5 – NE)	1.05 (1.02 – 1.08)	**0.003***	1.05 (1.02 – 1.08)	**0.003**
Tumour location	**Central**	11	3	NE (0.3 - NE)	1			
	Peripheral	176	43	NE (3.5 - NE)	0.63 (0.20 – 2.06)	0.449	-	-
Smoking status	**Never**	13	4	NE (0.9 – NE)	1			
	Former	134	31	NE (3.7 – NE)	0.58 (0.20 – 1.66)	0.313	-	-
	Current	40	**11**	3.5 (2.3 – NE)	0.88 (0.28 – 2.78)	0.833	-	-
	Overall					0.383	-	-
Lesion type	**Solid**	145	39	3.7 (3.3 - NE)	1			
	Part-Solid/ pGGO	42	7	NE (NE – NE)	0.52 (0.23 – 1.15)	**0.107**	-	-
FEV1 (% predicted)		182	44	NE (3.5 – NE)	1.00 (0.99 – 1.01)	0.679	-	-
DLCO (% predicted)		160	42	NE (3.5 – NE)	0.99 (0.98 – 1.01)	0.781	-	-
PD-L1 status	**<1%**	17	7	NE (1.2 – NE)	1			
	>=1%	12	8	1.8 (0.8 – NE)	1.80 (0.65 – 5.01)	0.260	-	-
Charlson index		186	46	NE (3.5 – NE)	1.01 (0.85 – 1.21)	0.905	-	-
Histology	**Pathology**	128	32	NE (3.5 – NE)	1			
	Radiology	59	14	NE (2.0 – NE)	0.98 (0.52 – 1.83)	0.939	-	-
Mediastinal Staging	**No**	179	42	NE (3.5 – NE)	1			
	Yes	8	4	1.98 (0.46 – NE)	2.39 (0.85 – 6.71)	**0.097**	-	-

Table 2.5: Time to relapse - Univariate and Multivariate Regression Analyses of factors associated with hazard of relapse. All factors with a $p < 0.2$ (bold) were taken forward to the multivariate model. * $p < 0.05$

2.6 Discussion

SBRT is now a well-established treatment for early stage NSCLC in patients that are unsuitable for surgical resection or in those who decline surgery. The potential survival advantage of sublobar lung resections over SBRT is the reason patients who are fit for surgery are still offered this as best primary treatment.[8, 19]

In this retrospective study of 187 patients, the median OS post-SBRT was 30 months and the 2-year OS rate was 69%. These figures are in line with previously reported outcomes. In the recently reported CHISEL trial, which randomised 101 patients to either SBRT or conventional radiotherapy for early stage NSCLC, the 2-year OS rate with SBRT was 77%.[1] Similarly, a large national patient registry study from the United States of America of 723 patients who had received SBRT, showed a median OS of 30 months for T1 tumours and 26 months for T2 tumours.[5]

We observed 47 episodes of recurrence of which only 14 (29.7%) were local relapses as defined as relapse within any part of the treatment lobe. The definition of local control can be complicated. The prospective CHISEL study defined local relapse using the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.0, as a 20% increase in the longest diameter relative to the previous smallest longest diameter recorded since (and including) the baseline longest diameter equal to an absolute increase of at least 5 mm, or the presence of any new disease (i.e. a new separate lesion) within 1·5 cm of the internal target volume.[1] This method may be complicated by radiation-induced lung damage which can be difficult to differentiate from local progression. SBRT is a truly local treatment and rates of regional and distal relapse are likely to reflect differences in accurate staging and patient selection. However, the overall local control rate throughout my study of 92.5% is comparable with previously reported studies that range from 85% to 95% for patients with stage I NSCLC treated with SBRT.[23] An advantage of my data is that with the exception of the earliest patients, standard protocol included an FDG-PET within 6 weeks of commencing treatment. A recently performed FDG-PET increases the accuracy of lung cancer staging, with a negative predictive value for lymph node metastatic disease of 91%, a

significant improvement over CT scanning alone.[12] This is particularly relevant for this cohort of patients, who rarely underwent invasive mediastinal staging. Several previous studies have assessed the significance of various parameters from pre-treatment PET scans on SBRT outcomes, with mixed results. While some studies found PET provided no prognostic information, other studies have identified the SUVmax, total lesion glycolysis and metabolic tumour volume as predictors of disease control and survival.[10] Data for the SUVmax of the target lesion is frequently included in the PET report and therefore could easily be retrospectively collected. A subsequent analysis of the RMH data looking at the significance of SUVmax on relapse and survival outcomes is warranted.

The disease-free survival at 1, 2 and 5 years was 78%, 59% and 25% respectively, also comparable to previously reported figures. A published meta-analysis of 11 reported studies of SBRT resulted in an unadjusted 1 year DFS of 87.1%.[24] A previous single-centre, retrospective, real-world study by Crabtree et al., which included 294 patients undergoing SBRT for stage I NSCLC, found 1 and 5 year DFS rates of 79% (72% to 85%) and 19% (10 to 30%) respectively.[3] A comparison summary of the RMH data with previously published figures is shown in Table 2.6.

	RMH Data	Chisel Trial[1]	US Registry Study[5]	Crabtree et al.[3]	Zheng et al. Meta-analysis[24]
Study Population	n = 187 ECOG PS: 0-2	n = 66 ECOG PS: 0 -1	n = 723 Karnofsky PS: 40 -100	n = 151 PS not reported	OS: 37 studies DFS: 11 studies PS not reported
Overall Survival (OS)					
Median (95% CI)	2.6 years (2.3 to 3.3)	5 years (3.4 to NA)	2.5 years (1.9 to 2.8)		2.3 years
1-year OS rate (95% CI)	87% (81% to 91%)			82% (75% to 87%)	83.4%
2-year OS rate (95% CI)	69% (62% to 76%)	77% (67% to 88%)			
5-year OS rate (95% CI)	29% (19% to 41%)			25% (14% to 37%)	41.2%
Disease Free Survival (DFS)					
Median (95% CI)	2.4 years (2.0 to 2.7)				
1-year DFS rate (95% CI)	78% (71% to 83%)			79% (72% to 85%)	87.1%
2-year DFS rate (95% CI)	59% (51% to 66%)				
5-year DFS rate (95% CI)	25% (16% to 35%)			19% (10 to 30%)	65.8%

Table 2.6: Comparison of the RMH data with previously published survival figures following SBRT for early stage NSCLC. ECOG = Eastern Conference Oncology Group; PS = Performance Status

In my study the rate of grade 2 or above pneumonitis was 9.6% for the 207 SBRT treatments. These were predominantly of grade two and could be managed as an outpatient, often with oral steroids. However, we did observe 2 patients (0.9%) who developed fatal complications following SBRT. This is similar to findings from previous studies which have reported life-threatening complications in up to 12% of cases.[11]

In the univariate regression analysis, I found no association between the risk of symptomatic pneumonitis and patient age, tumour size or pre-treatment lung function parameters. Having the mediastinum histologically staged was the only identified factor to be significantly associated with risk of developing symptomatic pneumonitis. The proportion of patients that received mediastinal staging was low and therefore this finding is statistically fragile with a correspondingly large confidence interval. Of note, in 5 of the 8 cases which were invasively staged, the tumours were greater than 30 mm in maximum diameter or centrally located. Invasive mediastinal staging was only performed in 7.6% of these high-risk cases, as described by the ESMO guidelines.[20] There is however good evidence that invasive mediastinal staging does not improve outcomes over FDG-PET alone, particularly for peripheral lesions and T1 lesions or those with a significant ground glass component.[7]

Patients selected for histological mediastinal staging are also likely to be relatively high-risk patients for undergoing SBRT. This is supported by my finding of a tendency for patients with mediastinal staging to have a shorter time to relapse (p =0.09), however no association was seen with overall survival. Nevertheless, the large hazard ratio (HR = 11.79) for the increased risk of developing pneumonitis following invasive mediastinal staging was an unexpected finding and requires further investigation. Although the sample size was small, with 50% of the patients that underwent mediastinal staging developing a minimum grade 2 pneumonitis, it remains possible that there is an associated causative link. This association has not previously been reported and it warrants repeating within a larger cohort of patients who undergo invasive mediastinal staging prior to SBRT for NSCLC. One possible explanation for this finding could be the presence of an obstructive rather than a radiation pneumonitis with an associated benign lymph node enlargement in these patients. The patients

included in this analysis, by definition, were found not to have metastatic disease after mediastinal node sampling. The reason for choosing to invasively stage the mediastinum was not assessed as part of this study, as these results were unexpected but enlarged lymph nodes would be a clear indication. An obstructive pneumonitis has previously been shown to be associated with benign lymph node enlargement at mediastinal sampling.[14] A deeper interrogation of the indications for sampling the mediastinum and the details regarding the development of pneumonitis in the patients from my analysis should be performed prior to designing a subsequent study.

My findings that lung function parameters do not increase the risk of pneumonitis support a previous study that found there was no association with lung function and the risk of developing either grade ≥ 2 or grade ≥ 3 pneumonitis.[9] Similarly, a retrospective study of patients with COPD and severe airways obstruction (Global Initiative for Obstructive Lung Disease (GOLD) III-IV) treated with SBRT demonstrated a low rate of grade 3 pneumonitis (1.7%).[18]

There was a suggestion that symptomatic pneumonitis may be more common in former or never smokers compared to current smokers, although this did not meet statistical significance (OR 5.27, $p = 0.11$). The possible inverse relationship between pneumonitis and pack-years smoked has previously been described.[22] Postulated biological explanations for this phenomenon include a reduction in radiation-induced inflammation in smokers and a smoking-induced increase in pulmonary glutathione, leading to prevention of oxidant lung injury.[2]

Low TLCO%pred was associated with worse overall survival in my study. For every 1% decrease in TLCO the hazard for death increased by 2%. My data supports the previous findings from Guckenberger et al. who found that while PFT parameters were not associated with cause-specific survival, TLCO and TLCO%pred were associated with overall survival.[9] It would thus appear that for patients undergoing SBRT, TLCO is the lung function parameter most associated with overall survival. However, low

TLCO is negatively associated with survival independent of SBRT in multiple lung pathologies including COPD[15] and idiopathic pulmonary fibrosis.[21]

Other patient factors that I found to be associated with worse overall survival were increasing age, male sex and a higher CCI. Larger and solid tumours were also associated with a worse prognosis. These findings support a similar study from a Japanese population which found tumour size, male sex and age were all negatively associated with overall survival in a multivariate analysis.[13] While higher CCI was associated with worse overall survival in the univariate analysis this was not significant in the multivariate model. Larger tumour size was also the only factor associated with shorter time to relapse. It is therefore likely that tumour recurrence is an important cause of the reduced overall survival seen in patients treated for larger cancers. My finding that patients with part-solid or pure ground glass opacities had a better prognosis than those with solid lesions is consistent with the known more indolent nature of sub-solid lesions.[16] Notably, there was no significant difference in either time to relapse or overall survival between radiologically and histologically diagnosed tumours. This supports the current accepted practice that lesions treated based on radiological criteria of both serial growth and avidity on FDG-PET are likely to be malignant.

Results of pre-treatment tumour PD-L1 expression were only available in a small sub-population of the analysed patients. PD-L1 quantification on tumour cells is a relatively new immunohistochemistry test. This test is now widely available due to the predictive significance provided by the degree of PD-L1 expression with relation to response to immune checkpoint inhibitors in advanced NSCLC. However, PD-L1 testing would not have historically been performed and is not standard of care in early stage, radically treatable disease. Due to the necessity in advanced disease for PD-L1 and molecular marker (EGFR, ALK and Ros-1) status to be established prior to any decision on systemic treatment, many laboratories are now performing ‘reflex’ PD-L1 assessments in all patients and for all stages of NSCLC. This provided an unselected cohort of tumours that had been tested for PD-L1 status despite not meeting criteria for immune-checkpoint inhibitors.

From my data, tumours had no expression of PD-L1 (TPS <1%) in 59% of the tested biopsies. High PD-L1 expression (TPS ≥ 50%) was seen in 3 (10%) biopsies. This suggests that the proportion of biopsies without PD-L1 expression may be higher in early stage disease than in metastatic disease. This is supported by a previous analysis of 289 resected tumours, which found that PD-L1 TPS was <1% in 176 (60.9%).[4] In this study, tumours with high expression of PD-L1 were correlated with higher tumour differentiation grades, suggesting more aggressive tumours. However, no association was seen between PD-L1 expression and overall survival. In locally advanced or metastatic disease, a global, real-world study found that only 48% of European NSCLC biopsies had a PD-L1 TPS of < 1%.[6]

Despite the small sample size in my data, there was a trend for patients with tumours that expressed PD-L1 on greater than 1% tumour cells to have worse overall survival suggesting it is a marker for poor prognosis. Rates of symptomatic pneumonitis in patients with tumours with positive PD-L1 expression tended to be higher than those with PD-L1 TPS < 1% (OR 8.0 $p = 0.083$). The induction of PD-L1 on tumour infiltrating T cells, B cells, and epithelial cells is a known mechanism for immune avoidance and subsequent tumour proliferation.[17] The more inflammatory nature of tumours with high PD-L1 expression may provide an explanation into the observed higher tendency to develop symptomatic pneumonitis. These findings would need to be confirmed in a prospective study with a larger sample size.

This study was limited by being retrospective in nature and therefore relied on clinic letters to assign episodes of symptomatic pneumonitis, calculate CCI and smoking status. Unrecorded episodes of symptomatic pneumonitis, for example when treated by the patient's GP, would not have been accounted for in this analysis. Radiotherapy treatment details including mean lung dose, V20 (the percentage of normal lung receiving at least 20 Gy) and conformity index were not available to be included in this analysis. These factors may be relevant to the risk of developing symptomatic pneumonitis.

Overall, my results support the published data that SBRT is a safe and effective treatment for early stage NSCLC with a low rate of local relapse. Significant pneumonitis is a rare side effect and does not appear to be correlated to baseline lung function parameters. Lower TLCO was associated with worse overall survival although it was unrelated to disease relapse suggesting SBRT is effective in patients with impaired lung function. Larger tumours were more likely to relapse and had worse overall survival, identifying this population as those most likely to need an active adjuvant therapy following SBRT. High PD-L1 expression on biopsied tumours was rarely seen in this small sample. However, I found a tendency for patients with PD-L1 expressing tumours to be more likely to develop significant pneumonitis. Patients with larger tumours and those with positive expression of PD-L1 may be the most suitable candidates for trials of combination radiotherapy/immunotherapy treatment regimens, but any associated increased risk of pulmonary toxicities must be established.

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Chapter 3: **Part 2A - Stereotactic Body Radiotherapy with Immunotherapy: Tolerability and Lung Effects – The STILE Trial**

3.1 Introduction

Any potential adjuvant treatment following SBRT that aims to improve rates of disease relapse and overall survival would need to have a tolerable toxicity profile and a treatment schedule that is acceptable to patients. The STILE trial aimed to assess the safety of using the immune checkpoint inhibitor nivolumab, as adjuvant therapy following SBRT. If tolerated, then a subsequent phase III placebo-controlled randomised trial could investigate the effect on outcomes of combining nivolumab with SBRT for early stage NSCLC.

I was a key contributor to the design of this study and was responsible for leading the protocol development. Approval for sponsorship of the study was obtained from the Royal Marsden Committee for Clinical Research (CCR). The study was approved by the National Institute for Health Research (NIHR) and the Health Research Authority (HRA) after discussion at a regional Research and Ethics Committee (REC). As the trial protocol included radiotherapy, an Administration of Radioactive Substances Advisory Committee (ARSAC) certificate was also obtained.

Funding for the study along with provision of nivolumab was secured from the pharmaceutical company, Bristol-Myers Squibb (BMS). The trial was sponsored by the Royal Marsden NHS Foundation Trust with trial oversight provided by the Royal Marsden Clinical Trials Unit (RM-CTU). RM-CTU had overall responsibility for facilitating and coordinating the conduct of the trial and was also responsible for collating data obtained and undertaking and reporting all analyses.

The STILE trial is currently open to recruitment. An independent data monitoring committee (IDMC) had their first scheduled meeting to discuss any ongoing safety

concerns when the first 5 patients had completed 3 months of follow up on study. I have received permission from the IDMC chair to report in this thesis the initial results from the first 5 patients recruited to the study.

3.2 Study Hypothesis

Nivolumab following SBRT for stage I-II NSCLC (T3 (≤5cm) N0M0 is a safe treatment and rates of pneumonitis at grade 3 or higher as defined by the Common Terminology Criteria for Adverse Events (CTCAE) v.4 within 6 months of the final fraction of SBRT occur at a rate that does not exceed 20%.

3.3 Study Objectives

3.3.1 Primary Objective

- To assess the lung toxicity of nivolumab after stereotactic body radiotherapy (SBRT) for early stage NSCLC (T1-3 (≤5cm) N0M0).

3.3.2 Secondary Objectives

- To assess the overall safety of nivolumab after SBRT
- To assess the tolerability of nivolumab after SBRT
- To assess local, local-regional and distant disease relapse rates
- To assess the overall survival rates at 6, 12 and 24 months
- To assess the disease-free survival (DFS) rates at 6, 12 and 24 months
- To measure health related quality of life (HRQoL)

3.3.3 Exploratory Objectives

- To describe the relationship between lung function and the tolerability of nivolumab after SBRT
- To assess relapse rates and survival rates in relation to tumour PD-L1 status
- To assess tumour biology and immune function changes with treatment

3.4 Trial Design

This was a single arm, phase Ib/II open label study of nivolumab administered on completion of SBRT to patients with early stage NSCLC. The study will aim to recruit 31 patients.

3.4.1 Inclusion and Exclusion Criteria

The study included only subjects with histologically verified NSCLC that had been deemed by a local MDT to have anatomical stage T1-3 [≤5cm] N0 M0 by means of CT and FDG-PET, amenable to radical treatment with SBRT and inoperable due to medical co-morbidity, being technically unresectable or patient declining surgery after a surgical assessment. This trial included only subjects with peripheral tumours i.e. outside a 2cm radius of main airways and proximal bronchial tree. This was defined as 2 cm from the bifurcation of the second order bronchus e.g. where the right upper lobe bronchus splits (see Figure 2.1). All subjects were required to have adequate renal, liver and bone marrow function. Women of childbearing potential were required to have a negative pregnancy tests during screening and to use contraception up to and including 23 weeks post their final nivolumab treatment. Exclusion criteria included any tumours that were not clearly definable on the treatment planning CT scan (e.g. due to surrounding atelectasis), significant co-morbidities including positive tests for HIV, hepatitis B or C and patients who had previously received an immune-checkpoint inhibitor. Patients with other current or previous malignancies that had not been in remission for a minimum of 2 years and those with evidence of interstitial lung disease were also excluded. Patients with active autoimmune conditions, with the exception of type 1 diabetes, residual hypothyroidism following an autoimmune condition or psoriasis were excluded. All patients that were on long-term immunosuppressive treatments including oral steroids were required to be on a stable maximum dose of 10 mg prednisolone (or equivalent) at the time of recruitment.

3.4.2 Treatment Schedule

SBRT was delivered as per standard of care with either 54 Gy in 3 fractions or 55 Gy in 5 fractions. Subjects received nivolumab every two weeks for up to a maximum of one year of treatment, unless any withdrawal criteria were met. The first nivolumab infusion was given after the final fraction of SBRT, within 24 hours and generally on the same day. (Figure 3.1) Tumour samples were analysed for PD-L1 expression using the Dako PD-L1 IHC 28-8 pharmaDx assay. Residual tumour from the diagnostic biopsy will be analysed within the study for further translational research. Research blood samples from the study may undergo proteomic, genomic and transcriptional analyses.

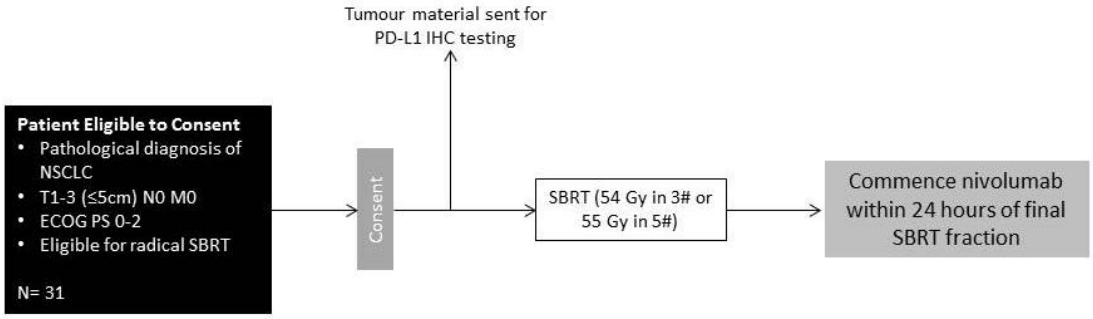

Figure 3.1: Trial schema

Dose interruptions were allowed up to a maximum of 12 weeks per dose for clinical reasons that did not meet the withdrawal criteria. Withdrawal criteria included disease relapse, significant inter-current illness, a positive pregnancy test or unacceptable adverse events (AEs). All AEs were graded using the CTCAE version 4.

Unacceptable adverse events were defined as:

- Grade 3 bronchopulmonary haemorrhage
- Grade ≥ 2 pulmonary fistula
- Grade ≥ 2 oesophageal perforation
- Grade ≥ 3 febrile neutropaenia

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Grade 3 drug-related uveitis, pneumonitis, bronchospasm, hypophysitis, adrenal insufficiency, hypersensitivity reaction or infusion reaction
- Any other Grade 3 non-skin, drug-related adverse event lasting > 7 days with the exception below:
    - **Exception:** Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation
- Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding
- Grade 3 drug related serum creatinine elevation
- Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
    - AST or ALT > 5 x ULN
    - Total bilirubin > 3 x ULN
    - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 3 immune-mediated adverse reaction that recurs
- Symptoms or signs of Steven-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)
- Any other life-threatening immune-mediated adverse reaction
- Any Grade 4 drug-related adverse event or laboratory abnormality, with the following exceptions, which do not require discontinuation:
    - **Exception:** Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset
    - **Exception:** Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
    - **Exception:** Grade 4 lymphopaenia or leucopaenia

- Any dosing interruption lasting > 12 weeks with the following exceptions:
    - Dosing interruptions were permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g. elective surgery, unrelated medical events, patient vacation, and/or holidays).

The protocol dictated that the first 5 patients to enroll must be of ECOG performance status < 2 at the time of first dose of investigational medical product (IMP). An IDMC was required when the first 5 patients had reached 3 months follow up from their first dose of nivolumab or had withdrawn consent to follow-up. The IDMC included an independent medical oncologist, clinical oncologist and statistician. The role of the IDMC was to review the ongoing safety information from the study and to ensure that the protocol and good clinical practice (GCP) principles were adhered to.

All patients were required to attend a safety follow up visit, 30 days after their final nivolumab treatment. Subjects were followed up every 3 months in the first year after completion of SBRT and every 6 months in the second year. These follow-up visits occured for all subjects including those no longer receiving nivolumab. AEs were captured up to 100 days from the last nivolumab treatment. Clinical follow up and investigations are as detailed in the schedule of events. (Figure 3.2)

		1 or 2 weeks	W1	W2	W3	W4	W5	W7	W9	Every 2 weeks	Every 6 weeks	**Follow Up Visits**						
Trial Phase	**Screening**	**Radiotherapy Phase**		**Nivolumab Phase**								**Safety Follow-Up**	**Follow-Up Phase**					
Treatment Cycle		1st #	Final #, C1, D1	C1, D8	C2, D1	C2, D8	C3, D1	C4, D1	C5, D1	C6 onwards D1 of each cycle	C7 D1 onwards	30 days post last dose[2]	3m post SBRT	6m post SBRT	9m post SBRT	12m post SBRT	18m post SBRT	24m post SBRT
Scheduling (Day)	-28 to -1										+/-7	+/-7	+/-14	+/-14	+/-14	+/-14	+/-14	+/-14
Informed Consent	**X**																	
Inclusion/Exclusion	**X**																	
Demographics & Past Medical History	**X**																	
Concomitant Medications	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**£**	**£**	**£**	**£**	**£**	**£**
Full physical exam	**X**																	
Directed physical exam		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**£**	**£**	**£**	**£**	**£**	**£**
Adverse Events	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**¥**	**¥**	**¥**	**¥**	**¥**	**¥**
ECOG PS	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**
MRC Dyspnoea score	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Vital signs & Weight[1]	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**£**	**£**	**£**	**£**	**£**	**£**
Height	**X**																	
Haem & Biochem[1]	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**£**	**£**	**£**	**£**	**£**	**£**
Thyroid Function Tests	**X**							**X**			**X**	**X**	**£**	**£**	**£**	**£**	**£**	**£**
Hepatitis Screen	**X**																	
Research Bloods	**X**		**X**				**X**		**X**									
Pregnancy Test (WOCBP)	**X**		**X**					**X**			**X**	**X**	**£**	**£**	**£**	**£**	**£**	
HRQoL Assessments	**X**												**X**	**X**	**X**	**X**	**X**	**X**
Pulmonary Function Tests	**X**													**X**		**X**		**X**
Bedside spirometry			**X**					**X**			**X**	**X**	**X**		**X**		**X**	
PDL-1 assessment	**X**																	
Radiotherapy Planning	**X**																	
SBRT		**X**	**X**															
Nivolumab infussion (mg)			**X**		**X**		**X**	**X**	**X**	**X**								
Imaging	**CXR (CT & PET)***						**CXR**		**CXR**			**CXR**	**CT**	**CT**	**CXR**	**CT**	**CT**	**CT**

Figure 3.2: Schedule of events

1 = Must be obtained ≤ 72 hours from dose administration

2 = Or before initiation of any new anti-cancer therapy

* = CT chest/abdomen within 8 weeks of first fraction of SBRT. FDG-PET within 6 weeks of SBRT

= Fraction of radiotherapy; ¥ = For 100 days (Adverse Events) after final dose of IMP: £ = If indicated

Pulmonary Function Tests only to correspond with 6, 12- and 24-month post SBRT follow-up

Pregnancy test to be performed within 24 hours of IMP for cycle 1 then minimum of every 6 weeks while on treatment.

Research blood samples at screening, and day 1 of cycles 1, 3 and 5 of nivolumab. An additional (optional) sample will be taken at time of relapse if the patient consents.

CXR to be performed during screening, at 4 weeks, 8 weeks and 9 months from final fraction of SBRT and at the safety follow-up visit

CT chest/abdomen at 3, 6, 12, 18 and 24 months from final fraction of SBRT

3.4.3 Endpoints & Assessments

The primary endpoint of the study was the rate of grade 3 or above pneumonitis within 6 months of the final fraction of radiotherapy. A rate that exceeds 20% will be deemed unacceptable and will lead to a rejection of the study hypothesis.

The overall safety of the study is a key secondary endpoint and therefore adverse events were collected throughout the study period and for 100 days after the final nivolumab treatment for each subject. The tolerability of the treatment was also assessed by the proportion of patients that receive 6 doses of nivolumab within 16 weeks of completing SBRT. The efficacy of the treatment was assessed by calculating the rates of local, loco-regional and distant relapse, disease-free survival and overall survival. The impact of the treatment on health-related quality of life was assessed throughout the study with regular completion of 3 questionnaires: the EORTC QLQ-C30, the EORTC QLQ-LC13 (lung module) and a patient's own subjective assessment of their performance status.

Exploratory endpoints include a breakdown of the rates of toxicity within bands of baseline FEV_1 and TLCO. On completion of the trial, outcome measures will also be stratified based on degree of tumour PD-L1 expression. Bedside spirometry was performed every 6 weeks while on nivolumab treatment with formal lung functions tests being performed at 6, 12 and 24 months after SBRT.

3.4.4 Sample Size Calculation

We aimed to show that the true population rate of grade 3 pneumonitis within 6 months of SBRT when combined with nivolumab is less than 20%. If the observed rate of pneumonitis (grade ≥ 3) is 10%, with 31 patients we would be able to estimate this with a one sided 95% confidence interval (CI) of 9% width. Table 3.1 demonstrates the widths of the 95% CIs given different rates of pneumonitis. Any patient that has enrolled on the study but does not receive at least 1 dose of nivolumab will be

replaced. Recruitment may continue until 31 patients have received at least one dose of nivolumab unless any study discontinuation criteria are met.

Observed proportion	Upper Bound of 95% C.I.
0.070	0.145
0.100	0.189
0.130	0.229
0.150	0.255

Table 3.1: Upper bound of a one-sided 95% confidence interval according to observed proportion assuming 31 patients

The upper limit of the CI should ideally exclude a rate of 20% for this treatment to be deemed acceptable, therefore with a total of 31 patients, if the observed rate of pneumonitis is higher than 10% then we would consider that the true population rate may exceed 20%.

3.4.5 Patient & Public Involvement (PPI)

For adjuvant nivolumab to be a successful treatment option it is essential for it to be an acceptable regimen to patients. The trial protocol and patient information sheet (PIS) were submitted to the Royal Marsden Hospital Patient and Public Involvement (PPI) panel for feedback. The PPI panel highlighted the importance of integrating quality of life measures as part of the study. The panel also provided invaluable feedback on the detail and wording of the PIS to ensure it was optimised for patient understanding. The PIS was further reviewed and updated following the ethics committee review, which included a patient representative.

3.5 Results

3.5.1 Recruitment

The STILE trial opened to recruitment on 20th February 2018. The first IDMC meeting was on 2nd October 2018. At this meeting, the findings from the first five patients recruited to the study, having completed a minimum of three months follow-up from their first dose of nivolumab were discussed. Recruitment to the study is described in Figure 3.3.

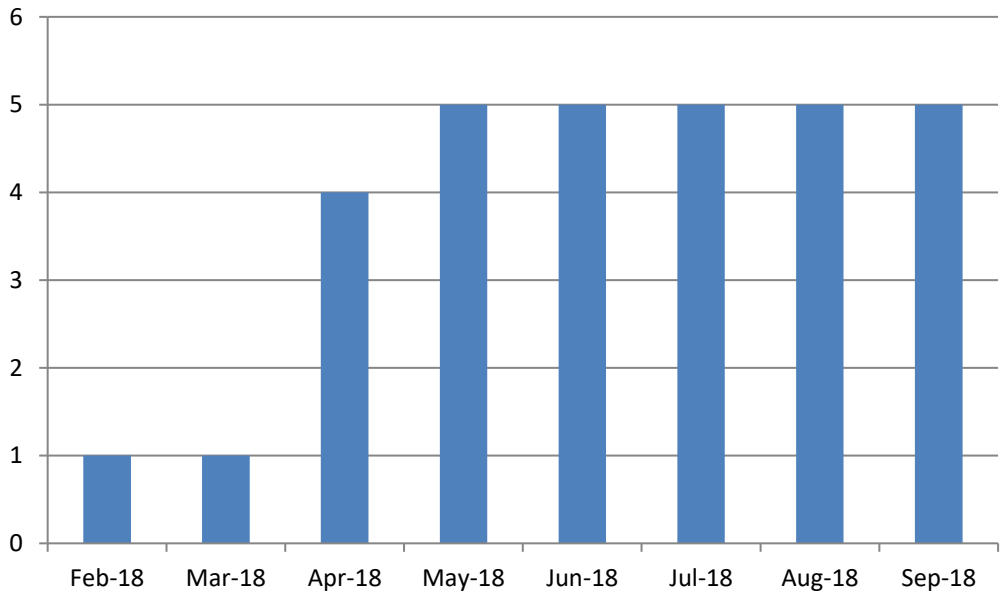

Figure 3.3: Cumulative number of patients recruited to STILE trial

Between the trial opening and 1st October 2018, 75 patients underwent SBRT for NSCLC at RMH across both sites. A PIS was offered to 6 patients of which 5 patients consented to screening. The reason patients were not eligible and thus not provided with a PIS, are described in Table 3.2. All 5 patients who were screened entered the study. The baseline characteristics of the first 5 patients enrolled onto the study are presented in Table 3.3. Lung cancer staging was defined in the protocol as per UICC version 7.

Reason for Ineligibility	Number (%)
Not Biopsy Proven	26 (47)
Performance Status ≥ 2	17 (31)
Cancer within 2 years	11 (20)
Synchronous Primary	4 (7)
Autoimmune Disease	8 (15)
Patient Declined PIS	2 (4)
Central Disease	1 (2)

Table 3.2: Breakdown of reasons for ineligibility (n =69)

	Frequency	%
Gender		
Females	3	60
Males	2	40
Histology		
Adenocarcinoma	5	100
Smoking Status		
Ex-smoker	4	80
Never Smoker	1	20
T stage at Diagnosis		
IA	1	20
IB	3	60
T2A	1	20
Stage at Diagnosis		
IA	4	80
IB	1	20
ECOG PS		
0	4	80
1	1	20

Table 3.3: Baseline characteristics of first 5 patients. ECOG PS = Eastern Conference Oncology Group performance status. NSCLC staging as per UICC version 7.

Of the 5 recruited patients, at the time of the IDMC meeting three remained on nivolumab treatment. Two patients had withdrawn consent for the study drug but remained in follow-up. The number of nivolumab treatments received is described in Table 3.4.

Maximum Cycle	**n = 5**		**Status**
	N	**(%)**	
1	1	20	Off drug: Patient withdrew
2	0	0	
3	0	0	
4	0	0	
5	0	0	
6	1	20	On treatment
7	1	20	On treatment
8	1	20	Off drug: Patient withdrew
9	1	20	On treatment

Table 3.4: Number of treatment cycles received at the time of first IDMC meeting

3.5.2 Adverse Events (AEs)

Treatment related AEs were seen in all subjects. A breakdown of all adverse events is shown in Table 3.5. Subjects who discontinued nivolumab cited treatment-related adverse events as a contributing factor into their decision to withdraw consent for further cycles of nivolumab (see below). However, no specific study drug discontinuation criteria were met, as laid out in the protocol.

Adverse Event Description	Grade 1	Grade 2	Grade 3	Grade 4
SBRT & Nivolumab Related Adverse Events				
Bronchial Infection		1 (20)	1 (20)	
Cough	1 (20)			
Dermatitis radiation	3 (60)			
Fatigue		2 (40)		
Fever	1 (20)			
Gastro-oesophageal reflux	1 (20)			
Pneumonitis	1 (20)	1 (20)		
Nivolumab Related Adverse Events				
Abdominal Pain	1 (20)			
Acneiform rash			1 (20)	
Adrenal insufficiency		1 (20)		
ALP increase	1 (20)			
ALT increase	2 (40)			
Anorexia	1 (20)			
Arthralgia		1 (20)		
AST increase	2 (40)			
Ataxia		1 (20)		
Bilirubin increased	1 (20)			
Blurred vision	1 (20)			
Bruising	1 (20)			
Confusion		1 (20)		
Constipation	1 (20)			
Dehydration		1 (20)		
Dizziness	1 (20)			
Dry mouth	1 (20)			
Dysarthria	1 (20)			
Dysgeusia	1 (20)			
Headache		1 (20)		
Hyperthyroidism	1 (20)			

Hypokalaemia			1 (20)	
Hyponatraemia				1 (20)
Hypophosphataemia			1 (20)	
Hypothyroidism		1 (20)		
Macular-papular rash	1 (20)			
Nausea		1 (20)		
Oral dysesthesia		1 (20)		
Pain of skin	1 (20)			
Palpitations	1 (20)			
Pruritus	1 (20)	1 (20)		
Vomiting	1 (20)			
Weight loss		1 (20)		
Unrelated Adverse Events				
Anxiety		1 (20)		
Dizziness	1 (20)			
Headache	2 (40)			
Oral candida		1 (20)		
Pain in extremity	1 (20)			
Tachycardia	1 (20)			

Table 3.5: Adverse events in all subjects divided by relatedness to SBRT and nivolumab, nivolumab or unrelated as classified in the adverse events log. Related adverse events included if classified as probably, likely or definitely related. Unrelated adverse events were those classified as unlikely or unrelated. All grades are as per CTCAE v.4

3.5.3 Severe Adverse Events (SAEs)

There were two SAEs on the study at the time of the first IDMC meeting. Both SAEs occurred in subject RM-4644-003, an 82-year-old female, ex-smoker with a past medical history of COPD and stage 3 chronic kidney disease who discontinued nivolumab following her first cycle. The withdrawal was preceded by 2 short hospital admissions meeting criteria for SAEs.

SAE1: RM-4644-003 received her final fraction of SBRT and first cycle of nivolumab on 11/5/18. She was admitted to her local hospital on the 18/5/18 (7 days post cycle 1 nivolumab) with headache (grade 2), dizziness (grade 1) and nausea (grade 1). She underwent a CT scan of the head which showed no acute abnormality. These symptoms were attributed to drug toxicity as no alternative explanation could be found. There was no evidence of an autoimmune cause including a normal pituitary hormone profile. She was treated with intravenous fluids and physiotherapy and was discharged on 22/5/18.

SAE2: Following RM-4644-003's discharge from her local hospital she was reviewed on several occasions by the trial team at RMH where she expressed that she was feeling more anxious (grade 2). She was subsequently admitted from clinic on 22/6/18 (32 days post cycle 1 nivolumab) with evidence of a chest infection (bronchial infection grade 3) with a productive cough, fever (grade 1), confusion (grade 1) and raised inflammatory markers. She improved with intravenous antibiotics and was discharged on 22/6/18. A CT scan was performed during this admission showed possible radiological evidence of pneumonitis and a short course of oral steroids was prescribed on discharge (grade 2 pneumonitis). Images from the baseline and the unscheduled CT scan performed 1 month following SBRT are shown in Figure 3.4. At the 3-month post SBRT follow up visit and CT scan she had returned to baseline function with no excessive shortness of breath and only evidence of an ongoing grade 1 pneumonitis.

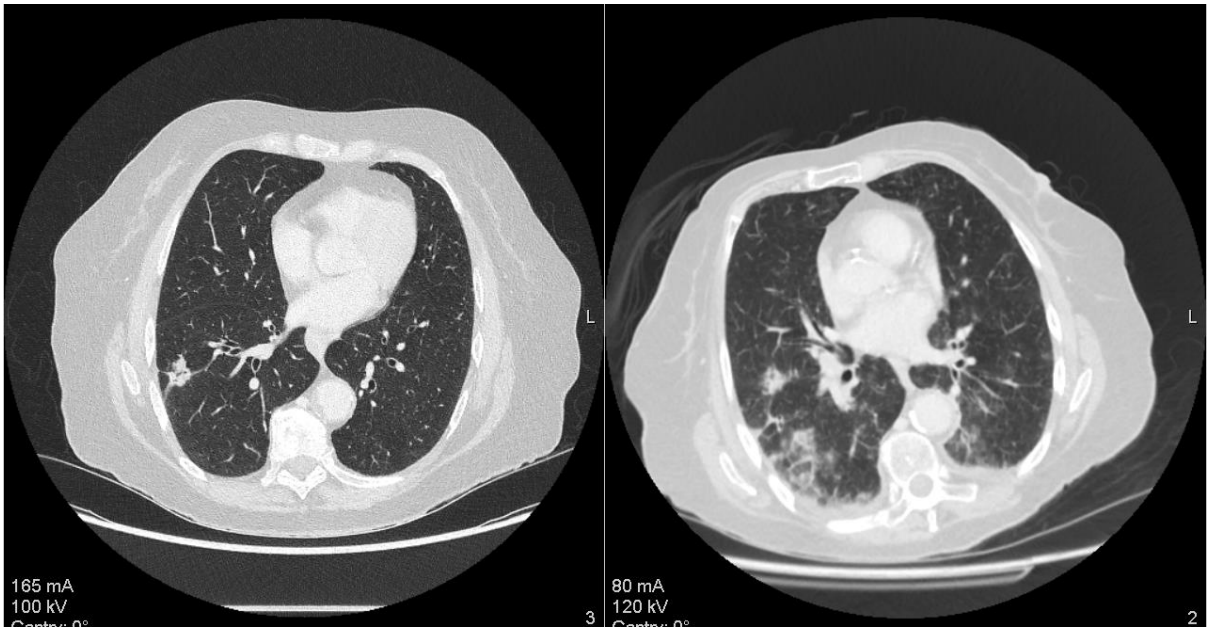

Figure 3.4: Subject RM-4644-003 axial CT images: (left) baseline i.e. pre-treatment showing 32 mm right lower lobe adenocarcinoma; (right) 1-month post SBRT and 1 cycle of nivolumab with evidence of bronchial infection and pneumonitis

3.5.4 Events leading to Discontinuation of Nivolumab

Two patients withdrew consent for further cycles of nivolumab, subjects RM-4644-001 and RM-4644-003. The adverse events leading to the withdrawal of subject RM-4644-003 have been discussed above in Section 3.5.3. In addition to the adverse events, this subject relocated during the screening process to a new house closer to her family but approximately 60 miles away from RMH. This made study visits more onerous for both her and her family and is likely to have made a significant contribution to her decision to withdraw from the study drug, which required visits every 2 weeks at minimum. She will continue in the follow up component of the study.

Subject-RM-4644-001, a 76-year-old male, ex-smoker with severe COPD chose to discontinue nivolumab after completing 8 cycles of nivolumab. Following his 8th cycle, he developed fatigue (grade 1) and he postponed his treatment. On subsequent review he was found to have developed significant hyponatraemia (grade 4) and worsening fatigue (grade 2). Investigations of his pituitary axis revealed evidence of adrenal insufficiency (grade 2). He was treated with oral prednisolone and was

regularly monitored for a response in his sodium. He was referred to an endocrinologist at his local hospital for ongoing management of his adrenal insufficiency. Grade 2 adrenal insufficiency does not meet criteria for study drug discontinuation and patients may continue nivolumab with steroid supplementation. However, given this side effect RM-4644-001 decided to withdraw from the study drug following this event. He will continue in the follow up component of the study. At his most recent study visit his fatigue had improved to grade 1. The trend in his serum sodium is shown in Figure 3.5.

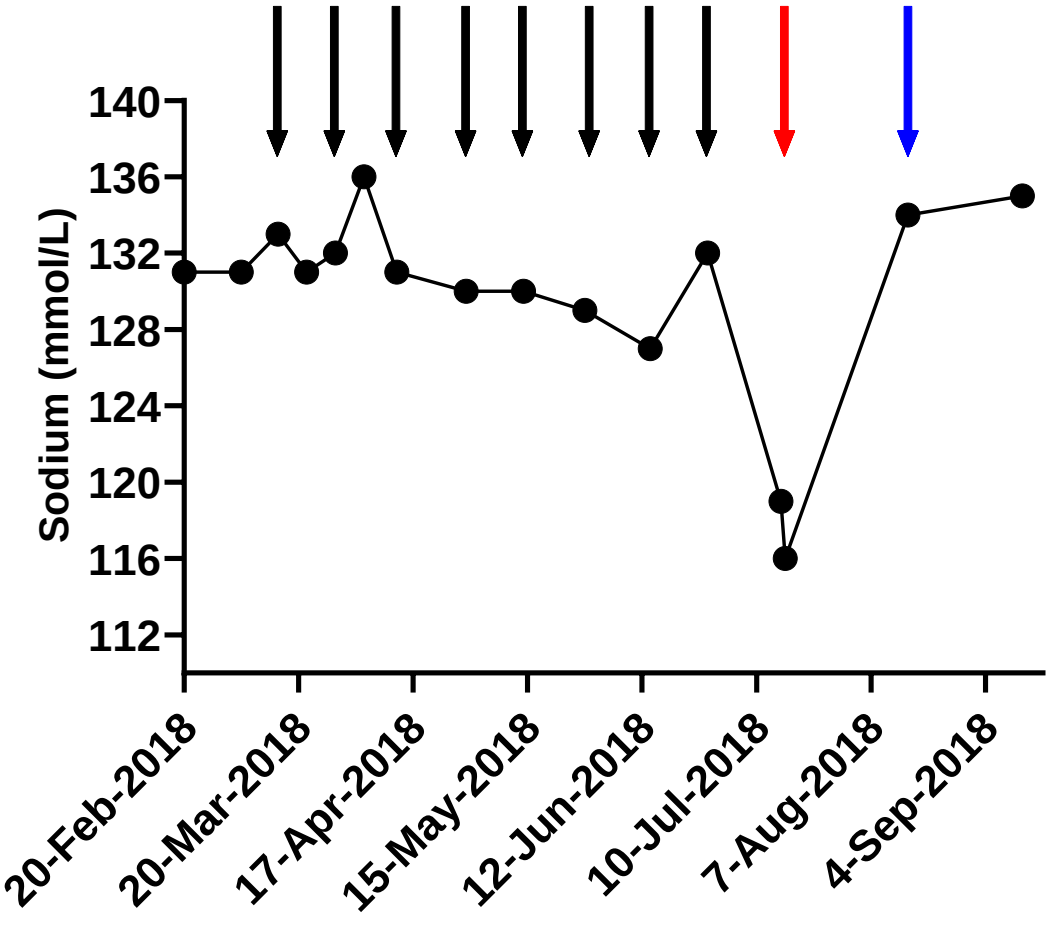

Figure 3.5: RM-4644-001 serum sodium throughout the study. Black arrows indicate treatment cycles of nivolumab. Red arrow indicates initiation of prednisolone 30 mg. Blue arrow indicates safety follow-up visit (30 days post final nivolumab treatment)

3.5.5 Primary Endpoint

There have been no events of grade 3 or above pneumonitis. This data was censored after the 5th subject had completed a minimum of 3 month follow up following SBRT. At this time, 3 of the 5 subjects had completed the primary endpoint timepoint of 6 months follow-up.

3.5.6 Secondary Endpoints

Tolerability: Four of the five subjects (80%) completed 6 cycles of nivolumab within 16 weeks of completing SBRT.

Relapse: There have been no occurrences of disease relapse. Tumour measurements are not part of the protocol. However, at the first scheduled CT, 3 months from the completion of SBRT, stable disease (as per RECIST criteria) was seen in four of the five subjects. One subject showed a partial response with the maximum tumour diameter on CT reducing from 25 mm to 13 mm, a reduction of 48%.

3.5.7 Other Adverse Events of Interest

Immune-mediated adverse events are of special interest in this trial. Subject RM-4644-004, a 69-year-old male with severe COPD and a history of cardiomyopathy, developed a grade 1 acneiform rash over his arms and torso following his 3rd cycle of nivolumab. He proceeded with the 4th cycle, but the rash progressed to grade 3 severity with more extensive spread and covering a minimum of 30% of his body surface area. Nivolumab treatment was paused for a short course of oral steroids. He was reviewed by a dermatologist who felt that this was likely to be an immune-mediated rash, typical of those seen with the use of checkpoint inhibitors. The rash improved significantly with oral prednisolone 30 mg, topical emollients and topical steroid. Nivolumab was restarted when he had weaned to oral prednisolone 10 mg.

3.5.8 Pulmonary Function Tests

Three of the five subjects (60%) had a diagnosis of COPD prior to entering the study. All subjects have baseline full PFTs including TLCO within 12 weeks of commencing SBRT. The results of the baseline PFTs are shown in Table 3.6. Bedside spirometry is performed every 6 weeks while on nivolumab treatment and at the routine follow-up appointments. Individual trends in FEV_1 and FVC through the study are shown in Figure 3.6. The largest decline from baseline in spirometry values were seen in subject RM-4644-002. FEV_1 declined from 65.4% at baseline to a nadir of 47% while FVC dropped from 82.3% to 52%.

	FEV_1	FEV_1%pred	FVC	FVC%pred	TLCO	TLCO%pred
RM-4644-001	0.88	32.2	2.1	58.2	3.28	40.4
RM-4644-002	1.25	65.4	1.91	81.4	4.36	63.7
RM-4644-003	1.1.8	71.7	1.79	82.3	-	-
RM-4644-004	0.96	27.0	2.88	62.6	4.53	44.6
RM-4644-005	1.46	61	2.69	95.0	4.40	57.0

Table 3.6: Baseline pulmonary function tests for each subject. RM-4644-003 was unable to perform the technique necessary to obtain TLCO values. FEV_1 = forced expiratory volume in 1-second; FEV_1%pred = FEV_1 as percentage of predicted normal; FVC = forced vital capacity; FVC%pred = FVC as percentage of predicted normal; TLCO = transfer factor of lung for carbon monoxide; TLCO%pre = TLCO as percentage of predicted normal

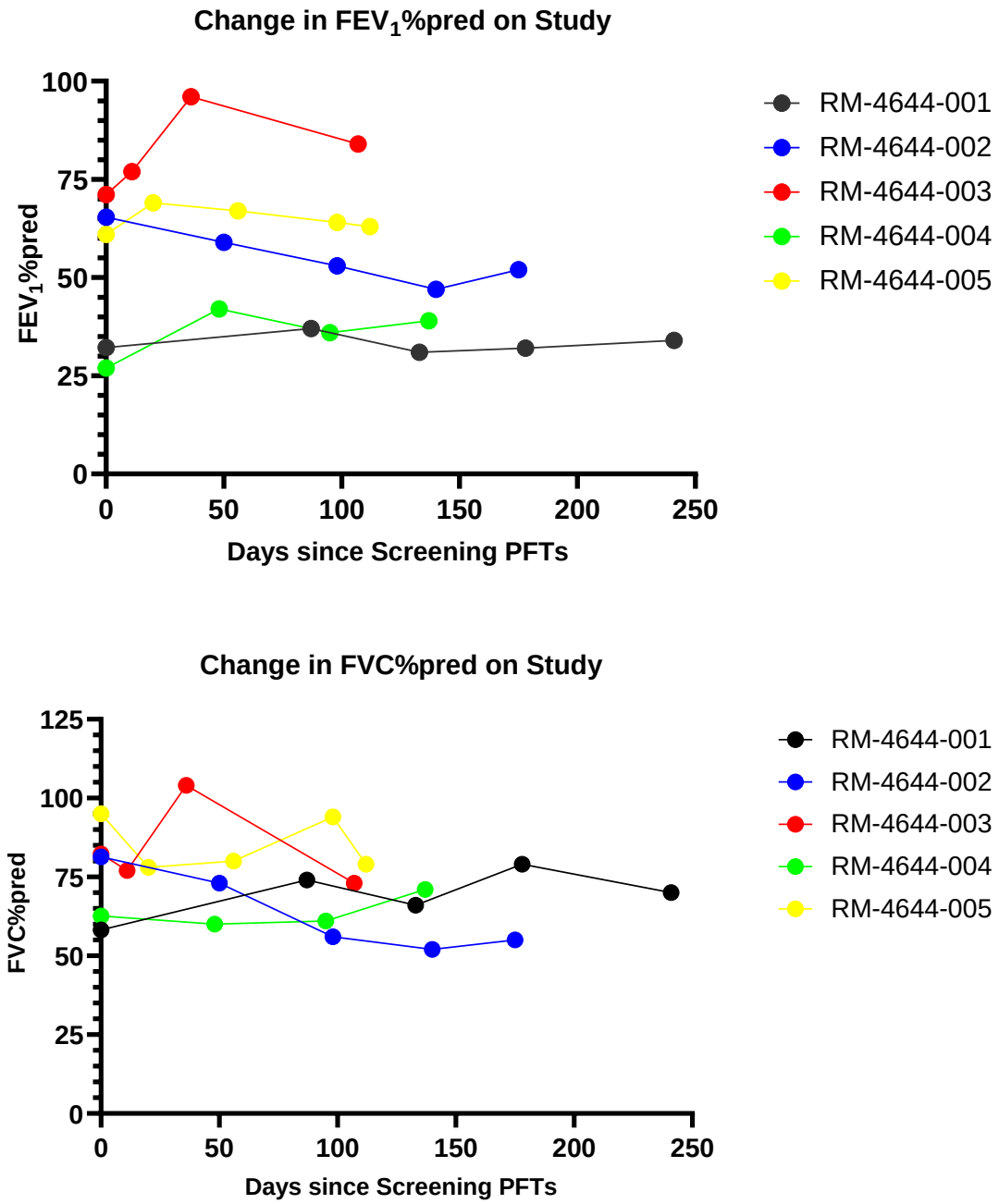

Figure 3.6: Scatter plots for each subject showing FEV_1%pred (top) and FVC%pred (bottom) against time since screening pulmonary function tests (PFTs). FEV_1%pred = FEV_1 as percentage of predicted normal; FVC%pred = FVC as percentage of predicted normal

3.6 Discussion

3.6.1 STILE Trial

The early results from the STILE trial are encouraging that the toxicity profile of combining the anti-PD-1 drug, nivolumab with SBRT in early stage NSCLC will be acceptable. If indicated, a subsequent phase III, placebo-controlled study would aim to establish the effect on relapse rates and overall survival of combining nivolumab with SBRT.

3.6.2 Recruitment

The STILE trial was initially able to recruit well with five subjects completing screening and entering the trial within the first three months after opening. The target recruitment is 31 subjects within 18 months and the initial recruitment rate was appropriate to meet this. Recruitment rate however has subsequently slowed with no subjects recruited in the following four months. All patients that were referred to RMH for SBRT for early stage NSCLC were screened as potential candidates for the STILE trial. From 75 patients who received SBRT there were only 8 patients that were deemed suitable for the trial of whom two declined a PIS and one failed screening due to having an ECOG performance status of 2. Poor performance status was the reason for ineligibility in nearly a third of cases. SBRT is commissioned within the NHS for patients with performance status ≤ 2. Therefore, all patients who were ineligible due to performance status would have been of performance status 2. Many patients with performance status 0-1 will be offered and will opt for surgical resection of early stage NSCLC, which remains the gold standard therapy. At the time of trial design, checkpoint inhibitors including nivolumab were only licenced for NSCLC in metastatic disease and for patients with performance status 0-1. For this reason, the research committee and ethics board insisted that the first patients enrolled onto the STILE trial should be of ECOG performance status 0-1. As per the protocol, the IDMC could escalate recruitment to include patients with ECOG performance status 2 if they were satisfied with the initial safety data from the first 5 patients. The IDMC were satisfied with the emerging safety data and the trial has now expanded to include patients with ECOG performance status 2, which should increase the rate of recruitment. Patients with a

minimum performance status of 2 may make up as much as 50% of those that undergo SBRT for NSCLC.[13] It will therefore be important for adjuvant nivolumab to be a safe treatment in all individuals that receive SBRT.

There is now emerging evidence that the use of checkpoint inhibitors in patients of performance status 2 is a safe and effective treatment for advanced NSCLC. The PePS2 study was a single arm phase 2 study of the PD-1 inhibitor pembrolizumab in patients with advanced NSCLC and performance status 2. This UK-based, multi-centre trial which evaluated 60 patients with performance status 2 found no increase in the rate of immune-mediated toxicities compared to the published data of patients of performance status 0-1.[16] Durable clinical benefit (DCB), as defined by complete response, partial response or stable disease at 18 weeks was 38% (95% CI: 21 -57) in first line patients and 36% (95% CI: 22-52) in subsequent line patients. The proportion of patients with a DCB increased with increasing proportion of PD-L1 expression on tumour biopsies. Treatment related toxicity leading to drug delay or discontinuation was seen in 28% (95% CI: 19-41%) of patients. From this study, pembrolizumab in patients with performance status 2 and advanced NSCLC appears to be a tolerable treatment and as efficacious as those with performance status (0-1).

The Checkmate 171 study was a phase 2 study of nivolumab in previously treated advanced squamous NSCLC. In this trial of 811 treated patients, 103 were ECOG performance status 2 at baseline.[9] There was no significant difference in the rates of toxicity in patients with PS 2 compared to those with PS 0-1. However, there was a difference in outcomes with median overall survival in PS 2 of 5.2 months, compared to 10.0 months in the overall trial population.

Two of the five subjects in STILE were not suitable for surgery, directly due to poor lung function. A further patient had COPD which contributed to the decision to not offer her surgery. One patient had had two previous lung resections for early stage NSCLC and therefore SBRT was deemed to be the most appropriate lung sparing treatment option. Performance status and poor lung function are two of the key reasons for

patients not to be offered lobectomy and regional lymph node resection in early stage NSCLC. It is important to improve the outcomes for these patients undergoing SBRT.

3.6.3 Drug Discontinuation

This trial aims to deliver nivolumab every two weeks for a maximum of 1 year following SBRT. The optimal duration of adjuvant treatment with checkpoint inhibitors such as nivolumab is not established. A duration of 1 year was chosen to align with other adjuvant studies including the ANVIL study of nivolumab following surgical resection in stage IB – IIIA NSCLC. The phase III PACIFIC trial has established the use of adjuvant immunotherapy, with the success of the PD-L1 inhibitor durvalumab, following chemo-radiotherapy for stage III NSCLC. In this trial of 713 patients with locally advanced NSCLC, progression-free survival was significantly longer with durvalumab every 2 weeks for up to 1 year than placebo and is now standard of care.[3] Overall survival has subsequently also been shown to be longer with a 3 year survival rate in the durvalumab arm of 57% versus 43.5% with placebo.[11] The median number of treatments received in the durvalumab arm was 20 (range 1 to 27) and 42.7% of patients completed 12 months of durvalumab treatment. Adverse events were the cause of discontinuation in 15.4% patients in the durvalumab arm and 9.7% in the placebo arm.[3] However, there is no published data on outcomes based on shorter treatment regimens and it remains plausible that such protocols would be as effective. The potential synergistic benefits of combining radiotherapy with immunotherapy are likely to be most effective when given concomitantly or in the short period after completing radiotherapy and the benefits of prolonged treatment may be only in controlling metastatic disease.

From the first five STILE subjects, two withdrew from further nivolumab treatments predominantly due to treatment-related toxicities. Immune-mediated toxicities are inherent with the use of checkpoint inhibitors and can frequently be managed with short courses of steroids. With adjuvant treatments however, the threshold for discontinuing treatment either by the patient or the treating physician will be lower. One patient, RM-4644-003 withdrew following multiple low-grade toxicities after the

first cycle, and a clear immune-mediated toxicity was not established. She improved with supportive measures only, without steroids, making the diagnosis of immune related toxicity highly unlikely.

RM-4644-001 withdrew because of the symptoms associated with nivolumab induced hypoadrenalism. Immune-related adverse events (ir-AE) can occur at any timepoint and in some cases may be as long as 1-year after completion of treatment. This is particularly true for pulmonary or hepatic toxicities.[14] In the pooled analysis from second line treatment of nivolumab in advanced NSCLC the median onset of immune related adverse events was between 4.9 and 30.3 weeks.[4] Endocrine abnormalities are one of the more common ir-AEs seen with immune check point inhibitors, with an incidence of 12%.[21] The median time of onset for endocrine toxicities with nivolumab in NSCLC is 12.2 weeks (range 1.9 - 88.1).[18] Primary adrenal insufficiency with nivolumab monotherapy is however a rare event (<1%).[6] In the case of RM-4644-001 there was no evidence of hypopituitarism as his thyroid function tests remained normal throughout. The random cortisol was low and there was evidence of increased vasopressin secretion with a typical pattern of paired osmolalities. The plasma osmolality was low at 261 mOsm/kg with a raised urine osmolality of 402 mOsm/kg; urine sodium was raised at 74 mmol/L.

3.6.4 Other Immune-Mediated Toxicities

Thyroid hormone abnormalities are a common immune-mediated toxicity from checkpoint inhibitors. Subject RM-4644-005 showed typical changes in thyroid function tests (TFTs) with an initial decrease in thyroid stimulating hormone (TSH) suggesting thyroiditis and a subsequent increase in TSH with evidence of low free T4 thyroxine, in keeping with hypothyroidism. A scoping review of case reports incorporating 79 cases of hypothyroidism related to checkpoint inhibitors found 50 (63%) developed hypothyroidism preceded by transient hyperthyroidism.[20] A systemic review and meta-analysis of published trials found the rate of hypothyroidism with immune checkpoint inhibitors to be 6.6% (95% CI: 5.5% - 7.8%).[5] The mean time from initiation of checkpoint inhibitors to onset of hypothyroidism with preceding

hyperthyroidism is 7.2 +/- 3.4 weeks.[20] RM-4644-005 had a low TSH after 36 days and developed biochemical evidence of hypothyroidism after 64 days (9 weeks and 1 day). We now know that autoimmune hypothyroidism with checkpoint inhibitors is frequently irreversible.[10]

Dermatological toxicities are the most prevalent immune-related AE associated with PD-1 inhibitors and include a wide range of clinical presentations. A pruritic maculopapular rash is the most common reaction with nivolumab with an incidence of 14.3% (95% CI 8.7-22.7).[19] Acneiform rashes which represent a papulopustular folliculitis are well described with anti-PD-1 agents, mainly affecting the torso as was seen with subject RM-4644-005.

3.6.5 Pneumonitis and PFTs

The results from the initial five patients are encouraging that a preclusive rate of symptomatic pneumonitis will not be seen by combining nivolumab and SBRT. The one episode of grade 2 pneumonitis observed, was complicated by co-existing infection at the time. The radiological appearances from the CT scan performed at this time were more suggestive of infection, although pneumonitis could not be excluded. Due to radiation pneumonitis occurring up to 6 months following treatment, my data will need further time to mature before I can be confident that radiation pneumonitis is not exacerbated by adjuvant nivolumab. The experience from the advanced disease setting is that immune-mediated pneumonitis from nivolumab can occur at any time point following initiation of treatment and it is expected that more subjects will experience this during the full length of the trial.

Symptomatic pneumonitis is a rare complication of SBRT monotherapy in early stage NSCLC. One large multi-centre study found the rate of grade 2 pneumonitis following SBRT to be 7%.[12] Immune-mediated pneumonitis as a complication from PD-1/PD-L1 inhibitors has a rate of 3-5%.[23] The incidence of pneumonitis is not equal across tumour types. In a meta-analysis of PD-1 inhibitors, the rate of pneumonitis was

amongst the highest in patients with NSCLC (all-grade 4.37% [95% CI: 2.97–6.39%]; grade 3-5 1.72% [95% CI: 0.09–3.28%]). In comparison, the rate of all grade and grade 3-5 pneumonitis in patients with melanoma was 1.44% (95% CI: 0.97–2.14%) and 0.90% (95% CI: 0.53–1.53%), respectively.[22] One explanation may be that pneumonitis appears to be more prevalent in patients with underlying COPD or having undergone previous thoracic radiation.[7] This is supported by the increased rate of pneumonitis seen in the durvalumab arm of the PACIFIC trial, which reported all grade pneumonitis rate of 33.9%, compared to 24.8% in those receiving placebo after radical chemo-radiotherapy in stage III NSCLC.[3]

A meta-analysis has compared the rates of pneumonitis for patients treated with nivolumab or pembrolizumab, check-point inhibitors that each target the PD-1 receptor.[22] The rate of all grade treatment-related pneumonitis with nivolumab was 3.20% (95% CI: 2.32–4.39%) compared to the pembrolizumab-related pneumonitis rate of 2.41% (95% CI: 1.19–4.83%).[22] There was no significant difference between the rates of pneumonitis using nivolumab or pembrolizumab, for either all grade (RR= 1.33, [95% CI: 0.62–2.88], $p = 0.15$) or grade 3-5 (RR= 1.07, [95% CI: 0.62–1.85], $p = 0.78$).[22] There is evidence that rates of pneumonitis are lower with the anti CTLA-4 agent, ipilimumab compared to PD-1 inhibitors although with associated higher rates of immune-mediated colitis and hypophysitis.[15] In a systematic review across tumour types, anti-CTLA-4 agents had significantly lower rates of pneumonitis compared to drugs acting via PD-1/PD-L1inhibiton (OR 0.03, 95% CI, 0.01–0.15, $p < 0.001$).[15] However, there is a significantly increased risk of ir-AEs when CTLA-4 inhibitors are used in combination with PD-1 inhibitors. The rate of pneumonitis in this setting may be as high as 10% and occurs earlier after initiation of treatment.[17]

Pneumonitis typically leads to a restrictive pattern on spirometry with reduced TLCO and lung volumes. In this trial spirometry is performed every 6 weeks while receiving nivolumab and then at all follow up visits. Full lung function testing with transfer factor calculation is performed at baseline and at 6, 12- and 24-months following completion of SBRT. Analysis of lung function test trends may facilitate early detection of a developing pneumonitis and will provide further insight into the impact of combining

nivolumab and SBRT. Notably, RM-4644-004, who had a baseline FEV_1%pred of only 27% tolerated the treatment well with no reported pulmonary toxicities. The only subject that showed significant decline in lung function values during the study, at the time of the first IDMC, was RM-4644-002 (

). The spirometry trends showed a progressive restrictive defect with FEV_1 and FVC declining at similar rates. This subject did not describe any respiratory symptoms and there was no radiological evidence of pneumonitis on the 3-month interval CT scan. The steepest decline was seen between the baseline and first on-study measurements. Baseline measurements were taken as part of full lung function testing which would include the use of nose clips, which may facilitate larger lung capacity measurements. However, this would not explain the ongoing decline on-study for this subject and this phenomenon was not seen in the other subjects. Spirometry is in part effort dependent and RM-4644-002 did complain of progressive fatigue during the study. The decline in spirometry may represent a correlation with declining patient frailty.

3.6.6 STILE in context of other trials

In this competitive and fast-moving field, similar trials are ongoing combining SBRT with checkpoint inhibitors in early stage NSCLC. Recently presented data from a phase I dose-escalation study of combining SBRT with the PD-L1 inhibitor atezolizumab, found no episodes of grade 3 pneumonitis amongst 12 evaluable subjects.[8] A subsequent phase III study is currently open to recruitment, comparing 8 cycles of atezolizumab and SBRT commencing on day 1 of cycle 3 (clinicaltrials.gov ref: NCT04214262) with SBRT alone. Similarly, a phase 1 study of the PD-1 inhibitor, avelumab, given concurrently and adjuvantly with SBRT for 6 cycles is open to recruitment (clinicaltrials.gov ref: NCT03050554).

MK-3475-867 is a phase III, randomised, placebo-controlled trial of pembrolizumab given concurrently and adjuvantly with SBRT for a maximum of 17 cycles (1-year duration). There is currently no consensus on the optimal sequencing of SBRT and checkpoint inhibitor combination regimens. A rationale for neo-adjuvant use of PD-

1/PD-L1 inhibitors prior to SBRT, is their ability to reinvigorate exhausted cytotoxic T-cells, which may be responsible for SBRT induced anti-tumour immunity.[2] However, the potential for immune-mediated toxicity, especially in the form of pneumonitis, runs the risk of having to abandon radical treatment in those treated with neo-adjuvant checkpoint inhibitors. Retrospective data from brain metastases in melanoma found no difference in efficacy, whether stereotactic radiosurgery was delivered prior to, during or after nivolumab administration.[1] While head-to-head trials comparing the sequencing of combination treatment are yet to be performed, it remains possible that there will be no significant impact on efficacy whether PD-1/PD-L1 inhibitors are administered prior to or shortly after SBRT.

3.6.7 Conclusions

The early data from this trial of nivolumab following SBRT for early stage NSCLC are encouraging that it will be a safe and tolerable treatment. After three months follow up of five patients there have been no episodes of grade 3 or above pneumonitis. The toxicity profile is within expected limits for patients receiving either SBRT or nivolumab in an advanced disease setting. The optimal treatment duration for adjuvant immunotherapy is not well established and we should not be discouraged by two patients withdrawing from the study drug due to toxicities. This however is only the early data and definitive statements regarding the efficacy and safety of adjuvant nivolumab following SBRT cannot be made at this stage.

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Chapter 4: **Part 2B - Immunogenomics of Patients with NSCLC and Impact on Response to Combination Radiotherapy and Immunotherapy**

4.1 Introduction

In order to fully harness the power of the immune system to treat cancers it is necessary to understand the tumour microenvironment, and specifically its immune cell component. The presence of tumour infiltrating lymphocytes (TILs) is known to be associated with a favourable response to immunotherapies across a range of tumour types including NSCLC.[28] The tumour microenvironment is infiltrated with varying phenotypes of T cells including naive, regulatory, cytotoxic and co-stimulatory. The varying abundance of these immune cells may help to predict response to immune-modulating therapies.[4]

Translational research in NSCLC is limited by the relative difficulty of obtaining large volume tissue samples and the lack of ease in serial sampling. Biopsy of the primary lung tumour, either via bronchoscopy or radiological guided biopsy frequently only provide small samples and are invasive tests which may not be accepted by patients for repeated sampling. Endobronchial ultrasound (EBUS) guided fine-needle aspiration (FNA) of metastatic lymph glands is a valuable diagnostic and staging investigation in NSCLC that can provide sufficient cytological material for molecular analyses and PD-L1 immunohistochemistry (IHC), but does not provide large volumes of archival tissue. A large proportion of biopsy sample is consumed during the necessary diagnostic immunohistochemistry and molecular tests. Therefore, it is necessary for researchers investigating the tumour microenvironment of NSCLC to work with small, often archival samples which have been stored in formalin-fixed paraffin-embedded (FFPE) blocks leading to more RNA degradation than with fresh, frozen samples.

RNA-sequencing (RNA-Seq) is an immunogenomic technique which uses next generation sequencing (NGS) to identify and quantify the presence of RNA in a

biological sample.[21] The development of this technique has overcome many of the technical issues of microarrays that required large volumes of high-quality RNA.[17] For a more focussed analysis, that does not require sequencing of the whole transcriptome, the NanoString nCounter technology is an effective alternative.

4.2 NanoString Technology

The NanoString Technologies nCounter platform is a relatively new technique that allows quantification of messenger RNA (mRNA), DNA and proteins from a single sample. This technique is particularly useful when working with small and archival tissue biopsies. NanoString provides a direct measurement of mRNA, without the need for amplification. When working with relatively degraded mRNA, the short length of the NanoString detector probes leads to improved detection of low abundance mRNA compared to other gene expression profiling methods such as quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

NanoString technology is an automated technique that allows the quantification of up to 800 gene targets in a single assay. Each gene target is identified by a 100 base pair region of interest. The technology involves using two adjacent 50 base pair probes that are complementary to the 100 base pair regions of interest from the sample RNA, which are then bound in a solution-based hybridisation reaction. One probe, known as the capture probe, is covalently linked to a biotin moiety which is subsequently used to immobilise the hybridised complex. The second 50 base unit is bonded to a uniquely colour coded molecular barcode and is known as the reporter probe. A hybridisation reaction is performed in the presence of excess probes to drive the reaction to completion. Up to 800 targets can be processed in a single reaction with each gene target having a unique colour coded sequence on the reporter probe. The complexes are then purified before being bound, immobilised and aligned on the imaging surface of the cell cartridge. A digital analyser that incorporates an automated fluorescence microscope scans the cartridge and counts the number of barcodes hybridised to each gene target. A schematic of this process is shown in Figure 4.1.

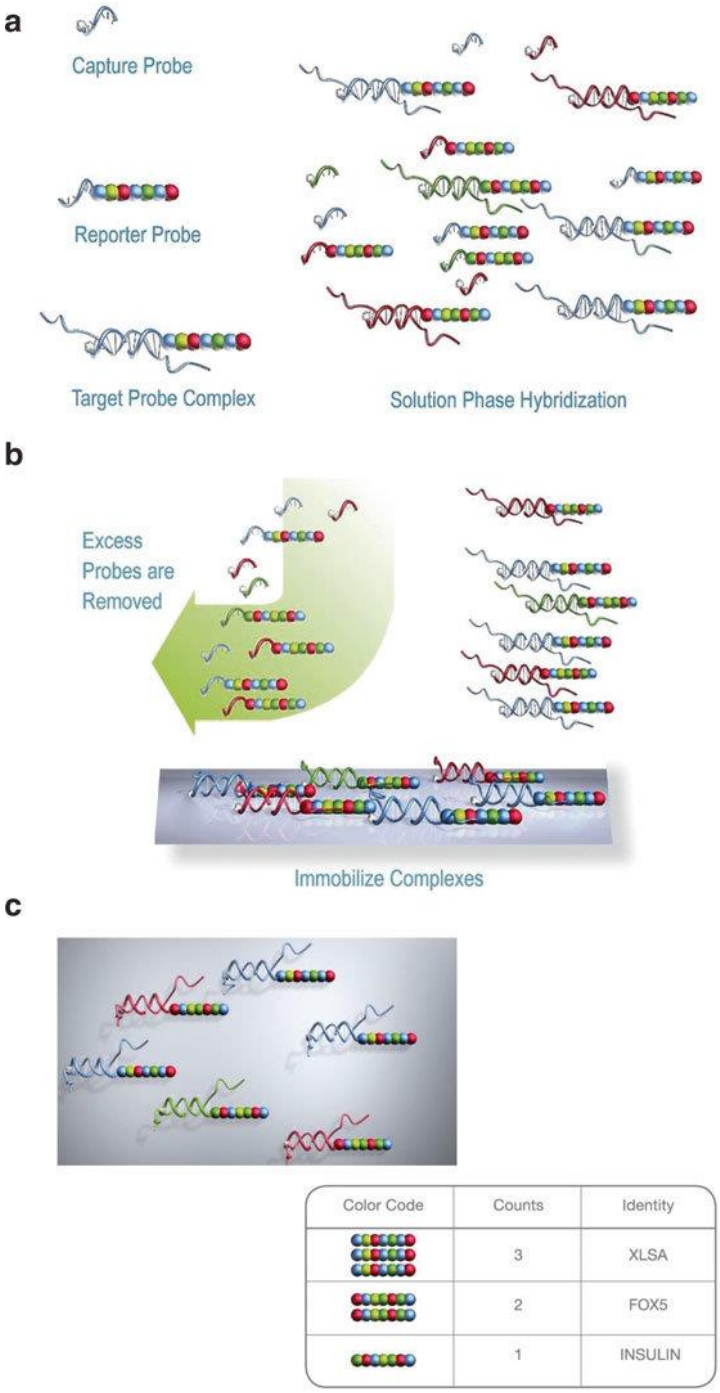


Figure 4.1: Schematic overview of the NanoString technology. (a) Targets of interest are hybridized with two juxtaposed probes: a biotinylated capture probe and uniquely fluorescently labelled reporter probe for each target. A target-probe complex is formed during hybridization with an RNA sample. (b) After removal of excess probes, the target-probe complexes are immobilized and aligned on the surface of a flow cell. These steps are automated on a liquid handler. (c) Images of nonoverlapping fields on the flow cell are taken. The unique sequences of the reporter probes are counted and translated into the number of counts per target.[18]

4.3 Trial Design

In this study, I performed a NanoString analysis of RNA extracted from NSCLC biopsy samples taken prior to treatment, to explore the immunogenomics of the tumour microenvironment. For this translational work, I was granted access to the diagnostic biopsy samples from patients recruited to the PEAR study, a phase 1 immunotherapy and radiotherapy combination trial running at the Royal Marsden Hospital. All patients recruited to this study had given consent for their excess diagnostic biopsy samples to be used for relevant translational research. Further samples were analysed from patients that had been recruited to the Lung MC study, a sister study to the PEAR trial to facilitate translational research. A brief synopsis of the PEAR trial and Lung MC protocols are described below.

PEAR Trial

The PEAR trial was a phase 1b open-label randomised trial to assess the safety and tolerability of the anti-PD-1 agent, pembrolizumab when given in combination with palliative radiotherapy to the lung for advanced NSCLC. All lines of systemic treatment were eligible, but patients were required to be naive to immune checkpoint inhibitors. This was a dose escalation study of pembrolizumab, at a starting dose of 100mg every 2 weeks prior to standard of care palliative radiotherapy. Radiotherapy was delivered either as 20 Gy in 5 fractions (low dose arm) or 36 Gy in 12 fractions (high dose arm) as per clinical need. On completion of the palliative radiotherapy all patients continued to receive pembrolizumab 200 mg every 3 weeks until disease progression or unacceptable toxicity. The trial was not enriched for PD-L1 status. In cohort 2, patients received a starting dose of 200 mg every 2 weeks prior to radiotherapy.

Lung MC Study

This study was a translational study that ran alongside the PEAR study to facilitate collection of circulating tumour cells (CTCs). Patients undergoing palliative radiotherapy or receiving pembrolizumab as standard of care were enrolled in this study to provide control translational samples of both sequential blood samples and use of any unexhausted biopsy samples. All patients in the Lung MC study had a diagnosis of NSCLC, were 18 years or older and had provided valid consent.

4.4 Aims

- Assess the ability to extract RNA and perform NanoString immunogenomic profiling from archival FFPE NSCLC biopsy samples
- Explore the immunogenomic profiles of baseline tumour biopsy samples of patients undergoing combination radiotherapy and immunotherapy and assess associations with response to treatment

4.5 Hypothesis

- The immune profile of the tumour microenvironment can predict response to combination radiotherapy and immunotherapy for advanced NSCLC

4.6 Methods

4.6.1 Sample Selection

All diagnostic biopsies from patients recruited to the PEAR study were reviewed. Tissue was not available for all patients as there were samples that had been exhausted from a combination of diagnostic IHC, PD-L1 quantification or other translational study work. One patient had two tissue samples, a lung resection specimen and a resected brain metastasis.

For all patients with a tissue block, a standard haematoxylin and eosin (H&E) slide was prepared. The slide was reviewed with support from a senior histopathologist to identify the presence of tumour cells and to delineate areas for removal by macrodissection, including necrotic tissue or normal lung parenchyma. After this process patients from the PEAR study provided 10 suitable samples. As the NanoString panel can incorporate 12 samples in one run, two control samples from the Lung MC study were included to avoid wasting of reagents.

4.6.2 Macrodissection

For each block, 2-30 slides (or until exhausted) of 8 µm thickness were cut, deparaffinised and stained with nuclear fast red. More slides were used in blocks with fewer microscopically visualised tumour cells present. These steps were performed by the histopathology technicians from the Breast Cancer Now (BCN) laboratory within the Institute for Cancer Research.

Each slide was then macrodissected by applying 5-10 µl of Buffer PKD (provided with Qiagen RNeasy® kit). Using the H&E slide as a template, the areas of interest on each slide were collected using a size 18-gauge needle and placed in a 1.5 ml collection tube with 240 µl of Buffer PKD. All slides for the same patient were combined in a single collection tube. The collection tubes were then mixed by vortexing.

4.6.3 RNA Collection

The Qiagen RNeasy® FFPE kit was used to collect and purify RNA from each sample. All buffers and RNase-Free DNase I were reconstituted as described in the provided Qiagen RNeasy® handbook. Each sample underwent RNA collection using the following protocol, which was adapted from the provided handbook

- Centrifuged for 1 minute at 10,000 rpm
- 10 µl proteinase K was added by pipette to the lower, clear phase. This was then mixed by pipetting up and down
- Incubated at 56 ^{0}C for 15 minutes

- Samples were removed to be kept at room temperature while the heat block was increased to 80 ^{0}C
- Incubated for 15 minutes at 80 ^{0}C
- The lower, uncoloured phase was transferred into a new 2 ml microcentrifuge tube
- Incubated on ice for 3 minutes
- Centrifuged for 15 minutes at 13,500 rpm
- Supernatant was transferred to a new microcentrifuge tube, taking care not to disturb the pellet
- 25 µl DNase Booster Buffer and 10 µl DNase I stock solution was added and mixed by inverting the tube
- Brief centrifugation to collect residual liquid from the sides of the tube
- Incubated at room temperature for 15 minutes
- 500 µl Buffer RBC was added to adjust the binding conditions and mixed thoroughly by pipetting up and down
- 1200 µl 100% ethanol was added and mixed well by pipetting
- 700 µl of the sample, including any precipitate that had formed was transferred to a RNeasy MinElute spin column in a 2 ml collection tube
- Centrifuged at 10,000 rpm for 15 seconds
- Follow through discarded
- The previous three steps were repeated until the entire sample had passed through the same RNeasy MinElute spin column
- 500 µl Buffer RPE was added to the RNeasy MinElute spin column
- Centrifuged at 10,000 rpm for 15 seconds
- Follow through discarded
- 500 µl Buffer RPE was added to the RNeasy MinElute spin column
- Centrifuged at 10,000 rpm for 2 minutes
- RNeasy MinElute spin column was carefully removed, taking care not to contact the follow through and placed in a new 2 ml collection tube
- Centrifuged at 13,500 rpm for 5 minutes
- Collection tube and follow through discarded
- RNeasy MinElute spin column was placed in a new 1.5 ml collection tube
- 20 µl RNase-free water was applied directly to the spin column membrane
- Incubated at room temperature for 30 minutes

- Centrifuged at 13,500 rpm for 1 minute to elute the RNA
- Follow-through pipetted and reapplied to the spin column membrane
- Centrifuged at 13,500 rpm for 1 minute to elute the RNA
- RNeasy MinElute spin column was discarded, and the eluted RNA was stored in a freezer at -80 ^{0}C until required

4.6.4 RNA Quantification

Each sample had the RNA quantified using the Qubit® RNA high sensitivity assay kit with the standard methodology. This technique uses fluorescence measurement following the binding of RNA to target-sensitive dyes. A Qubit® working solution was made by diluting the provided Qubit® reagent 1:200 in Qubit® buffer. Two standard samples were made by diluting 10 µl of each of the provided RNA standards with 190 µl of Qubit® working solution. For each of the experiment RNA samples, 1 µl of sample was added to 199 µl of Qubit® working solution. After vortexing, the tubes were incubated for 2 minutes at room temperature. The two standard samples were then inserted into the fluorometer for calibration before each experimental sample was inserted and the RNA quantified.

4.6.5 RNA Bioanalysis

The quality of the RNA collected was assessed using the Agilent 2100 Bioanalyzer. The purpose of the bioanalysis was to assess the level of degradation of the RNA. The information from the bioanalysis when combined with the RNA concentration was used to guide the volume of solution containing optimal amount of RNA to input into the NanoString analyser. The Agilent 2100 Bioanalyzer uses electrophoresis to separate the RNA samples before reading the chip via laser induced fluorescence detection. The electropherogram provides a visual representation of the quality of the data. Intact RNA will provide two peaks on the electropherogram corresponding to 18S and 28S ribosomal bands. As RNA degrades the ratio of 18S to 28S will reduce and there will be an increase in the baseline signal between the two ribosomal peaks (see Figure 4.2). The Agilent 2100 Bioanalyzer software provides an algorithmic interpretation of the data, which removes individual interpretation of the RNA quality. The RNA integrity

number (RIN) classifies the total RNA with a numbering system between 1 and 10, with 1 being the most degraded and 10 being the most intact.[19] An alternative measure of the degree of degradation can be made by quantifying the percentage of RNA made up of fragments between 50 and 300 base pairs.

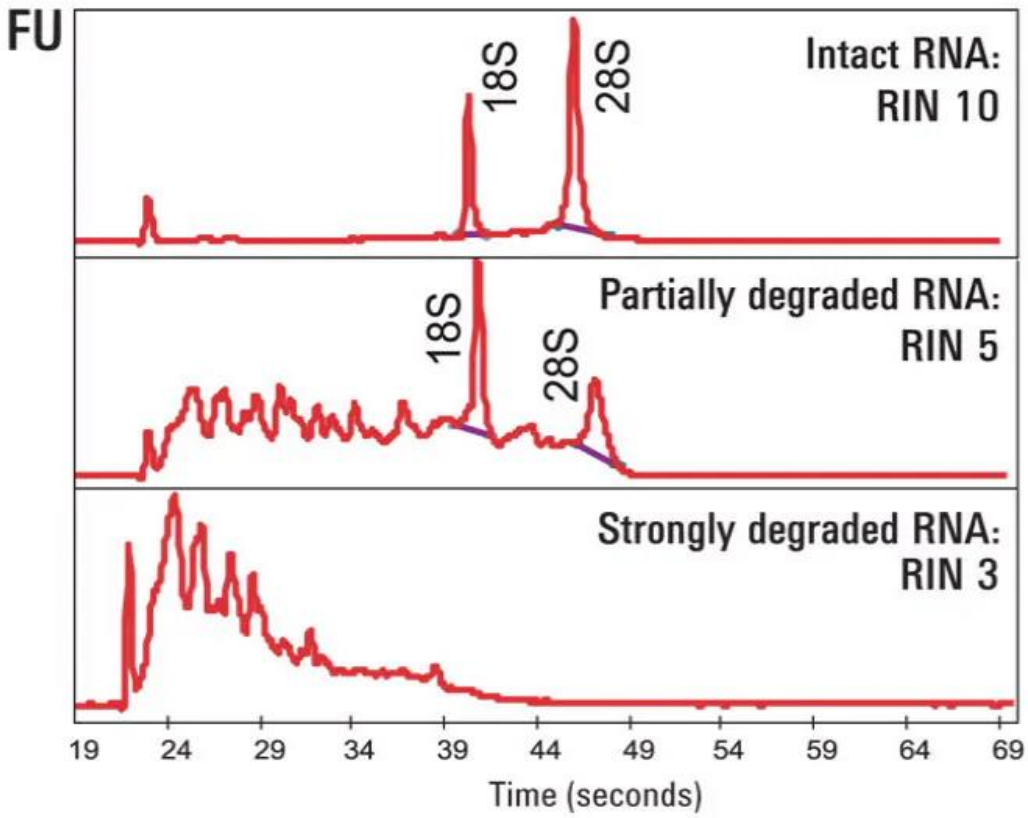


Figure 4.2: Impact of RNA degradation on the electropherogram from the Agilent 2100 Bioanalyzer. RIN range from 1 – 10 with 1 being the most degraded and 10 being the most intact[19] The small peak to the left in the top panel shows the marker peak

The bioanalysis was performed as per the provided Agilent RNA 6000 Pico protocol. The abbreviated steps are outlined below:

1. Prepare a gel-dye matrix
    - Add 1 µl of RNA 6000 Pico dye concentrate to 65 µl of filtered gel matrix
    - Vortex to mix
    - Centrifuge at 14,000 rpm for 10 minutes at room temperature
2. Load the gel-dye mix to RNA Chip (see Figure 4.3)
    - Pipette 9 µl of gel-dye matrix directly to the bottom of the loading well (marked G in a black circle) in the bottom right corner of the RNA Pico chip
    - Load RNA Pico chip in the chip priming station and plunge for exactly 30 seconds before releasing the plunger
    - Pipette 9 µl of gel-dye matrix directly to the bottom of each of the two wells marked with a G
3. Loading the RNA 6000 Pico Conditioning Solution and Marker
    - Pipette 9 µl of the RNA 6000 Pico conditioning solution into the well marked CS
    - Pipette 5 µl of the RNA 6000 Pico marker into the well marked with a ladder and each of the 11 sample wells
4. Loading the diluted ladder and samples
    - Pipette 1 µl of the diluted RNA 6000 Pico ladder into the well marked with the ladder symbol
    - Pipette 1 µl of each sample into each of the 11 sample wells
    - Place the chip horizontally in the adapter of the IKA vortex mixer
    - Vortex at 2000 rpm for 60 seconds
5. Load the chip in the Agilent 2100 Bioanalyzer and start the chip run

Figure 4.3: RNA Pico Chip used for the Agilent 2100 Bioanalyzer[10]

4.6.6 NanoString Analysis

The NanoString assay run was performed by the BCN NanoString Facility. The specific quantity of RNA solution for each sample was aliquoted into labelled, RNase-free tubes and provided to the BCN NanoString Facility. The NanoString analysis was run using NanoString's PanCancer immune panel, which includes 770 genes, plus an additional 30 gene spike. The gene spike included additional genes of interest that are not included in the standard panel and was provided with thanks by Dr Anna Wilkins. The additional genes included in the spike are listed in appendix A.

4.6.7 Statistical Analysis

Raw data from the NanoString readout was interpreted with the use of the nSolver Analysis 4.0 software. Immune cell subsets analysis was interpreted through the nSolver Advanced Analysis software. As a component of the advanced software, a Benjamini-Hochberg procedure was performed to correct for the multiple comparisons and reduce the false discovery rate (FDR).

4.7 Results

4.7.1 RNA Collection, Quantification and Bioanalysis

RNA was extracted from 12 biopsy samples of which 10 were samples from the PEAR study and 2 samples from Lung MC. The two patients in the lung MC study received pembrolizumab without combination radiotherapy. A description of the samples that were amenable to RNA extraction, including baseline characteristics and the patients' best treatment responses are provided in Table 4.1.

The analysis included a total of 11 patients of which one patient, 4282-18, had two biopsy samples tested, one from a surgical lung resection and the other from a resected brain metastasis. Three biopsy samples were of squamous cell subtype and nine were adenocarcinoma. At the time of diagnosis, two of the patients included were current smokers, five were ex-smokers and four were never smokers. From the nine patients included from the PEAR study, three were from the low dose radiotherapy arm and six received high dose palliative radiotherapy. Two patients were included from cohort 2 of the PEAR study which had a starting pembrolizumab dose of 200 mg.

Sample	Study	Smoking Status	Biopsy Type	Histology	PD-L1 TPS	Prior Treatment	Radiotherapy	Pembrolizumab Starting dose	Best Response
4282-01	PEAR 01	Current	Lung Bronch Bx	Adeno	<1%	Carbo-Pem	36 Gy in 12#	100 mg	Partial Response
4282-03	PEAR 03	Ex-Smoker	Lung Surgical	Adeno	>50%	Carbo-Pem	36 Gy in 12#	100 mg	Progressive Disease
4282-05	PEAR 05	Never	Lung CT Bx	Adeno	>50%	Carbo-Pem	36 Gy in 12#	100 mg	Partial Response
4282-06	PEAR 06	Ex-Smoker	Lung CT Bx	Adeno	1-49%	Cis-Pem Docetaxel	20 Gy in 5#	100 mg	Progressive Disease
4282-07	PEAR 07	Ex-Smoker	Lung Bronch Bx	Squam	>50%	Carbo-Vin	20 Gy in 5#	100 mg	Progressive Disease
4282-11	PEAR 11	Ex-Smoker	Lung Bronch Bx	Squam	>50%	Carbo-Vin	36 Gy in 12#	100 mg	Progressive Disease
4282-13	PEAR 13	Never	Lung CT Bx	Adeno	1-49%	Cis-Pem Pem Maint	36 Gy in 12#	100 mg	Progressive Disease
4282-16	PEAR 16	Current	Lung Bronch Bx	Squam	1-49%	Carbo-Vin	20 Gy in 5#	200 mg	Partial Response
4282-18A	PEAR 18A	Ex-Smoker	Lung Surgical	Adeno	N/A	Cis-Pem Pem Maint	36 Gy in 12#	200 mg	Partial Response
4282-18B	PEAR 18B	See PEAR 18A	Brain Surgical	Adeno	<1%	See PEAR 18A	See PEAR 18A	See PEAR 18A	See PEAR 18A
4506-016	LUNG MC 16	Never	Lung CT Bx	Adeno	70%	Cis-Pem Pem Maint	-	200 mg	Progressive Disease
4506-084	LUNG MC 84	Never	Lung CT Bx	Adeno	3-5%	Carbo-Pem	-	200 mg	Stable Disease

Table 4.1: Descriptions of biopsy samples used for RNA extraction including smoking status at time of diagnosis, biopsy type, histology, prior treatments for advanced NSCLC, palliative radiotherapy dose, starting dose of pembrolizumab and best response to treatment. Bx = biopsy, Adeno = adenocarcinoma, Squam = squamous cell carcinoma, Cis = Cisplatin, Carbo = Carboplatin, Pem = Pemetrexed, Vin = Vinorelbine, Maint = maintenance therapy, Gy = Gray, # = fraction

The mRNA quantification for each sample via Qubit fluoroscopy is shown in Table 4.2. The Qubit concentration is calculated from the concentration of the reference standards and adjusted for the dilution in the Qubit buffer to produce the sample concentration. The degree of fragmentation was calculated with the bioanalyzer, the outputs of which are displayed in Figure 4.4 and Figure 4.5. The RIN ranged from 1.1 to 2.3 representing highly degraded RNA. The highly degraded RNA has a large peak to the left of the electropherogram and the distinct ribosomal peaks are not seen. In three cases, the degraded RNA obliterated the marker peak causing the analyser to misalign the marker and therefore lead to inaccurate quantification of the degradation of the sample. For these samples, a quantification of the percentage of samples between 50 and 300 base pairs could not be accurately measured. Despite the degraded nature of the RNA, the samples were reviewed by the experienced team within the BCN NanoString Facility and it was agreed that the samples should be of high enough quality to run on the NanoString panel. Therefore, an estimate of the degree of degradation was made by using the mean values from the other samples. This assumption was also used for sample 4506-084 which did not undergo bioanalysis (11 wells for bioanalysis cartridge and 12 slots in NanoString assay).

The target quantity of mRNA to use in a NanoString analysis from a fresh biopsy sample would be 80 ng. A more fragmented and degraded RNA, as seen from FFPE samples, will require a proportionally greater total amount of RNA to be inputted into the NanoString analyser to provide a good signal. Hence, for a set concentration of RNA, more degraded samples will require a larger volume of solution to be used in the NanoString analyser. A minimum of 5 µl of solution is required to be inputted, however the maximum volume that can be used is 10 µl. The degree of fragmentation, calculated target mRNA quantity and volume of solution inputted into the NanoString analyser are shown in Table 4.2.

The concentration of RNA eluted from the surgical resected specimens was higher than for other specimens. The quantity of RNA inputted into the NanoString analyser is an attempt to achieve optimum conditions. If the quantity of RNA is too high, then the NanoString probes may be exhausted or overlap leading to image saturation. If

this occurs, then significant data may be lost. If the RNA quantity is too low, then low abundance genes may not be detected. When the data is normalised in the analysis package, a correction is made for the relative concentrations of RNA, which allows for between sample comparisons.

						NanoString Inputs		
Sample	**Number of 8 µm slides**	**Sample RNA Concentration (ng/µl)**	**Percentage of RNA between 50 -300 base pairs**	**Adjusted Target RNA (ng)**	**Required Volume of Sample Solution (µl)**	**RNA Aliquoted (µl)**	**Water Added (µl)**	**Total Volume (µl)**
Sample: 4282-01	25	9.6	70	266.7	27.8	10.0	0.0	10.0
Sample: 4282-03	4	69.2	64*	222.2	3.2	3.2	1.8	5.0
Sample: 4282-05	20	25.0	64	222.2	8.9	8.9	0.0	8.9
Sample: 4282-06	30	7.1	67	242.4	34.0	10.0	0.0	10.0
Sample: 4282-07	25	18.0	72	285.7	15.9	10.0	0.0	10.0
Sample: 4282-11	18	50.0	53	170.2	3.4	3.4	1.6	5.0
Sample: 4282-13	18	22.0	57	186.0	8.5	8.5	0.0	8.5
Sample: 4282-16	6	10.3	56	181.8	17.7	10.0	0.0	10.0
Sample: 4282-18A	2	164.0	64*	222.2	1.4	1.4	3.6	5.0
Sample: 4282-18B	2	110.0	64*	222.2	2.0	2.0	3.0	5.0
Sample: 4506-016	3	75.4	71	275.9	3.7	3.7	1.3	5.0
Sample: 4506-084	2	42.2	64*	222.2	5.3	5.3	0.0	5.3

Table 4.2: Quantification of RNA for each sample using Qubit fluorescence. The degree of fragmentation as described by percentage of RNA in the 50-300 base pair range from the Agilent 2100 Bioanalyzer. The adjusted target RNA and the subsequent volumes of RNA solution and water used in the NanoString analyser. * = estimate based on mean of recorded samples

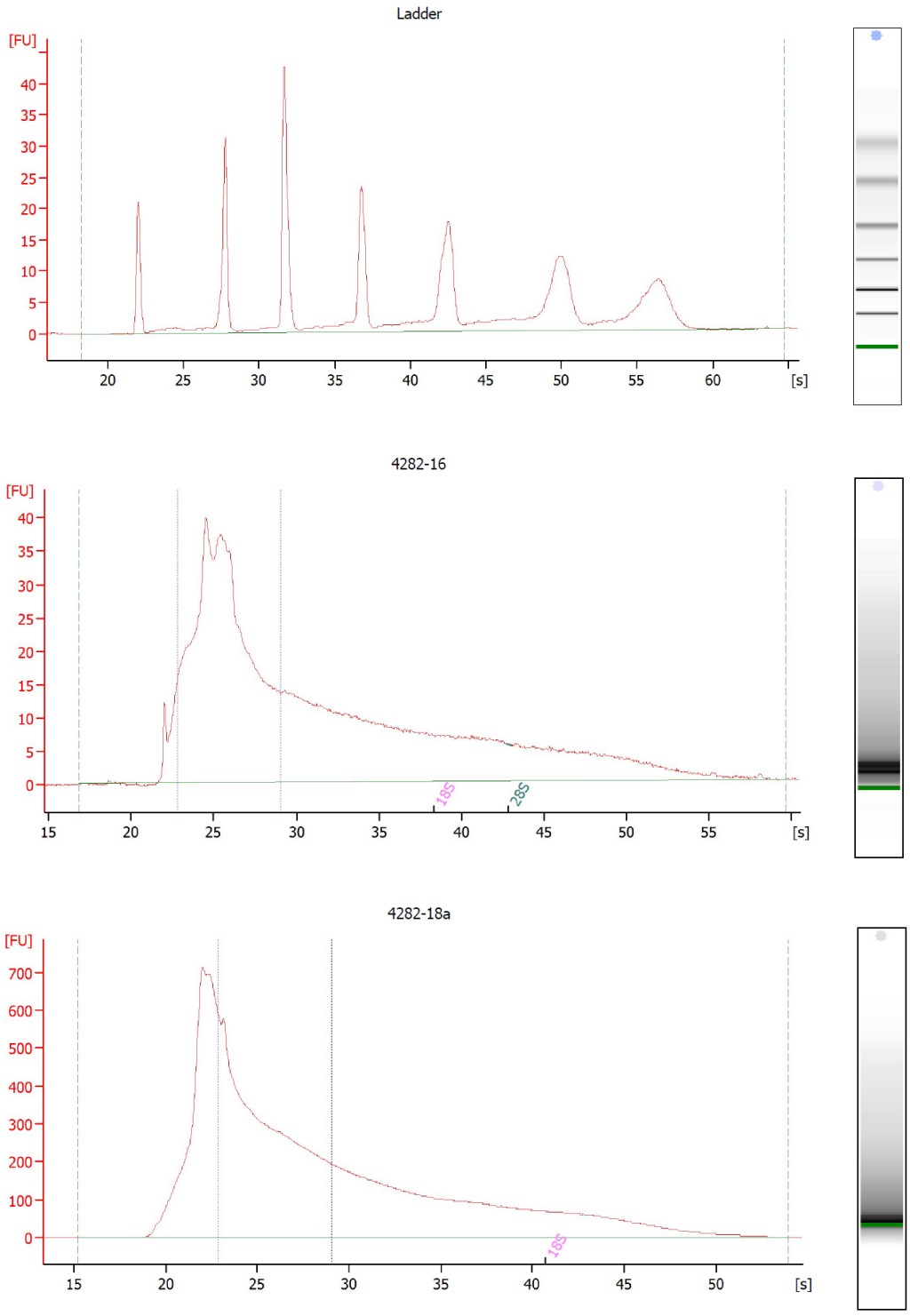


Figure 4.4: Bioanalysis results using electrophoresis in the Agilent 2100 Bioanalyzer. The top panel shows the standard ladder to establish times to peak concentration of standardised fragments of 25, 200, 500, 1000, 2000 and 4000 base pairs. The middle panel shows sample 4282-16 with an aligned marker peak. The bottom panel shows sample 4282-18A with the misaligned peak and therefore failure to establish degree of fragmentation.

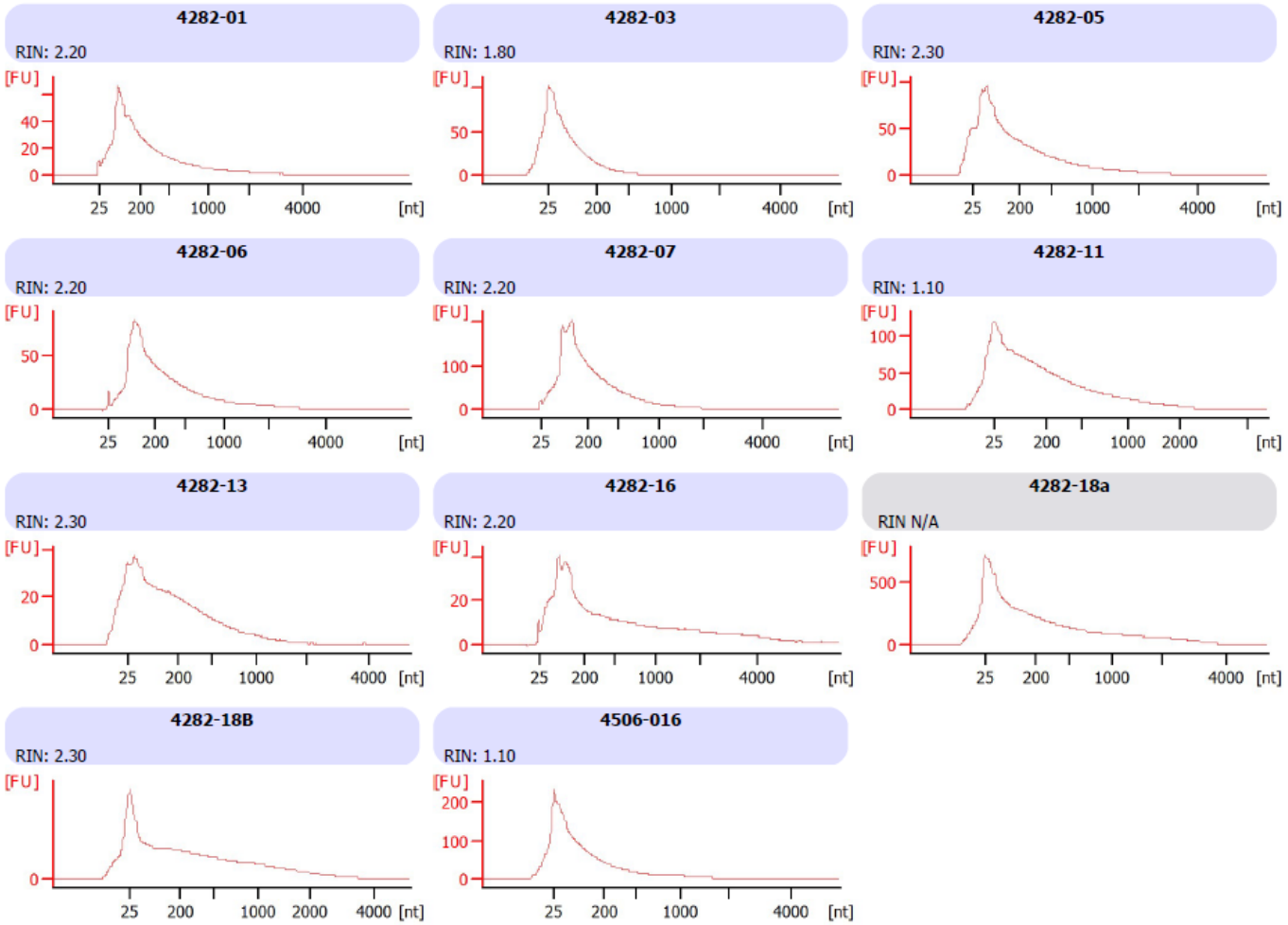


Figure 4.5: Bioanalysis results using electrophoresis in the Agilent 2100 Bioanalyzer for the 11 samples analysed. The RNA integrity number (RIN) is an objective measure of the integrity of the RNA provided by the software with higher RIN denoting less fragmented RNA

4.7.2 NanoString Analysis

All 12 samples were successfully run on the NanoString analysis assay and provided adequate quality data with few quality control (QC) fails. One QC metric is the binding density which describes the abundance of signal overall on a per sample basis. (Table 4.3).

Sample	Binding density
Sample: 4282-01	0.25
Sample: 4282-03	0.4
Sample: 4282-05	0.78
Sample: 4282-06	0.23
Sample: 4282-07	0.81
Sample: 4282-11	1.27
Sample: 4282-13	0.93
Sample: 4282-16	0.38
Sample: 4282-18A	2.08
Sample: 4282-18B	1.69
Sample: 4506-016	1.27
Sample: 4506-084	1

Table 4.3: NanoString binding density for each sample. Samples with lower binding densities will be less sensitive for detecting low abundance genes

There was a range of binding densities observed across the samples, as anticipated from the RNA quantification analysis. Four samples had less than the optimal amount of RNA used in the NanoString analysis after correction for degradation, due to 10 µL being the maximum volume of solution that can be used. Despite this, the binding density for the samples ranged from 0.25 to 2.08. The nSolver 4.0 software identifies a binding density of between 0.1 and 1.8 to be the ideal range to avoid overlapping of reporter probes. Sample 4282-18A was the only sample that had a binding density outside of this range. This was a surgical sample that had failed to identify the marker

peak on the bioanalyzer and hence an estimated fragmentation ratio, based on the other samples was used. Given the binding density results, it is likely that this sample was less degraded than the mean of the other samples. A visual review of the electropherograms for the surgically resected samples 4282-03, 4282-18A and 4282-18B, suggests that these samples were less degraded with a larger area under the curve after 30 seconds than the smaller biopsy samples. Samples with a binding density that falls outside of the ideal range should be reviewed with some caution as overlapping of probes may cause inaccuracies in the gene counts. However, in this descriptive analysis and with the binding density only just lying outside the ideal range it is appropriate to keep sample 4282-18A in the experimental analyses.

4.7.3 Heatmaps

An initial review of the data was performed by creation of heatmaps. This provides agglomerative clustering of both genes and samples. The software automatically clusters similar samples together to facilitate review of the data (See Figure 4.6). An explorative experiment was performed to investigate any potential differences between samples from patients that responded to immunotherapy +/- radiotherapy and those that did not. Samples were denoted as either responders (best response of partial response or stable disease) or non-responders (best response of progressive disease). Figure 4.7 describes the samples when aligned for response.

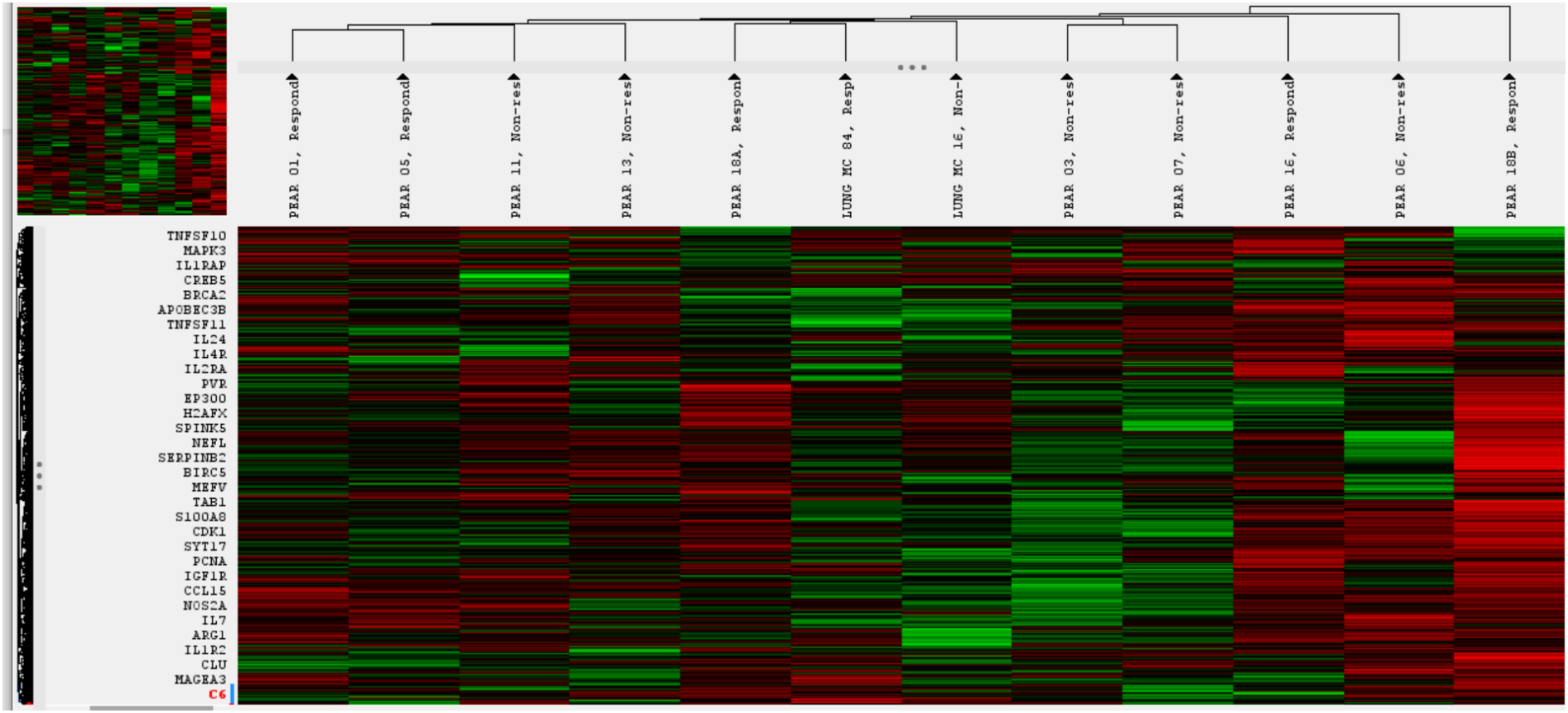


Figure 4.6: Heat map showing agglomerative clustering of all genes and samples. Genes with an increased expression are displayed in green and genes with a decreased expression are displayed in red. The intensity of the colour indicates the degree of differential expression

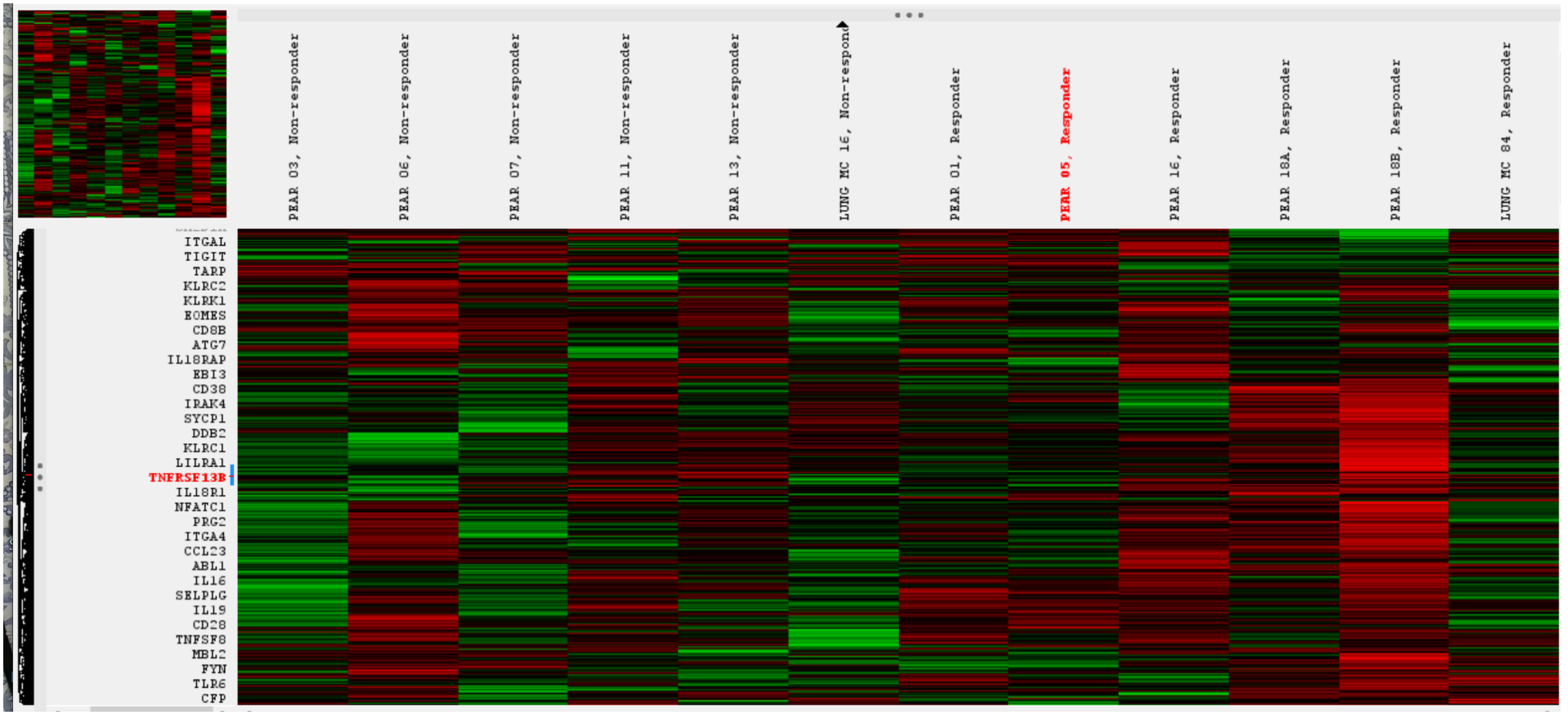


Figure 4.7: Heat map showing clustering of genes with the samples fixed for response to treatment. The six samples to the left are non-responders and the six samples to the right are responders. Genes with an increased expression are displayed in green and genes with a decreased expression are displayed in red. The intensity of the colour indicates the degree of differential expression

4.7.4 Experiment: PEAR Response

I tested the hypothesis that there was a difference in the baseline immunogenomic tumour microenvironment between responders and non-responders to the combination of radiotherapy and immunotherapy. For this experiment I excluded the samples from the Lung MC study as they did not receive combination therapy. After reviewing the heatmaps it was apparent that sample 4282-18B had a significantly different immunogenomic makeup compared to the other samples. This sample was a brain metastasis resection specimen, while all other samples were taken from the lung. This sample was also from the same patient as sample 4282-18A and therefore to avoid duplication in the experiment, 4282-18B was removed from the analysis. A comparison was made between responders (4 samples) and non-responders (5 samples) for the normalised mean gene counts and standard deviation for each gene. From these results, a Welch's t-test was performed which generated *p*-values for each gene. All genes with a *p*-value less than 0.05 are displayed in Table 4.4.

Using the nSolver advanced analysis software, a correction was made for multiple covariates using the Benjamini-Hochberg procedure. After this correction, there were no genes with significantly different counts (p-value < 0.05) between responders and non-responders. A volcano plot identifying the 40 most statistically significant genes is displayed in Figure 4.8.

Individual genes were compared for normalised abundance between responders and non-responders. Violin plots for the most significantly differentially expressed genes MICB and IRF8 along with the related gene IFNB are displayed in Figure 4.9. Violin plots for other genes of interest cGAS, STING and Trex1 and are displayed in Figure 4.10.

Cell type profiling was performed using the nSolver Advanced Analysis software. In this module the software measures the abundance of various cell populations based on marker genes that are stably expressed and specific to given cell types. These marker genes act as reference genes, as they are expressed only in the nominal cell

type. Cytotoxic cells and T cells showed significant correlation between marker genes and would be the most robust cell types from which to draw conclusions (Figure 4.11). Relative abundance of immune cells was compared between responders and non-responders. A summary of the differential expression of each cell type between responders and non-responders is shown in Figure 4.12.

Box and whiskers plots for the cell types with best correlation for marker genes (cytotoxic cells and T cells) is displayed in Figure 4.13. The relative abundance of Exhausted CD8+, TILs and Treg cells as a proportion of TILs is displayed in Figure 4.14.

Gene	Responder Mean	Responder SD	Non-responder Mean	Non-responder SD	Relative Ratio	p value	t score
TFE3	82.29	2.99	59.67	6.45	1.38	0.001	6.21
MICB	92.86	19.15	170.98	39.96	-1.84	0.005	-4.05
LILRA4	11.29	3.13	21.01	4.05	-1.86	0.008	-4.08
IL10	24.62	3.81	35.35	4.74	-1.44	0.009	-3.76
BMI1	275.17	38.03	198.68	27.6	1.39	0.009	3.61
SYK	181.75	29.19	371.36	165.67	-2.04	0.013	-3.63
IRF8	113.72	43.94	241.22	40.16	-2.12	0.015	-4.02
CD14	358.14	126.17	822.06	405.32	-2.3	0.016	-3.15
TNFRSF9	9.02	3.36	26.32	18.11	-2.92	0.019	-3.04
CARD11	96.7	34.49	208.42	94.39	-2.16	0.020	-3
CTSL	287.21	177.91	798.78	269.66	-2.78	0.020	-3.38
IL2RG	140.84	47.88	333.01	135.73	-2.36	0.021	-2.98
CCL17	69.53	31.5	31.11	11.91	2.24	0.023	3.09
ARG2	80.31	27.38	40.92	14.09	1.96	0.024	2.97
CD99	2401.33	337.27	3572.96	998.55	-1.49	0.030	-2.78
IL2RA	97.62	9.21	178.12	100.69	-1.82	0.032	-3.05
RIPK2	105.81	33.68	179.45	45.1	-1.7	0.033	-2.78
APOE	825.58	528.92	2838.13	2552.93	-3.44	0.034	-2.67
HAMP	12.95	4.29	32.66	23.56	-2.52	0.035	-2.71
IRF5	82.83	18.43	135.67	43.72	-1.64	0.038	-2.57
ICOSLG	80.32	18.16	155.97	80.66	-1.94	0.038	-2.66
CX3CR1	75.83	25.87	47.35	14.1	1.6	0.044	2.53
TNFRSF1B	172.74	90.34	376.92	141.17	-2.18	0.045	-2.62
BLNK	25.34	8.08	42.94	14.39	-1.69	0.047	-2.42
FUT7	11.88	3.98	20.93	7.07	-1.76	0.049	-2.43

Table 4.4: Normalised mean gene counts and standard deviations for responder and non-responder samples. Relative ratio based on response, t-score and unadjusted *p*-value based on Welch's t-test. *p*-values are not adjusted for multiple testing. Genes are listed in order of statistical significance; only genes with a *p* value less than 0.05 are included

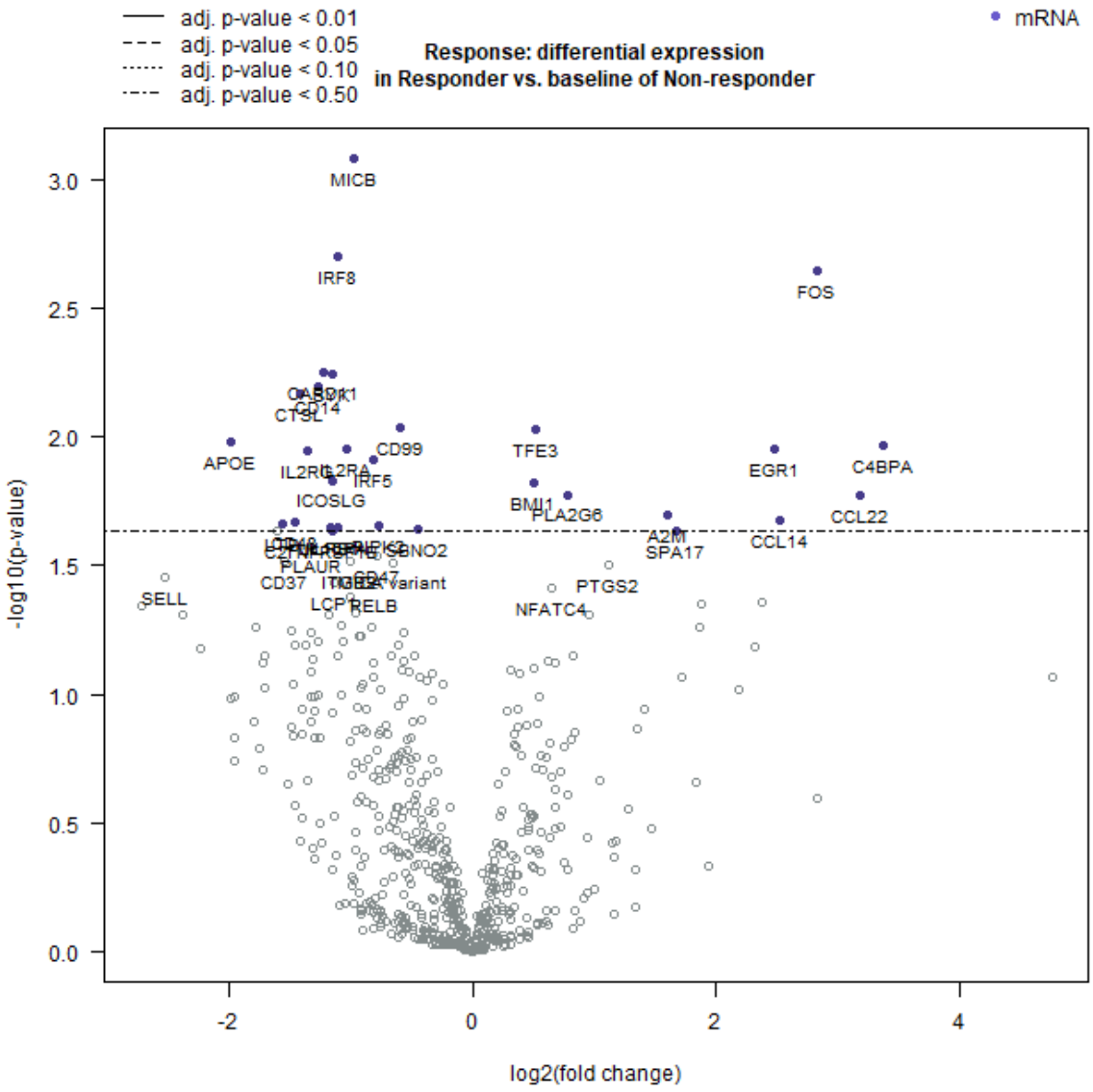
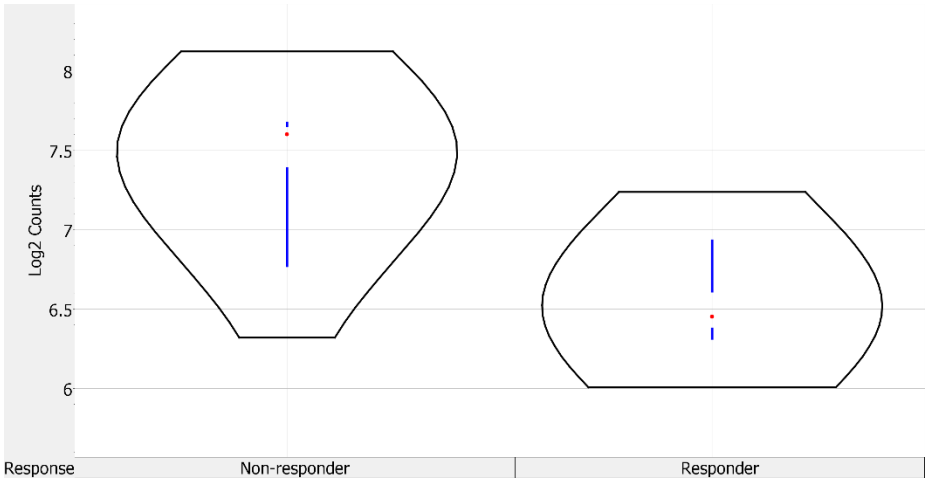
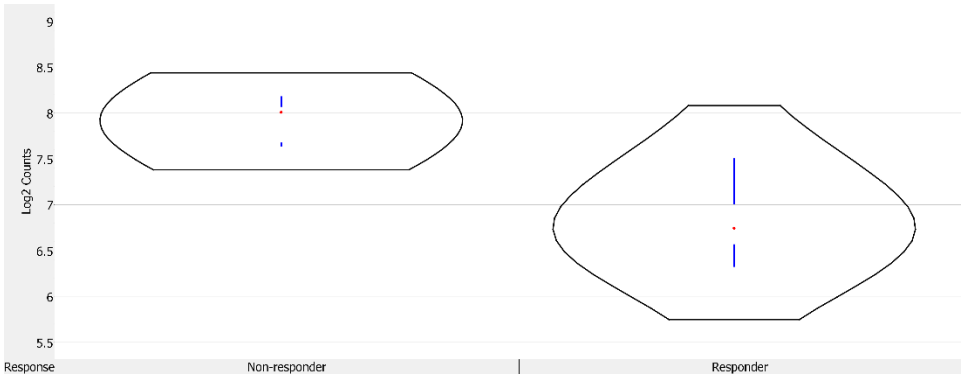


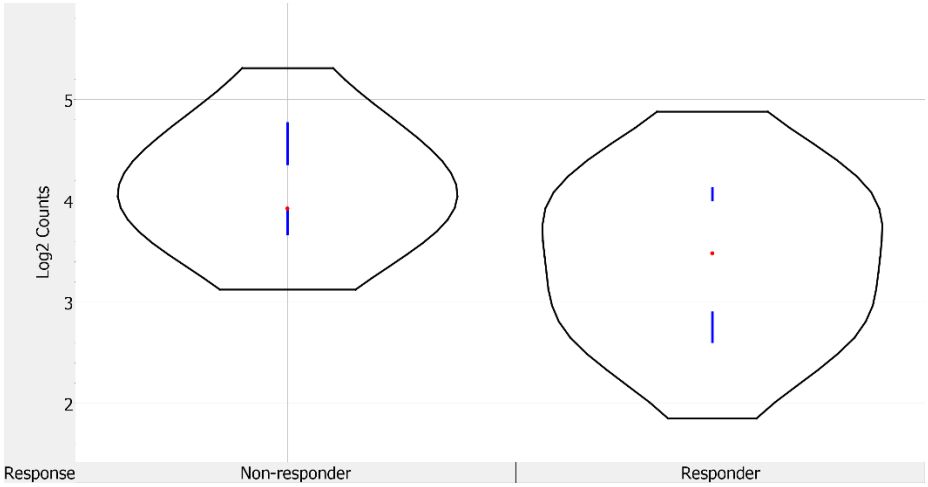
Figure 4.8: Volcano plot showing the difference in normalised mean gene count for responders compared to baseline of non-responders. Y axis displays each gene's -$\log_{10}$(p-value) after adjustment for multiple testing and x axis displays $\log_2$ fold change. More statistically significant genes fall at the top of the plot above the horizontal lines denoted in the key; highly differentially expressed genes fall to either side.



MICB



IRF8



IFNB

Figure 4.9: Violin plot of normalised gene abundance by response for genes MICB, IRF8 and IFNB. Median (red dot), blue lines represent first and third quartile with adjustment. The curve around the plot shows the rounded distribution of data

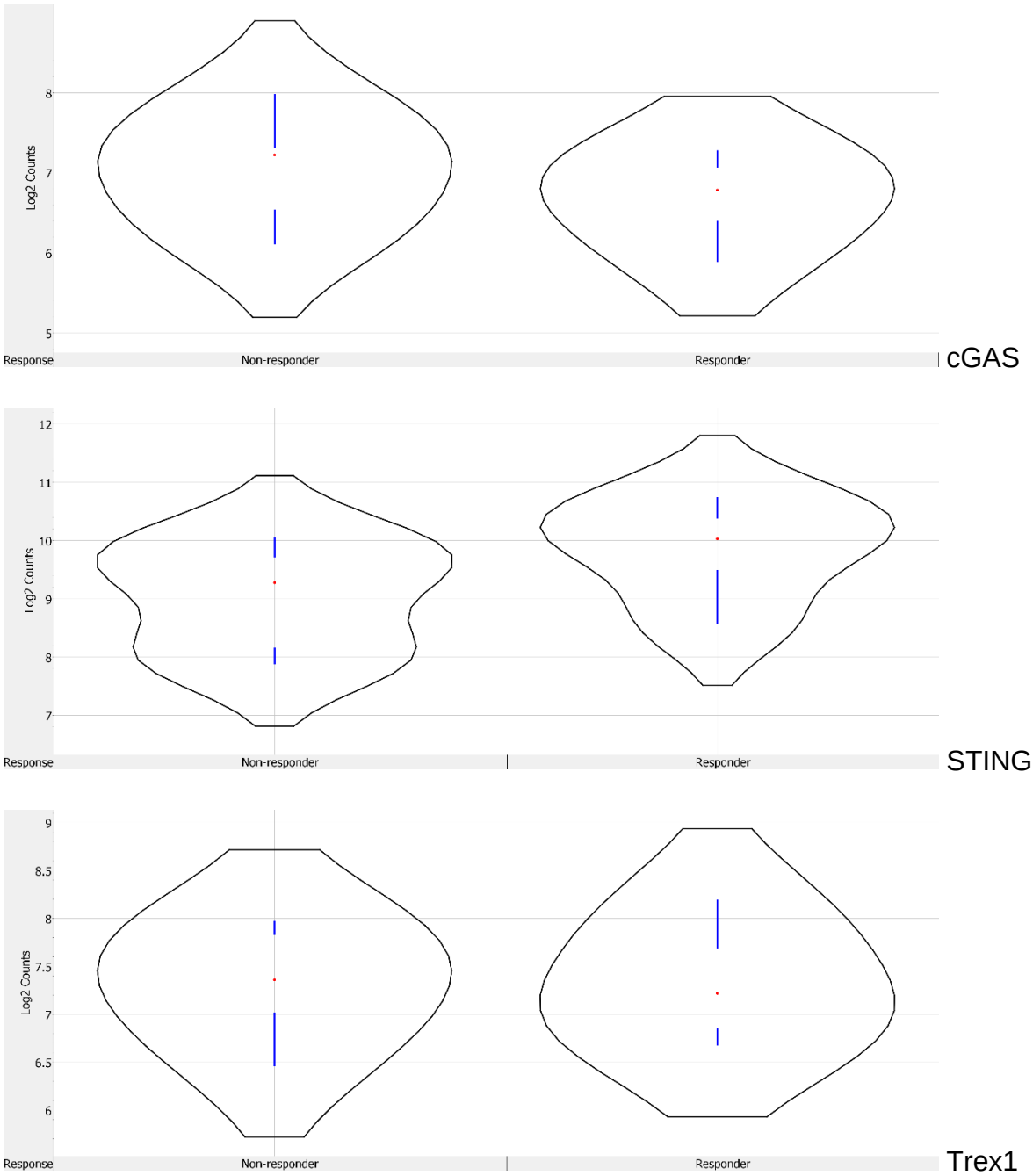


Figure 4.10: Violin plot of normalised gene abundance by response for three genes of interest: cGAS, STING and Trex1. Median (red dot), blue lines represent first and third quartile with adjustment. The curve around the plot shows the rounded distribution of data

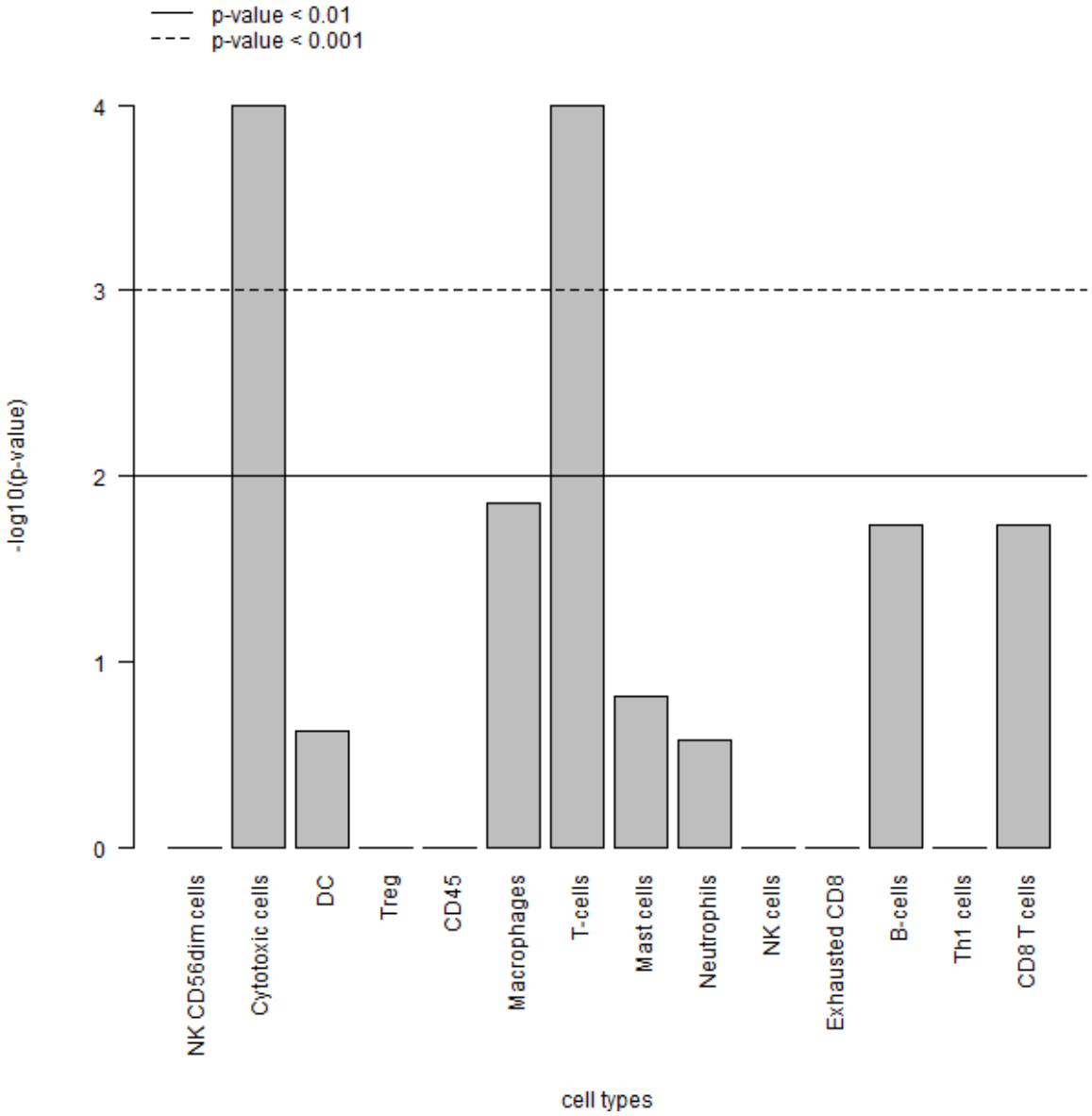


Figure 4.11: QC bar plot of -$\log_{10}$(*p*-values) for correlation of marker gene expression across cell types. Cell types with better correlation of marker genes (lower *p*-value) can be more robustly interpreted. Bars above the solid black line indicate statistically significant cell types at a *p*-value threshold of 0.01. Bars above the dashed black line indicate statistically significant cell types at a p-value threshold of 0.001

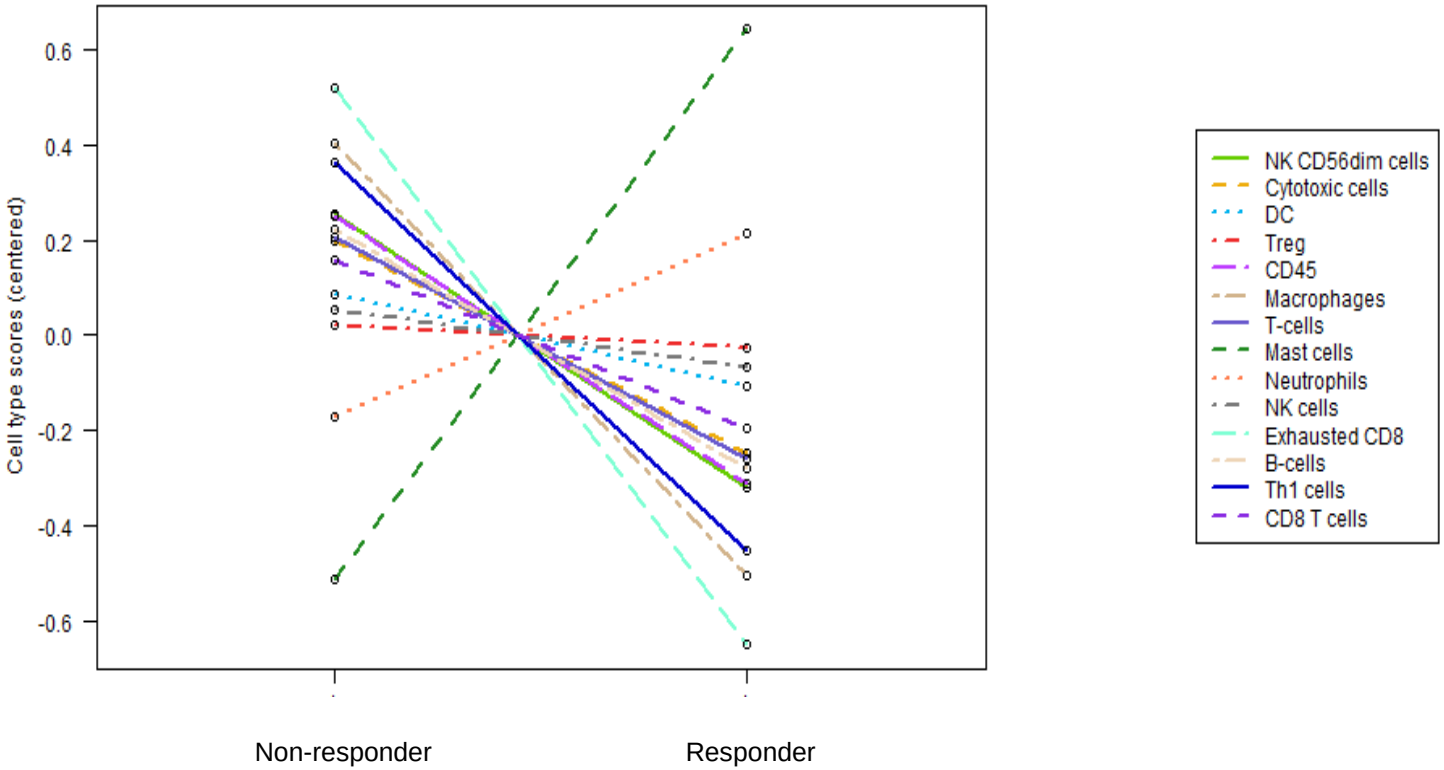


Figure 4.12: The relative abundance of immune cell subsets between non-responders (left) and responders (right). Each cell type's score has been centered to have mean 0. As abundance estimates (cell type scores) are calculated in $\log_2$ scale, an increase of 1 on the vertical axis corresponds to a doubling in abundance.

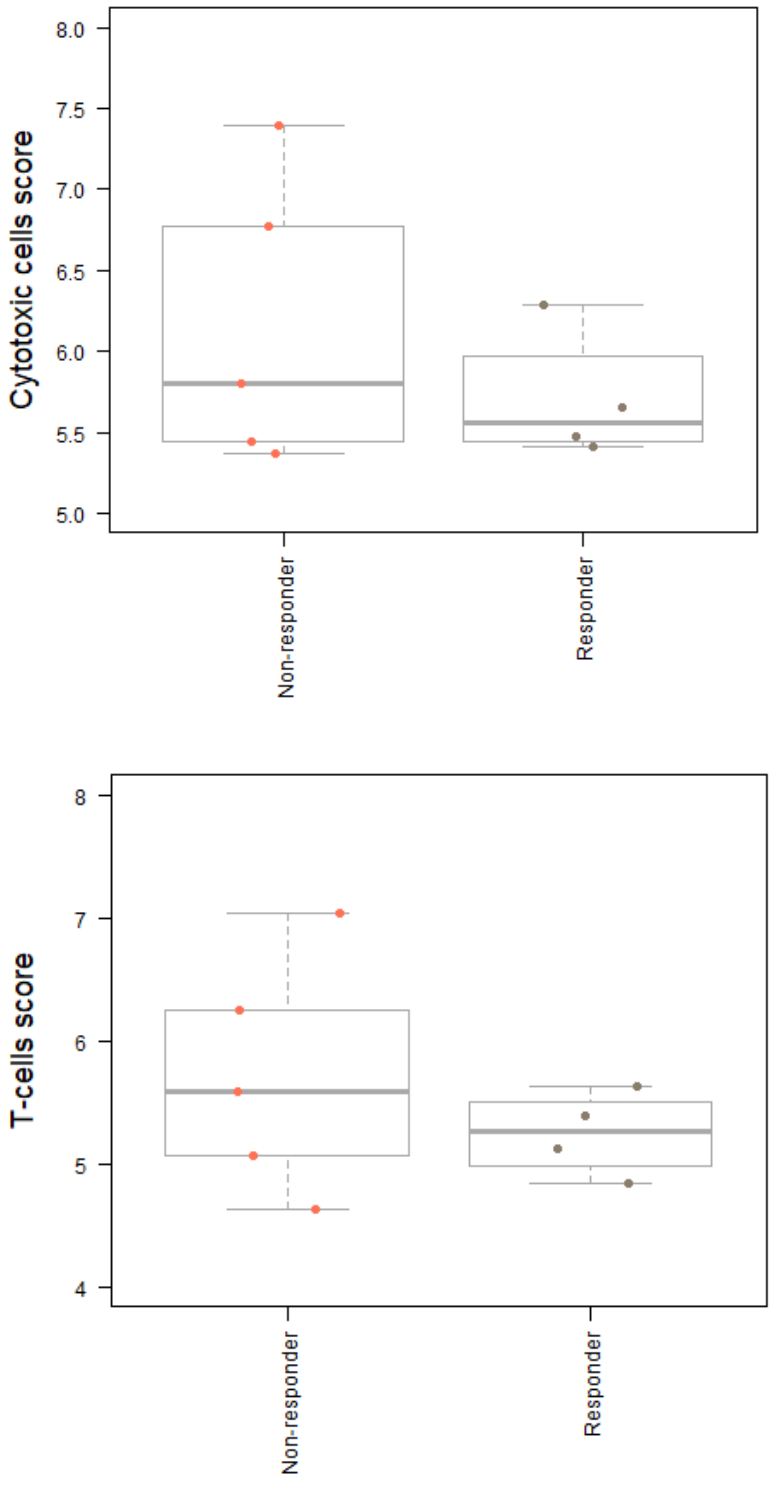
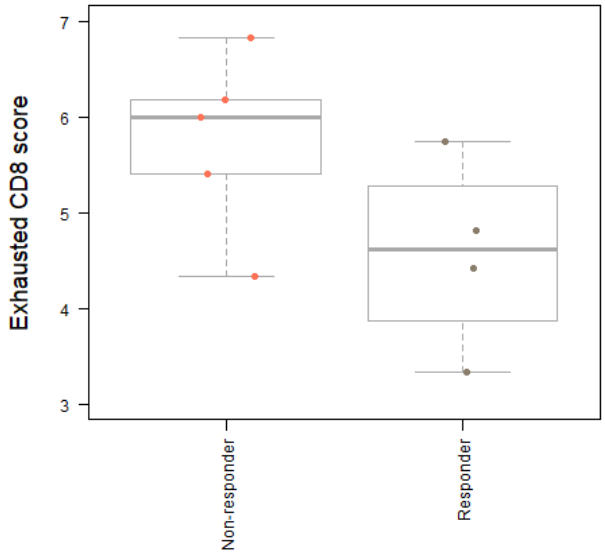
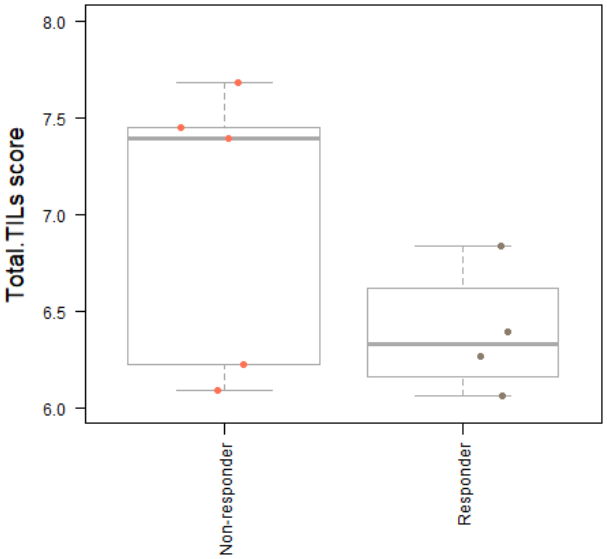


Figure 4.13: Box and whiskers plots for relative abundance of cytotoxic cells and T cells between non-responders and responders. As abundance estimates (cell type scores) are calculated in $\log_2$ scale, an increase of 1 on the vertical axis corresponds to a doubling in abundance





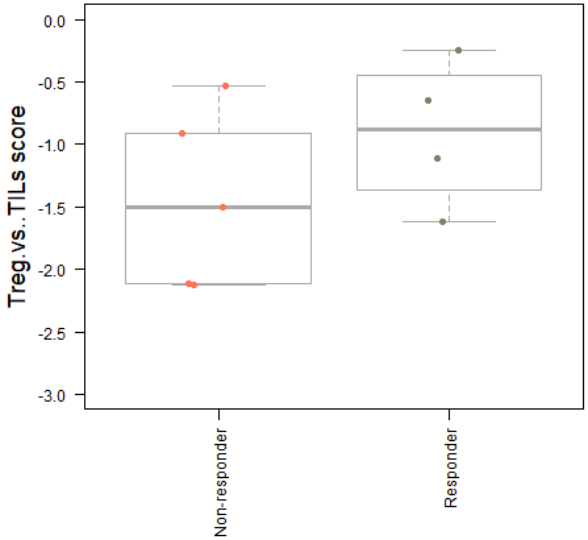


Figure 4.14: Box and whiskers plots for relative abundance of exhausted CD8+ cells, TILs and Tregs as a proportion of TILs between non-responders and responders. As abundance estimates (cell type scores) are calculated in $\log_2$ scale, an increase of 1 on the vertical axis corresponds to a doubling in abundance

4.8 Discussion

4.8.1 NanoString Technology and NSCLC Immunogenomics

In this study I have shown that NanoString analysis can be performed on archival heavily pre-used, lung biopsy samples. Although statistical significance was not shown in this small series, it appears that a more exhausted immune tumour microenvironment was seen in the biopsies from patients who failed to respond to the combination of radiotherapy and immunotherapy.

Despite the majority of these FFPE samples being from small biopsy samples, sufficient mRNA for NanoString analysis was extracted from every sample with histologically proven tumour cells. Using Qiagen RNeasy® methodology, it is feasible to elute DNA separately from RNA using the same extraction process. DNA extraction was not performed in this experiment due to concerns over the potential for reducing the RNA yield. The effective yields achieved in this experiment should encourage future studies to attempt both RNA and DNA extraction. The concentration of the RNA yield will be affected by the quality of the samples but also the number of slides macrodissected and combined in a single collection tube. As expected, surgically resected specimens had much higher concentration of RNA and fewer slides were used from these higher quality specimens. Specimens that required a high number of slides and especially those that were used to exhaustion may have included a higher proportion of surrounding stroma compared to tumour cells.

In these exploratory and descriptive experiments, samples were included from various histological subtypes and for patients undergoing different lines of therapy. The 12 samples analysed did not receive identical treatment, including both high dose and low dose palliative radiotherapy and two patients from the PEAR study being included from cohort 2, with a higher starting dose of pembrolizumab. Therefore, inferences from these experiments should be drawn with caution and used as signals to pursue further research.

The bioanalysis results were used to guide the quantity of RNA to use in the NanoString analysis. All samples, both surgically resected specimens and smaller biopsy samples were heavily degraded. FFPE processing and storage will lead to degradation of RNA which will reduce the confidence on inferences that can be made from these samples. However, high quality, intact RNA is less imperative when working with NanoString technology gene measurement than with microarrays or RNA-Seq.

Twelve samples were successfully run using the NanoString PanCancer immune panel which includes 770 genes covering 14 immune cell types along with common checkpoint inhibitors, CT antigens, and genes covering both the adaptive and innate immune response. An additional 30 gene spike was added which had previously been used to investigate potential pathways of abscopal effects in prostate cancer models treated with radiotherapy and immunotherapy.

4.8.2 Heatmaps

The heatmap of the 12 samples demonstrated the wide variety in the expression of immune genes in an RNA analysis of NSCLC biopsies that include the tumour microenvironment. While the automatic clustering performed by the nSolver software did not perfectly group the samples by response (Figure 4.4), when the samples were aligned by response (Figure 4.5) it appears that the samples from the patients that went on to not respond to either pembrolizumab and radiotherapy (PEAR samples) or Pembrolizumab alone (Lung MC samples), had generally higher counts of immune function related genes. This may not be what we would expect from previous studies of patients treated with single agent immunotherapies, which are preferentially more effective in patients with a pre-existing T cell mediated anti-tumour response.[11] The hallmark of a previous immune response is the presence of CD8+ T cells in the tumour microenvironment. Tumours that activate signalling pathways to avoid immune destruction may have a lower immune cell infiltration at baseline. In melanomas for example, β-catenin expression inhibits dendritic cell and T cell infiltration of the tumour by preventing expression of CCL4.[23]

Sample 4282-18B, a brain metastasis resection sample and the only extra thoracic tumour sample included, had a notably more inert immune makeup. This sample also had a PD-L1 TPS of <1%. Lower counts of PD-L1^{+} TILs in brain metastasis biopsies compared to the corresponding lung primary has previously been described.[13] The presence of the blood brain barrier means that immune responses to brain metastases may be dampened, despite activated T cells being able to infiltrate brain metastases.[22] However, durable responses with checkpoint inhibitors in patients with brain metastases from NSCLC are now well recognised.[8]

4.8.3 Single Gene Analysis

When comparing the normalised, single gene counts between responders and non-responders there was a statistical difference in 25 genes (Table 4.4). Due to the large number of tested genes, a Benjamini-Hochberg procedure was performed to reduce the false discovery rate. After this correction, there were no significant differences in gene counts between responders and non-responders below an adjusted *p*-value of 0.05 (Figure 4.8). This may not be surprising from the small number of samples tested, with relatively large standard deviations. However, it is notable that several of the genes with the most significant differential counts were genes that have previously been described with relevance to the interaction between radiotherapy and the immune system. I therefore went on to explore these further.

I found a trend towards MICB expression being lower in responders than non-responders (Figure 4.9). All biopsies analysed were taken prior to radiotherapy and therefore are baseline samples. It should be noted that all the patients had received chemotherapy between the biopsy being taken and commencing the PEAR trial which may have had an impact on the immune make-up of the tumour microenvironment. MICA and MICB are transmembrane proteins that are ligands for the natural killer group 2, member D (NKG2D) receptor. This receptor acts as a “master switch” to activate NK cells[9] but is also expressed on other immune cells, including NK T cells and activated CD8+ T cells. Binding of the NKG2D receptor with its ligands plays an important role in the immune response.[15] The interaction between the NKG2D receptor

and its ligands can lead to direct tumour cell killing effect through cytolytic proteins along with activation of synergistic immune effectors.[16]

Stressed and malignantly transformed cells have been found to express higher levels of MICA and MICB on their surface and MICB levels decrease in late stage tumours.[15] There is evidence that both chemotherapy and radiotherapy may be able to induce increased expression of these NKG2D ligands. Cisplatin-based chemotherapy has been shown to upregulate expression of MICA and MICB in NSCLC and is associated with a good prognosis.[20] Radiotherapy has also been shown to increase the expression of NKG2D ligands in both *in vitro* and *in vivo* glioblastoma models.[27] This enhanced the immunogenicity of the glioma cells and provided survival benefit that was decreased upon inhibition of the NKG2D pathway.[27] The suggestion of lower gene counts of MICB seen in the baseline biopsies of responders in my analysis may be significant. A lower baseline expression may provide an opportunity for radiotherapy to induce stress and to induce MICB in previously immunologically inert tumours. In combination with immune checkpoint inhibitors, this may lead to increased immune-mediated tumour cell destruction. To explore this further it will be necessary to measure the immunogenicity of the tumour microenvironment longitudinally, through sequential biopsies following treatment. In the expansion phase of the PEAR study, sequential biopsies are included in the protocol and this should facilitate the exploration of this hypothesis.

There was also a suggestion of lower counts of the IRF8 gene in responders compared to non-responders (Figure 4.9). IRF8 is an interferon regulatory factor that promotes common myeloid precursor cells to differentiate to monocyte precursor cells.[14] Interferon regulatory factors play a critical role in regulating the expression of IFN-α and IFN-β gene expression. I found IFN-β expression also tended to be lower in responders at baseline than in non-responders (Figure 4.9). IFN-β production by epithelial cells can be triggered by DNA damage as well as from viral infections.[1] IFN-β stimulates the recruitment of Batf3-dependent dendritic cells leading to antigen presentation and priming of CD8+ T cells for anti-tumour response.[23] In an elegant experiment in murine models, Vanpouille-Box et al. showed that radiotherapy could induce IFN-β driven anti-tumour immunity and that the effect was radiotherapy dose-

dependent due to the upstream regulator Trex1.[26] Trex1 plays an essential role in the clearance of DNA from the cytoplasm of damaged cells and hence dampening activation of the type-I interferon (IFN-I) pathway. The IFN-I pathway is mediated via cyclic GMP-AMP synthase (cGAS) and STING, a downstream adaptor stimulator of interferon genes.[3] In a mouse mammary carcinoma model known to be refractory to checkpoint inhibitors, radiation given as 3 fractions of 8 Gy in combination with anti-CTLA4 was able to induce T-cell mediated rejection of both the irradiated tumours and synchronous non-irradiated tumours. This abscopal effect was abrogated when a single 20 Gy fraction was used.[5] Notably, a significant increase in Trex1 expression was detected in cells treated with the single 20 Gy fraction compared to those treated with either single or multiple 8 Gy fractions.[26] However, IFN-I stimulating genes (ISG) were only induced with the multiple 8 Gy fraction regimen. Activation of the cGAS-STING pathway in tumour infiltrating dendritic cells leads to the activation of ISG.[24] The authors concluded that radiotherapy given at a high dose leads to induction of Trex1, enhanced clearance of DNA and therefore reduced activation of the cGAS-STING pathway and subsequently a dampened IFN-I mediated anti-tumour response. Radiation given below a threshold that stimulates Trex1 can induce abscopal effects when given in combination with anti-CTLA4 in tumours that are refractory to single agent checkpoint inhibitors.

Furthermore in NSCLC patients, Formenti et al. have demonstrated promising responses to anti-CTLA4 immunotherapy when combined with palliative radiotherapy at two fractionation schedules, 3 x 9.5 Gy over three days and 5 x 6 Gy delivered over five days.[6] Of the twenty-one evaluable patients, seven showed a radiological response and a further five patients showed stable disease. Circulating soluble markers and immune cells were measured longitudinally in this study. A strong association was seen between change in serum IFN-β and clinical response with the seven patients with a radiological response showing the largest rise in IFN-β.

In my study, RNA from the baseline tumour biopsy microenvironment did not show any difference in the expression of cGAS, STING or Trex1 between responders and non-responders (Figure 4.10). The fractionation schedule used in either arm of the

PEAR study would appear to be favourable for induction of IFN-β and without increasing expression of Trex1. Exploration of this would ideally require sequential tumour sampling as planned in the next phase of the study. Higher levels of IFN-β and its regulator genes in baseline biopsies of non-responders, if a true difference, may represent a more exhausted immune microenvironment with less potential for an augmented response via combination radiotherapy and checkpoint blockade. Sequential measurement of peripheral blood T cell clones, as is being performed in the PEAR study, may provide further evidence for the ability of radiotherapy to induce immune mediated, anti-tumour responses.

4.8.4 Cell Type Profiling

The cell type profiling analysis provided further evidence of a more exhausted immune tumour microenvironment in the biopsies of non-responders. In keeping with my previous findings, baseline biopsies from patients that went on to respond to combination radiotherapy and immunotherapy had a lower abundance of immune cells in the tumour microenvironment (Figure 4.12). Several cell types showed uncorrelated marker genes, which is a reported common finding, but more caution is required when interpreting further analyses from these cells (Figure 4.11). T cells, which showed strong correlation between marker genes, tended to be less abundant in responders than non-responders (Figure 4.13). Notably when T cells were subdivided further, it was exhausted CD8+ cells that showed the largest difference between responders and non-responders (Figure 4.14). Persistent antigen presentation leads to exhaustion of CD8+ cells which have decreased effector function and proliferative capacity. Exhausted T cells can arise from chronic interferon signalling and therefore this corresponds with my earlier finding of higher levels of IFN-β amongst the non-responders.[2] This mechanism may also be driven by inhibitory receptors, including tumour expression of PD-L1, leading to avoidance of immune-mediated cell death.[7]

Tumour infiltrating lymphocytes (TILs) tended to be less abundant amongst responders. However, in the biopsies of responders Tregs made up a greater proportion of the TILs. Tumour infiltration with Tregs is known to confer unfavourable

prognosis across a range of cancers, including NSCLC.[25] Although statistical power was not shown in this experiment, these paradoxical findings may be explained by the ability of radiotherapy to act as an *in situ* vaccine and to prime the immune system for response to immunotherapy.

Antigen-specific T cell responses plays a central role in the interaction between tumour and host. Genetic evaluation of TILs as part of the lung TRACERx (Tracking Cancer Evolution Through Therapy) study has shown complex T Cell Receptor (TCR) spatial heterogeneity.[12] This study used single-cell RNA-seq to characterise the TCR repertoire in NSCLC tumour biopsies. They found that an expanded cohort of T cells, with TCRs that could recognise tumour neo-antigens, formed around 20% of the T cells within the tumour microenvironment.[12] Furthermore, they showed that spatial TCR heterogeneity within the tumour was correlated with genetic mutational heterogeneity, which suggests that the TCR repertoire is sculpted by the intratumoural neoantigen composition. However, they found no evidence that a higher number of expanded TCR had any protective effect.[12] This suggests that in tumours that have escaped immune destruction, the expanded T cells are dysfunctional. This supports my findings of having a relatively more enriched T cell population in non-responders compared to responders, but with a higher proportion of exhausted T cells. Therapeutic interventions that can reinvigorate exhausted intratumoural T cells by correcting dysfunctional TCRs are attractive potential treatments.

4.8.5 Conclusions

In this analysis of baseline tumour biopsies, I have shown it is possible to perform NanoString analysis on purified mRNA, collected from small FFPE biopsy samples. Although there was significant RNA degradation, acceptable binding densities were obtained with few quality control flags raised in the NanoString analysis. In the explorative analysis, there appeared to be differences in the immunogenomics between those patients that responded to the combination of radiotherapy and pembrolizumab and those that primarily progressed. While statistical significance between differential individual gene counts was not achieved in these small numbers,

responders tended towards lower counts of MICB and IFN-β at baseline. Additionally, non-responders tended to have higher counts of exhausted CD8 T cells in the tumour microenvironment, potentially driven by chronic interferon signalling and this may explain their lack of response to immune check point inhibition. Pathways involving proteins encoded from these genes have previously been highlighted as potential mechanisms by which radiotherapy can induce abscopal effects. Further work, with sequential sampling to assess change in tumour microenvironment immunogenomics throughout treatment will be required to explore these hypotheses and are justified based on this study.

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4.10 Appendix A

List of genes in the 30 gene spike:

PARP1	H2AX	Trex1
APEX1	PCNA	BATF3
XRCC4	RPA3	CLEC9A
LIG4	DDB2	MICA variant
CTIP	ATR	APOBEC3B
RAD51	cGAS	CFLAR
BRCA1	STING	MLH1
BRCA2	ifit3	TRADD
MDC1	OAS1	NLRP9
NBN	OAS2	STK39

Chapter 5: **Predicting Toxicity and Outcome from Docetaxel in NSCLC**

5.1 Introduction

I attempted to explore the impact that lung function parameters have on the tolerability and outcomes with the chemotherapy agent, docetaxel in advanced NSCLC. In NSCLC, docetaxel is primarily used in the second-line cytotoxic setting, in patients who have progressed on platinum-doublet chemotherapy. Prognostic markers for toxicity and survival in second-line chemotherapy for NSCLC are not well established.

With only a modest survival advantage conferred by docetaxel in NSCLC compared to best supportive care and with its significant side effect profile, it is imperative to identify prognostic factors with which to identify which patients are most likely to benefit from second and subsequent line chemotherapy.

5.2 Aim

- To understand the factors that affect the tolerability and outcomes for patients receiving the second-line chemotherapy, docetaxel for NSCLC

5.3 Objectives

- Assess the impact of prophylactic granulocyte-colony stimulating factor on toxicity from docetaxel in metastatic NSCLC

- Perform a multivariate analysis of the patient and disease factors which affect tolerability of docetaxel as measured by relative dose intensity and the number of cycles received

- Perform a multivariate analysis of the patient and disease factors (including pulmonary function tests) which affect patient outcome as measured by progression free survival and overall survival

5.4 Study Hypotheses

- Patients with poorer lung function parameters are less tolerant to second-line docetaxel for NSCLC and have worse survival outcomes

5.5 Study Design

5.5.1 Patient Selection & Data Collection

I designed a retrospective analysis study of patients that had received docetaxel for NSCLC at the Royal Marsden Hospital including the Fulham Road, Kingston and Sutton sites. The study was carried out as part of a service evaluation and was approved by the local service evaluation board. All patients meeting the inclusion criteria were included in the analysis.

Inclusion Criteria

- Aged 18 years or over
- NHS standard of care patients only (patients receiving docetaxel within a clinical trial or as private prescription were excluded)
- Cytological or histologically confirmed NSCLC - all subtypes
- Received treatment for NSCLC within a palliative paradigm
- Received first dose of docetaxel between 15th July 2015 and 15th July 2017
- Docetaxel given as single cytotoxic agent. Patients receiving docetaxel in combination with nintedanib or other targeted treatments were eligible

The inclusion dates were chosen due to the change that occurred to the trust prescription proforma on 15th July 2015. This change was due to the updated NICE guidance on prescribing docetaxel. From this date, the antibiotic ciprofloxacin was added as standard to the treatment regimen at 500mg BD for 7 days, starting on Day

5 of the cycle. As this was likely to have a positive impact on the tolerability of the treatment, I excluded patients when docetaxel was given prior to this date. The end date was chosen to include 2 years' worth of patients and to allow enough follow-up time for the outcome measures to mature.

I performed a retrospective notes review to obtain the required data fields. This included reviewing the chemotherapy prescriptions, oncology clinic letters and referral documents. All data was as recorded at the time of the patient's first cycle of docetaxel. The list of factors included in the data collection for subsequent analysis was generated by consensus from literature review and to be attainable within the scope of a retrospective review. Specifically, lung function testing was not taken contemporaneously to docetaxel treatment in most cases. Lung function test results within 2 years of the patient's first cycle of docetaxel were included in the analysis. The list of patients receiving docetaxel at the Royal Marsden Hospital within the specified dates was supplied by the hospital's service planning department.

5.5.2 Outcome Measures and Statistical Analysis

Tolerability

The tolerability of docetaxel was assessed by two outcome measures; the number of cycles received and the relative dose intensity. These outcome measures were chosen because they assess different aspects of tolerance. Relative dose intensity incorporates dose adjustments and treatment delays. The number of cycles received assesses whether patients needed to discontinue early. As the planned number of treatments is not necessarily set it would not be possible to combine these outcome measures. Relative dose intensity (RDI) was calculated as the received dose intensity as a percentage of the planned dose intensity. Dose intensity was calculated by the total amount of drug given (mg/kg) divided by the total treatment length (days). Linear regression models were used to test the effect of each potential factor on the number of cycles of docetaxel received and the relative dose intensity.

Outcome

Progression free survival (PFS) was measured as the time from the date of first docetaxel dose to the date of radiological, clinical progression (as deemed by the

medical team) or death due to any cause. Overall survival (OS) was measured as the time from the date of first docetaxel dose until death from any cause. Response rate was not assessed as the objective response rate in the original published studies was less than 10%.[20] Thus, in this small group of patients, response rate would be unreliable. Patients without an event (progression or death) were censored at their last follow up visit. I used the Kaplan-Meier method to summarise the survival estimates with Cox proportional hazards models to compare the survival rates between groups and for prognostic modelling.

For both Cox proportional hazards and linear regression models, factors were tested by univariate analysis and all factors with a p value < 0.25 were taken forward into the multivariate analysis. The forward conditional approach was used with only factors with a $p < 0.05$ remaining in the final multivariate model. A two-side alpha of 5% was used to declare statistical significance with no adjustment for multiplicity.

5.6 Results

5.6.1 Patient Characteristics

Between July 2015 and July 2017, fifty-two patients received docetaxel chemotherapy for NSCLC at the Royal Marsden in a palliative setting. The patients' baseline characteristics are described in Table 5.1. The median age of the patients was 64 years with twenty-eight (54%) being male. Fifty-one (98%) patients were graded as ECOG performance status 0 or 1 at the start of treatment. All patients had prior chemotherapy exposure. There were four patients who received docetaxel as a first line palliative chemotherapy regimen. In these cases, the patients had progressed shortly after adjuvant chemotherapy, used within a radical treatment plan and therefore repeat platinum doublet therapy was not appropriate. Two previous lines of palliative chemotherapy had been used in nine (17.3%) patients and three prior lines in three (5.8%) patients. Most patients were either current (ten (19%)) or former smokers (thirty-seven (71%) and twenty-three (44%) had been formally diagnosed with COPD. Spirometry results were available for thirty-four (65%) of the patients and TLCO data was available in twenty-seven (52%) patients. The mean FEV_1 for patients with spirometry results was 1.98 L and 73.7% of predicted normal. There were twenty-three (65%) patients with an FEV_1 at least less than 80% of predicted normal and five patients (15%) with an FEV_1 less than 50% of predicted normal.

Non-squamous histology was more commonly treated with only ten (25%) patients having squamous cell cancer. Concomitant nintedanib was prescribed from cycle 1 in twenty-three patients which is equivalent to 56% of the non-squamous group, the reasons for not prescribing nintedanib were predominantly due to bleeding risk.

Age	
Median	64
Range	50-85
Gender - Male	28 (54)
ECOG Performance Status	
0	3 (5.8)
1	48 (92.3)
2	1 (1.9)
Mean BMI (SD)	25.8 (4.1)
Wight loss >5% in last 3/12	13 (25)
Smoking Status	
Current	10 (19.2)
Former	37 (71.2)
Never	5 (9.6)
Ethnicity	
White	44 (84.6)
Black	2 (3.8)
Asian	5 (9.6)
Other	1 (1.9)
COPD	23 (44.2)
Diabetes Mellitus	3 (5.8)
Histology	
Squamous	11 (21.2)
Non-Squamous	41 (78.8)
Baseline Stage	
IIIA	1 (1.9)
IIIB	3 (5.8)
IV	48 (92.3)
Line of Therapy	
1st	4 (7.7)
2nd	36 (69.2)
3rd	9 (17.3)
4th	3 (5.8)
1st line Response	
Partial Response	26 (50)
Stable Disease	12 (23.1)
Progressive Disease	11 (21.2)
N/A	3 (5.7)
Lung Function	
Mean ± SD FEV_1	1.98 ± 0.62
Mean ± SD FEV_1% predicted	73.7 ± 19.9
FEV1 <80% predicted	22/34 (64.7)
FEV1 <50% predicted	5/34 (14.7)
Mean ± SD TLCO	4.89 ± 1.72
Mean ± SD TLCO% predicted	62.7 ± 19.9
TLCO <50% predicted	8/27 (29.6)

Table 5.1: Baseline characteristics of patients receiving docetaxel. Figures given as number of patients and as percentage of the total except where stated. SD = standard deviation, FEV_1 = forced expiratory volume in 1 second, TLCO = Transfer factor for Carbon Monoxide

5.6.2 Toxicity

A total of 160 cycles of docetaxel were administered to the fifty-two patients, with a median of 3 cycles per patient and a mean of 3.1 cycles. Supportive treatment with granulocyte-colony stimulating factor (G-CSF) was used in 53 of the 160 cycles. Progressive disease (44.2%) was the most common reason for discontinuing docetaxel with only 20% of patients completing a minimum of 4 planned cycles.

I compared rates of toxicity following docetaxel from treatment cycles with or without G-CSF prophylaxis in each of the 160 cycles. Myelosuppression was a common side effect of treatment, with 22 cycles being complicated by CTCAE grade 3-4 neutropaenia. Not receiving G-CSF significantly increased the risk of having grade 3-4 neutropaenia (p = 0.05, Fisher exact test) but there was no significant difference in rates of febrile neutropaenia of grade 3-4 (p = 0.72, Fisher exact test). There was no significant difference between the number of admissions for patients that received G-CSF prophylaxis and those that did not (p = 0.135, Chi squared) or length of admissions (p = 0.73, Wilcoxon Rank Sum). Treatment cycles that included prophylactic G-CSF required fewer clinic attendances per cycle (p <0.0001, Wilcoxon Rank Sum). G-CSF prophylaxis had no significant effect on the cause of docetaxel discontinuation. The toxicity results are displayed in Table 5.2.

	All Prophylaxis	No Prophylaxis	*p*-value
Grade 3-4 Neutropaenia	3 (5.3)	19 (17.8)	0.05
Grade 3-4 Febrile Neutropaenia	2 (3.5)	7 (7.5)	0.72
Admission (n)	6	22	0.135
Median admission (days)	4	6.5	0.73
Median clinics per cycle **Mean clinics per cycle (SD)**	1 1.15 (0.41)	1 1.56 (0.64)	**<0.0001**
Reason for discontinuation (n) PD Toxicity Completed	 3 3 1	 20 14 11	0.77

Table 5.2: Comparison of rates of toxicity in patients treated with docetaxel with or without granulocyte-colony stimulating factor prophylaxis. SD = standard deviation, PD = progressive disease

The mean relative dose intensity for the fifty-two patients was 93.4 +/- 8.9% with a range of 61.5 to 100%. In the univariate analysis, weight loss of greater than 5% in the preceding three months, diabetes mellitus and response to first line therapy were associated with a significantly lower relative dose intensity. Using the forward selection method, in the multivariate model only recent weight loss and response to first line therapy had a significant impact on relative dose intensity. In the multivariate model, if response to first line treatment was kept constant, patients who had weight loss would be expected to receive a 6% lower (95% C.I. -11.6 to -0.4) relative dose intensity. A best response to first line therapy of progressive disease or partial response were associated with a lower relative dose intensity compared to stable disease. In the multivariate model, if weight loss was kept constant a response to first line therapy of partial response was associated with a 7.2% lower relative dose intensity compared to patients who achieved stable disease (95% C.I. -13 to -1.4%). Patients who had primary progressive disease to first line therapy had a 9.2% lower relative dose intensity with docetaxel compared to those achieving stable disease, if recent weight loss was kept constant (95% C.I. -16.2 to -2.2%). The results from the univariate and multivariate analysis of relative dose intensity of docetaxel are laid out in Table 5.3.

		Univariate Model		Multivariate Model	
Variable	N	Coef. (Std Err)	*p*-value	Coef. (Std Err)	*p*-value
G-CSF use: Prophylaxis vs. No prophylaxis	7/45	3.03 (3.61)	0.41	-	-
Performance Status >0 vs. 0	49/3	-2.64 (5.32)	0.622	-	-
COPD (yes vs. no)	23/29	-3.12 (2.46)	**0.211***		
FEV_1% predicted (cont.)	-	0.01 (0.86)	0.932	-	-
FEV_1% (>80% vs. ≤80%)	12/22	-0.94 (3.56)	0.792	-	-
FEV_1% (>50% vs. ≤50%)	29/5	1.04 (4.80)	0.829	-	-
TLCO% predicted (cont.)	-	-0.02 (0.11)	0.869	-	-
TLCO% (>50% vs. ≤ 50%)	19/8	1.04 (4.54)	0.820	-	-
Histology (squamous vs. non-squamous)	11/41	-1.31 (3.04)	0.667	-	-
Nintedanib (yes vs. no)	23/29	-1.37 (2.50)	0.586	-	-
Initial Dose (75 vs. <75 mg/m^2)	29/23	1.66 (2.49)	0.509	-	-
Smoking status					
Ex-smoker vs. Never	37/5	-0.35 (4.23)	0.935	-	-
Current vs. Never	10/5	3.28 (4.89)	0.506	-	-
BMI (> 18.5 vs. ≤18.5)	50/2	-6.88 (6.39)	0.287	-	-
Weight loss in 3/12 > 5% (yes vs. no)	13/39	-4.47 (2.80)	**0.117***	-6.00 (2.77)	**0.036****
Diabetes Mellitus (yes vs. no)	3/49	-6.52 (5.25)	**0.220***		
Response to 1st line					
PR vs. SD	26/12	-6.61 (3.00)	**0.033***	-7.23 (2.90)	**0.016****
PD vs. SD	11/12	-8.28 (3.59)	**0.220***	-9.19 (3.48)	**0.011****
Variable Overall			**0.048***		

Table 5.3: Relative dose intensity multiple regression analysis. Factors with a *p*-value < 0.25 (*) were included in the multivariate analysis. In the multivariate analysis factors with a *p*-value < 0.05 (**) were deemed significant. FEV_1 = forced expiratory volume in 1 second, TLCO = Transfer factor for Carbon Monoxide, BMI = Body Mass Index, PR = partial response, SD = stable disease, PD = progressive disease

In the univariate analysis, factors associated with worse tolerability of docetaxel, as measured by number of treatment cycles received, were squamous cell histology, a lower percentage of predicted FEV_1, having an FEV_1 of less than 50% predicted and not receiving concomitant nintedanib. When these were taken forward to the multivariate model only concomitant nintedanib impacted on the number of docetaxel cycles received. In the multiple regression model, a patient who had nintedanib at cycle 1, would expect to have 1.1 (95% C.I. 0.1 to 2.1, p = 0.03) more cycles of docetaxel than a patient without nintedanib. (Table 5.4)

5.6.3 Outcome

Docetaxel efficacy was measured by progression free survival (PFS) and overall survival using the Kaplan-Meier method. Of the fifty-two patients included in the study, forty-four had either progressed or died at the point of data censure. Median PFS was 3.2 months (95% CI: 2.0 to 4.2) in those not receiving G-CSF prophylaxis, which accounted for forty-five of the fifty-two patients. For the seven patients with G-CSF prophylaxis from cycle 1, the median PFS was 2.3 months (95% CI: 0.4 to 4.2). In the univariate analysis decreasing FEV_1, squamous histology, not receiving nintedanib, and recent weight loss were associated with a significantly worse PFS. In the multivariate model, only squamous histology predicted a poorer PFS (HR: 2.3, 95% CI (1.0 - 5.2 p = 0.039) (Table 5.5).

At the time the data was censored, thirty-one (60%) of the patients had died. Median overall survival was 6.9 months in those receiving G-CSF compared to 6.7 months in those without G-CSF prophylaxis. Factors that were associated with a worse overall survival included FEV_1 as either a continuous variable or as a binary cut-off of less than 80% predicted or less than 50% predicted, no concomitant nintedanib use, previous smoking history or recent weight loss. However, in the multivariate model only FEV_1%pred as a continuous variable (HR 0.96 CI: 0.93 - 0.99) or having an FEV_1%pred less than 50% predicted (HR 0.15 CI: 0.04 – 0.57) were associated with worse overall survival. We would expect for every 1% decrease in FEV_1%pred the hazard of death would increase by 4% (95% CI 1 – 7%) (Table 5.6).

		Univariate Model		Multivariate Model	
Variable	N	Coef. (Std Err)	*p*-value	Coef. (Std Err)	*p*-value
G-CSF use: Prophylaxis vs. No prophylaxis	7/45	-0.08 (0.57)	0.85	-	-
Performance Status >0 vs. 0	49/3	-0.27 (0.84)	0.747	-	-
COPD (yes vs. no)	23/29	-0.60 (0.39)	0.880	-	-
FEV_1% predicted (cont.)	-	0.018 (0.01)	**0.164***		
FEV_1% (>80% vs. ≤80%)	12/22	0.45 (0.54)	0.405	-	-
FEV_1% (>50% vs. ≤50%)	29/5	0.945 (0.71)	**0.196***		
TLCO% predicted (cont.)	-	0.00 (0.01)	0.886	-	-
TLCO% (>50% vs. ≤ 50%)	19/8	0.56 (0.56)	0.319	-	-
Histology (squamous vs. non-squamous)	11/41	-0.79 (0.47)	**0.097***		
Nintedanib (yes vs. no)	23/29	0.72 (0.38)	**0.065***	1.11 (0.49)	**0.031****
Initial Dose (75 vs. <75 mg/m^2)	29/23	0.45 (0.39)	0.253	-	-
Smoking status Ex-smoker vs. Never Current vs. Never	 37/5 10/5	 -0.52 (0.67) -0.8 (0.77)	 0.44 0.36	 - -	 - -
BMI (> 18.5 vs. ≤18.5)	50/2	0.6 (1.01)	0.557	-	-
Weight loss in 3/12 > 5% (yes vs. no)	13/39	-0.31 (0.45)	0.497	-	-
Diabetes Mellitus (yes vs. no)	3/49	-0.08 (0.84)	0.923	-	-
Response to 1st line PR vs. SD PD vs. SD Variable Overall	 26/12 11/12	 -0.10 (0.48) -0.79 (0.58)	 0.835 0.184 0.327	 - - -	 - - -

Table 5.4: Number of treatment cycles multiple regression analysis. Factors with a *p*-value < 0.25 (*) were included in the multivariate analysis. In the multivariate analysis factors with a *p*-value < 0.05 (**) were deemed significant. FEV1 = forced expiratory volume in 1 second, TLCO = Transfer factor for Carbon Monoxide, BMI = Body Mass Index, PR = partial response, SD = stable disease, PD = progressive disease

		Univariate Model		Multivariate Model	
Variable	N	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
G-CSF use: Prophylaxis vs. No prophylaxis	7/45	1.50 (0.6 - 3.3)	0.369	-	-
Performance Status >0 vs. 0	49/3	1.30 (0.4 - 4.4)	0.622	-	-
COPD (yes vs. no)	23/29	1.10 (0.6 - 2.0)	0.708	-	-
FEV_1% predicted (cont.)	-	0.99 (0.97 – 1.01)	**0.225***		
FEV_1% (>80% vs. ≤80%)	12/22	0.70 (0.3 – 1.5)	0.341	-	-
FEV_1% (>50% vs. ≤50%)	29/5	0.6 (0.2 – 1.7)	0.327	-	-
TLCO% predicted (cont.)	-	1.01 (0.99 – 1.03)	0.411	-	-
TLCO% (>50% vs. ≤ 50%)	19/8	1.2 (0.5 – 2.9)	0.729	-	-
Histology (squamous vs. non-squamous)	11/41	1.7 (0.8 – 3.4)	**0.166***	2.3 (1.0 – 5.2)	**0.039****
Nintedanib (yes vs. no)	23/29	0.7 (0.4 – 1.2)	**0.207***		
Initial Dose (75 vs. <75 mg/m^2)	29/23	0.9 (0.5 – 1.6)	0.652	-	-
Smoking status					
Ex-smoker vs. Never	37/5	0.7 (0.3 – 1.8)	0.447	-	-
Current vs. Never	10/5	0.8 (0.3 – 2.4)	0.652	-	-
BMI (> 18.5 vs. ≤18.5)	50/2	0.9 (0.2 – 3.7)	0.860	-	-
Weight loss in 3/12 > 5% (yes vs. no)	13/39	1.6 (0.8 – 3.2)	**0.146***		
Diabetes Mellitus (yes vs. no)	3/49	0.9 (0.2 – 3.9)	0.916	-	-
Response to 1st line					
PR vs. SD	26/12	0.9 (0.4 - 1.9)	0.745	-	-
PD vs. SD	11/12	1.5 (0.6 – 3.5)	0.380	-	-

Table 5.5: Progression Free Survival multiple Cox regression analysis. Factors with a *p*-value < 0.25 (*) were included in the multivariate analysis. In the multivariate analysis factors with a *p*-value < 0.05 (**) were deemed significant. FEV1 = forced expiratory volume in 1 second, TLCO = Transfer factor for Carbon Monoxide, BMI = Body Mass Index, PR = partial response, SD = stable disease, PD = progressive disease

		Univariate Model		Multivariate Model	
Variable	N	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
G-CSF use: Prophylaxis vs. No prophylaxis	7/45	0.99 (0.35 – 2.88)	0.998	-	-
Performance Status >0 vs. 0	49/3	3.7 (0.5 – 28)	**0.210***		
COPD (yes vs. no)	23/29	1.5 (0.7 – 3.1)	0.262	-	-
FEV_1% predicted (cont.)	-	0.96 (0.93 – 0.99)	**0.009***	0.96 (0.93 – 0.99)	**0.009*****
FEV_1% (>80% vs. ≤80%)	12/22	0.5 (0.2 – 1.5)	**0.209***	-	-
FEV_1% (>50% vs. ≤50%)	29/5	0.15 (0.04 – 0.57)	**0.005***	0.15 (0.04 – 0.57)	**0.005*****
TLCO% predicted (cont.)	-	1.01 (0.98 – 1.03)	0.578	-	-
TLCO% (>50% vs. ≤ 50%)	19/8	2.0 (0.6 – 7.3)	0.292	-	-
Histology (squamous vs. non-squamous)	11/41	1.4 (0.6 – 3.2)	0.483	-	-
Nintedanib (yes vs. no)	23/29	0.6 (0.3 – 1.3)	**0.193***		
Initial Dose (75 vs. <75 mg/m^2)	29/23	0.8 (0.4 – 1.7)	0.621	-	-
Smoking status					
Ex-smoker vs. Never	37/5	0.4 (0.1 – 1.2)	**0.103***		
Current vs. Never	10/5	0.6 (0.2 – 2.1)	0.447	-	-
BMI (> 18.5 vs. ≤18.5)	50/2	0.99 (0.14 – 7.38)	0.999	-	-
Weight loss in 3/12 > 5% (yes vs. no)	13/39	2.0 (0.9 – 4.3)	**0.075***		
Diabetes Mellitus (yes vs. no)	3/49	0.6 (0.1 – 4.8)	0.673	-	-
Response to 1st line					
PR vs. SD	26/12	1.05 (0.43 – 2.58)	0.916	-	-
PD vs. SD	11/12	0.98 (0.34 – 2.79)	0.963	-	-

Table 5.6 : Overall Survival multiple Cox regression analysis. Factors with a *p*-value < 0.25 (*) were included in the multivariate analysis. In the multivariate analysis factors with a *p*-value < 0.05 (**) were deemed significant, factors with a *p*-value < 0.01 (***). FEV1 = forced expiratory volume in 1 second, TLCO = Transfer factor for Carbon Monoxide, BMI = Body Mass Index, PR = partial response, SD = stable disease, PD = progressive disease

Due to my findings that poor lung function impacted on toxicity and outcomes, I performed a post hoc analysis to look at the impact of FEV_1 and transfer factor with regards the proportion of patients discontinuing docetaxel due to toxicity. The proportion of patients that discontinued due to toxicity increased as FEV_1 decreased. This was significant when using a cut-off FEV_1 of less than 50% predicted. While there was also a trend for patients with a TLCO%pred to be more likely to discontinue docetaxel, this did not reach statistical significance (Table 5.7).

	Toxicity	Progression/ Complete	Total	*p*-value
$FEV_1 \geq 80\%$ predicted	1	11	12	-
$FEV_1 \leq 80\%$ predicted	10	12	22	0.053
$FEV_1 \leq 50\%$ predicted	4	1	5	**0.023***
$TLCO \geq 50\%$ predicted	5	14	19	-
$TLCO \leq 50\%$ predicted	5	3	8	0.101

Table 5.7: Post-hoc analysis: Reason for discontinuation of treatment for differing parameters of lung function. FEV1 = forced expiratory volume in 1 second, TLCO = Transfer factor for Carbon Monoxide. Chi-squared analysis with a *p*-value < 0.05 deemed significant (*).

5.7 Discussion

This real-world data, of docetaxel given as second-line chemotherapy for advanced NSCLC provides evidence for the importance of lung function as a prognostic marker. In this group of patients, FEV_1 was the patient characteristic most associated with overall survival following docetaxel. Patients with an FEV_1%pred less than 50% were significantly more likely to have a poor prognosis. Furthermore, patients with lower FEV_1 and especially those with an FEV_1%pred less than 50% appear to be the most likely to discontinue docetaxel due to toxicity.

This retrospective data, which would not have the same level of patient selection as the trial data, reassuringly still produced similar PFS results as the original randomised trials. The median PFS in my data for those without G-CSF prophylaxis was 3.2 months (95% CI: 2.0 to 4.2) which compares to 3.4 months and 2.7 months for docetaxel plus nintedanib and docetaxel alone respectively in the LUME-1 study.[17] In my data I included all histology subtypes and both treatment cycles with and without concomitant nintedanib. The LUME-1 trial was in adenocarcinoma patients only. I found median overall survival however to be shorter at 6.7 months (95% CI: 4.1 to 9.8), compared to 10.1 and 9.1 months for with and without nintedanib respectively in LUME-1.[17] The inferior overall survival of my data is likely to be due to including patients with squamous histology and the fact that we included twelve patients (23%) who were on their third or fourth line of treatment. However, given that my data has similar results in terms of PFS, it does provide a good sample to study potential factors that may affect tolerability and outcome with docetaxel.

Primary prophylaxis against febrile neutropaenia with G-CSF is not standard of care as per NICE guidelines for docetaxel for advanced NSCLC. However, it is approved if there is concern regarding the high-risk nature of lung cancer patients or due to prior episodes of febrile neutropenia in previous lines of treatment. G-CSF has come down significantly in price due to biosimilars and is now considered a low-cost intervention (one injection per day around £10). I therefore aimed to interrogate what effect G-CSF would have on rates of febrile neutropaenia as this could potentially be a significant contributor to the tolerability and outcomes from docetaxel. Only seven of the fifty-two

patients had G-CSF prescribed from cycle 1, so called primary prophylaxis. However, G-CSF was also prescribed as secondary prophylaxis following an episode of documented neutropaenia resulting in a total of 53 of the 160 docetaxel cycles receiving either form of prophylaxis. The rate of grade 3-4 febrile neutropaenia was significantly higher than in the trial data, with nine of the fifty-two patients (17.3%) suffering such an episode, compared to only 5.9% in LUME-Lung 1 across both arms of the study. While rates of grade 3-4 neutropaenia were significantly higher in those not receiving G-CSF, prophylaxis had no significant impact on the rate of grade 3-4 febrile neutropaenia in my study. Notably, two patients suffered an episode of febrile neutropaenia despite G-CSF prophylaxis. A sub-population of patients not receiving primary G-CSF prophylaxis, that were perceived by clinicians to be at higher risk of toxicity, were scheduled for extra appointments at day 7 of their cycle for a toxicity check. This toxicity check would include a full blood count which showed asymptomatic neutropaenia in some patients. These extra blood tests, which were not required in patients receiving prophylaxis, will skew the data to increase the rate of grade 3-4 neutropaenia in the non-prophylactic group. The anticipated neutrophil nadir with docetaxel would be at around day 7 to 8.[16] It is therefore not surprising that patients not receiving G-CSF prophylaxis had more clinic appointments (mean 1.56 / cycle) than those who had prophylaxis (mean 1.15 / cycle). However, G-CSF prophylaxis had no impact on the number of admissions, the length of hospital admissions or reason for discontinuation of docetaxel. These findings support the NICE guidelines that G-CSF prophylaxis is not warranted with docetaxel in this patient group and that extra appointments to check toxicities are not routinely necessary. Avoiding unnecessary appointments is likely to be of benefit to both clinicians working in busy clinics and for patients who already will be weighing up the burden of treatment intensity on quality of life. It should be noted that the studied population were relatively young (median age 64 years) and of good performance status (ECOG PS < 2), which are known protective factors regarding risk of developing febrile neutropaenia.[15] An individualised risk assessment should be performed by clinicians considering prophylactic G-CSF with docetaxel chemotherapy for patients with metastatic NSCLC.

In this study, I was particularly interested to understand the impact of lung function on the tolerability and efficacy of second line chemotherapy. In the multiple regression analyses I also included previously described patient and tumour specific factors that

might affect these outcomes. Performance status was an independent prognostic factor for overall survival in several previous second line studies in advanced NSCLC.[10, 22, 23] Performance status was also prognostic for progression free survival in one retrospective second line study.[10] In these studies there was a significant proportion of patients being graded as performance status 0 or 2. In our patient group, 92.3% of the patients were graded as ECOG performance status 1 at the start of treatment and therefore we were less likely to see performance status as an impact factor for either tolerability or efficacy of treatment.

Lung cancer is a disease predominantly of current or former smokers who have a high prevalence of lung and cardiac co-morbidities and these comorbidities may be associated with poor tolerance of treatment. It was my theory that lung function parameters may be a simple, measurable marker of this perceived fragility to second line chemotherapy.

As this was a retrospective study, lung function data was not available for all the patients and was not taken contemporaneously to the start of second line treatment in most cases. Of the fifty-two patients that received docetaxel, spirometry data was available for thirty-four patients and transfer factor in twenty-seven patients. A documented diagnosis of COPD was found in twenty-three (44.2%) patients. Spirometry was not cross-referenced with this data as the diagnosis of COPD requires knowledge of symptoms along with spirometry criteria. As lung function parameters to examine, I chose FEV_1 and TLCO. FEV_1%pred is a marker of airflow obstruction severity in COPD. In the GOLD guidelines, airflow obstruction is classified as moderate if FEV_1 < 80% but ≥ 50% predicted and severe if <50% but ≥ 33% predicted (Table 5.8). The degree of airflow obstruction, as measured by FEV_1%pred is a component of the BODE index, which is a known prognostic marker in COPD.[5] While COPD may not be the only disease that would impact on FEV_1 in our patient group it is likely to be the biggest factor. Restrictive lung disease which include fibrotic lung disease, obesity and pleural effusions are other potential contributors to impairment in FEV_1. It should be noted that in idiopathic pulmonary fibrosis, trends in forced vital capacity (FVC) rather than FEV_1 is the strongest marker of disease mortality.[12]

Classification of airflow limitation severity in COPD (based on post-bronchodilator FEV_1)		
In patients with $FEV_1/FVC < 0.7$		
GOLD 1:	Mild	$FEV_1 \geq 80\%$ predicted
GOLD 2:	Moderate	$50\% \geq FEV_1 < 80\%$ predicted
GOLD 3:	Severe	$30\%\% \geq FEV_1 < 50\%$ predicted
GOLD 4:	Very Severe	$FEV_1 <30\%$ predicted

Table 5.8: GOLD classification of airflow obstruction in COPD[6]

TLCO is a composite measure of alveolar volume (VA) and gas transport across the alveolar-capillary membrane. Lung volume increases are seen in COPD when there has been alveolar breakdown and the lungs have become hyperexpanded as a result of air trapping and poor elastic recoil. The carbon monoxide coefficient (KCO) is the measured index of efficiency of alveolar transfer of carbon monoxide. As COPD incorporates both small airways disease obstruction, as measured by FEV_1, and lung parenchymal destruction I also wanted to assess whether impairment in TLCO may impact on the tolerability and outcomes from chemotherapy in NSCLC. In a previous outpatient study of 604 COPD patients, the strongest predictors of survival were age, arterial oxygen partial pressure on room air (PaO_2) and TLCO percentage of predicted. In this study FEV_1%pred was not prognostic.[3] In their Cox regression analysis, patients in the first quartile of TLCO predicted (>51% (HR: 0.332 (0.172 – 0.639) p = 0.001)) and those in the second quartile (51 – 37.3%(HR: 0.515 (0.332 – 0.825) p = 0.006)) had significantly lower mortality risk than those in the lower quartile (<27.3%).[3] I therefore chose TLCO%pred as a continuous variable along with a cut-off of 50% as the parameters to use in this analysis.

In my study, in the cox-regression analysis for overall survival, factors which were significantly associated with poorer survival were performance status >0, low FEV_1%pred as a continuous variable, FEV_1%pred less than 80% and less than 50%, not having concomitant nintedanib (aligning with squamous histology), being an ex-smoker and weight loss of over 5% in the last 3 months. However, when these factors were taken forward into the multivariate model only FEV_1%pred as a continuous variable and with a cut-off of 50% predicted were significant. For patients with an FEV_1 greater than 50% predicted the hazard risk of death decreased by 85% (HR 0.15 (95% CI: 0.04 to 0.57) p = 0.005) compared to those with FEV_1 less than 50% predicted.

Similarly, when FEV_1%pred is looked at as a continuous variable, for each additional unit increase in FEV_1 percentage predicted the hazard of death decreases by 4% (HR: 0.96 (95% CI: 0.93 to 0.99) p = 0.009). TLCO%pred, either as a continuous variable or with a cut-off of 50% predicted was not a prognostic factor for overall survival.

My data is consistent with previous studies of the impact of lung function as a prognostic factor in advanced NSCLC. A Korean study which looked at spirometry prior to first line therapy found that FEV_1 less than 50% of predicted was an independent poor prognostic factor (HR 2.74 (95% CI: 1.516 to 4.823) p = 0.001).[13] Similar to my data, Lee at al. found that COPD on its own was not a prognostic factor for overall survival.[14] However, FEV_1 less than 80% predicted with COPD was associated with worse overall survival in the multivariate model for patients who went on to have chemotherapy. Of note, FEV_1 was not a prognostic factor in patients who received a targeted agent. This difference may be due to these patients having less severe airflow obstruction, as targetable mutations are more common in patients who are never or light smokers. In the Lee et al. study, the proportion of patients who died from pulmonary complications was not different between COPD and non-COPD patients, suggesting that FEV_1 may be a marker of a more global frailty to chemotherapy. In contrast, Izquierdo et al. showed that there was a poor correlation between FEV_1%pred and overall survival (r=0.12) in patients with advanced NSCLC treated with chemotherapy and/or tyrosine kinase inhibitors.[11] In this study, COPD was not found to be an independent prognostic factor in the Cox regression model but poor performance status and advanced clinical stage were negatively associated.

Another study which looked at absolute values of FEV_1 found that a cut-off of ≥ 2 litres was an independent prognostic factor for survival (HR 0.61 (95% CI: 0.45 to 0.84) p = 0.002).[1] Similarly, in a study of locally advanced NSCLC, in patients who underwent concurrent chemoradiation an FEV_1 of greater than 2 litres was associated with improved survival.[2] FEV_1%pred is likely to be a better marker than an absolute value as the patient's age and sex is incorporated into the value and these are both known prognostic factors. An absolute value may have more relevance in local or locally advanced disease where surgery or radical radiotherapy are potential treatment modalities. Loss of viable lung from lobectomy or radiation pneumonitis may have a

significant impact on post treatment morbidity and mortality especially in those patients with less lung function reserve.

Lung function parameters were not shown to be significantly associated with progression free survival in my multivariate model. FEV_1%pred as a continuous variable was significant at the 0.25 significance level in the univariate analysis but the effect was small (HR: 0.99 (95% CI: 0.97 to 1.01) p = 0.225). The only factor that was shown to be an independent prognostic factor was histology, with patients with squamous cell carcinoma being 2.3 times more likely to have progression compared to those in the non-squamous group (HR 2.3 (95% CI: 1.0 to 5.2) p = 0.039). As expected, concomitant nintedanib, available only to patients with non-squamous histology, was a good prognostic marker for PFS in the univariate analysis.

FEV_1%pred was not associated with either RDI or number of treatment cycles received in the multivariate analysis. It appears that these markers of treatment tolerability do not fully explain why patients with a lower FEV_1%pred had worse overall survival. However, both lower FEV_1%pred as a continuous variable and at a cut-off of ≤ 50% predicted were associated with fewer cycles in the univariate analysis. It may be that the sample size in this study was too small to show a significant effect on the number of cycles. There is further evidence that patients with lower FEV_1%pred tolerated the treatment less well from the post-hoc analysis which showed that patients with an FEV_1 ≤ 50% predicted were more likely to discontinue the treatment due to toxicity rather than disease progression or completing the planned treatment regimen (p = 0.023). In the multivariate regression model, weight loss of > 5% in the last 3 months and response to first line treatment were associated with a lower RDI. In a previous study on the use of taxanes in breast cancer, a significantly low RDI of <85% was shown to be predicted by increased age, episodes of febrile neutropaenia and grade III or IV hypersensitivity reactions.[18] I calculated the relative dose intensity based on the Hryniuk method where the measured dose intensity is given as a percentage of the standard dose intensity.[9] The mean relative dose intensity for all patients was high at 93.4% (SD: 8.9) The calculation of RDI is complicated in the advanced disease setting where the total planned dose is not set and treatment may be discontinued due to disease progression or toxicity. For patients that discontinue treatment early due to patient choice or toxicity but do not undergo treatment delays, the Hryniuk method

may overestimate the relative dose intensity compared to those patients that persevere for more cycles but with treatment delays.

The cause for the worse overall survival in patients with lower FEV_1%pred in my study is not clear. FEV_1 is known to be associated with mortality in the general population. Two large prospective observational studies both found that FEV_1%pred was inversely correlated to all-cause mortality in both men and women.[8, 19] In the US-based, Buffalo health study, 554 men and 641 women, aged 20 to 89 years were randomly selected, underwent baseline spirometry and were followed up for 29 years. Except for men who survived longer than 25 years, there was a negative association between baseline FEV_1%pred and all-cause mortality in all groups. FEV_1%pred was also inversely related to ischaemic heart disease mortality.[19] Similarly, the Renfrew and Paisley prospective population study in the UK assessed risk factors for mortality in the middle aged population. This study found that reduced FEV_1%pred was second only to cigarette smoking in predicting all-cause mortality.[8] Both of these studies looked at mortality over a long time period, yet I saw a divergence based on FEV_1%pred even with a treatment that had a median overall survival for all patients of only 6.9 months.

In my study, I did not establish the percentage of deaths that were attributable to respiratory pathology. Docetaxel is known to cause an interstitial pneumonitis in some patients treated for NSCLC.[7] The rate of grade 3-4 interstitial pneumonitis with docetaxel is estimated to be between 7 and 47% and may be underestimated due to radiological changes being attributed to confounding factors such as prior radiation, infection or progressive disease. However, when administered 3-weekly at 75 mg/m^2 without concurrent radiotherapy rates of pulmonary toxicity are likely to be less than 5%.[21] The treatment schedule rather than the overall dose appears to be the largest factor for predicting pulmonary toxicity with higher rates seen when docetaxel is delivered weekly rather than every three weeks.[4] Although the occurrence of docetaxel-related pneumonitis is rare it can lead to rapid respiratory failure and death. In patients without lung cancer or lung pathology FEV_1, FEV_1/FVC, TLCO and TLCO%pred are all significantly reduced following a docetaxel containing regimen.[24] It is likely that docetaxel related pneumonitis would be more common in patients with architectural lung distortion as is seen with radiation-pneumonitis.[7] Furthermore, any deterioration in lung function is likely to have a more serious impact and cause more

symptoms in those with a lower respiratory reserve such as those with severe airflow obstruction.

My study was limited by being a retrospective analysis with the associated disadvantages of this method of data collection. Importantly, lung function data was not collected contemporaneously and in most cases was performed prior to first line palliative treatment. Lung function may decline with progressive lung pathologies or as a result of treatments. To minimise such errors, the inclusion criteria for lung function was therefore set as to be within a maximum of 2 years prior to the first cycle of docetaxel. It is likely that lung function would have been no better and, in most cases, worse than previously recorded at the time of commencing second-line docetaxel. As this was a single-centre study, which was limited by the change to prophylactic antibiotic prescribing, it included an analysis of only fifty-two patients. This small sample size increases the chance of type 2 errors, with significant differences between groups not being shown statistically. The surprising finding that only one patient was graded as ECOG performance status 2 at the start of docetaxel therapy means that these results may not be applicable to patients of this performance status.

This study highlights the significance of a low FEV_1%pred on overall survival in patients receiving chemotherapy for NSCLC. In my data, a decreasing FEV_1 as percentage of predicted and especially patients with an FEV_1 ≤50% predicted was more strongly negatively associated with overall survival than performance status, low BMI, recent weight loss or smoking status. If this data can be prospectively validated and applied to first line therapies including combined chemotherapy and immunotherapy, it could be an important factor in deciding on the intensity of treatment and indeed whether any treatment could be safely delivered. Whether improving FEV_1 with bronchodilators or lung-volume reducing surgery could improve overall survival in lung cancer patients undergoing chemotherapy is yet to be shown and was outside the scope of this study. However, bronchodilators have not been shown to improve overall mortality in COPD patients without lung cancers. Spirometry is a quick, non-invasive, repeatable bedside test that should be included in the oncologist's decision-making algorithm when considering second-line chemotherapy with docetaxel.

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Chapter 6: **Optimal Management of Breathlessness in Co-Existing Undiagnosed COPD and Lung Cancer – The ADOPT Study**

6.1 Introduction

Finally, I looked at the impact of impaired function with regards to symptom burden in patients with lung cancers. The ADOPT study was an open label, randomised, controlled trial on the effectiveness of adding inhaled therapies to best supportive care to improve breathlessness in co-existing untreated COPD and lung cancers. This trial was set-up and was actively recruiting when I commenced my MD (Res). From this point I was responsible for all aspects of the study, including patient recruitment, performing trial observations and assessments, data entry, engagement in sponsor monitoring visits, closing the study and analysing the primary and secondary outcomes. I proposed and helped to carry out the post hoc analyses including generating further baseline characteristic data to fully understand the implications of the data. I was responsible for the recruitment of nine patients prior to the decision by the principal investigator and to close the study. A planned interim results analysis of a single secondary outcome has previously been reported and will not be repeated here.

6.2 Breathlessness in Lung Cancer

Approximately 80% of lung cancer patients will suffer from the symptom of breathlessness at some point during their illness.[13, 14, 20, 23] Progressive breathlessness leads to restricted activities of normal living, reduced quality of life and social isolation.[18] Reversible causes of breathlessness include bronchial obstruction, pleural or pericardial effusions and pulmonary emboli. Co-existing cardio-pulmonary disease, lymphangitis carcinomatosis and respiratory muscle fatigue secondary to cachexia may also be contributory factors. The mainstay of the management of breathlessness in oncology remains to be opiate medications. Opiates reduce the sensation of breathlessness and are an effective treatment in patients with lung cancer[15] but have associated side effects including drowsiness, dysphoria, nausea and constipation.[3] Opioids can also act as a cough suppressant, which is a common symptom in both

lung cancer and COPD and can add to the sensation of breathlessness. There are several international consensus statements to support the use of opiates to palliate chronic breathlessness in advanced disease.[13, 14, 20]

COPD is characterised by progressive loss of lung function over time. Breathlessness is one of the cardinal clinical features of COPD, with greater than 40% of patients suffering with moderate to severe breathlessness.[16] While COPD optimisation and prehabilitation is recommended practice for lung cancer patients undergoing surgery, there is a paucity of evidence that medical COPD treatments improve outcomes in either radical treatment paradigms or in the advanced disease setting.[17] Medical management of COPD includes bronchodilator therapies, which reduce breathlessness[9], improve exercise tolerance[1] and reduce rates of exacerbations.[5, 9, 22] Inhaled corticosteroids, usually prescribed as a combination inhaler with long-acting bronchodilators reduce rates of COPD exacerbation.[4]

COPD may be over diagnosed in patients with lung cancer due to structural changes leading to obstructive spirometry measurements. However, Young et al. showed there was only a small and insignificant increase in the prevalence of COPD (56% to 61%) in patients who had undergone spirometry before and after the diagnosis of lung cancer.[25] A previous single-arm study, including fifteen lung-cancer patients of mixed staging and airflow obstruction suggested that subjective breathlessness and FEV_1 could be improved with inhaled therapies,[6] although the small sample size and the lack of a control arm were limiting factors in this study.

There is therefore no previous convincing evidence that using inhaled therapies in patients with lung cancer and untreated COPD, is a successful strategy to improve breathlessness.

6.3 Aim

To understand the effects that the addition of inhaled therapies has on improving breathlessness over best supportive care alone in patients with lung cancer and untreated or undertreated COPD

6.4 Objectives

- To assess the effect of inhaled therapies on the proportion of patients with a clinically significant improvement in subjective breathlessness compared to best supportive care alone
- To assess the effect of inhaled therapies on lung function parameters in patients with co-existing COPD and lung cancer
- To assess the effect of inhaled therapies on exercise tolerance in patients with co-existing COPD and lung cancer
- To assess the effect of inhaled therapies on quality of life in patients with co-existing COPD and lung cancer

6.5 Hypothesis

- The addition of appropriate inhaled therapies to best supportive care will improve breathlessness for patients with co-existing COPD and lung cancer

6.6 Methods

6.6.1 Patients

Eligible adult patients had a histological diagnosis of NSCLC, small cell lung cancer (SCLC) or mesothelioma, had received a minimum of 2 cycles of chemotherapy or biological treatment, with subjective breathlessness evidenced by a baseline visual analogue scale (VAS) of greater or equal to 4 on a 10 point scale (with greater numbers indicating worse breathlessness) and a diagnosis of COPD.

The diagnosis of COPD was made as per the British Thoracic Society (BTS) guidelines which include being a current or ex-smoker, suggestive symptoms and baseline spirometry of FEV_1/FVC less than 0.7.[10] All other reversible causes of breathlessness were excluded or treated prior to enrolment in the study. Patients with previous or proposed radiotherapy or surgery were eligible if the treatment was not planned within 4 weeks of enrolment. Eligible patients were not taking long-acting inhaled or oral bronchodilators, anti-cholinergic containing drugs or potent CYP30 inhibitors but short-acting bronchodilators were permitted. Patients taking oral steroids on stable doses for a minimum of one week prior to enrolment were eligible. Patients with asthma, severe cardiovascular disorders, urinary dysfunction, cardiac arrhythmias, previous or current tuberculosis, renal failure, thyrotoxicosis or uncorrected hypokalaemia were excluded. Written informed consent was provided by all the patients before enrolment.

6.6.2 Trial Design and Treatment

Eligible patients were randomly assigned in a 1:1 ratio to the intervention group of inhaled therapies with best supportive care or to best supportive care alone. Randomisation was stratified for baseline use of short-acting bronchodilators. Randomisation was performed by the Institute for Cancer Research Clinical Trials Unit. Patients allocated to inhaled therapy were commenced on salbutamol (Ventolin™ Evohaler™) 100 micrograms; two puffs up to four times a day and tiotropium (Spiriva© Handihaler®) 18 mcg; one puff, once daily. Patients with an FEV_1 less than 50% predicted normal and a history of frequent exacerbations were additionally commenced on a long acting beta agonist and corticosteroid combination inhaler

(salmeterol/fluticasone (Seretide® Accuhaler® 50/500)); one puff, twice daily. Best supportive care was as per local guidelines to include pharmacological and non-pharmacological measures. Oral morphine solution was offered to all patients at a dose of 2.5 mg, as required at 4 hourly intervals if opiate naïve, with the dose increased to 5 mg if already on regular opiate medication.

6.6.3 End Points and Assessments

The primary end point was a 2-point or more decrease in VAS breathlessness at 4 weeks compared to baseline. Secondary end points included the change in 6-minute walk distance (6MWD) at 2 and 4 weeks from baseline, change in FEV_1 at 2 and 4 weeks from baseline, change in peak expiratory flow rate (PEFR) at 2 and 4 weeks from baseline, change in Medical Research Council (MRC) Dyspnoea score at 2 and 4 weeks from baseline and change in quality of life (QoL) assessments at 4 weeks from baseline. Global QoL and breathlessness were assessed using the European Organisation for Research and Treatment of Cancer Core Quality of Life Questionnaire with the addition of the Lung Cancer Module (EORTC C-30 and L-13). Physical activity was assessed with the St. George's Respiratory Questionnaire (SGRQ) activity scale.

Further secondary assessments included the relationship between VAS breathlessness and FEV_1 at baseline, 2 and 4 weeks, relationship between VAS breathlessness and tumour position at baseline and the relationship between VAS breathlessness and BODE index (a composite prognostic index in COPD comprising Body Mass Index (BMI), FEV_1, 6MWD and MRC Dyspnoea Score) at baseline, 2 and 4 weeks of treatment. Tumour position was defined as central if located proximal to the lobar bronchus, or peripheral if located in a segmental bronchus or beyond or was non-endobronchial, as determined by CT criteria and bronchoscopy reports where available.

All spirometry measurements were performed by an experienced operator as the best of three attempts. Measurements were taken at a minimum of 4 hours after bronchodilator therapy. Patients randomized to best supportive care alone, whose VAS increased by 2 points at the 2-week assessment were deemed to be treatment

failures and were offered COPD optimisation. At the end of the study period all patients had their symptoms and medications reviewed and were offered optimisation of their COPD management.

6.6.4 Statistical Analysis

The initial sample-size estimation for the primary efficacy analysis population (2-point decrease in VAS breathlessness at 4 weeks) was based on an expected response rate in the inhaled therapy arm of 40% and in the best supportive care alone group of 10%. We estimated that a sample size of seventy-two patients would provide the trial with 80% power to detect a difference in treatment effect on the primary outcome, with a two-sided significance of 5% (Fisher's Exact Test). Due to a slow rate of recruitment the protocol was amended, and the trial was closed after sixty-four patients had been recruited. With sixty-four patients, to maintain an 80% power to detect a treatment effect it would require a response rate in the primary end point in 42.1% of patients receiving optimised inhaled therapies and 10% for those receiving best supportive care alone. Patients who failed to complete the week 4 assessment were deemed non-responders.

All secondary end points were considered exploratory and a significance level of 1% was used. The between-group comparisons of change in 6MWD, FEV_1, PEFR, QoL assessments and MRC dyspnoea score were performed by means of t-test or Mann-Whitney test. The relationship between VAS breathlessness and FEV_1 was assessed with Pearson's correlation. The relationship between VAS breathless and tumour position was assessed using the Kruskal-Wallis test. The relationship between VAS breathlessness and BODE index was assessed with Spearman's correlation.

6.6.5 Patient & Public Involvement (PPI)

The ADOPT trial was conceived following a pilot study at the Royal Marsden Hospital, which found that 59% of patients with lung cancer undergoing chemotherapy and with no prior diagnosis of COPD, had symptoms and spirometry values consistent with COPD. Another key finding from this pilot study was the recurrent feedback from patients that breathlessness was a common and debilitating symptom and they

supported further research into treatments that could improve breathlessness associated with lung cancer.

6.7 Results

6.7.1 Patients and Treatment

From sixty-five patients enrolled to the study, sixty-four patients were randomized with one patient failing to meet the inclusion criteria of VAS dyspnoea ≥ 4. One patient was subsequently ineligible due to not having spirometry evidence of COPD. Of the sixty-three patients in the intention to treat population, thirty-two patients were randomised to the intervention arm (inhalers and best supportive care) and thirty-one patients randomised to the control arm of best supportive care alone. The consort diagram is shown in Figure 6.1. There were two patients in the intervention arm and three patients in the control arm withdrawn from the study prior to completing the week 4 assessment. One patient in the intervention arm withdrew from study due to drug-related toxicity (grade 2 insomnia, possibly related to tiotropium).

Among all the eligible patients, there were no significant differences in the baseline characteristics between the groups although there were numerically a higher proportion of current smokers in the control group than in the intervention group (26% vs. 13%) (Table 6.1). NSCLC was the most common histological subtype (84%) with 64% of these patients having locally advanced (Stage III 16/53 (30%)) or metastatic (Stage IV 18/53 (34%)) disease. Of the ten patients with small cell lung cancer (SCLC), 30% had extensive stage disease. The median baseline FEV_1 was 1.5 L and 63% (range 53-74%) of predicted normal.

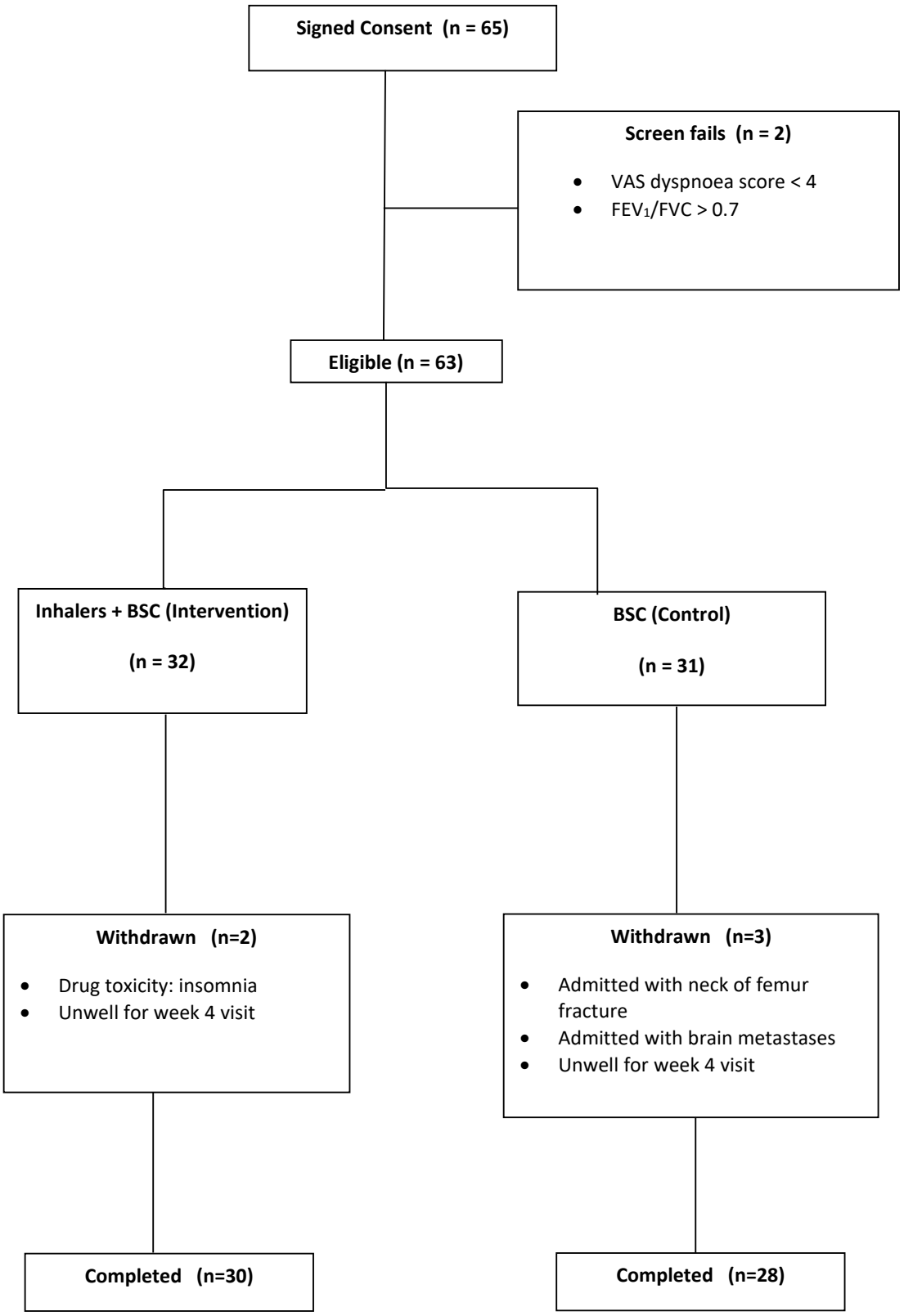

Figure 6.1: ADOPT Study Consort Diagram

	Inhalers + BSC (n = 32)	BSC Alone n = 31	All (n =63)
Female	21 (66)	19 (61)	40 (63)
Male	11 (34)	12 (39)	23 (37)
Age			
Median (IQR)	68 (59 - 75)	67 (61 - 71)	67 (60 - 73)
Smoking history			
Current smoker	4 (13)	8 (26)	12 (19)
Ex-smoker	28 (88)	23 (74)	51 (81)
ECOG Performance status			
0	1 (3)	0 (0)	1 (2)
1	25 (78)	25 (81)	50 (79)
2	6 (19)	6 (19)	12 (19)
Histology/Cytology			
NSCLC	28 (88)	25 (81)	53 (84)
SCLC	4 (12)	6 (19)	10 (16)
Mesothelioma	0 (0)	0 (0%)	0 (0)
Staging			
NSCLC: 1-2	7 (22)	12 (39)	19 (30)
3	11(34)	5 (16)	16 (25)
4	10 (31)	8 (26)	18 (29)
SCLC: Limited	3 (9)	4 (13)	7 (11)
Extensive	1 (3)	2 (6)	3 (5)
Treatment Paradigm			
Radical	16 (50)	13 (42)	29 (46)
Palliative	16 (50)	18 (58)	34 (54)
Prior Thoracic Surgery	9 (28)	13 (42)	22 (35)
Prior Thoracic Radiotherapy	15 (47)	16 (52)	31 (49)
Tumour position			
Large airway	14 (45)	10 (33)	24 (39)
Peripheral	17 (55)	20 (67)	37 (61)
VAS dyspnoea			
Median (IQR)	7.1 (5.4 - 7.7)	7.1 (4.9 - 7.7)	7.1 (5.2 - 7.7)
6MWD			
Median (IQR)	375 (325 - 450)	396.5 (333 - 450)	392 (325 - 450)
MRC dyspnoea			
Median (IQR)	3 (2 - 4)	3 (2 - 3)	3 (2 - 3)
BODE index			
Median (IQR)	3 (2 - 4)	3.5 (2.5 - 4.5)	3 (2 - 4)
FEV1 (L)			
Median (IQR)	1.5 (1.2 - 1.9)	1.5 (1.2 - 2.1)	1.5 (1.2 - 1.9)
FEV1 (% predicted normal)			
Median (IQR)	64 (53 - 74.5)	63 (53 - 73)	63 (53 - 74)
PEFR (L/min)			
Median (IQR)	198.5 (154 - 283)	204 (172 - 292)	204 (160 - 283)
QoL Global Health			
Median (IQR)	66.7 (50 - 75)	66.7 (50 - 83.3)	66.7 (50 - 75)
QoL Dyspnoea			
Median (IQR)	38.9 (33.3 - 44.4)	33.3 (22.2 - 55.6)	33.3 (22.2 - 44.4)
SGRQ Activity			
Median (IQR)	66.3 (53.5 - 79.1)	66.2 (47.7 - 73.2)	66.2 (53.5 - 76.4)

Table 6.1: Baseline characteristics of all patients in the intention to treat population. All figures as total number of patients (percentage) unless stated

IQR = Interquartile range; VAS = visual analogue scale (higher score indicates greater breathlessness); 6MWD = 6-minute walk distance; The Medical Research Council (MRC) dyspnoea score is assessed on a 5-point scale, with higher numbers indicating greater breathlessness; BODE index is a predictive scale for survival in patients with COPD, with higher scores having lower survival rates; QoL = Quality of life Questionnaire (EORTC C-30 and L-13 modules) - Global Health Score where a higher score indicates greater quality of life and Dyspnoea Score where a higher score indicates greater symptomatology; SGRQ = St. George's Respiratory Questionnaire Activity Score where higher score indicates greater limitation of activities due to breathlessness.

6.7.2 Efficacy

For the primary efficacy analysis population (2-point improvement in VAS breathlessness at 4 weeks from baseline) there was a significant difference in the proportion of responders in the intervention group compared to the control group. The response rate was 53% (95% confidence interval [CI]: 35% to 71%) in the intervention group and 26% (95% CI 12% to 45%) in the control group (p = 0.027) (Figure 6.2). The response rate after 2 weeks was 59% (95% CI: 41% to 76%) in the intervention group and 29% (95% CI: 14% to 48%) in the control group.

The improvement in FEV_1 was also significantly greater in the intervention group than the control group at 4 weeks from baseline (0.05L vs. -0.06L, p = 0.002). There was a strong tendency to improvement after 2 weeks (median change 0.09L vs. -0.01L, p = 0.016) although this did not meet statistical significance at the 1% level. Change in PEFR was also significantly greater in the intervention group than the control group at 2 weeks from baseline (median 21L vs. 4L, P= 0.009) however, the difference was not statistically significant at 4 weeks (median change 10.5L vs. -8.5L, p = 0.150).

There was no statistically significant difference between the treatment arms for change from baseline in 6MWD at 4 weeks (p = 0.766). The median improvement in 6-minute walk distance at 4 weeks from baseline was 28 metres (range -34m to 145m) in the intervention group and 34 metres in the control group (range -45m to 444m).

Quality of life measures for dyspnoea, global health or physical activity at 4 weeks from baseline were not improved by the addition of inhalers. Similarly, there was no significant difference between the groups for change in MRC dyspnoea score at either

2 or 4 weeks from baseline. The results for the secondary endpoint results are displayed in Table 6.2.

VAS breathlessness did not correlate with FEV_1 at baseline (r = -0.1, p = 0.39) (Table 6.3A). However, change in VAS at 2 weeks from baseline showed a moderate and significant correlation to change in FEV1 (r = -0.4, p = 0.002)(Table 6.3B); the correlation was not significant at 4 weeks from baseline (r = -0.2, p = 0.22) (Table 6.3C). There was only a weak correlation (r = 0.2, p = 0.25) between baseline VAS breathlessness and baseline BODE index although this was not significant. There was no significant correlation between change in VAS breathlessness and change in BODE index at 2 weeks from baseline (r = 0.3, p = 0.07) or at 4 weeks from baseline (r = 0.2, p = 0.32).

Tumour position had no significant effect on level of VAS breathlessness at baseline (p = 0.622). I therefore performed a post hoc analysis to assess if the addition of inhalers had similar effects in both patients with centrally located and peripheral tumours. A minimum two-point reduction in VAS dyspnoea at 4 weeks was seen in 43% (95%CI: 18% to 71%) for centrally located tumours in the intervention arm compared to 10% (95% CI: 0.3% to 45%) in the control arm. For patients with peripheral tumours the response rate in the intervention group was 65% (95% CI: 38% to 86%) and 30% (95% CI: 12% to 54%) in those receiving BSC alone. After logistic regression modelling, patients with central tumours were nearly seven times more likely to respond with the addition of inhalers (Odds ratio (OR) 6.8 95% CI: 0.7 to 68.8, p = 0.107) than with best supportive care alone although this did not meet statistical significance (Table 6.3 & Table 6.4). For patients with peripheral tumours the odds ratio to respond to intervention was 4.3 (95% CI: 1.1 to 17.0, p = 0.039), which was also not significant at the 1% level. Improvement in FEV_1 was also seen with the addition of inhalers in both patients with central tumours (adjusted mean change (SE) 0.05L (0.04) vs -0.03L (0.05), p = 0.268) and peripheral tumours (adjusted mean change (SE) 0.09L (0.04) vs -0.08L (0.04), p = 0.003) but was only significant for those with peripheral tumours. (Table 6.7)

I performed a further post-hoc subgroup analysis to assess if a treatment response was seen in both radically treatable and palliatively treated advanced disease. Patients being treated in a palliative paradigm were 3.3 times more likely to respond to inhalers with BSC than to BSC alone (OR: 3.3 95%CI: 0.8 to 13.6, p =0.094), while patients treated radically were over four times more likely to respond (OR: 4.3 95%CI: 0.7 to 25.9, p = 0.114), however these did not reach statistical significance. (Table 6.5 & Table 6.6)

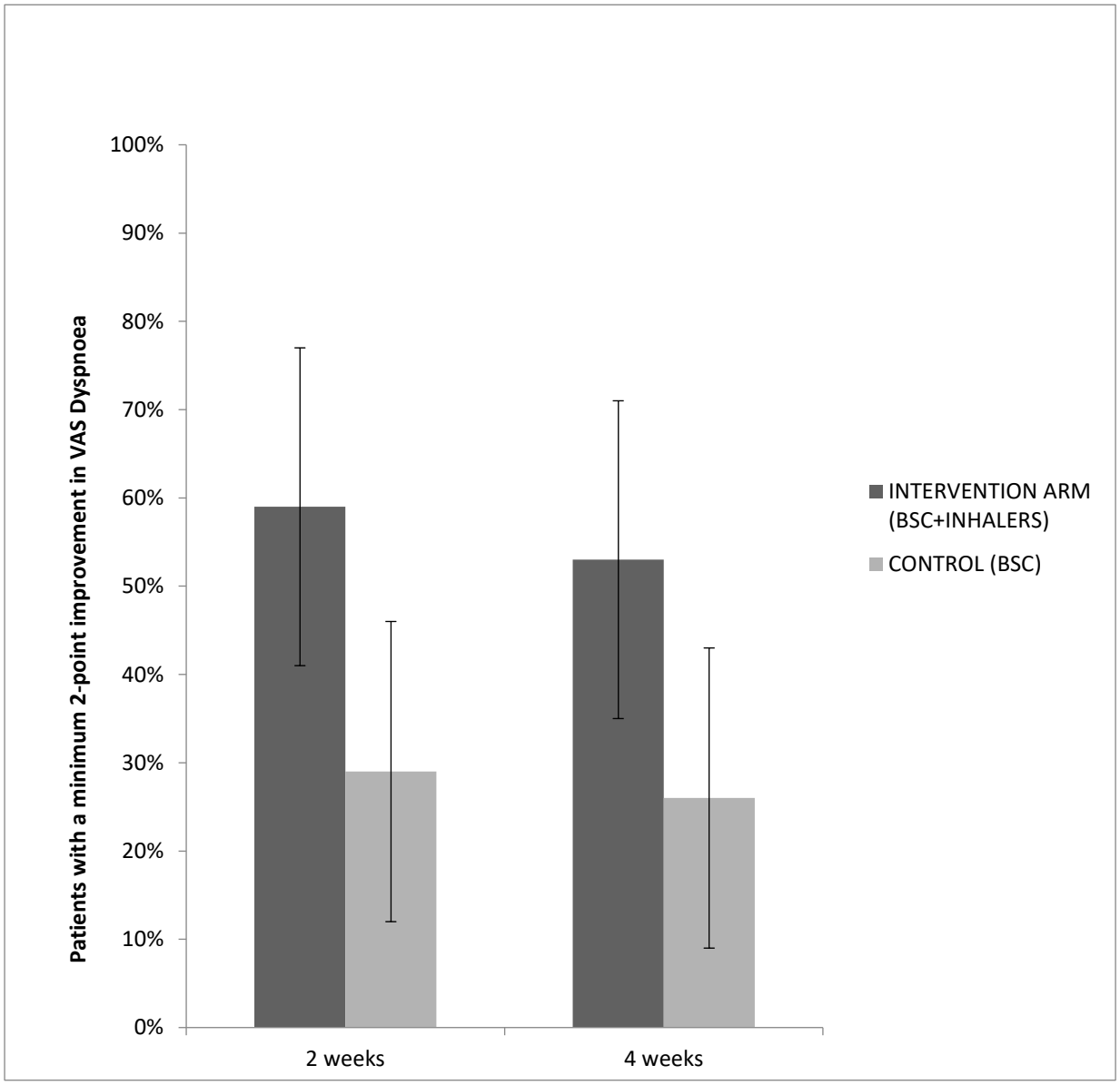

Figure 6.2: Response rate of a minimum 2-point improvement in VAS dyspnoea. Error bar denotes 95% confidence interval.

	BSC Alone	Inhalers + BSC	p-value
Median (range) Δ 6MWD (m) - 2 wk	+29.75 (-67 to +408)	+3 (-61 to +120)	0.246
- 4 wk	+ 34 (-45 to 444)	+28 (-34 to +145)	0.766
Median (range) Δ FEV_1 (L) - 2 wk	-0.01 (-0.59 to +0.75)	+ 0.09 (-0.33 to +0.65)	0.016
- 4 wk	-0.06 (-0.65 to +0.22)	+ 0.05 (-0.31 to +0.38)	0.002*
Median (range) Δ PEFR (L/min) - 2 wk	+4 (-100 to +69)	+21 (-89 to +114)	0.009*
- 4 wk	-8.5 (-65 to +164)	+ 10.5 (-67 to +100)	0.150
Median (range) Δ MRC Dyspnoea score - 2 wk	0 (-2 to 1)	0 (-2 to 0)	0.063
- 4 wk	0 (-2 to 1)	0 (-1 to 2)	0.620
Mean (SD) Δ QoL Dyspnoea Score at 4 wks	+0.51 (15.1)	-8.7 (15.8)	0.042
Mean (SD) Δ QoL Global Health Score at 4 wks	-0.30 (18.2)	-0.57 (19.7)	0.956
Median (range) Δ SGRQ Activity Score at 4 wks	-0.02 (-47.7 to +16.2)	-6.74 (-36.8 to +60.5)	0.392

Table 6.2: Secondary endpoint analysis of median change (Δ) in 6-minute walk distance (6MWD), FEV_1, PEFR and MRC Dyspnoea score after 2 and 4 weeks of treatment. Mean change in quality of life (QoL) scores at 4 weeks from baseline using EORTC C-30 and L-13 modules for Dyspnoea Score where a higher score indicates greater symptomatology and for Global Health Score where a higher score indicates greater quality of life; Median change in SGRQ = St. George's Respiratory Questionnaire Activity Score where higher score indicates greater limitation of activities due to breathlessness. Statistical significance was considered to be at the 1% level.

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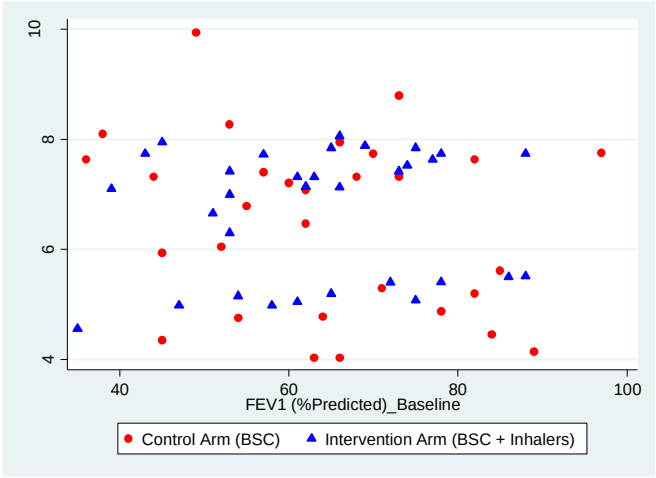

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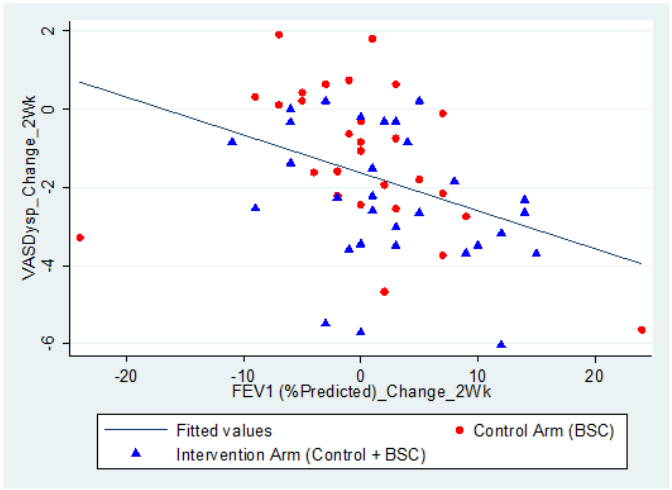

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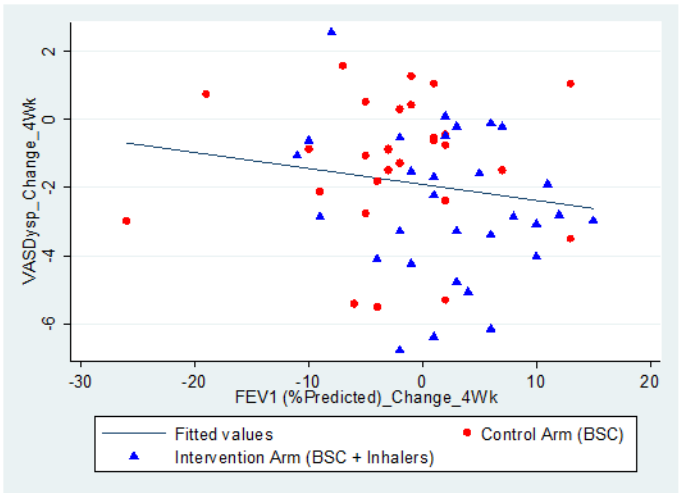

Figure 6.3: Relationship between VAS and FEV_1 percentage of predicted normal at baseline (r = -0.1, *p* = 0.39) (A). Figure B and C show the relationship between change in FEV_1 percentage of predicted normal and change in VAS at 2 and 4 weeks respectively. The fitted line in B represent best fit correlation for all patients after 2 weeks treatment (r= -0.4, *p* = 0.002) and in C after 4 weeks (r = -0.2 *p* = 0.22)

Tumour Position – Central i.e. Large Airway			
	BSC alone	**Inhalers + BSC**	**All patients**
Response	1	6	7
Non-response	9	8	17
Total	10	14	24
Response rate (95% CI)	10% (95% CI: 0.3% to 45%)	43% (95% CI: 18% to 71%)	29% (95% CI: 13% to 51%)
Tumour Position - Peripheral			
	BSC alone	**Inhalers + BSC**	**All patients**
Response	6	11	17
Non-response	14	6	20
Total	20	17	37
Response rate (95% CI)	30% (95% CI: 12% to 54%)	65% (95% CI: 38% to 86%)	46% (95% CI: 29% to 63%)

Table 6.3: VAS Dyspnoea response by tumour position and treatment arm. BSC = Best Supportive Care

Tumour position sub-group: Central (Large Airway)	**Odds Ratio (95% CI)**	***p*-value**
Treatment Arm: Control (BSC) Intervention (BSC + Inhalers)	 1 6.8 (0.7 – 68.8)	 0.107
Tumour position sub-group: Peripheral	**Odds Ratio (95% CI)**	***p*-value**
Treatment Arm: Control (BSC) Intervention (BSC + Inhalers)	 1 4.3 (1.1 – 17.0)	 0.039

Table 6.4: Logistic regression model fitted for response outcome, adjusted by treatment arm for tumour position sub-groups. BSC = Best Supportive Care

Treatment Paradigm - Radical			
	BSC alone	**Inhalers + BSC**	**All patients**
Response	2	7	9
Non-response	11	9	20
Total	13	16	29
Response rate (95% CI)	15% (95% CI: 2% to 45%)	44% (95% CI: 20% to 70%)	31% (95% CI: 15% to 51%)
Treatment Paradigm - Palliative			
	BSC alone	**Inhalers + BSC**	**All patients**
Response	6	10	16
Non-response	12	6	18
Total	18	16	34
Response rate (95% CI)	33% (95% CI: 13% to 59%)	63% (95% CI: 35% to 85%)	47% (95% CI: 30% to 65%)

Table 6.5: VAS Dyspnoea response by treatment paradigm and treatment arm. BSC = Best Supportive Care

Treatment paradigm sub-group: Palliative	**Odds Ratio (95% CI)**	**p-value**
Treatment Arm: Control (BSC) Intervention (BSC + Inhalers)	 1 3.3 (0.8 – 13.6)	 0.094
Treatment paradigm sub-group: Radical	**Odds Ratio (95% CI)**	**p-value**
Treatment Arm: Control (BSC) Intervention (BSC + Inhalers)	 1 4.3 (0.7 – 25.9)	 0.114

Table 6.6: Logistic regression model fitted for response outcome, adjusted by treatment arm for treatment paradigm sub-groups. BSC = Best Supportive Care

	BSC Alone	Inhalers + BSC	p value
Central: Adjusted mean Δ FEV_1 (SE) at 4 weeks	-0.03 (0.05)	0.05 (0.04)	0.268
Peripheral: Adjusted mean Δ FEV_1 (SE) at 4 weeks	-0.08 (0.04)	0.09 (0.04)	0.003
Radical: Adjusted mean Δ FEV_1 (SE) at 4 weeks	-0.09 (0.06)	0.04 (0.05)	0.095
Palliative: Adjusted mean Δ FEV_1 (SE) at 4 weeks	-0.07 (0.04)	0.08 (0.04)	0.018

Table 6.7: Adjusted means (with standard error) after running analysis of covariance model, for change in FEV1 at 4 weeks from baseline reported by treatment arm and by tumour position (central/peripheral) or treatment paradigm sub-group (palliative/radical). BSC = Best Supportive Care; FEV_1 = forced expiratory volume in 1 second

6.8 Discussion

In this randomized, controlled study of patients with co-existing or newly diagnosed COPD and lung cancer, the addition of inhaled therapies for 4 weeks led to an improvement in patient-reported breathlessness, as evidenced by a minimum 2 point improvement in VAS dyspnoea in significantly more patients than in those that received best supportive care alone. A previously reported secondary endpoint from an interim analysis of this trial showed an increased proportion of patients with a 1-point improvement in VAS breathlessness at 4 weeks from baseline by the addition of inhaled therapies. My findings go further and provide more compelling evidence for the use of inhalers in this patient group.

The VAS is a validated assessment tool that is ideally suited to within-subject comparisons. While few studies have evaluated VAS in terms of minimal clinical important difference, it is generally accepted that a 10% change (or 1-point reduction) in VAS is indicative of a meaningful clinical improvement.[7] A 2-point reduction was chosen in this study as for this population, with potentially multifactorial breathlessness, we felt a large difference would need to be demonstrated to change practice. In our study, 53% of the patients who had their COPD medications optimised had a clinically significant improvement in subjective breathlessness after 4 weeks compared to only 26% in the control arm. Both treatment arms outperformed our predictions. The high response rate in the control arm demonstrates the importance of inclusion of a control arm and of the benefits achieved by anti-cancer therapies.

FEV_1 was also significantly increased by inhalers compared to best supportive care alone after 4 weeks use. While the mean size of the increase was modest (50ml) and less than the accepted minimal clinically significant threshold of 100ml,[24] the ability to maintain lung function that would otherwise decline may be the important role of inhalers in this patient group. PEFR was significantly higher after 2 weeks inhaler use and with a strong trend towards a greater change after 4 weeks. In the exploratory analysis, the change in VAS was moderately negatively correlated to the change in FEV_1. This relationship was strongest after 2 weeks of treatment ($r = -0.4$). This spirometry data supports the hypothesis that patients' perception of breathlessness improved due to an increase in their lung function rather than a potential placebo effect

from the inhaler use. In this study, the use of sham-inhalers for the control group was deemed not ethically appropriate. Our findings support a previous single arm study by Congleton and Muers that showed FEV_1, PEFR and perception of breathlessness could be improved by the use of inhalers in lung cancer patients with obstructive spirometry.[6] It would thus appear that VAS at 2 or 4 weeks is a simple measure and an effective tool to assess dyspnoea in future trials and correlates with spirometry.

The improvement that patients receiving inhalers gained in subjective breathlessness and spirometry did not translate into improved exercise tolerance, as measured by the 6-minute walk distance. The 6-minute walk test is a commonly used assessment tool of sub-maximal exercise capacity in patients with cardiorespiratory disease and correlates well with a patient's functional exercise tolerance.[10,11] Short-term bronchodilators have been shown to improve the 6MWD in patients with COPD.[1] In one study, inhaled corticosteroids improved the 6MWD by a mean of 33 m.[19] However, this fell below the accepted minimum 50 m required to meet a clinically meaningful improvement.[1] Our findings are in keeping with previous studies that have shown that the addition of tiotropium has minimal impact on exercise tolerance.[2] Other treatment modalities such as pulmonary rehabilitation may be more effective at improving exercise capacity.[26]

Quality of life measures were also not improved by the addition of inhaled therapies. The short observation period of 4 weeks in this study may be responsible for not identifying a treatment effect. In addition, the intrinsic effects of lung cancer and related treatments are likely to be the largest contributors to these measures. While this study endeavoured to treat all other underlying reversible causes, it may not be surprising that the addition of inhalers could not impact significantly on these more global measures in the short observation period. Previous studies of the impact of inhalers to improve quality of life have been over longer time periods,[21] typically of up to a year of follow up, and would be more likely to incorporate the prolonged benefits of stabilising the patient's COPD. In keeping with previous studies,[4,5,12,22] we observed a very low rate of adverse events with only one patient discontinuing the trial medication due to insomnia, making the use of inhalers a safe and beneficial treatment in this patient group.

Of note, all the patients recruited to this trial were of COPD GOLD stage 2, indicating only moderate airflow limitation. The optimisation of COPD medications is likely to have a larger impact on patients with more severe airflow obstruction. Patients identified to have more severe airway obstruction would be more likely to receive maximum inhaled therapy immediately rather than entering a trial that could delay this by a minimum of 2 weeks.

The trial was slow to recruit reflecting a trend towards wider prescribing of long-acting bronchodilators by primary care physicians in recent years and thus a lack of eligible patients. Since this study completed, the national and international guidelines for the management of COPD have been updated with a greater emphasis on dual bronchodilator therapy and a trend away from inhaled corticosteroids, unless there is a clear asthmatic component or frequent exacerbations.[10] Combination long-acting beta agonist and antimuscarinic (LABA/LAMA) inhalers have a greater impact on FEV_1 and patient symptoms than monotherapy devices,[8] suggesting that greater treatment effects may be seen in patients treated in accordance with the updated guidance.

This study was limited by the heterogeneous population that included both radically treatable patients and those with advanced disease. However, this is typical of the range of cases of breathlessness that are seen in the oncology outpatient clinic. While the subpopulations were not powered sufficiently to show clinical significance there was a strong tendency towards inhaler therapy being of benefit in both radically treatable and advanced disease patients, treated palliatively.

There was a strong tendency for patients with both peripherally and centrally located tumours to be more likely to improve their breathlessness by the addition of inhaled therapies. FEV_1 was significantly improved in patients with peripheral tumours and to a non-significant level in patients with central tumours. This supports the hypothesis that it is the underlying COPD contributing to the breathlessness rather than physical obstruction from a central mass. A larger study of centrally located tumours would be necessary to clarify this statistically, however I suggest that from these results a pragmatic approach should be taken and all patients with evidence of COPD and lung cancer should be treated with the appropriate inhaled therapies.

Together, these findings highlight the importance of identifying co-existing COPD in all lung cancer patients and treating it appropriately to improve the symptom of breathlessness. Although not assessed in this study, other potential benefits of optimising COPD treatment for this patient group may include reduced frequency of COPD exacerbations and fewer interruptions to anti-cancer therapies. A proportion of patients will not undergo spirometry as part of their diagnostic work-up and COPD should therefore be considered in the differential diagnosis of breathlessness in lung cancer patients. With recent advances and new treatment modalities in lung cancer, patients may develop COPD while under the care of the oncology team and this treatable cause of breathlessness should be identified and treated. The findings from this study would support a firm recommendation in the guidelines for the optimal lung cancer diagnostic pathway, to include spirometry assessment in all patients, not only those investigated within a radical test bundle. Lung cancer clinics provide a diagnostic opportunity to screen patients for the possibility of underlying COPD, a treatable cause of breathlessness in many patients with lung cancer.

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Chapter 7: **Conclusions and Recommendations**

In this thesis I have explored the significance that impaired lung function has on patients with lung cancer. The focus of this thesis has been on the management of patients with NSCLC, although many of my findings will be translatable across tumour types. Impairment in lung function is a common finding in patients with lung cancer and it presents challenges for delivering optimal management.

Impairment in lung function is one of the most frequent medical reasons why patients with early stage NSCLC are not offered the gold-standard of surgery. Stereotactic Body Radiotherapy (SBRT) is the treatment of choice for inoperable patients with early stage NSCLC without nodal spread and hence it is frequently used in patients with impaired lung function. Outcomes from SBRT appear to be inferior compared to surgical options of lobectomy or wedge resection. In a series of experiments, I have provided insights to help predict outcomes from SBRT and have built on the evidence for combining SBRT with adjuvant immunotherapy.

7.1 Radiotherapy

In a retrospective analysis of 187 patients with early stage NSCLC treated with SBRT, I found the overall median survival to be 30 months and that the 2-year survival rate to be 69%. Symptomatic pneumonitis within 6 months of treatment was a rare event, occurring in less than 10% of cases. Reassuringly, there was no association between pre-treatment lung function parameters (FEV_1 or TLCO) and rates of symptomatic pneumonitis. Patients who had undergone mediastinal staging appeared more likely to develop pneumonitis, although the number of cases who underwent this were small and therefore may be subject to confounding factors. If there is an increase in the use of invasive mediastinal staging prior to SBRT in everyday practice, then its relationship with the development of pneumonitis should be studied further. I found pre-treatment TLCO was negatively associated with overall survival following SBRT (HR: 0.98 (0.97 – 0.99, p = 0.010) as was increased tumour size (HR: 1.03 (1.01 – 1.06) p = 0.010)

and increased age (HR: 1.05 (1.01 – 1.09)*p* = 0.023). Part-solid or pure ground glass lesions were associated with better overall survival than solid nodules (HR: 0.48 (0.24 – 0.93) *p* = 0.031). Increased tumour size was the only factor associated with time to relapse (HR: 1.05 (1.02 – 1.08) *p* = 0.003). I found lack of expression of PD-L1 on lung cancer biopsy samples from patients who received SBRT (TPS <1%) more commonly than in the reported figures in advanced NSCLC. This has not previously been reported, although there are similar findings from surgically resected early stage NSCLCs. Notably, I found a tendency for patients whose tumours had positive expression of PD-L1 (TPS >1%) to be more likely to suffer symptomatic pneumonitis. Thus, I conclude that patients with larger tumours and those with PD-L1 expression may potentially benefit from adjuvant immunotherapy following SBRT, however they may also be particularly at risk of developing significant pneumonitis. Patients with lower TLCO have a worse prognosis although it is unclear if this is specific to the SBRT treatment or simply a poor prognostic marker in chronic lung disease.

7.2 Radiotherapy / Immunotherapy Combination

To explore the potential for adjuvant immunotherapy after SBRT further, I helped develop a phase 1b/II study of adjuvant nivolumab following SBRT for early stage NSCLC, the STILE trial. In chapter 3, I presented the findings from the first five patients enrolled in the study, who had completed 3 months follow-up post SBRT. Recruitment to this study was slow due to the trial initially only being open to patients with ECOG performance status < 2. After 3 months of follow up, there was no episodes of grade 3 pneumonitis, the primary endpoint in this study. The toxicity profile seen so far in this trial is as expected from the published data with the use of nivolumab in advanced NSCLC. Following the first Independent Data Monitoring Committee (IDMC) review the trial has been expanded to include patients with ECOG performance status 2. This should facilitate an increase in the recruitment rate to the study and ultimately provide robust evidence as to the safety of adjuvant nivolumab following SBRT. A run-in phase 3 study could then attempt to clarify if adjuvant immunotherapy after SBRT can improve outcomes for patients ineligible for surgical resection. The impact on lung function from combining nivolumab with SBRT is not yet established. From the early results of five patients, one patient did show a deterioration in spirometry values, with

both FEV_1 and FVC declining on study. The decline was most precipitous earlier in her treatment. Close monitoring of lung function parameters is paramount in this study to ensure that the proposed treatment regimen does not cause unacceptable lung toxicity.

To complement the STILE trial, I undertook translational work on NSCLC biopsy samples from patients enrolled in another radiotherapy/immunotherapy combination trial, the PEAR study. Despite the small volume of tissue collected from predominantly CT-guided lung biopsies or bronchoscopy biopsies, which had been heavily pre-used for diagnostic and translational studies, I was able to successfully extract and purify RNA from the tumour microenvironment. Each section was macrodissected to include only relevant tissue and to reduce the noise in the assay. Despite the RNA being highly degraded, by using NanoString technology I was able to perform counts of 800 genes relevant to the relationship between cancer and the immune system from the tumour microenvironment of my samples. I subsequently compared baseline gene counts between responders and non-responders in the PEAR study. This research was intended to be exploratory and hypothesis generating due to the small sample size and unequal treatments between patients. However, I found several signals which support previous evidence on the mechanisms behind the interaction of radiotherapy and the immune system. I found that there did appear to be a different immunogenetic profile between responders and non-responders. Notably, gene counts related to MICA and IFNβ tended to be lower in the baseline biopsies of responders. Through estimated cell counts, exhausted CD8 cells appeared relatively more abundant in non-responders. Together these findings lead to the hypothesis that a less exhausted immune tumour microenvironment may be the most likely to respond to combination radiotherapy and immunotherapy regimens and that this is mediated through the interferon-1 pathway. Further comparative trials, using larger sample sizes and including sequential biopsies is justified following this study.

7.3 Chemotherapy

In advanced NSCLC, I investigated patient and tumour markers that may predict for tolerance and survival with the use of the second-line chemotherapy agent, docetaxel. In a retrospective review of fifty-two patients who underwent 160 cycles of docetaxel I found that FEV_1 was the factor most associated with overall survival (HR: 0.96 (0.93 - 0.99 p = 0.009). Patients with an FEV_1%pred less than 50% had significantly worse survival (HR: 0.15 (0.04 – 0.57 p =0.005) and were also more likely to discontinue treatment due to toxicity (p = 0.023). Use of G-CSF did not significantly reduce the rate of febrile neutropaenia when given alongside docetaxel for advanced NSCLC and its use as standard of care is not supported. My finding that FEV_1 is the best predictor of overall survival with second line docetaxel, if validated in a prospective trial, would support the use of spirometry as a simple test to guide oncologists and patients as to the expected benefit from undergoing treatment.

7.4 Symptom Control

Finally, I have presented the findings from an open-label, controlled trial which found that the addition of inhaled therapies improves breathlessness over best supportive care (BSC) alone in patients with co-existing COPD and lung cancer. A minimum 2-point improvement in VAS breathlessness at 4 weeks was achieved in 53% (95% CI: 35 -71) of those receiving inhaled therapies compared to 26% (95% CI:12 -45) in the group that received BSC alone (p = 0.027). There was strong evidence that this effect was due to bronchodilation as a significant difference was observed in the change in FEV_1 after 2 weeks of treatment (median change 0.09L (inhalers) vs. -0.01L (BSC), p = 0.016). Although statistical significance was not achieved in the post-hoc analyses, it appears that this effect is seen for patients in both radical and palliative treatment paradigms. With this study I have highlighted the importance of using the lung cancer clinic as an opportunity to diagnose COPD and that inhaled therapies are an effective treatment in reducing breathlessness in this patient group.

7.5 Recommendations

In this thesis I have added to the evidence that SBRT can be used safely in patients even with significantly impaired lung function. In line with previous studies,[2,3] I have found that the rate of symptomatic radiation pneumonitis following SBRT was not associated with pre-treatment FEV_1 or TLCO. I found that a lack of expression of PD-L1 on early stage tumour biopsies was more common than the reported data in the metastatic disease setting.[1] This may be important, as I found a tendency towards a higher rate of pneumonitis in patients undergoing SBRT in those whose biopsies had higher expression of PD-L1. Future work should look to confirm this novel finding as it may be particularly relevant when combing SBRT with immunotherapies, as in the STILE trial. A phase 3 trial of combining SBRT and nivolumab may have sufficient patient numbers to establish if PD-L1 expression on pre-treatment biopsies impacts on treatment efficacy and toxicity. It is imperative that such studies incorporate sequential tumour tissue biopsies and blood sampling to further our understanding of the interplay between the patient's immune system and cancer development and progression. A greater understanding of the impact that combining radiotherapy with immunotherapies has on the immunology may lead to the development of new treatment targets and improved patient selection.

I have shown through this thesis the relationship between lung function test results and outcomes for patients undergoing NSCLC anti-cancer treatments. These simple measures are rarely considered by oncologists when making treatment decisions. I postulate that in this context, it is probable that the impaired lung function is predominantly a marker of patient frailty rather than a direct effect of the underlying lung pathology. However, as an objective measure, it has advantages over other techniques such as grip strength as it can also help diagnose chronic lung conditions such as COPD. Significantly, I have shown for the first time that treating undiagnosed COPD in patients with lung cancer can significantly improve breathlessness over best supportive care alone. Routine regular spirometry in oncology lung cancer clinics is a feasible, low-tech, repeatable measurement that would allow prospective collaboration of my findings and would provide valuable information to treating physicians.

After drawing conclusions from the studies within this thesis, I make the following recommendations:

- Low TLCO is a poor prognostic indicator for patients undergoing SBRT

- Tumour size is the most important factor for predicting relapse following SBRT. Thus, adjuvant immunotherapy trials following SBRT should focus on the largest tumours in order to maximise the potential of seeing a beneficial effect

- The early data from a trial combining the immunotherapy agent nivolumab with SBRT suggests that severe unacceptable lung toxicities are probably less than 20%, but more data is needed including patients with ECOG performance status 2

- NanoString provides an effective technique to quantify the immune makeup in NSCLC, even from small, FFPE biopsy samples and degraded RNA.

- The immune makeup of the tumour microenvironment may help to predict responses to combination immunotherapy and radiotherapy treatment regimens, in particular the MICA/MICB and interferon pathways.

- An FEV_1 less than 50% predicted is a poor prognostic marker in patients being considered for docetaxel chemotherapy for advanced NSCLC.

- There is no indication for the routine use of G-CSF as primary prophylaxis for febrile neutropaenia in patients receiving docetaxel for advanced NSCLC

- Spirometry performed in the lung cancer clinic can identify undiagnosed COPD and treating this with inhaled therapies improves breathlessness.

7.6 References

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