RESEARCH PROTOCOL

HIGH **I**NTENSITY **F**UNCTIONAL **I**MAGE GUIDED **V**MAT LUNG **E**VASION

Abbreviated Title: HI-FIVE

Coordinating Principal Investigator: Dr Nicholas Bucknell ∑
Supervisor: A/Prof Shankar Siva ∑

Associate Investigators:

Dr Nicholas Hardcastle >	Dr Price Jackson >	Prof Tomas Kron >
Ms Rhonda Lawrence ∑	Mr Nigel Anderson ∑	Ms Lisa Selbie ∑
A/Prof Michael Hofman ∞	Dr Amir Iravani ∞	Dr Beverley Woon ∞
A/Prof Olga Martin ∑	Mr Mathias Bressel ∏	Prof Benjamin Solomon #
A/Prof Daniel Steinfort £	A/Prof Douglas Johnson *	Dr Benjamin Blyth ∑
Dr William Wilson £	Prof David Ball ∑	Prof Rod Hicks ∞

Short title:	HI-FIVE Phase II protocol
Protocol number:	1
Protocol version:	1.0
UTN:	U1111-1208-1546
HREC reference number	HREC/18/PMCC/23
Date submitted:	03/04/2018
Sponsor:	Peter MacCallum Cancer Centre

PRINCIPAL INVESTIGATOR DETAILS:
Nicholas Bucknell,
Department of Radiation Oncology
Peter Maccallum Cancer Centre
Phone: +61385595000 Fax: +61 385597729
Email: **nick.bucknell@petermac.org**

∑Department of Radiation Oncology, Peter MacCallum Cancer Centre, Melbourne Australia
> Department of Medical Physics, Peter MacCallum Cancer Centre, Melbourne Australia
∞ Department of Cancer Imaging, Peter MacCallum Cancer Centre, Melbourne Australia,
£ Department of Medicine, Royal Melbourne Hospital, Melbourne Australia
* Department of Medicine, Austin Hospital, Melbourne Australia
# Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne Australia
∏ Department of Biostatistics and Clinical Trials, Melbourne Australia

FOREWORD

This document contains confidential information. This information should not be disclosed, other than to those involved in the execution or ethical review of the study, without written authorisation from sponsor.

COMPLIANCE STATEMENT

This trial will be conducted in accordance with the protocol, International Conference on Harmonization (ICH) and Good Clinical Practice (GCP). In addition, the trial will be conducted in compliance will all applicable laws and regulatory requirements relevant to the use of new diagnostic agents (Galligas and ^{68}Ga-MAA) for V/Q PET in Australia and any other participating country. Agreement of the investigator(s) to conduct and administer this trial in accordance with the protocol and associated regulations will be documented in the trial agreements with the Sponsor and other forms required by national authorities in the country where the trial site is located.

The Investigator(s) is responsible for ensuring the privacy, safety and welfare of the patients during and after the trial.

The Principal Investigator at each site has the overall responsibility for the conduct and administration of the trial at their site, and for conduct with the trial site management, the Independent Ethics Committee (IEC) / Institutional Review Board (IRB), and local authorities.

VARIATIONS TO THE PROTOCOL

No changes from the final approved (signed) protocol will be initiated without the ethics committee's prior written approval of favourable opinion of a written amendment, except when necessary to eliminate immediate hazards to the patients or when the change involves only the logistics or administration.

PROTOCOL HISTORY

Version No	Date	Author	Reason
1.0	03/04/18	Nicholas Bucknell	

Bucknell N, *et al. BMJ Open* 2020; 10:e042465. doi: 10.1136/bmjopen-2020-042465

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Principal Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the NHMRC's *National Statement on Ethical Conduct of Research in Humans*, the TGA's *Clinical Trial Handbook*, Good Clinical Practice, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature	Date
Name (please print)	
Position	

Principal Investigator:

Signature	Date
Name (please print)	

Bucknell N, *et al. BMJ Open* 2020; 10:e042465. doi: 10.1136/bmjopen-2020-042465

ABBREVIATIONS

^{99m}Tc	Technetium-99m
^{68}Ga	Gallium-68
4DCT	Four dimensional computed tomography
AUC	Area under the curve
BaCT	Centre for Biostatistics & Clinical Trials
Boost	Use of a Simultaneous Integrated Boost technique, where a higher dose per fraction is given to a defined sub-volume of the treated region for the entire overall length of treatment.
CRF	Case Report Forms
CTCAE	Common Terminology Criteria for Adverse Events
CTV	Clinical Target Volume
DECT	Dual Energy Computed Tomography
DLCO	Monoxide diffusion capacity of the lung
ECG	Electrocardiogram
Echo	Transthoracic echocardiogram
ECOG	Eastern Co-operative Oncology Group
FBE	Full blood examination
FDG-PET	Fluorodeoxyglucose – Positron Emission Tomography
FDG-PET-CT	FDG-PET computed tomography
FEV1	Forced expiratory volume over 1 second
fMLD	Mean dose (in Gy) received by the functional lung subvolume
Functional lung	The volume of anatomical lung tissue with a specific unit value (SUV) of ≥ 30% of the maximum threshold for ventilation and/or perfusion as defined on ^{68}Gallium ventilation-perfusion 4D PET/CT
fV5	Volume of lung (percentage) receiving ≥ 5 Gy
fV20	Volume of lung (percentage) receiving ≥ 20 Gy
fV30	Volume of lung (percentage) receiving ≥ 30 Gy
FVC	Forced vital capacity
Galligas	Gallium-68 aerosol
GCP	Good Clinical Practice
GTV	Gross Tumour Volume
Hb	Haemoglobin
HREC	Human Research Ethics Committee
HRQOL	Health-related Quality of Life
IB	Investigator's Brochure
IDSMC	Independent Safety Data Monitoring Committee
IGTV	Internal Gross Tumour Volume – a radiotherapy volume that takes the gross extent of the tumour on imaging in addition to tumour motion through respiration
ITV	Internal Target Volume – a radiotherapy volume that takes into account the gross extent of tumour, tumour motion and a margin for subclinical spread
MAA	Macroaggregated albumin
MDT	Multi-Disciplinary Team
MLD	Mean dose (in Gy) received by the anatomical lung subvolume
NSCLC	Non small cell lung cancer
OS	Overall survival
PET/CT	Positron emission tomography / computed tomography
PFTs	Pulmonary function tests

PICF	Patient Information Sheet and Consent Form
PTV	Planning Target Volume – a radiotherapy volume that takes into account the ITV in addition to an additional margin to ensure the prescribed dose is given to the ITV, taking into account the physical uncertainties in planning or treatment delivery.
QA	Quality Assurance
Reduction in functional lung volume irradiated	The reduction in the volume of functional lung receiving a significant dose of radiation expressed using the functional dose metrics: fV5, fV20, fV30 and fMLD.
RT	Radiation Therapy
RTP	Radiotherapy Treatment Planning
SAE	Serious Adverse Event
SPECT/CT	Single-photon emission computed tomography/computed tomography
SUSAR	Serious unexpected suspected adverse events
SUV	Standard uptake value
Technegas	Technetium-99m aerosol
TLC	Total lung capacity
TMC	Trial Management Committee
VMAT	Volumetric Modulated Arc Therapy
V/Q	Ventilation/Perfusion
V/Q PET/CT	^{68}Gallium ventilation-perfusion PET/CT
V5	Volume of lung (percentage) receiving ≥ 5 Gy
V20	Volume of lung (percentage) receiving ≥ 20 Gy
V30	Volume of lung (percentage) receiving ≥ 30 Gy

TABLE OF CONTENTS

1. SYNOPSIS

Title: High **I**ntensity **F**unctional **I**mage Guided **V**mat Lung **E**vasion **Short title:** HI-FIVE
Sponsor: Peter MacCallum Cancer Centre **Study design:** Single arm interventional pilot study
Background and rationale: Radiotherapy has an essential role in the curative treatment of locally advanced lung cancer however radiation dose to delivered to healthy lung can result in radiation induced lung injury. This can results in significant treatment related morbidity with symptomatic pneumonitis occurring in 1 in 3 patients and fatal pneumonitis occurring in 2% of these patients.[1,2] This risk of damage to the lungs limits the dose that can be safely delivered and as a result local failure occurs in 1 in 3 patients. Radiation induced lung injury physiologically manifests as reduction in air-flow (ventilation) and blood flow (perfusion).[1] Functional lung imaging using 68Ga 4D V/Q PET has enhanced our understanding of underlying lung function and enables personalised lung radiotherapy.[3-8] Functional lung can be now be defined using this imaging technique before radiotherapy commences and planning studies have demonstrated this allows significant reductions in dose to functioning lung.[5,6] CT is already a standard of care imaging test used in radiotherapy planning and treatment response assessment. CT ventilation and dual energy CT iodine mapping (as a surrogate for pulmonary perfusion) may be a future useful tool in as an alternative to V/Q PET/CT to expand access to functional lung radiotherapy planning without the need for additional investigations. Volumetric Modulated Arc Therapy (VMAT) is an advanced radiotherapy planning and delivery technology that now makes it technically possible to increase dose to tumour while reducing dose to normal tissues.[9] Phase 3 dose-escalation trials to date have failed to improve overall survival and have demonstrated increased rates of normal tissue toxicity.[2,10,11] Using advanced techniques such as VMAT functionally adapted radiotherapy may enable safe moderate dose escalation with an aim of improving local control and concurrently decrease treatment related toxicity.
Study Objectives **Primary Objective:** To assess the technical feasibility of the delivery of personalised functional lung radiotherapy. This study will be considered feasible if all feasibility crieteria defined within the protocol are achieved for ≥15 out of 20 patients. **Secondary Objectives** 1. To determine the incidence of grade ≥ 2 clinical or radiological pneumonitis after high dose functionally adapted radiotherapy 2. To determine the incidence of grade ≥ 2 acute and late toxicities 3. To quantify regional ventilation loss and regional perfusion loss on post treatment V/Q PET/CT following functionally adapted lung radiotherapy and its relationship to respiratory function testing 4. To assess the relationships of cytokine release in patient's plasma with grade ≥ 2 radiation pneumonitis 5. To assess the associations; a) Ventilation PET/CT with inhale/exhale CT ventilation b) Perfusion PET/CT with dual energy CT iodine mapping (a surrogate for pulmonary perfusion) 6. To assess patient reported quality of life outcomes using the FACT-L quality of life questionnaire 7. To assess incidence of complete metabolic response on 3 month post treatment FDG-PET/CT 8. To assess progression free survival at 12 months following completion of trial radiotherapy (defined by RECIST 1.1) 9. To assess overall survival at 12 months following completion of trial radiotherapy
Number of sites: 2 - Parkville and Sunshine campuses **Recruiting Period:** 2 years **Sample Size:** 20 patients stage IIIa-c non-small cell lung cancer for curative intent radiotherapy +- chemotherapy +- adjuvant immunotherapy

Interventions: All patients will receive functional lung adapted 60 Gy in 30 fractions to the primary and nodal planning target volume with a simultaneous integrated boost to the primary tumour to a total dose of 69Gy in 30 fractions.

Inclusion Criteria

- Age ≥ 18 years;
- Written informed consent has been provided.
- Histologically or cytologically confirmed Non-Small Cell Lung Cancer
- ECOG performance status 0-2 within 2 weeks prior to registration (see **appendix 2**)
- Locally advanced disease (stage IIIA, IIIB, IIIC AJCC, 8th ed.) as confirmed on staging FDG-PET/CT
- Willing to participate in the full follow up schedule
- Planned for treatment with curative intent
- No evidence of metastatic intracranial disease on CT brain with contrast or MRI

Exclusion Criteria

- Participant is not able to tolerate supine position on PET/CT bed for the duration of the PET/CT acquisitions, is not cooperative, or needs continuous nursing (e.g. patient from Intensive Care Unit) or is unable to attend full course of follow up visits
- Pregnancy or Breast-feeding
- If history of a prior extra-thoracic invasive malignancy (except non-melanomatous skin cancer) must be free from recurrence for a minimum of 3 years at the time of registration
- Prior radiotherapy to the lungs or mediastinum
- Prior known history of interstitial lung disease

Primary endpoints:

Feasibility will be considered to have been achieved for a given patient if all of the following criteria is met: a) Reduction in mean functional lung dose of ≥2% and functional lung volume receiving 20Gy of ≥4% b) Mean heart dose is ≤30 Gy and relative heart volume receiving 50 Gy is <25%.

This study will be considered feasible if the treatment was feasible for ≥15 out of 20 patients

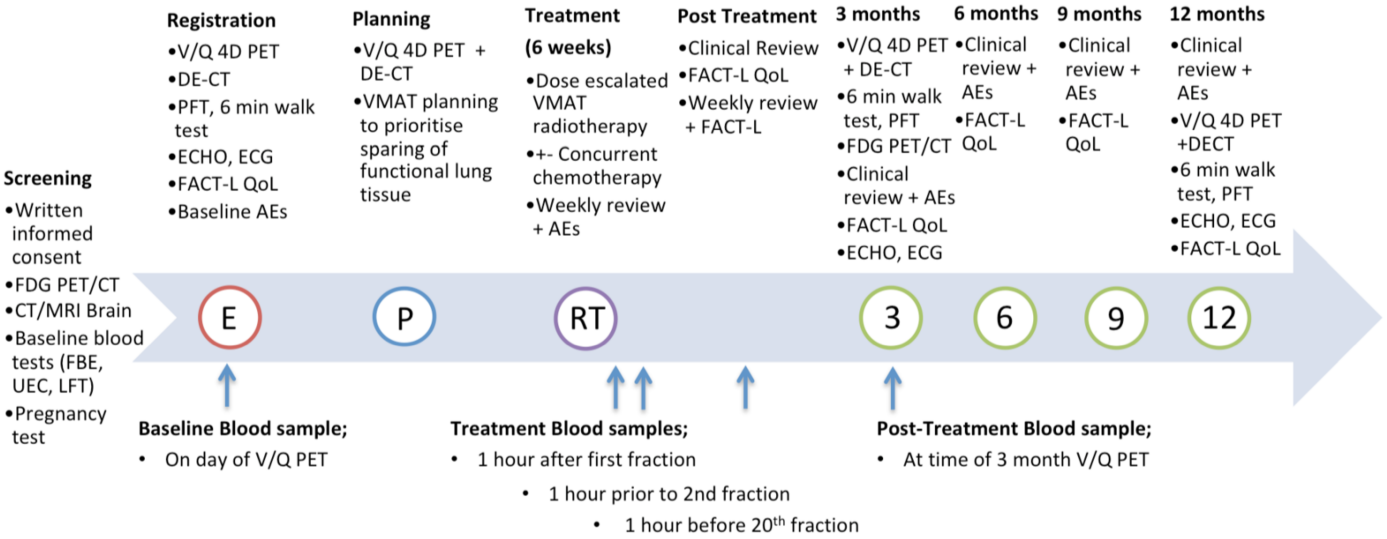
Secondary endpoints:

1. Radiation pneumonitis will be assessed and graded using CTCAE v4.03 (**appendix 4**).
2. Acute toxicities are defined as any adverse event (AE) occurring from the time of treatment commencement to 4 weeks after treatment completion. Late toxicities are defined as any AE occurring after 4 weeks post end of treatment.
3. Regional ventilation loss and regional perfusion loss will be assessed as the difference in regional ventilation and regional perfusion assessed on V/Q PET/CT imaging from baseline to 3 and 12 months post treatment using the quantitative and qualitative measures and assessed with respiratory function testing
4. To determine the incidence of grade ≥ 2 toxicities with cardiac function measured by TTE, ECG, and coronary calcium scoring
5. The association between levels of inflammatory cytokines will be assessed using a broad cytokine panel such as the Ray-biotech platform. Radiation pneumonitis ≥ grade 2 will be assessed and graded using CTCAE v4.03.
6. Association between V/Q PET/CT and CT Ventilation and DECT perfusion will performed at registration and at 3 and 12 months following radiotherapy treatment;
7. Patient reported quality of life outcomes using the FACT-L quality of life questionnaire
8. Complete metabolic response will be assessed at 3 month post treatment on FDG-PET/CT and determined using: a) the Peter Mac Visual response criteria, and b) PERCIST 1.0 criteria.
9. Progression-free survival will be measured from the date of registration to first disease progression at any site, or death due to any cause for patients without progression. Progression will be defined using RECIST 1.1 for CT based imaging.

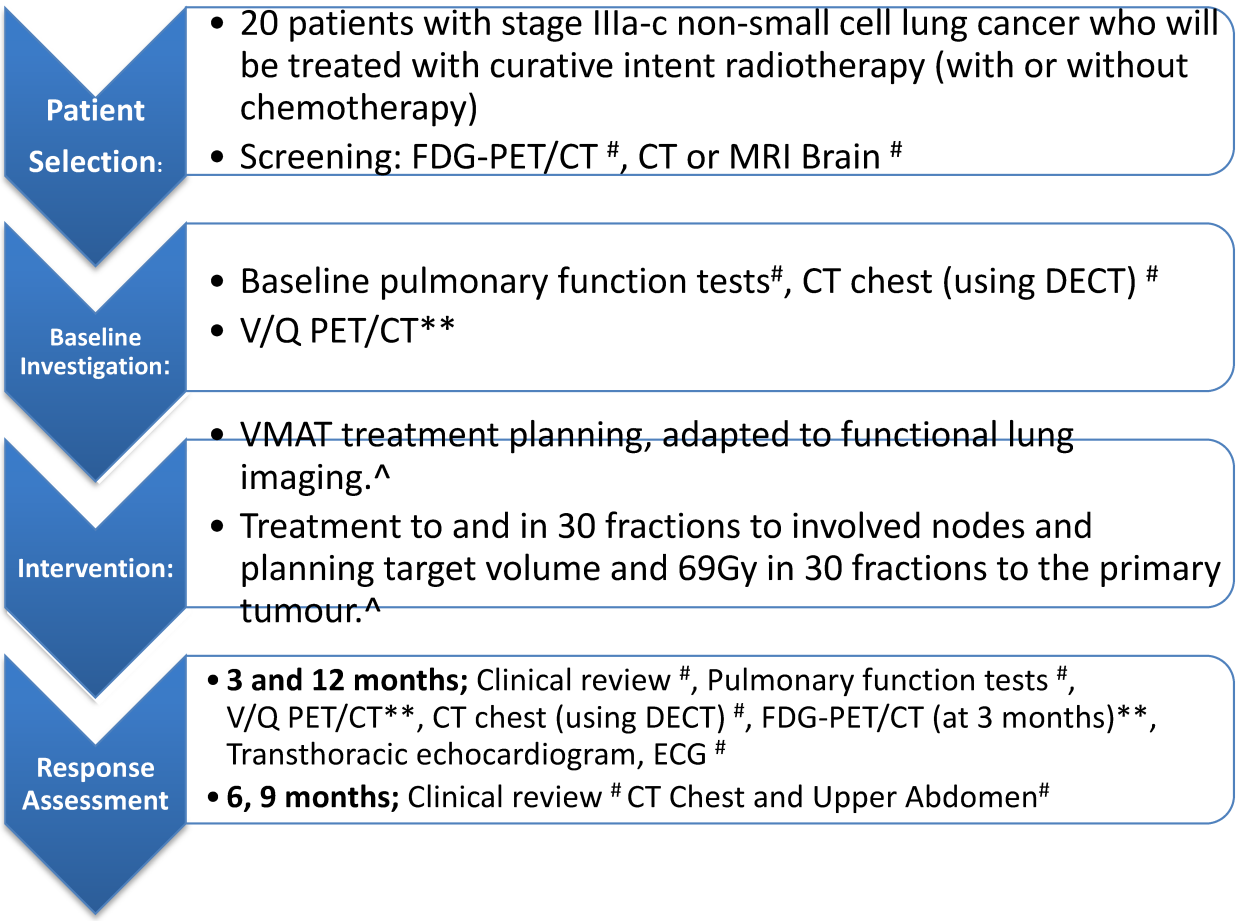
10. Overall Survival will be measured from date of registration to date of death from any cause
Treatment duration: Treatment will be administered 5 fractions per week over 6 weeks
Follow up schedule: 3 monthly until 1 year after the last participant completed treatment
Efficacy assessments: 3 month post treatment FDG-PET/CT, 3 monthly CT chest and upper abdomen
Safety assessments: Weekly treatment review during treatment, post treatment review 4 weeks post treatment. 3 monthly follow up until 12 months after last participant completed treatment.

2. TRIAL SCHEMA AND TIMELINES

2.1 PATIENT TIMELINE



2.2 TRIAL SCHEMA



Key:
Tests that can be considered standard of care and are not considered to be additional study investigations
** Tests that are additional study investigations
^ Additional study interventions, not current standard of care

3. BACKGROUND

Radiotherapy is the standard of care for locally advanced non-small cell lung cancer (NSCLC). Technological advances in the planning and delivery of radiotherapy have improved accuracy of target definition, motion management and quality assurance. Despite these advances, local failure still occurs in a third of patients.[2] Recent phase 3 clinical trials have demonstrated that dose escalation to improve outcomes is limited by the risk of lung toxicity and dose to other normal tissues.[2,10-12] Excess dose to healthy lung can result in the complication of radiation pneumonitis, which physiologically manifests as reduction in air-flow (ventilation) and blood flow (perfusion).[1] Symptomatic pneumonitis occurs in a third of patients treated with curative intent lung radiotherapy with fatal pneumonitis occurring in 2% of these patients.[1] A number of factors increase this risk further including the use concurrent chemotherapy, prior lung function and age.[1] Pioneering work with 68Ga 4D V/Q PET has allowed us to accurately predict for risk of lung injury to ventilation and perfusion with a dose-response relationship seen in functional imaging of both ventilation and perfusion.[3,4] We have shown that we can use this information in radiotherapy planning to significantly reduce dose to functional lung.[5,6] In this research, we use our prior body of work to investigate the safe delivery of dose-escalated radiotherapy by sparing functional lung.

Functional Lung Identification

Functional lung imaging has the ability to enhance our understanding of underlying lung function, which can supplement anatomical imaging. Currently lung is defined anatomically which results in our treatment planning regarding lung as uniformly functional. A series of studies at the Peter MacCallum Cancer Centre using V/Q PET/CT has shown that this is not an accurate depiction of lung function. Functional lung imaging can enhance our understanding of radiation induced normal tissue complication beyond the current anatomical based dose volume constraints and can be integrated into treatment planning to personalise lung radiotherapy so as to minimize lung toxicity.

Our team recently performed a systematic review and meta-analysis on the use of functional lung imaging in radiotherapy for lung cancer (manuscript submitted for publication, *IJROBP*). This found that several techniques were available for the imaging of functional lung using perfusion, ventilation or gas exchange including CT, MRI, SPECT and PET. The majority of the publications used nuclear medicine imaging with SPECT enabling three-dimensional imaging and direct interrogation of physiologic ventilation through inhaled nanoparticles or gases and/or perfusion after intravenous injection of small particles that are trapped only in the terminal bronchial arterioles. PET uses bio-identical molecules but offers improved spatial and temporal imaging resolution with ability to

perform respiratory gating.[8,13]

Future use of V/Q PET/CT is limited by the cost; the need for an additional investigation to identify functional lung and that Peter MacCallum Cancer Centre is the only institution currently offering these investigations. Therefore exploring the use of CT surrogates of ventilation and perfusion and their comparison to the accuracy of V/Q PET/CT to CT to make a larger scale multi-centre trial possible without the need for additional investigations. CT is already a standard of care imaging test used in radiotherapy planning and treatment response assessment. CT ventilation is another common functional imaging modality that relies on using the change in density of lung as a surrogate for ventilation.[14] At this stage, CT ventilation has been shown to not have a high correlation with PET ventilation and there are considerable variations between each ventilation algorithm.[15] Acquisition of iodine maps which are a surrogate for pulmonary perfusion is now also possible with dual energy CT.[16-18] Although iodine mapping (as a surrogate for pulmonary perfusion) has been used in radiotherapy planning its accuracy as not yet been compared with PET MAA perfusion with one published study comparing this modality to pulmonary scinitigraphy which lacks the special resolution of PET.[16]

Based on our institutions experience, we have optimised the 4D V/Q PET technique - our functional imaging modality - and established a robust methodology to identify areas of ventilated and perfused lung tissue both before and after radiation therapy.[3,4,6] This technique provides both structural and temporal information (using 4D-CT) in addition to functional information on ventilation and perfusion identified on PET. This is a significant advance over traditional V/Q SPECT scans with higher resolution fully tomographic images with the potential for better regional quantification of lung function.[4] The GalliPET study at the Peter Mac was a prospective observational study of 60 patients, which demonstrated dose-dependent changes in lung ventilation and perfusion prior to and during radiotherapy.[3] One of the major setbacks to the wide scale use of such functional imaging is the cost and additional patient and staff time involved. From this perspective, CT is most attractive as functional imaging can be derived at the same time as the radiotherapy planning CT. We have therefore integrated CT ventilation and DECT (for perfusion) imaging investigations at the same time points as our V/Q PET scans to describe the correlations between these imaging modalities.

Dose Escalation

The role of dose escalation in locally advanced lung cancer remains undefined and there are a number of studies underway to address this question. Multiple promising phase II trials and a large cohort study by Brower et al. have demonstrated improved overall survival with higher radiotherapy

doses.[19] Phase 3 trials to date have not supported this hypothesis with the largest study, the RTOG 0617 showing significantly worse overall survival in the high dose arm (74Gy in 37 fractions to tumour and nodes) compared to the 60Gy in 30 fraction conventional arm (5 year overall survival 23% vs. 32.1%).[2] Extensive analysis was undertaken to evaluate potential differences in quality of radiation and other potentially confounding factors. In multivariable analysis heart dose was found to impact on overall survival.[2] It was also noted in RTOG 0617 that lung V20 and MLD were significantly higher in the group receiving the higher dose.[20] Although there were not considerably higher rates of radiation pneumonitis in the high dose arm, it has been hypothesised that this higher dose to functional lung may relate to longer-term cardiopulmonary toxicity.[20] Another important consideration for dose escalation is the potential for damage to the proximal bronchial tree and vessels, similar to the concerns about using stereotactic radiation to centrally located tumours. In an isotoxic dose-escalation study, dose given was stratified by risk of developing radiation pneumonitis with total doses between 57 to 85.5 Gy in 25 daily fractions over 5 week.[12] At 3.42Gy per day, the highest dose arm delivered a considerably higher dose per day compared to the standard 2Gy per day. This dose was uniformly prescribed to both primary and nodes regardless of size, proximity to vessels or other structures.[12] The investigators found 6 grade 4 or 5 toxicities out of a total of 79 patients.[12] 5 of these severe toxicities related to damage to peri bronchial structures and all of these had primary tumours encasing or abutting a main stem or proximal lobar bronchus.[12] The authors concluded future dose escalation studies should have strict dose constraints applied to the proximal bronchial tree.[12] Due to this risk of toxicity the current RTOG 1106 trial has placed dose constraints on the dose to the proximal bronchial tree.[21]

Radiobiological Basis for Dose Escalation

74Gy in 37 fractions at 2 Gy per fraction has been established as the maximum tolerated safe dose in multiple phase 1/2 dose escalation studies and the ideal dose to achieve optimal local control.[22] Phase 3 trials such as RTOG 0617 have failed to show a survival benefit. Further analysis of dose escalation trials suggest there may be an overall survival benefit to a dose escalated approach without treatment prolongation.[23] To achieve 74Gy (EQD2 equivalence) to the primary tumour, factoring in overall treatment time; 74Gy in 37# is approximately equivalent to 69Gy in 30# (2.3 Gy per fraction).[24] A number of dose escalation studies are currently underway, each using different techniques of identifying tumour sub volumes to escalate dose and methods of reducing dose to organs at risk

- RTOG 1106: Adaptive radiation therapy using an interim FDG-PET/CT to escalate dose to as high as 80.4 Gy in 30 fractions.

- PET Boost Study patients are treated to 66Gy in 24# (2.75Gy per fraction) with randomisation between a simultaneous integrated boot to the whole tumour to 72Gy in 24# and a simultaneous integrated boot of 72Gy to the 50% SUV max area of the tumour.
- FLARE-RT where non responders on mid-treatment PET are given 74Gy in 30#

Advances in Radiotherapy Planning

Volumetric Modulated Arc Therapy (VMAT) is an advanced radiotherapy planning and delivery technology that now makes it technically possible to increase dose to tumour while reducing dose to normal tissues. A number of studies have demonstrated improved dosimetry compared to other radiotherapy techniques for lung cancer.[9] VMAT may enable safe dose escalation above the standard dose of 60 Gy with accurate normal tissue definition, motion management and avoidance. Another advance to VMAT planning is the introduction of non-coplanar arcs. This has been shown to significantly decrease heart dose by 20-30% in patients with lower lobe tumours treated with 74Gy in 37 fractions.[25]

Integration of functional information into advanced radiotherapy planning combined with advanced planning techniques has the potential to significantly reduce dose to functional lung. Our recently conducted meta-analysis showed the mean (95% CI) functional mean lung dose was reduced by 1.98Gy [0.57; 3.39] and the mean functional volume receiving 20Gy was reduced by 4.19% [2.34; 6.04]. In most cases planning was performed using IMRT or VMAT and many of the planning studies showed there was no significant additional doses to organ at risk.

Imaging as a Predictor of Cardiac Damage

Radiation induced cardiac disease is a well known radiotherapy toxicity and the significance well recognized factor affecting both overall survival and rates of cardiac disease in the treatment of breast cancer and lymphoma. Previously it was thought that cardiac was not a significant issue due to poor prognosis of locally advanced lung cancer.[10] Radiation doses delivered to cardiac structures have now been recognised as a significant predictor of post treatment mortality and morbidity.[26] In the RTOG 0617 study higher heart doses were associated with worse overall survival.[2] Analysis of 127 patients in 6 trials at a single institution receiving dose-escalated radiotherapy found that 23% of patients had cardiovascular events (arterial, pericarditis, arrhythmia) within 5 years of treatment. This is much earlier than those toxicities typically seen in breast and lymphoma examples).[10] Competing risk analysis was performed, adjusting for the competing risks of cancer progression and the authors found that mean heart dose was significantly associated with rates of symptomatic cardiac events with incidence of events 4% if the mean heart dose (MHD) was less than 10Gy, 7% with MHD of between

10-20Gy and 21% if MHD was greater than 20Gy.[10] In this pooled analysis, heart doses were not associated with a change in overall survival.[10]

Cardiac damage induced by radiation in multifactorial affecting the pericardium, myocardium, valves, conduction system and coronary arteries though mostly fibrotic processes.[27-29] At present, an individual's risk of radiation induced cardiac damage and how this relates to dose is currently unknown. Coronary cardiac scoring is a marker of atherosclerotic plaque burden and has been shown in healthy populations to be an independent predictor of future myocardial infarction and mortality.[30] It allows for individualized coronary risk scoring, superior to population-based models such as the Framingham Risk Score.[30] It may be useful in predicting those patients at greater risk of radiation induced heart damage by giving an indication of pre-treatment heart disease. Its major advantage is that scoring can be performed on the thoracic CT images already used for radiotherapy planning. Integrating this data into a normal tissue complication probability model may enable more precise, personalised treatment planning adjusted for individual's risk of cardiac disease.

Imaging as a Predictor of Treatment Response

Dual-Energy CT-Based Iodine Tumour Quantitation may be an effective imaging tool in the response assessment of NSCLC. A preliminary study of 11 patients found that semi-automatic iodine-related quantitation in DECT correlated well with metabolism-based measurements in FDG-PET/CT.[31] This has significant potential in improving the response assessment of patients with NSCLC where the use of FDG-PET/CT does not currently attract Medicare reimbursement and is already a common assessment in the follow up of patients with locally advanced lung cancer.

Current Functional Lung Trials

There are three prospective interventional trials currently underway investigating functional lung imaging:

- 'Functional Lung Avoidance and REsponse-adaptive escalation' (FLARE) study which uses perfusion SPECT to identify functional (perfused) lung and randomises patients to proton pencil beam scanning or volumetric arc therapy (VMAT).[32] A concomitant boost to PET non responders (on a mid treatment FDG-PET) is given to 74Gy in 30 fractions to a FDG-PET defined sub-volume.[32]
- 'Functional Lung Avoidance for Individualized Radiotherapy' (FLAIR) which uses hyperpolarised Helium MRI to identify and IMRT/VMAT planning to avoid functional (ventilated) lung.[33,34]

- The 'novel lung functional imaging for personalized radiotherapy' study has also published their first patient treated with functional lung avoidance. This study uses IMRT or VMAT to spare CT ventilation functional lung identified on CT ventilation.[35]

Weaknesses in the current literature

There are a number of areas identified in the current literature that require further investigation. There is weak correlation between current methods of functional lung determination with few studies comparing the different methods with robust statistical comparisons. This study will address this by the use of V/Q PET in addition to DECT and ventilation CT, comparing these modalities at pre and post treatment time points. Additionally although dose-response relationships have been demonstrated in post treatment perfusion imaging there is little data available on dose-response relationships in ventilation imaging. The role of dose escalation to areas at high risk of local relapse is also currently unknown. Despite the results of RTOG 0617, there may be still some role for dose escalation without treatment prolongation and with strict normal tissue constraints. This study will assess the feasibility and tolerability of performing this using advanced radiotherapy planning techniques. The recent development of Dual Energy CT and CT cardiac imaging (using calcium scoring) has future potential roles in both radiotherapy planning by providing measures functional information and radiotherapy response assessment. This study will further our knowledge on the potential uses of DECT and cardiac imaging by integrating these modalities into treatment planning and assessing their correlation with current imaging techniques.

This prospective study combines 4D pre-treatment functional and structural information on lung function and tumour definition (with FDG PET). Other important organs at risk including heart, oesophagus and the proximal bronchial tree will also be precisely defined. Identification of functional lung and organs at risk will be combined with VMAT planning which will optimise radiotherapy delivery to avoid functional lung and minimise dose to important organs at risk whilst increasing dose to the tumour. This may improve local control while reducing risk to functional lung and other organs at risk. The translational laboratory component described in **section 13** will enhance our understanding of the mechanisms behind radiation damage and develop predictive biomarkers for treatment response and normal tissue damage. In doing this, the study will address a number of unanswered questions regarding the personalisation of lung radiotherapy in locally advanced disease in addition to assessing the feasibility of further implementing this in a larger scale clinical trial.

4. TRIAL OBJECTIVES

4.1 HYPOTHESIS

That functionally adapted lung radiotherapy using V/Q PET/CT imaging and VMAT planning is technically feasible for a) sparing functional regions and b) delivering a simultaneous integrated boost to the primary tumour in patients with stage 3a-c NSCLC.

4.2 OBJECTIVES

4.2.1 PRIMARY OBJECTIVES

1. To assess the technical feasibility of the delivery of personalised functional lung radiotherapy. This study will be considered feasible if all planning parameters defined in the protocol can be achieved for ≥15 out of 20 patients.

4.2.2 SECONDARY OBJECTIVES

1. To determine the incidence of grade ≥ 2 clinical or radiological pneumonitis after high dose functionally adapted radiotherapy
2. To determine the incidence of grade ≥ 2 acute and late toxicities
3. To quantify regional ventilation loss and regional perfusion loss on post treatment V/Q PET/CT following functionally adapted lung radiotherapy and its relationship to respiratory function testing
4. To assess the relationships of cytokine release in patient's plasma with grade ≥ 2 radiation pneumonitis
5. To assess the associations between;
    a. Ventilation PET/CT with inhale/exhale CT ventilation
    b. Perfusion PET/CT with dual energy CT iodine mapping (DECT iodine mapping is regarded as a suggogate for pulmonary perfusion)
6. To assess patient reported quality of life outcomes using the FACT-L quality of life questionnaire
7. To assess incidence of complete metabolic response on 3 month post treatment FDG-PET/CT

8. To assess progression free survival at 12 months following completion of trial radiotherapy (defined by RECIST 1.1)
9. To assess overall survival at 12 months following completion of trial radiotherapy

4.2.3 EXPLORATORY OBJECTIVES

1. To assess the mechanisms behind enhanced DNA damage repair during a course of fractionated radiotherapy treatment
2. To investigate the utility of mid-treatment cardiac biomarker testing and pre-treatment coronary calcium scoring to predict patients at greater risk of radiation induced cardiac toxicity
3. To correlate primary and nodal disease seen on pre and post-treatment FDG-PET/CT with dual energy CT.
4. To assess ct-DNA levels as a predictor of treatment response

4.3 ENDPOINTS

4.3.1 PRIMARY ENDPOINTS

Feasibility will be considered to have been achieved for a given patient if all of the following criteria is met: a) Reduction in mean functional lung dose of ≥2% and functional lung volume receiving 20Gy of ≥4%; b) Mean heart dose is ≤30 Gy and relative heart volume receiving 50 Gy is <25.

This study will be considered feasible if the treatment was feasible for ≥15 out of 20 patients

4.3.2 SECONDARY ENDPOINTS

1. Radiation pneumonitis will be assessed and graded using CTCAE v4.03 (**appendix 4**).
2. Acute toxicities are defined as any adverse event (AE) occurring from the time of treatment commencement to 4 weeks after treatment completion. Late toxicities are defined as any AE occurring after 4 weeks post end of treatment.
3. Regional ventilation loss and regional perfusion loss will be assessed as the difference in regional ventilation and regional perfusion assessed on V/Q PET/CT imaging from baseline to 3 months post treatment and from baseline to 12 months post completion of radiotherapy using the quantitative and qualitative measures (section 9.1). Quantitative V/Q PET/CT measures will be end-inspiratory and end-expiratory volume

for each lung and lobe, contoured using semi-automatic threshold based on the operator's discretion and compared with the pre-treatment V/Q PET/CT. Respiratory function testing will be measured by the 6 minute walk test at baseline, 3 months post treatment and 12 months post completion of radiotherapy.

4. Grade ≥ 2 cardiac toxicity will be assessed and graded using CTCAE v4.03. This will be assessed by pre, 3 and 12 month post treatment transthoracic echocardiograms and ECG investigations. Coronary calcium scoing (the Agatston score) will be scored on the DECT investigation at these time points. Ventricular dysfunction seen on the 3 and 12 month post-treatment transthoracic echocardiogram will be scored using the enrolment TTE as a baseline.
5. The association between levels of inflammatory cytokines will be assessed using a broad cytokine panel such as the Ray biotech platform. Radiation pneumonitis will be assessed and graded using CTCAE v4.03.
6. V/Q PET/CT, CT Ventilation and DECT iodine mapping (as a surrogate for pulmonary perfusion) will be assessed at registration and 3 and 12 months following radiotherapy treatment.

   a. A qualified radiologist and nuclear medicine physician will perform a qualitative assessment of each modality as described in section 9.2

   b. Quantitative assessment of;

      a) PET/CT Ventilation will be performed using the methods described in section 9.1
      b) PET/CT Perfusion will be performed using the methods described in section 9.1
      c) CT Ventilation undergo quantitative voxel-wise assessment against PET/CT Ventilation
      d) CT iodine mapping (as a surrogate for pulmonary perfusion) undergo quantitative voxel-wise assessment against PET/CT Perfusion
7. Patient reported quality of life outcomes using the FACT-L quality of life questionnaire
8. Complete metabolic response will be assessed at 3 month post treatment on FDG-PET/CT and determined using: a) the Peter Mac Visual response criteria, and b) PERCIST 1.0 criteria.
9. Progression-free survival will be measured from the date of registration to first disease progression at any site, or death due to any cause for patients without progression. Progression will be defined using RECIST 1.1 for CT based imaging.
10. Overall Survival will be measured from the date of registration to the date of death from any cause

4.3.3 EXPLORATORY ENDPOINTS

1. Identify the genetic mechanisms behind enhanced DNA damage repair in circulating lymphocytes during a course of fractionated radiotherapy treatment by gene expression analysis at samples taken prior to radiotherapy, 1 after the first fraction, prior to the second fraction, prior to the 20th fraction and at 3 months following completion of radiotherapy.
2. Mid-treatment cardiac biomarkers including highly sensitive troponin and NT-pro BNP (or BNP) taken at before treatment, 1 hour after the first fraction, prior to the 2nd fraction and 1 hour prior to the 20th fraction will be measured.
3. Qualitative assessment of primary and nodal disease seen on pre and post-treatment FDG-PET/CT and dual energy CT will occur. A radiologist and nuclear medicine physician will perform a qualitative response assessment of the primary tumour and metastasis between the DECT and FDG-PET. This is classified as suspicious residual disease seen on PET only, suspicious residual disease seen on DECT only or suspicious residual disease seen on both modalities.
4. ct-DNA levels will be assessed at the 3-month post treatment time interval. Treatment response will be defined as complete metabolic response at 3-month post treatment FDG-PET/CT using the Peter Mac Visual response criteria.

5. TRIAL DESIGN

HI-FIVE is a single-arm prospective interventional feasibility study. 20 patients with locally advanced (stage 3a-c) NSCLC will undergo functional lung adapted radiation therapy to 60Gy in 30 fractions with a simultaneous integrated boost to the primary tumour to 69Gy in 30 fractions. Patients will undergo concurrent chemotherapy if deemed to be suitable by the treating medical oncologist. Where possible, dependent on contemporary access programs, eligible patients will be offered adjuvant immunotherapy if deemed to be suitable by the treating medical oncologist. Anticipated total duration of accrual is approximately 24 months, with all patients expected to complete all protocol treatment within 3 months. Patients will be followed until the last patients complete their 12-month post treatment follow-up assessment.

6. STUDY POPULATION

Patients with a diagnosis of stage 3a-c NSCLC who meet all the inclusion and exclusion criteria will be eligible for participation in this study.

6.1 INCLUSION CRITERIA

All of the following criteria must apply:

- Age ≥ 18 years;
- Written informed consent has been provided.
- Histologically or cytologically confirmed Non-Small Cell Lung Cancer
- ECOG performance status 0-2 within 2 weeks prior to registration (see **appendix 2**)
- Locally advanced disease (stage IIIA, IIIB, IIIC AJCC, 8th ed.) as confirmed on staging FDG-PET/CT (see **appendix 1**)
- No evidence of metastatic intracranial disease on CT brain with contrast or MRI
- Willing to participate in the full follow up schedule
- Planned for treatment with curative intent

6.2 EXCLUSION CRITERIA

None of the following must apply:

- Participant is not able to tolerate supine position on PET/CT bed for the duration of the PET/CT acquisitions, is not cooperative, or needs continuous nursing (e.g. patient from Intensive Care Unit) or is unable to attend full course of follow up visits
- Pregnancy or Breast-feeding
- If history of a prior extra thoracic invasive malignancy (except non-melanomatous skin cancer) must be free from recurrence for a minimum of 3 years at the time of registration
- Prior radiotherapy to the lungs or mediastinum (a history of prior breast radiotherapy is not an exclusion)
- Prior known history of interstitial lung disease

* A history of renal impairment or reaction to iodine contrast is not an exclusion criteria, if a patient has medical comorbidities that exclude the use of iodine contrasts, these exploratory investigations can be omitted.

6.3 PATIENT REGISTRATION

Prior to patient registration, the site principal investigator should ensure that all of the following requirements are met:

- The patient meets all inclusion criteria and none of the exclusion criteria should apply.
- The patient has signed and dated all applicable consent forms.

- All screening assessments and investigations have been performed.
- The eligibility checklist has been completed, signed and dated.

A patient will not be registered if treatment has commenced or if consent has not been given. Once a patient is registered on a trial registration will not be cancelled.

To register a patient onto the trial, an adequately qualified and authorized member of the research team at the trial site must complete the registration and eligibility Case Report Forms (CRFs) and forward them to the trials coordinator, at the Peter MacCallum Centre Department of Radiation Oncology and Cancer Imaging.

Following registration, patients should begin protocol treatment within 30 days

6.4 SCREENING LOG

A screening log will be created, to record the number of patients referred for consideration of registration onto the trial, and reasons they were excluded or ineligible. This screening log will aid in identifying factors that may impede recruitment and impede the escalation of the trial to a larger multi-institutional setting.

6.5 STUDY ASSESSMENTS

The following assessments will occur during the trial. A schedule of assessments is provided on page 34.

6.6 INFORMED CONSENT

All patients registered must meet selection criteria as specified in inclusion and exclusion criteria outlined in section 6. In addition, the patient must be thoroughly informed about all aspects of the trial, including the trial visit schedule, commitments and required evaluations, and all regulatory requirements for informed consent. A thorough medical history and examination must be performed. Disease stage and site must be documented. The written informed consent must be obtained from the patient prior to registration into the trial. Informed consent must be obtained from the patient by a treating clinician as designated in the Trial Delegation Log. Registration paperwork must be completed

from a qualified and authorized member of the research team as designated in the Trial Delegation Log.

6.7 PRE-REGISTRATION/SCREENING ASSESSMENTS

The following assessments must be performed within 28 days prior to registration.

- Written informed consent – must be given before registration may proceed
- Comprehensive medical history and demographics
- Documentation of concomitant medications at time of screening
- Physical examination, weight and documentation of ECOG performance status
- Laboratory studies (if not already performed) including FBE, UECr, LFTs
- Radiological evaluation with a FDG-PET/CT and CT or MRI brain
- Negative serum / urine pregnancy test within 1 week prior to registration for women of childbearing potential
- Women of childbearing potential and male participants must agree to use a medically effective means of birth control throughout their participation in the treatment and follow-up phase of the study.

6.8 PRE-TREATMENT/ REGISTRATION ASSESSMENTS

The following assessments must be performed within 30 days prior to start of treatment.

- FACT-L QoL
- Adverse events (baseline abnormalities)
- Concomitant medications
- V/Q PET scan and DECT
- Pulmonary Function Tests
- Transthoracic echocardiogram
- Samples taken for translational studies, ECG, cardiac symptoms

6.9 TREATMENT ASSESSMENTS

A qualified member of the radiation oncology team must undertake weekly treatment review.

- Recording of any adverse events
- Physical examination, weight and documentation of ECOG performance status
- Bloods (FBP, UEC, LFTs) will be performed weekly
- Samples taken for translational studies 1-hour post first fraction, 1 hour before the 2nd fraction, 1 hour prior to the 20th fraction. A history will be taken for the presence of any cardiac symptoms.

6.9.1 POST-TREATMENT ASSESSMENT

A post treatment review should also be performed within 4 weeks following radiotherapy completion. The timing of this assessment will be dictated by any treatment related toxicities the patient is experiencing i.e. oesophagitis. Multiple post treatment assessments may be required. At one of these assessments within 4 weeks post treatment;

- Recording of any adverse events
- Concomitant medications
- Physical examination, weight and documentation of ECOG performance status
- Bloods (FBP, UEC, LFTs)
- FACT-L QoL

6.9.2 DEFINITIVE RESPONSE ASSESSMENT

This shall be performed 3 months following completion of radiotherapy treatment (+- 10 days)

- Recording of any adverse events
- Concomitant medications
- Physical examination, weight and documentation of ECOG performance status
- Bloods (FBP, UEC, LFTs)
- Blood sample taken for translational studies, ECG, cardiac symptoms
- FACT-L QoL
- FDG-PET/CT scan
- DECT chest and upper abdomen
- V/Q PET scan
- Pulmonary Function Tests
- Survival status

- Transthoracic echocardiogram

6.9.3 FOLLOW-UP

The following assessments will occur 3 monthly (+- 10 days) from 6 months after the end of treatment:

- Recording and reporting of any adverse events
- Concomitant medications
- Physical examination, weight and documentation of ECOG performance status
- Adverse events / toxicities
- CT of chest and upper abdomen
- Survival status

6.9.4 12 MONTH POST- TREATMENT ASSESSMENT

This shall be performed 12 months following completion of radiotherapy treatment (+- 10 days)

- Recording and reporting of any adverse events
- Concomitant medications
- Physical examination, weight and documentation of ECOG performance status
- Bloods (FBP, UEC, LFTs)
- FACT-L QoL
- DECT chest and upper abdomen
- V/Q PET scan
- Transthoracic echocardiogram, ECG
- Pulmonary Function Tests
- Survival status

6.9.5 V/Q PET/CT PROCEDURE

The radiopharmaceuticals used in this study will be synthesized onsite by a qualified radio pharmacist using methods we have previously validated by our group[36,37]. ^{68}Ga will be eluted from our ^{68}Ge/^{68}Ga generator and used to label the appropriate precursor. ^{68}Ga-Galligas is prepared using a Technegas generator except that the radionuclide Technetium-99m is replaced with Gallium-68 in the carbon crucible inserted into the Technegas synthesis unit. ^{68}Ga-macroaggregated albumin (MAA) is prepared as follows: A commercially available kit of MAA is washed three times with 0.1 M acetate

buffer at pH 5 and dispensed into 1 mL aliquots with each aliquot containing MAA particles of between 250 to 700 thousand particles. Gallium-68 obtained from the generator is buffered with acetate buffer to pH 5 before adding to the MAA aliquot. The suspension mixture is allowed to incubate for 5 minutes at 37^{o}C after the addition of radioactivity for the radiolabeling process. Quality assurance tests will be performed in accordance with the British Pharmacopoeia before the compound is released for clinical use.

A contemporaneous 4D-CT of the chest will be performed in order to allow co-registration with the PET scan. This will allow respiratory-gated attenuation correction. After cannulation, an additional blood sample will be drawn for translational research described in section 10.

The methodology of the V/Q PET/CT will be as follows:

1. A peripheral intravenous catheter is installed in the arm.
2. Blood is drawn for storage and processing for translational research.
3. Participant inhales approximately 5 MBq of ^{68}Ga-Galligas, in semi-supine position, using the same technique as for Technegas.
4. For the initial (radiotherapy planning) V/Q PET/CT the participant is placed in the radiotherapy planning position on the PET/CT camera bed, arms up with the Varian respiratory tracking box in place. For follow up V/Q PET/CT scans; the participant does not need to be in the radiotherapy planning position.
5. A scout acquisition is performed to determine the limits of the PET and CT acquisitions.
6. A chest 4D-CT acquisition is performed (140 kVp, 30-40 mA, axial scan time is breathing period + 1sec.
7. Lung ventilation 3D List-mode Respiratory gated PET acquisition is started (2-3 bed positions, 5 minutes per bed position). This acquisition will be reconstructed as both a respiratory gated and un-gated scan.
8. Without moving, approximately 20-40 MBq^{68}Ga-MAA is injected intravenously, as a bolus, via the catheter. The syringe is then flushed with normal saline.
9. The lung perfusion 3D List-mode Respiratory gated PET acquisition is started (2-3 bed positions, 5 minutes per bed position, exactly the same bed positions as for the ventilation study). This acquisition will be reconstructed as both a gated and un-gated scan.
10. The total scan time will be approximately 30-40 mins (CT: 5 mins, Vent-PET: 15mins, Perf-PET: 15mins).

The V/Q PET/CT study will be performed no longer than 30 days before the commencement of radiotherapy and at 3 and 12 months following completion of treatment (+-10 days). If at clinical assessment at 6 months or 9 months, there is a significant deterioration in the patient's clinical symptoms or PFTs, then at clinician discretion an earlier Gallium-PET study may be requested.

6.9.6 FDG-PET/CT PROCEDURE

At the Peter MacCallum Cancer Centre the FDG-PET scans are performed using a GE Discovery 710 or 690. These are dedicated PET/CT scanners with 64-slice MDCT on the GE-690 and Biograph. Patients are fasted for at least 6 hours prior to intravenous injection of Fluorodeoxyglucose F-18 (^{18}F-FDG). The administered radioactivity of ^{18}F-FDG is adjusted for patient weight and camera using according to standard protocol. After 60 minutes of resting supine, a whole body PET/CT scan is acquired with the arms position above the head. A standard whole body scan extends from the base of the brain to the proximal thighs. The CT scan uses lower exposure factors than a standard diagnostic CT so it is considered a low-dose CT scan. The whole body PET scan is taken in a series of bed steps with the time per bed adjusted for patient weight and scanner. A whole body PET scan takes from between 15-30 minutes depending on the length of scan and patient body habitus.

This will be performed at the 3-month response assessment time point.

6.9.7 DECT PROCEDURE

A high-resolution dual energy CT scan of the chest with intravenous contrast will be performed on the Siemens SOMATOM Definition Force. This will occur in the pre and post treatment (3, 12 month) interval settings. If the patient has sufficient renal function and no history of allergic reactions iodinated contrast will be used (Omnipaque-350), injected via a peripheral intravenous cannula installed in the arm. Following intravenous cannula placement, blood will be drawn for processing and storage for translational research described in section 10. Contrast dosing used and precautions will be as per the radiology departmental protocol.

This CT chest will be performed on the morning of the planned V/Q PET to minimise patient visits and minimise risk of occupational radiation exposure.

The CT scans will be acquired in the supine position with the arms elevated. CT ventilation will be acquired non-contrast in full inspiration and full expiration phase using visual and audio coaching.

Patients will be provided with coaching before the CT scan. A DECT iodine map (as a surrogate for pulmonary perfusion) will then be acquired using iodinated contrast and will be post processed on the Syngo Via console. The addition of contrast will allow the acquisition of an iodine map using the dual energy feature of the CT. At the two time points where FDG-PET correlation is required (registration and at the 3 month response assessment) patients will undergo an additional scan in the portal venous phase to provide nodal information.

Additional analysis of CT imaging will occur to correlate CT images with V/Q PET and FDG PET.

One radiologist will review the DECT CT and issue a standard of care report. In addition to this, in the post-treatment setting the report will include criteria as per RESICT 1.1 outlined in **appendix 3**. One radiologist and one nuclear medicine physician will together perform the qualitative and data analysis assessment.

6.9.8 PULMONARY FUNCTION TEST PROCEDURES

Performance of respiratory function testing comprises measurement of Spiro metric lung volumes as well as gas diffusion capacity.

Spirometry

Participants inhale to maximum capacity then exhale as forcefully as possible for a minimum of 6 seconds into a closed system which measures the volume of air exhaled as a function of time.

DLCO (Carbon monoxide diffusion capacity of the lung)

Participants exhale completely, and then inhale a standard composition gas containing 0.3% carbon monoxide (CO). The inhaled gas also contains trace amounts of helium to allow measurement of Alveolar volume (V_A). The remainder of the test gas mixture contains oxygen (O_2) and nitrogen (N_2) at normal atmospheric concentrations. Participants hold their breath for 10 seconds and then exhale completely into a mouthpiece attached to a gas composition analyser. The reduction in CO concentration of exhaled air allow the gas diffusion capacity to be derived through standard mathematic equations[38].

Six minute walk test

Subjects will walk continuously for six minutes, in the presence of a technician or clinician and monitored continuously with an oxygen saturation finger monitor or equivalent. The distance covered

in six minutes is a commonly used measure of integrated pulmonary capacity. [39]

These pulmonary function tests will be performed at baseline (no longer than 30 days weeks before commencement of radiotherapy) and at 3 and 12 months after completion of radiotherapy.

6.9.9 CARDIOVASCULAR INVESTIGATIONS

Transthoracic echocardiograms will be performed prior to radiotherapy treatment and at 3 and 12 months following completion of radiotherapy. Due to the incidence of cardiac disease and associated mortality in this population this test is regarded as a standard of care procedure.[10,29,40-42] This procedure will be performed at a private facility external to Peter MacCallum Cancer Centre. The patient will be positioned by a sonographer in the supine position with the left arm abducted and leaning to the left side, ultrasound gel will be applied and a series of images including Doppler ultrasound will be taken to visualise the cardiac structures and assess cardiac physiology. These investigations will be reported by a cardiologist and utilised to investigate potential for radiotherapy induced cardiac toxicity.

A cardiac symptom score will be performed at the same time as the translational blood tests to exclude patients who have symptomatic cardiac disease (which could indicate an acute myocardial infarction or pulmonary embolism) from cardiac biomarker testing and refer them to receive urgent medical attention. In addition to this a standard 12 lead ECG will be performed at enrolment and at 3 and 12 months post treatment.

6.9.10 QUALITY OF LIFE QUESTIONNAIRE

Quality of life will be measured at multiple time points throughout the study. This will occur pre treatment, in the final week of treatment and at 3 and 12 months post treatment. Quality of life will be collected using the Functional-Assessment of Cancer Therapy-Lung (FACT-Lung) scale. The FACT-Lung assessment tool is attached in **appendix 5**

6.10 PATIENT WITHDRAWAL/DISCONTINUATION

Each patient has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a patient from the study at any time if the Investigator considers it necessary for any

reason.

6.10.1 PATIENT WITHDRAWAL

Trial Participants have the option to either completely or partially withdraw from the trial at any time without giving a reason. An example of 'partial withdrawal' is agreement to be followed up for survival, but withdrawal of consent to further scans and other tests. The Trial Participants' rights must be respected and should not prejudice their further treatment.

6.10.2 PROTOCOL THERAPY DISCONTINUATION OR WITHDRAWAL

Trial Participants have the option to withdraw from trial participation completely. The Trial Participants' rights must be respected and should not prejudice further treatment.

A Trial Participant may be discontinued from trial treatment for any of the following reasons:

- Unacceptable toxicity
- Inter-current illness which prevents further treatment
- Withdrawal of consent for treatment by participant
- Any alterations in the participant's condition which justifies the discontinuation of treatment in the investigator's opinion

All reasons for stopping protocol therapy must be documented. Discontinuation of treatment does not necessarily indicate withdrawal from the trial.

6.11 PROTOCOL TREATMENT DISCONTINUATION

A participant would be considered to have discontinued treatment where trial related treatment is ceased. However the participant may still agree to further follow-up assessments. Under these circumstances the participant's discontinuation of treatment must be documented on the relevant case report form. Follow-up visits will continue as scheduled. Patient's data should still be collected using the provided CRFs.

6.11.1 DISCONTINUATION OF TRIAL

If any grade 5 toxicities are recorded as a direct consequence of the investigational treatment, then the trial must be suspended pending investigation into the cause of death. An independent specialist

will conduct the Investigation with expertise in radiation oncology. If a direct causal link between the investigational treatment and the grade 5 toxicities recorded which is believed to be independent of extraneous factors not associated with the trial, then this is grounds for early termination of the trial. If the independent expert deems no causal link between the grade 5 toxicities and the investigational treatment, the trial may recommence as planned. If the Data Safety Monitoring committee, on review of SAE's, deems that the trial is not safe, then the trial should also be discontinued.

6.12 SUPPORTIVE CARE MEDICATION

Patients should receive appropriate supportive care measures as deemed necessary by the treating investigator. Supportive care medications should be documented on the CRFs.

Bucknell N, *et al. BMJ Open* 2020; 10:e042465. doi: 10.1136/bmjopen-2020-042465

7. SCHEDULE OF EVENTS

Assessment	Screening (-28 to -1 days)	Registration	Treatment Weeks 1-6 weekly	Acute Toxicity Follow-up	Late Toxicity/ Response Assessment Follow-up
Time point post RT				4 weeks	3 monthly ≅
Written informed consent	X				
Demographics		X			
Medical history		X			
Prior and concomitant medications		X	X	X	X
Adverse events		X	X	X	X
Physical exam/ weight/ ECOG		X	X	X	X
Full blood count	X*		X	X	X
Serum biochemistry	X*		X	X	X
Daily Radiotherapy			X		
Pregnancy test (if required)	X				
V/Q PET scan		X			X∧
CT (chest and upper abdomen)		X			X∞
FDG-PET/CT scan	X*				X°
CT/MRI Brain	X*				
Pulmonary Function Tests (PFTs)		X§			X§
Response assessment					X¶
Quality of Life		X¥		X¥	X¥
Survival Status					Xμ
Blood sample for translational studies		X≠	X≠		X≠
Cardiac symptom history		X∑	X∑		X∑
Transthoracic echocardiogram and ECG		X=			X=

Footnotes:
* Standard of care investigation
≅ Late Toxicity/ Response Assessment Follow-up will occur 3 monthly (+-10 days) until 12 months after the last participant has completed treatment.
∧ V/Q PET scan to be performed at 3 and 12 months following completion of radiotherapy
∞ CT (chest and upper abdomen) to be performed at 3 monthly following completion of radiotherapy for the duration of follow-up. 3 and 12 months this is a dual energy CT.
°FDG-PET scan to be performed at 3 and 12 months following completion of radiotherapy
§ PFTs to be performed at 3 and 12 months following completion of radiotherapy
¶ Response assessment to be performed at 3 and 12 months following completion of radiotherapy
¥ Quality of Life to be performed at 3 and 12 months following completion of radiotherapy
μ Survival Status to be performed at 3 monthly following completion of radiotherapy for the duration of follow-up
≠ Blood sample for translational studies to be at enrolment, 1 hour to first fraction, 1 hour prior to the 2nd fraction and 1 hour prior to the 20th fraction during radiotherapy and at 3 months following completion of radiotherapy. A cardiac symptom history to be performed at each time point.
= Transthoracic echocardiogram and ECG to be performed 3 and 12 months following completion of radiotherapy

8. TRIAL TREATMENT

8.1 SUMMARY OF TREATMENT REGIMEN

The investigational treatment will be prescribed ensuring that 98% of the 60Gy PTV is covered by 100% of the dose. When this cannot be achieved coverage of 95% of the 60Gy PTV acceptable with 100% of the dose is acceptable. The volume that receives 57Gy should be 100% (V95=100%). The 69Gy in 30 fractions (9Gy) boost sub-volume is defined as the primary tumour IGTV, ensuring that 95% of the IGTV receives 100% of the boost dose (D95=100%). The maximum dose to PTV (PTV max) must be contained within the IGTV. This boost dose will be reduced if organ at risk planning constraints cannot be met. This will be at the discretion of the treating clinician. If dose reduction is necessary, this event should be recorded and the D95% reported. All treatments will use be delivered using megavoltage photons delivered with a VMAT technique using partial arcs to avoid the contralateral lung. Non-coplanar arcs may be considered if technically feasible and if this improves normal tissue sparing. It is expected that majority of patients will receive concurrent cytotoxic chemotherapy; the treating medical oncologist will determine the agents. It is expected that a proportion of patients will also undertake adjuvant immunotherapy. At present this will be dependent on access, the treating medical oncologist will determine the agents and specific regime.

8.2 TREATMENT SCHEDULE

Treatment should begin within 3 weeks of the simulation scan.

8.3 RADIOTHERAPY PLANNING SIMULATION AND TECHNIQUE

8.3.1 PATIENT POSITIONING

The patient will be positioned supine with arms above head, head to gantry. The patient will be scanned to encompass the entire lung volume typically from C3 to L2.

8.3.2 IMMOBILISATION

Due to the VMAT planning technique, an upper half body evacuated vacuum bag must be used for patient immobilisation. Patient comfort is to be considered during the positioning process to ensure adequate immobilisation.

8.3.3 MOTION MANAGEMENT

Treatment delivery will occur in free breathing, to account for respiratory motion patients will undergo a 4D planning CT (the low dose 4D V/Q PET/CT will be used for planning). The Varian Respiratory Patient Management (RPM) system will be used to monitor patients breathing pattern as well as to

trigger the 4D CT acquisition. A staff familiar with 4D CT acquisition will then assess each patient's breathing trace. If the breathing trace is deemed to be irregular, the amplitude of the patient's respiratory cycle seems abnormally large, staff should intervene in order to allow a more accurate 4D CT scan.

8.3.4 IMAGE FUSION

The patients FDG-PET and V/Q PET will be fused with the 4D CT to enable radiotherapy planning

8.3.5 TARGET VOLUME DEFINITIONS

Target Volumes must be defined as per ICRU 50 and 62, with clear definitions, individual contouring and specific labelling,[43,44]. These include: Tumour (IGTV, ITV and PTV) and nodal (GTV, ITV and PTV). Internal Target Volume (ITV) as defined by ICRU70 will be used to take into account tumour movement through respiration and a margin for subclinical spread.[45] Target delineation and margins applied to primary tumour and nodal volumes are as per institutional protocol; described in the lung unit clinical guidelines (DRO_06.21.00). Boost Volume Definition: The proximal bronchial tree will have an isotropic 3mm PRV named proxbronch_PRV. The boost volume (IGTV_6900) will be given to the IGTV of the primary tumour alone minus the proxbronch_PRV.

8.3.6 DOSE PRESCRIPTION, FRACTIONATION AND DURATION

Treatment should be given daily at 5 fractions per week over 6 weeks. Treatment interruptions should be avoided where possible and if safe any missed treatments should be compensated so as not to increase the overall treatment time longer than 6 weeks.

8.4 TREATMENT PLANNING AND DOSIMETRY

8.4.1 PLANNING SYSTEM

The Varian Eclipse 3D computerized planning system will be used to plan the radiotherapy. Arcs will be used to deliver the radiotherapy. 360-degree arcs are to be avoided to minimise dose to the contralateral lung. It is expected arcs will typically span between 180 to 240 degrees.

8.4.2 BEAM ARRANGEMENTS

Radiation beams are expected to be of megavoltage quality and of 6 MV energy.

8.4.3 DOSE DISTRIBUTION AND REPORTING

Dose to 98% of the PTV (D98) should be reported. Near maximum absorbed dose to 2% (D2) of the PTV should be reported (ICRU82). The median absorbed dose specified by D50% should be reported as defined in ICRU 82. The conformity index will be reported. This is defined as the ratio between the volume encompassed by the prescription isodose and the target volume (ICRU 62). The homogeneity index should also be reported as per ICRU-83, which characterises the uniformity of the absorbed dose distribution within the target.

8.4.4 NORMAL TISSUE CONTOURING

Normal tissue contouring will follow the RTOG 1106 contouring atlas.[46] An additional 3mm isotropic expansion will be added to the proximal bronchial tree to create a proximal bronchial tree PRV .

8.5 RADIOTHERAPY DOSE CONSTRAINTS

Structure	Metric	Per Protocol	Source
PTV (primary and nodal)	V60	98%	[6]
	V60*	95%	
IGTV_6900 (p)	V69	95%	
Bony Spinal Canal	Max dose 0.03cc	≤ 50.0 Gy	[6]
Oesophagus	Max dose 0.03cc	< 63 Gy	[6]
	Mean	< 34 Gy	[6]
Heart	V40	< 30 %	
	V50	< 25%	[47]
	Mean	< 20 Gy	[48]
	Max dose 0.03cc	< 70 Gy	[6]
Brachial Plexus	Max dose 0.03cc	< 63 Gy	[6]
Proximal Bronchial Tree	Max dose 1.0 cc	< 64.5Gy	[12]
Great Vessels (Normal)	Max dose 0.03cc	< 80Gy	[49]
Great Vessels (Tumour involved)	Max dose 0.03cc	< 70Gy	[49]

* If D98 constraint unable to be met

Lung Dose Constraints

Structure		Metric	Per Protocol	Definition	Source
Lungs (anatomic)		Mean	< 20 Gy		[6]
Left + Right lung – IGTV		V30	< 30 %		[6]
		V20	< 35 %		[6]
		V5	< 66 %		[6]
Lungs (functional)	High functioning	HF		Intersection of V (>70%), Q (>70%) and lung, excluding PTV	
	Functioning	F		Intersection of V and Q contours > 30% max excluding HF	
	Perfused	Q		Q > 30% max excluding HF & F	
	Ventilated	V		V > 30% max SUV threshold, excluding HF & F & P	

The spinal cord dose constraint cannot be exceeded. Parameters for all other dose constraints should be met however if constraints must be exceeded to achieve adequate tumour coverage, the treating clinician may approve this.

8.6 TREATMENT EQUIPMENT SPECIFICATIONS/PHYSICAL FACTORS

All patients will be treated on a linear accelerator with megavoltage photon beams of a nominal energy of typically between 6MV, and 10MV (10MV energy beams should be avoided where possible). The linear accelerator must be equipped with multi-leaf collimator of central leaf widths of 5mm or smaller projected to the isocentre. The linear accelerator must be also equipped with verification imaging that allows visualization of the target volume. This must be on board kV quality imaging, which is expected to be cone beam CT.

8.7 TREATMENT VERIFICATION AND DELIVERY

Daily CBCT (Cone Beam CT) will be performed with online soft tissue matching will ensure that the target is within the PTV. Radiation therapists in accordance with the institutional Lung Soft Tissue IGRT protocol will perform this.

8.8 QUALITY ASSURANCE

All patients should have a pre-treatment dosimetric quality assurance according to departmental VMAT QA guidelines. In vivo dosimetry is not mandatory.

9. ASSESSMENT OF EFFICACY

The data analysis of investigations in this study will be qualitative and quantitative.

9.1 QUANTITATIVE DATA ANALYSIS

Quantitative CT measures

- Patients will be assessed according to the Response Evaluation Criteria In Solid Tumours (RECIST) 1.1 criteria. This published guide that defines when tumours are deemed to have responded to treatment or progressed following treatment (**appendix 3**).

Quantitative FDG-PET/CT measures

- Response measures to FDG-PET/CT will be assessed by the PERCIST and Peter Mac Criteria. Both are semi quantitative methods of response assessment to treatment using FDG-PET/CT imaging (**appendix 3**).

Quantitative FDG-PET/CT measures will be standardised uptake value (SUV) units or metabolic response as defined below:

- FDG-PET measures will be SUV maximum, SUV minimum and SUV average. Post-treatment changes in the tumour ^{18}F-FDG pattern are scored as per **appendix 3**
- Quantitative PET count density at V/Q PET/CT will be correlated to SUV parameters CT density at 3 months post-therapy, to assess any relationship between vascular, metabolic and radiological surrogates for radiation pneumonitis. These measures will also tested for association with local progression.

Quantitative Gallium PET measures will be end-inspiratory and end-expiratory volume for each lung and lobe. The following measurements will be made:

- 4-D Ventilation PET
  1. The lungs on the end-inspiration and expiration Ventilation PET scans will be contoured using semi-automatic threshold based on the operator's discretion.
- 4D-Perfusion PET
  1. The lungs on the end-inspiration and expiration Ventilation PET scans will be contoured to derive volumes
  2. The relative perfusion counts of each lung and lobe will be measured
- Lung volume measured by V/Q PET/CT will be compared with volumes measured by PFTs. Change in count density in aerated lung at end-expiration will be assessed for correlation with the dose from the radiotherapy plan to assess whether there is dose-dependence in the severity of post-radiation change.
- Functional lung dose parameters (fMLD, fV5, fV20, fV30) will be compared to the rate of ≥2 radiation pneumonitis using AUC and Spearman's rank order correlation
- Pulmonary function measures for ventilation will be forced vital capacity (FVC), forced expiratory volume over 1 second (FEV1), and total lung capacity (TLC). The pulmonary function measure for perfusion will be DLCO. These will be correlated to regional and global changes in pulmonary perfusion and ventilation as recorded by V/Q PET/CT. Routine measurements of FEV1/FVC ratio, forced expiratory flow (FEF), and Tidal Volume (TV) will be recorded, and these measurements at baseline in addition to FVC, FEV1, TLC and DLCO will be tested for association with V/Q PET/CT measures.

9.2 QUALITATIVE ANALYSIS

V/Q PET

Two nuclear medicine specialists will review the V/Q PET/CT scans and reports. Qualitative data analysis will be descriptive in nature, and based on:

- Quality of co-registration between CT with PET, characterised as adequate or not adequate
- Appearance of global lung ventilation and perfusion on PET/CT, characterised as normal, abnormal or non-diagnostic
- Visual dose-effect relationship between irradiated lung and changes in pulmonary perfusion

DECT

A radiologist will review the DECT scans and issue a standard of care report. Qualitative data analysis will be descriptive in nature, and based on:

- Qualitative difference between the ventilation CT and ventilation PET, characterised as significantly different, similar or the same.
- Qualitative difference between the iodine map of the CT (as a surrogate for pulmonary perfusion) and perfusion PET, characterised as significantly different, similar or the same.
- Qualitative between the ventilation CT and ventilation PET, characterised as significantly different, similar or the same.
- Qualitative response assessment of the primary tumour and metastasis between the DECT and FDG-PET. Classified as suspicious residual disease seen on PET only, suspicious residual disease seen on DECT only or suspicious residual disease seen on both modalities.

10. TRANSLATIONAL SUB-STUDY

10.1 CYTOKINES AS MEDIATORS OF RADIATION INDUCED NORMAL TISSUE TOXICITY

Radiation induced inflammatory cytokine release is a well-documented phenomenon. Radiation pneumonitis is a biphasic phenomenon characterised by an early inflammatory response within 12 weeks and a late fibrotic response often evident around 12 months after radiotherapy.[50] Increased levels of plasma TGF-β have been shown to predict for the risk of developing radiation pneumonitis.[51] A cytokine panel performed during the prospective observational GalliPET study demonstrated early changes in plasma IP-10, MCP-1, Eotaxin, IL-6 and TIMP-1 were associated with higher grades of radiation induced lung toxicity and these cytokines have been associated with accumulation of DNA damage in normal tissues outside of the irradiated volume.[52,53]

Chemokine (C-C) ligand 2 (CCL2/MCP-1) is a cytokine that has been associated with many inflammation-related diseases and has been implicated in the progression and prognosis of several cancers [54]. The levels of CCL2 increase in irradiated tissues and cells or in serum after single dose or fractionated low-dose irradiation in a dose-dependent manner.[55,56] CCL2 increase in serum has been associated with excess risk of cardiovascular disease. [57]

This study will aim to prospectively validate the previous GalliPET findings regarding the association of IP-10, MCP-1, Eotaxin, IL-6 and TIMP-1 with the toxicity endpoint of radiation pneumonitis. In addition to this HI-FIVE will explore the potential link between radiation-induced cytokines and the development of post-treatment clinically significant cardiac disease using the same cytokine biomarker panel including CCL2 and TGF-β. Using a broad cytokine panel such as the Ray biotech platform and an increased cohort size has the potential to reveal other significant cytokine mediators of normal tissue toxicity.

10.2 CT-DNA AS A PREDICTOR OF TUMOUR RESPONSE

Circulating tumour DNA (ct-DNA) is an evolving predictive biomarker to assess response to cancer therapies.[58] Personalised cancer profiling with deep sequencing (CAPP-seq) of ct-DNA has been recently developed and is currently the most sensitive methodology to predict response to treatment in NSCLC.[58] This study will prospectively validate these associations and build on this clinical data using our advanced imaging capabilities to correlate findings with ct-DNA levels. We envision this will enabling the development of a comprehensive toxicity risk model integrating patient risk factors, treatment risk factions, biochemical predictors of lung toxicity and tumour response and imaging predictors of lung toxicity to enable a future personalised risk adapted radiation planning strategy.

10.3 DNA DAMAGE REPAIR KINETICS AND MECHANISMS

DNA is the most significant target of radiation exposure for survival and carcinogenesis. The GalliPET study involved a translational component where during radiotherapy treatment, blood samples and eyebrow hair follicles were collected. In 16 patients γ-H2AX assay was used to monitor DNA damage in peripheral blood lymphocytes and hairs. The γ-H2AX response correlated to dose delivered to lung in circulating lymphocytes (r=0.739 p=0.009) but not in out of field hair follicles (r=0.684 p=0.062).[52] Non-linear regression analysis of DNA damage repair kinetics in a subset of 11 patients demonstrated

improved DNA damage repair efficiency during and after radiotherapy. Repair efficiency and rate of incomplete responses on FDG-PET were compared however this did not reach statistical significance (p= 0.124). DNA repair efficiency chances potentially underlie a normal tissue defence against continuous damaging effects of radiation, and radioresistance. In this study an extended panel of DNA damage and repair pathway(s) and factors involved (in addition to γ-H2AX) in the radiation treatment response will be identified. This study will prospectively validate these associations and build on this clinical data in enhancing our knowledge of how radiation toxicity and potentially treatment response and outcomes can be predicted though mid-treatment tests which can then be used to develop a personalised risk adapted radiation treatment strategy.

10.4 CARDIAC BIOMARKERS AS EARLY MARKERS OF CARDIAC TOXICITY

Cardiac radiotherapy dose as been shown to be an independent predictor of worse overall survival and increased rates of multiple different cardiac diseases.[10,26,29,40] At present there are no established methods for predicting patients that have increased cardiac radio sensitivity. Cardiac biomarkers including brain natriuretic peptide (BNP)/ NT-pro BNP and troponins may be useful serum biomarkers that could act early and perhaps pre-clinical markers of myocardial damage.[29]

Although these makers have been used extensively in the chemotherapy and targeted therapy setting, one published study by Nellessen et al. has evaluated these markers in the setting of radiation therapy.[59] This study of 23 patients, 18 with lung cancer found significant increases in both troponin and brain natriuretic peptide during a 6 week course of radiation therapy.[59] This physiologically reflects myocardial cell injury and changes in left ventricular function. Although there was a time-dependent increase in cardiac levels, levels of both BNP and troponin remained below limits usually seen in acute cardiac event or in patients with heart failure.[59] Two patients in this study had significantly reduced ejection fraction between immediately pre and immediately post treatment echocardiograms.[59] There was no long term follow up reported for the patients described in the study.

An elevation of cardiac troponin indicates the presence of myocardial injury but not the underlying cause.[60] Increased troponin levels have been shown to correlate with worse outcomes in critical care and perioperative.[61] However, at present there is no data from randomized, controlled trials to assess the efficacy of interventions or pharmacotherapies aimed at reducing the risk of adverse events among patients with troponin elevations in the absence of an acute coronary syndrome.[62]

For a diagnosis of myocardial infarction to be confirmed patients must have an elevated cardiac biomarker with at least one of the following present: "symptoms of ischemia, new or presumed new significant ST-segment-T wave changes or new left bundle branch block, development of pathological Q waves on the electrocardiogram, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, or identification of an intracoronary thrombus by angiography or autopsy".[61]

Using a chest pain history patients experiencing any features that could indicate a clinically significant elevated troponin will be excluded from a non-clinical point of test biomarker troponin and instead undergo urgent medical review by a medical officer and if appropriate, referred for the necessary clinical investigation and management of their symptoms.

The availability of rapid result, point of care cardiac biomarker testing has made mid-treatment cardiac biomarker testing technically feasible. Patients who have early signs of cardiac injury demonstrated by raised cardiac biomarkers may be at increased risk of developing late cardiac toxicity. Further study is needed to establish if this link between early cardiac biomarkers and late clinically significant cardiac toxicity exists. If there is a correlation, mid-treatment cardiac biomarker testing can be used to drive a risk-adapted radiotherapy approach where strategies such as re-planning with breath hold techniques, pharmaco-prevention of myocardial injury with medications such as ACE inhibitors or beta blocker or other strategies of cardiac dose reduction including the use of non-coplanar arcs or proton therapy could be considered.[59]

10.5 METHODOLOGY

Blood samples will be collected and processed at the following time points:

- At baseline before treatment. (This will be taken at the time of blood collection prior to injection of the Ga-68 tracer for the baseline PET scan)
- Within 1-hour after the first fraction of radiotherapy
- Within 1 hour prior to the second fraction radiotherapy. Patients will be scheduled so that the 2nd fraction of radiotherapy occurs approximately 24 hours following the first fraction of radiotherapy (+- 2 hours).
- Mid-treatment (within 1 hour before the 20th fraction)
- 3-months post-treatment (this will be taken at the time of blood collection prior to injection of the Ga-68 tracer for the post-treatment PET scan)

- At each time point that blood samples are taken a history of cardiac symptoms will be taken to exclude patients who are at risk of an intercurrent clinically significant cardiac or pulmonary event

Approximately 28-36ml of blood samples (4x 9ml EDTA tubes) will be drawn at each of the specified time points above.

Collection of lymphocytes by Ficoll gradient separation. To process the blood sample for biodosimetric analysis, the following methodology will be used:

- Fixing and immunofluorescent staining using a mouse γ-H2AX primary antibody (Abcam) and secondary anti-mouse antibody labelled with Alexa488 fluorescent dye (Millipore). Further immunofluorescent markers of the DNA damage response will be identified and acquired to investigate alternate pathways.
- Kinetics of DNA repair factors and their co-localization will be analysed using imaging with microscopy techniques established in the group, to identify the repair pathway involved indicating the respective DNA damage repair pathways and kinetics.
- A genomic analysis of DNA repair factors will occur following this microscopy study, using a customised panel of genes.

To process the blood samples for assessment of cytokine release and potential future ct-DNA analysis, the following methodology will be used:

- Serum will be stored for future point of care cardiac biomarker testing.
- Serum (2 separate samples) will be separated and frozen at -80ºC until analysis within 1 hour of blood collection for samples undergoing future ct-DNA analysis (pre-treatment and 3 month post treatment timepoints). All other samples must be separated and frozen at -80ºC until analysis within 2 hours of blood collection.
- The samples stored (pending further grant funding) then will be sent in batch to Ray biotech for a human inflammatory cytokines panel (Austin, TX) for cytokine screening as described in the pilot cytokine study[53]
- At a future date, dependent on further funding, ct-DNA samples will be assessed for ct-DNA levels (in collaboration with Professor Diehn's group at Stanford university). The ct-DNA samples that will be processed will be the pre-treatment and 3 month post-treatment blood samples.

11. ADVERSE EVENTS

11.1 ADVERSE EVENT DEFINITION

An Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product (or any other protocol specified intervention including radiation therapy, surgery or use of a device) and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign, symptom or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product (or associated with the use of any other protocol specified intervention including radiation therapy, surgery or use of a device).

AEs include: 'Adverse Drug Reactions', i.e. a reaction, in contrast to an event, is characterised by the fact that a causal relationship between the drug and the occurrence is suspected.

Adverse events are graded according to CTCAE v4.03 **see appendix 4**

For unapproved medicines: any noxious and unintended response to a medicinal product, related to any dose. The phrase "response to an unapproved medicinal product" means that a causal relationship between the product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out. ('Unapproved medicinal product' here includes approved products used at levels or in ways that are unapproved).

Regarding marketed medical products: a noxious and unintended response to a drug that occurs at doses normally used in humans for prophylaxis, diagnosis or therapy of diseases or for modification of physiological function.

11.2 UNEXPECTED ADVERSE EVENT DEFINITION

An unexpected adverse event (UAE) is an AE for which the nature or severity of the event is not consistent with the information in the relevant source documents e.g. the IB, published information, product information (or with the applicable side effect risk profile for radiation therapy, surgery or use of a device).

UAEs also include unexpected adverse drug reactions (UADR) - The nature and severity of the ADR is not consistent with the information in the Investigators Brochure for an unapproved investigational product, or the product information/package insert/summary of product characteristics for an approved product.

11.3 SERIOUS ADVERSE EVENT DEFINITION

Adverse events and adverse drug reactions are considered 'serious' if they threaten life or function.

Due to the significant information they provide, serious adverse events (SAE) (including Serious Adverse Drug Reactions) require expedited reporting. SAEs are defined as any adverse event or adverse drug reaction (including radiopharmaceuticals) which:

- Results in death (i.e. fatal/grade 5 CTC AE) **see appendix 4**
- Is life-threatening (i.e. grade 4 CTC AE) **see appendix 4**
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity; or
- Is a congenital anomaly/birth defect
- Other significant medical event*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

The following are NOT considered SAEs:

- Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term care facility).
- Surgery or procedure planned before entry into the study. Note: Hospitalizations that were planned before the signing of the PICF, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is

to be reported as a new serious adverse event.

- Disease progression should NOT be reported as an adverse event or serious adverse event term; instead, signs and symptoms of clinical sequelae resulting from disease progression/lack of drug efficacy will be reported if they fulfill the serious adverse event definition.
- A standard procedure for protocol therapy administration will not be reported as a serious adverse event. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a serious adverse event.
- The administration of blood or platelet transfusion. Hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable serious adverse event.
- A procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling, pharmacokinetic or biomarker blood sampling). Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
- Prolonged hospitalization for technical, practical, or social reasons in the absence of an adverse event.

Radiation overdoses should be reported in an expedited fashion if the events associated with the overdose meet the SAE definitions listed above. If no serious adverse events are experienced the overdose must be reported on the relevant trial forms.

11.4 ATTRIBUTION

Attribution of cause requires at least a reasonable possibility of a causal relationship between the event and the use of the investigational drug or any other protocol-specified intervention.

All protocol-specified interventions (including pharmaceutical products, radiation therapy, surgery or use of a device) administered prior to the date of the event must be attributed a degree of causality from one of the following codes:

RELATIONSHIP	ATTRIBUTION	DESCRIPTION
Unrelated	Unrelated	The AE *is clearly **NOT** related* to the intervention
	Unlikely	The AE *is **doubtfully related*** to the intervention

Related	*Possible*	The AE ***may be related*** to the intervention
	Probable	The AE *is* ***likely related*** to the intervention
	Definite	The AE *is* ***clearly related*** to the intervention

11.5 SEVERITY CRITERIA

An assessment of severity grade will be made using the NCI-CTCAE (version 4.03). Where parameters are not addressed within the criteria, severity of AEs should be graded as:

Mild = Aware of sign or symptom, but easily tolerated

Moderate = Discomfort enough to cause interference with usual activities

Severe = Incapacitating with inability to work or perform usual activities

Life-threatening = Patient is ***at immediate risk of death***

Fatal = Death

11.6 ADVERSE EVENTS REPORTING

All adverse events, which occur whilst the patient is enrolled on the trial, must be reported in the patients' medical records and recorded on the relevant CRF.

11.7 EVALUATING ADVERSE EVENTS

An investigator who is a qualified medical doctor will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.03. All adverse events regardless of CTCAE grade must also be evaluated for seriousness. Laboratory values need reporting as AEs only if abnormal and deemed clinically significant by the investigator.

11.8 SERIOUS ADVERSE EVENT REPORTING

11.8.1 TRIAL SITES/INVESTIGATORS

All SAEs that occur from the time a patient has signed consent for the Trial to 24 months of the final protocol-specified treatment, intervention or procedure are required to be reported to the Sponsor

whether or not considered related to the treatment under investigation.

Serious adverse events should be reported to the Principal Investigator and Sponsor within 24 hours as per the PI Flow chart Safety reporting in Peter Mac sponsored studies.

The Principal Investigator (PI) must:

- Determine whether an AE is 'Serious' (refer to section xx)
- For SAEs, the PI must then ascertain the suspected cause
- The attribution to the SAE must be recorded in the patients' medical records and reported on the SAE form.

SAEs must be reported by completing the Trial SAE form and emailing to the following:

Email To:	
Sponsor (Peter MacCallum Cancer Centre)	safetyreporting@petermac.org

SAE forms are required at the following points:

Initial Report	Within one working day/24 hours of discovery or notification of the event. If the reporting of an SAE is delayed by more than 24 hours, an explanation must be provided in the comments section of the SAE form.
Incomplete Reports	If all details are not available at the time of the initial report a completed report must be sent within the next 10 days.
Updated Report	If the event is not resolved (or 'on-going') at the time of the initial report, the SAE Form must be submitted every 30 days until the event is resolved, death has occurred or the condition has stabilised. If a change occurs in a stable condition (i.e. either worsens or improves), then a new SAE Form should be faxed

The Investigator is ultimately responsible for reporting the SAE and must sign the final SAE report(s). Should this Investigator not be available to sign the initial SAE form within the 24-hour period, a comment to this effect must be written on the form and the form signed by the clinician attending to the patient at the time and faxed to the Sponsor. The investigator must sign the SAE form as soon as possible and re-fax to the Sponsor.

The Investigator at the Trial Site is responsible for determining the local SAE reporting requirements of the responsible HREC and subsequently notifying the HREC of SAEs as required.

All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the patients participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (patient or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

11.8.2 SPONSOR

The Sponsor is responsible for:

- Implementing and maintaining a suitable recording system to record information from all SAEs received from Trial Sites.
- Ensuring that the Coordinating Principal Investigator (CPI) is notified of each SAE to enable the SAE to be assessed by the CPI and any other appropriate reviewers for nature (expected/unexpected), causality and whether the TGA needs to be notified of the SAE.
- Under the direction of the CPI, notifying the TGA (Australia) in accordance with the regulatory authority's detailed guidance of any SUSARs that are fatal or life threatening as soon as possible but no later than 7 days after the site gained first knowledge of the event. Incomplete reports must be completed and forwarded as soon as possible within 8 additional calendar days. All other serious, unexpected ADRs should be reported to the TGA within 15 days after the site gained first knowledge of the event.
- Considering information provided by (non-serious) adverse event data.
- Informing each trial site of new information arising from serious and non-serious adverse events and adverse drug reactions that may affect the conduct of the Trial, or the rights, interests, safety or wellbeing of trial patients.
- Under the direction of the CPI, notifying the TGA of any significant issue that has arisen from analysis of overseas reports or action that has been taken by another country's regulatory authority within 72 hours of first knowledge.

11.9 OTHER SITUATIONS REQUIRING EXPEDITED REPORTING

11.9.1 OVERDOSES

Radiation Overdoses must be reported to the Principal Investigator if the event(s) associated with the overdose meet the SAE definitions. If no serious adverse events are experienced the overdose must be reported in the patients' medical record and transcribed onto the relevant trial CRF.

11.9.2 NEW CANCERS

The development of new cancers at any time during the trial must be reported in the patients' medical record and transcribed onto the relevant trial CRF. If any events associated with the new cancer meet the SAE definitions, then they should also be reported in an expedited fashion.

11.9.3 PREGNANCY

Because the effect of the radiopharmaceuticals on sperm is unknown, pregnancies in partners of male patients during therapy or within 90 days of stopping treatment will be reported by the study-site personnel within 24 hours of their knowledge of the event. Written informed consent for release of medical information from the partner must be collected prior to collection of any pregnancy-specific information and the pregnancy will be followed to outcome. In all cases, follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

All initial reports of pregnancy must be reported by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g., spontaneous abortion, stillbirth, and congenital anomaly) are considered serious adverse events and must be reported as a Serious Adverse Event. Any patient who becomes pregnant during the study must discontinue further study treatment.

12. STATISTICAL CONSIDERATIONS

This is a single arm interventional feasibility study of patients with primary non-small cell lung cancer

who are treated with curative intent radiation / chemoradiation therapy.

12.1 ANALYSIS POPULATION

- Enrolled participant population includes all participants registered to the study
- Evaluable patient population includes all registered participants who commenced protocol treatment. This is the primary population for analysis. Non-evaluable patients will be replaced.

12.2 STATISTICAL METHODS

Descriptive statistics of baseline characteristics of all evaluable patients will be reported. Continuous variables will be described as mean, standard deviation, median, minimum and maximum, and qualitative variables will be described as counts and percentages. Unless stated otherwise, the calculation of proportions will not include the missing category in the denominator. No imputation for missing value is intended and all confidence intervals provided will be 95% two-sided.

Feasibility rate, rate of grade ≥ 2 radiation pneumonitis, rate of grade ≥ 2 acute and late toxicities and CMR rate (PeterMac Visual and PERCIST 1.0) will be described as percentages with 95% confidence intervals using exact methods.

PFS and OS curves will be described using Kaplan-Meier methods. The curves will be presented with 95% confidence intervals. A cut-off date for follow-up will be determined at the time of analysis. The cut-off date will be chosen to enable data on follow-up to that date to be collected, where possible, on all living patients. All events occurring after this date will be ignored in the analysis in order to minimise reporting bias.

Inflammatory cytokines release of patients with grade ≥ 2-radiation pneumonitis will be compared with patients with grade <2-radiation pneumonitis using the Wilcoxon rank sum test.

Regional ventilation loss and regional perfusion loss will be described as mean, standard deviation median, minimum and maximum. The correlation of change in respiratory function testing with regional ventilation loss and regional perfusion loss will be assessed using Spearman's correlation.

The association between PET/CT ventilation and CT Ventilation and between PET/CR perfusion and DECT perfusion will be assessed using Spearman's correlation.

Quality of life will be analysed using linear mixed models (LMM) with time (as factor) included as a

fixed effect and patient included as a random effect. No within-group correlations will be assumed, with the model being fitted by maximizing the restricted log-likelihood (REML). No imputation for missing values is intended. Means and 95% confidence intervals will be estimated from the LMM contrasts for each time point.

12.3 SAMPLE SIZE CALCULATION AND EXPECTED DURATION

The study sample size is pragmatic and is based on the clinically relevant number of patients needed to determine the technical feasibility of the functional lung sparing VMAT radiotherapy technique.

We plan to recruit 20 participants with NSCLC who have been referred for curative intent radiotherapy. Approximately 1 new patient commences treatment across the Parkville and Sunshine Peter MacCallum Cancer Centre per week. It is therefore expected accrual to the study should take no more than 2 years to complete.

The Table below shows the exact 95% confidence intervals for different scenarios of feasibility rates.

Confidence intervals for different feasibility rate scenarios.

Number of feasible cases	*Feasibility rate*	*Exact 95% confidence intervals for rate estimate*	
		Lower limit	Upper limit
15	75%	51%	91%
16	80%	56%	94%
17	85%	62%	97%
18	90%	68%	99%
19	95%	75%	100%
20	100%	83%	100%

The study follow up duration was designed to be pragmatic for a feasibility study whilst having enough follow up duration to detect clinically significant toxicity. In the context of lung cancer radiotherapy, late cardiac and lung toxicity typically presents within a 12 month time period. This significantly shorter timeframe compared with late radiotherapy toxicities from the treatment of breast or hematological malignancies is potentially due to the considerably higher doses delivered to

the organs at risk.

A recent publication by Wang et. al. has demonstrated 23% of patients had clinically significant late cardiac radiation toxicity at a median time of 26 months post treatment.[10] Later onset pulmonary toxicities including pulmonary fibrosis are typically detected within the 12-month timeframe. Our recent systematic review showed that functional lung imaging dose-response relationships plateau around 6-12 months post treatment.[63]

Due to the aggressive nature of locally advanced NSCLC median progression free survival ranges from 6-11 months. FIVE plans to recruit over 24 months and with a 12-month follow up period, we expect the median potential follow-up of surviving patients to be over 24 months.

12.4 ANALYSIS PLAN

There are 2 analysis planned for the study: main analysis and final analysis.

Main analysis

The main analysis will be performed after all patients have been registered and completed their 3-month follow-up assessment. Baseline patient and tumour characteristics, treatment details, rate of complete metabolic response on FDG-PET/CT, qualitative comparison of response assessment of FDG-PET/CT with DECT, the rate of radiation pneumonitis and other acute toxicities analysis will be provided.

Final analysis

The final analysis will be performed at the completion of the study, which will be 12 months after the final patient completes treatment. Overall survival, progression free survival, rate of complete metabolic response, associations of cardiac biomarkers and coronary calcium scoring with cardiac toxicity, the associations of cytokine levels with radiation pneumonitis, rate of acute and late toxicity and associations between PET Ventilation and CT ventilation, PET Perfusion and DECT iodine maps (as a surrogate for pulmonary perfusion) will be provided. Updated treatment and safety results will also be provided.

12.5 EARLY TERMINATION CRITERIA

Adverse Events (AEs) must be reported on the relevant trial case report forms (CRFs). Documentation of an adverse event requires specific information regarding the signs, symptoms, or disease. The Common Terminology Criteria for Adverse Events (CTCAE version 4.03) must be used to grade the severity of an event. Regular analyses of cumulative AE data should be undertaken by the trial statistician and discussed at the Data Safety Monitoring Committee meeting when convened. Any grade 3 or 4 AE's that do not meet criteria for an SAE (see Serious Adverse Event) should be sent to the principal investigator for evaluation.

SAE's will be forwarded to the Data Monitoring Committee. If three SAE's are recorded during the trial, the Data Monitoring Committee must convene and determine the causal link between SAE's and the research. An assessment must be made regarding early termination of the trial, and recommendations forwarded to the Peter MacCallum Ethics Committee. The Data Monitoring Committee should also convene at the completion of the trial to assess safety of this project.

12.6 DEVIATIONS

Any deviations from the statistical plan should be described and justified in a protocol amendment or in the final report.

13. TRIAL MANAGEMENT & ADMINISTRATIVE REQUIREMENTS

13.1 PROTOCOL AMENDMENTS

There are no protocol amendments to date

13.2 MONITORING AND QUALITY ASSURANCE

13.2.1 INFORMATION OF TRIAL PERSONNEL

The Investigator(s) is responsible for ensuring that all trial personnel are qualified for their designated roles and provides information about the trial to all staff members involved in the trial or any element of patient management, both before starting the practical performance of the trial and during the course of the trial (e.g. when new staff become involved).

Additional information available during the trial should be given, as agreed upon, either by the

investigator or delegate and always when a new staff member becomes involved in the trial.

13.3 INDEPENDENT DATA MONITORING COMMITTEE

An independent Data Monitoring Committee will be appointed and will convene with the purpose of:

- Assessing quality issues related to RT.
- Assessing the conduct and progress of the trial – accrual, non-eligibility, treatment toxicity and serious adverse events.

SAE's will be forwarded to the Data Monitoring Committee. If three SAE's are recorded during the trial, the Data Monitoring Committee must convene and determine the causal link between SAE's and the research. An assessment must be made regarding early termination of the trial, and recommendations forwarded to the Peter MacCallum Ethics Committee. The Data Monitoring Committee should also convene at the completion of the trial to assess safety of this project.

13.4 AUDIT AND INSPECTION

According to ICH/GCP Guidelines, the Sponsor may audit the investigational site to compare raw data, source data and associated records with the interim (if applicable) or final report of the trial to assure that data have been accurately reported. The Sponsor's Clinical Quality Assurance department is responsible for the auditing of the trial.

The Investigator(s) must accept that regulatory authorities may conduct an inspection to verify compliance of the trial with GCP.

13.5 PROTOCOL DEVIATIONS

The protocol must be read thoroughly and the instructions must be followed. However, exceptions will be made in emergency situations when the protection, safety and wellbeing of the patient requires immediate intervention based on the judgement of the Investigator or a responsible, appropriately trained and credentialed professional(s) designated by the Investigator as a sub-investigator.

In the event of a significant deviation due to an emergency, accident or error, the Investigator or designee must contact the Principal Investigator at the earliest possible time by telephone. This allows for an early joint decision to be made as to whether or not the patient should continue in the trial. The

Investigator and the Sponsor will document the decision. HREC must be notified of the deviation if the patient's safety is compromised.

14. DATA HANDLING AND RECORD KEEPING

14.1 CASE RECORD FORM (CRF)

In this trial the CRF will be electronic (eCRF). The investigator or the designated site person must complete the CRF and supporting documentation for each patient within a timely manner of the visit occurring.

The Clinical Trial Manager(s) will review the completed data for accuracy, completeness and consistency. The Clinical Trial Manager will submit requests for correction / clarification of data (e.g. queries) to the Investigator or designee when inconsistencies are identified during review, monitoring (if applicable) or during the edit check process.

All corrections and alterations to eCRF data must be made by the investigator or by the designated site personnel in a timely manner and in according to the instructions provided. Completed eCRFs should be reviewed and electronically signed by the Principal Investigator or designated site personnel. All persons appointed by the Investigator to participate in the trial must be indicated on the delegation of authority log.

14.2 SOURCE DOCUMENTS

The investigator is required to prepare and maintain adequate and accurate case histories (i.e. medicals) designed to record all observations and other data pertinent to the trial for each trial patient. The medical records must contain adequate information to allow for verification of patient identity throughout the trial.

Any date recorded directly on the CRF, as agreed by the Sponsor for which no other written or electronic record will be maintained in the patient's medical record, will be considered source data (e.g. results from physical examinations, vital signs testing or the drug administration procedure).
The CRF and the patient's medical records pertinent to the trial may be reviewed by a designated monitor, auditors and possibly by representatives from the IRB/IEC and regulatory bodies such as the TGA to the extent permitted by regulation.

The investigator is required to retain a patient identification code list to allow unambiguous identification of each patient included in the trial. This list should contain the patient's full name, data of birth, and dates of participation and trial identification number. This list is password protected and stored at the Investigator site.

14.3 ARCHIVING OF TRIAL DOCUMENTS

Trial data and other essential documentation will be retained for a period of at least 15 years.

The original source documents and CRFs will be archived by the Investigator for 15 years. No trial document or image will be destroyed without prior written agreement between the Sponsor and the Investigator(s). Should the Investigator(s) wish to assign the trial records to another party or move to another location, advance written notice will be given to the Sponsor.

15. SAFETY CONSIDERATIONS

Radiotherapy plans will be adapted based on the pre-treatment V/Q PET/CT and there will be a boost given to the primary tumour. This will therefore modify the treatment patients will receive. Despite these modifications, strict dose constraints will be observed and patients will be monitored closely for adverse events. The two components of intervention: functional lung adaptation and dose escalation are similar to current prospective trials being conducted internationally.[21,32,33,35,64]

Potential Benefits to Patients from Trial

Although planning modifications will be made with an aim to minimise risk of radiation induced lung toxicity these toxicities are relatively uncommon and therefore individual participants are unlikely to draw any direct benefit from participation in this study. VMAT planning and delivery is commonly used the treat this type of cancer at a number of Peter MacCallum centres although the integration of functional lung information a new component of the planning process. The minimum prescribed radiation dose and fractions in this trial is the current considered standard of care and is acceptable to achieve potential cure (60 Gy in 30 fractions). Based on available phase 3 evidence (from the RTOG 0617 trial) there is no evidence that dose escalation improves overall survival.[11] In this trial dose escalation to the entire treatment volume resulted in decreased overall survival and increased normal tissue toxicity. The results of this trial were contrary to multiple phase 2 studies and large retrospective pooled data analysis.[19] Due to this evidence it is therefore unlikely that dose escalation in this trial will be of any benefit to individual participants.

The HI-FIVE trial places strict dose constraints on the heart due to recent evidence from dosimetric analysis of RTOG 0617 and other dose escalation trials showing increase in cardiac toxicity and reduced survival with increased heart doses. The VMAT planning used in HI-FIVE does significantly reduce cardiac dose compared to conventional radiotherapy planning methods. Given this evidence, decreased cardiac dose may benefit individual participants.

Additional cardiac investigations may assist in the earlier detection of cardiac injury or toxicity and prompt earlier interventions than the standard of care investigations.

Potential Benefits to Society from Trial

The investigations included in the study have the potential to produce substantial societal benefit. If dual energy CT and ventilation CT methods are shown to be reliable at providing ventilation and perfusion information, a large scale clinical trial using these methods to reduce radiotherapy dose to

the functional lung and reduce radiation related toxicity. Our collaborating radiologist has also indicated that these CT ventilation and perfusion methods have the potential to significantly change CT chest practices for other non-oncologic conditions including COPD and asthma.

The results of the radiotherapy treatment component of this trial have the potential to significantly benefit society (in particular other patients with locally advanced NSCLC). If dose escalation or functional lung adaptation is shown to be feasible, a larger scale trial investigating these interventions further will be conducted. The biomarkers being investigated in this trial will provide information regarding how these biomarkers can be used to help personalise and radiotherapy to an individual patients risk of future complications.

Dose Escalation

The minimum prescribed radiation dose and fractionation in this trial is the current considered standard of care and is acceptable to achieve potential cure (60 Gy in 30 fractions). There is a potential increased risk of clinical toxicity secondary to the intensified radiation therapy (additional 9Gy in 30 fractions to the primary tumour – 69Gy in 30 fractions total dose). All efforts will be made to minimise dose to normal tissues and specified dose constraints should be met. In particular Cannon et. al. demonstrated risk of grade 4 or 5 toxicity caused by damage to the proximal bronchial tree from hypo fractionated, dose escalated radiation therapy. For this reason, dose escalation will occur to the primary tumour ITV alone avoiding nodal disease in close proximity to this structure. In addition, a strict dose constraint is placed on this structure. If the dose constraints cannot be met the boost dose will be reduced. Using strict normal tissue dose constraints it is not expected that there will be any clinically detectable excess toxicity. Potential additional risks to the patient from increased radiotherapy dose include an increased risks of; radiation pneumonitis, oesophagitis, damage to the proximal bronchial tree causing grade 4 or 5 toxicity, dermatitis, dyspnoea, cough, brachial plexopathy cardiac toxicity and myelopathy.

Functional Lung Avoidance

It is not expected that introducing additional functional lung information into the planning process would result in any additional toxicity. VMAT planning and delivery is commonly used the treat this type of cancer at a number of Peter MacCallum centres although the integration of functional lung information a new component of the planning process. Normal anatomical lung constraints will be reported and these will be secondary objectives in the planning process. Particular attention will be paid to the other organs at risk to ensure that additional dose is not being distributed to normal tissues to avoid functional lung.

V/Q PET/ CT

V/Q PET: Our institution has now performed over 311 V/Q PET scans using Galligas and ^{68}Ga-MAA in patients since 2010 under the GalliPET clinical trial (HREC 11/64). There have been no adverse events related to the administration of these imaging agents to date. Therefore risk of adverse event related to Galligas and ^{68}Ga-MAA administration in this study is minimal. Should any adverse event occur, the participant would be treated promptly in a hospital setting. These tracers are almost identical (except for the radiolabel itself) to their ^{99m}Tc analogues (Technegas and ^{99m}Tc-MAA, respectively), which have been used extensively worldwide, including at Peter MacCallum Cancer Centre for several decades. Cases of transient hypoxia have been reported in the literature occurring upon initial inhalation of Technegas. Specific adverse reactions attributable to MAA have not been noted but there exists literature reports of adverse reactions in patients with pre-existing severe pulmonary hypertension. Instances of hemodynamic or idiosyncratic reactions have also been reported (product insert, Pulmolite, Pharmalucence Inc, Bedford, MA, USA). ^{68}Ga is chemically identical to ^{67}Ga, which has been used in nuclear medicine for over 50 years.

The main risk associated with this imaging procedure is the low of additional radiation exposure from the V/Q PET/CT procedure mainly from the 4D-CT component (up to 17 mSv). Gallium-68 ventilation and perfusion PET results in radiation exposure similar to conventional SECT scintigraphy with ^{99m}Tc-labelled compounds. 20 MBq of Technegas and 100 MBq of ^{99m}Tc-MAA results in an effective dose of about 2.04 mSv[65] and this is comparable to the use of 5 MBq Galligas and 20 MBq GaMAA in this study[65]. Additional dose will be delivered to the patient as a result of the contemporaneous 4D CT scan performed at the time of the Gallium PET/CT. This 4D CT has a longer acquisition time compared to a standard CT in order to take into account multiple breathing cycles in a similar way to a PET scan. The 4D CT scan will take ~1 minute to acquire, and should account for <15mSV of dose. Thus, the combined effective radiation dose of Gallium PET/CT (~2mSv) with co-registered 4DCT (<15mSV) is typically no more than 17mSv. This is comparable to that of a CT pulmonary angiogram study (about 16 mSv), and is significantly less than the dose incurred during radiation treatment.

These doses are comparable to many other diagnostic imaging procedures, and significantly lower than the lower the radiation dose associated with radiotherapy. The principal source of inconvenience for the participant will be to spend about 45 minutes of additional time for each V/Q PET/CT scan. Our technologists always take care to maximize the comfort of the patients.

Participants in this study will be monitored with the same level of medical attention as every patient undergoing routine FDG-PET/CT or V/Q SPECT/CT scanning at our facility. In the unlikely event of

adverse reaction to ^{68}Ga-MAA injection or Galligas, prompt medical attention will be provided, and the PET/CT procedure will be stopped if required.

FDG-PET/CT

An additional FDG PET/CT scan will be performed post-treatment. The FDG PET/CT a routinely performed investigation in the staging of lung cancer and frequently performed following radical radiotherapy, particularly to plan early salvage surgery in patients who may fail the radiotherapy treatment. Post-treatment FDG-PET provides important prognostic information to the patient, as our group has previously reported that post-therapy metabolic response is more predictive for patient outcomes then any other known pre-treatment factors[66]. The CT component of this study will account for 4mSv per scan and the FDG PET component gives an additional 4mSv per scan in total the dose will be approximately 8mSV. Risks of anaphylaxis or other adverse events from the tracer itself are rare and even more so in this patient group due to their prior exposure to the FDG tracer in the staging of their lung cancer.

Dual Energy Computed Tomography

CT of the chest with contrast is commonly used in the re-staging of lung cancer and is considered a standard of care. The use of IV contrast does involve risk of anaphylaxis, other allergic reactions and risk of renal impairment. This is not an additional risk with DECT in particular given that patients would be receiving a contrast enhanced CT scan of the chest and abdomen as part of the follow up process. An additional consideration is the use of three separate phases in the DECT to provide additional ventilation and perfusion information. This does add a small increased dose of radiation, this is approximately 9mSv per scan and an additional 3mSV at the two nodal response assessment time points due to the ability of the SOMATOM Definition Force device to considerably minimise radiation dose.[67]

Additional Ionising Radiation Dose

The expected additional total dose due to investigational procedures will be less than 92 mSV (Table 1- Total doses). Due the theoretical potential risk of ionising radiation to induce malignancy, these doses are minimised using the ALARA (as low as reasonably achievable) principle. The unit mSv is a quantity, which takes radiation quality and the body parts irradiated into account to derive a relative risk quantity. For a whole body irradiation with photons, 1mGy of dose is equivalent to 1mSv. The excess dose received during the course of the study needs to be taken in the context of a standard of care radiation treatment dose of 60,000 mGy to the lungs. The radiation doses incurred by the investigational imaging are small in relation to the therapeutic dose delivered to the target.

Table 1 Total Doses

	V/Q 4DCT-PET	DECT + CT Vent	DECT Nodal response	FDG –PET/CT
Incremental Dose per Scan	17mSv	9mSV	3mSV	8mSV
Number of procedures	3	3	2	1
Total additional dose in protocol	51mSV	27mSV	6mSV	8mSV

Pulmonary Function Tests and Echocardiogram

There are no risks to the patient anticipated in the conduct of the outlined pulmonary function tests or echocardiograms.

16. ETHICAL ASPECTS

The protocol is designed to comply with the Declaration of Helsinki and ICH GCP guidelines and adheres to the principles outlined in the NHMRC National Statement on Ethical Conduct of Human Research. The Principal Investigator is responsible for ensuring that written informed consent is obtained from the patient before trial entry.

16.1 INFORMED CONSENT

Written informed consent will be obtained from each patient before any procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. Explanation will also be provided to the patients that they are free to refuse entry into the trial and free to withdraw from the trial at any time without prejudice to future treatment.

The patient's willingness to participate in the trial will be documented in writing on a consent form, which will be signed by the patient with the date of that signature recorded. The Investigator(s) will keep the original consent forms and copy will be given to the patient. In addition, the person conducting the informed consent discussion will document the process of obtaining consent in the patient's medical record.

Bucknell N, *et al. BMJ Open* 2020; 10:e042465. doi: 10.1136/bmjopen-2020-042465

16.2 CONFIDENTIALITY REGARDING TRIAL PATIENTS

The Investigator must ensure the privacy of the patients, including their personal identity and all personal medical information, will be maintained at all times. In CRFs and other documents submitted, patients will not be identified by their names, but by an identification code (e.g. patient ID number).

An exception is where the trial participant has provided written consent for his/her records to be included in source document verification. In this instance, the records may be inspected by the investigator(s) for the purposes of source document verification or quality audit as stipulated in the ICH GCP Guidelines, or (b) a representative of a government regulatory authority for the purposes of official inspection. Records must be made available for inspection on the understanding that all information relating to trial participants will be treated in strict professional confidence.

17. PUBLICATION AND PRESENTATION POLICY

17.1 REPORTING OF RESULTS

The Trial Management Committee will be responsible for decisions regarding presentations and publications arising from this trial according to the Sponsor Authorship, Publication and Spokesmanship Guidelines.

The statistician will perform the primary analysis of trial results, for publication. The principal investigator will publish the primary trial results.
Publications and abstracts must be presented to the TMC for review and approved prior to submission.

The results of this study will be published in a peer reviewed medical journal. On request, this publication will be made available to participants in the trial. Should for unforeseen circumstances, the results of this study not be published in a peer reviewed journal, then upon request a patient information sheet containing the broad findings of the trial will be provided to the participants.

17.2 TRIAL REGISTRY

The Trial Chair (TC) is responsible for registering all trials with an appropriate clinical trials registry prior to the accrual of the first patient.

18. FINANCIAL CONSIDERATIONS

A Peter MacCallum Foundation Grant has funded the cost of V/Q PET/CT. This grant also includes funding for statistical analysis. A RANZCR (Royal Australian and New Zealand College of Radiologists) Research Grant has funded laboratory consumables and staff. Laboratory Data analysis will be conducted by the investigators.

19. REFERENCES

[1] Palma DA, Senan S, Tsujino K, Barriger RB, et al. Predicting radiation pneumonitis after chemoradiation therapy for lung cancer: An international individual patient data meta-analysis. *Int J Radiat Oncol Biol Phys.* 2013;85:444-450.DOI: 10.1016/j.ijrobp.2012.04.043.

[2] Bradley JD, Hu C, Komaki RU, Masters G, et al. Long-term results of rtog 0617: A randomized phase 3 comparison of standard dose versus high dose conformal chemoradiation therapy +/- cetuximab for stage iii nsclc. *Int J Radiat Oncol Biol Phys.* 2017;99:S105.DOI: 10.1016/j.ijrobp.2017.06.250.

[3] Siva S, Hardcastle N, Kron T, Bressel M, et al. Ventilation/perfusion positron emission tomography-based assessment of radiation injury to lung. *Int J Radiat Oncol Biol Phys.* 2015;93:408-417.

[4] Siva S, Callahan J, Kron T, Martin OA, et al. A prospective observational study of Gallium-68 Ventilation and Perfusion PET/CT during and after radiotherapy in patients with non-small cell lung cancer. *BMC Cancer* 2014;14:740.

[5] Siva S, Devereux T, Ball DL, MacManus MP, et al. Ga-68 MAA perfusion 4d-PET/CT scanning allows for functional lung avoidance using conformal radiation therapy planning. *Technology in Cancer Research & Treatment* 2016;15:114-121.

[6] Siva S, Thomas R, Callahan J, Hardcastle N, et al. High-resolution pulmonary ventilation and perfusion pet/ct allows for functionally adapted intensity modulated radiotherapy in lung cancer. *Radiotherapy & Oncology* 2015;115:157-162.

[7] Hardcastle N, Hofman MS, Hicks RJ, Callahan J, et al. Accuracy and utility of deformable image registration in 68Ga 4D PET/CT assessment of pulmonary perfusion changes during and after lung radiation therapy. *Int J Radiat Oncol Biol Phys.* 2015;93:196-204.DOI: 10.1016/j.ijrobp.2015.05.011.

[8] Callahan J, Hofman MS, Siva S, Kron T, et al. High-resolution imaging of pulmonary ventilation and perfusion with 68Ga-VQ respiratory gated (4-D) PET/CT. *Eur J Nucl Med Mol Imaging* 2014;41:343-349.DOI: 10.1007/s00259-013-2607-4.

[9] Jiang X, Li T, Liu Y, Zhou L, et al. Planning analysis for locally advanced lung cancer: Dosimetric and efficiency comparisons between intensity-modulated radiotherapy (IMRT), single-arc/partial-arc volumetric modulated arc therapy (sa/pa-vmat). *Radiation Oncology* 2011;6:140.DOI: 10.1186/1748-717x-6-140.

[10] Wang K, Eblan MJ, Deal AM, Lipner M, et al. Cardiac toxicity after radiotherapy for stage III non–small-cell lung cancer: Pooled analysis of dose-escalation trials delivering 70 to 90 gy. *J. Clin. Oncol.* 2017;35:1387-1394.DOI: 10.1200/jco.2016.70.0229.

[11] Bradley JD, Paulus R, Komaki R, Masters G, et al. Standard-dose versus high-dose conformal radiotherapy with concurrent and consolidation carboplatin plus paclitaxel with or without cetuximab for patients with stage IIIa or IIIb non-small-cell lung cancer (ROTG 0617): A randomised, two-by-two factorial phase 3 study. *The Lancet Oncology* 2015;16:187-199.DOI: 10.1016/S1470-2045(14)71207-0.

[12] Cannon DM, Mehta MP, Adkison JB, Khuntia D, et al. Dose-limiting toxicity after hypofractionated dose-escalated radiotherapy in non–small-cell lung cancer. *Journal of Clinical Oncology* 2013;31:4343-4348.DOI: 10.1200/jco.2013.51.5353.

[13] Hicks RJ Hofman MS. Is there still a role for SPECT–CT in oncology in the PET–CT era? *Nat Rev Clin Oncol.* 2012;9:712.DOI: 10.1038/nrclinonc.2012.188.

[14] Hegi-Johnson F, Keall P, Barber J, Bui C, et al. Evaluating the accuracy of 4D-CT ventilation imaging: First comparison with technegas spect ventilation. *Medical Physics* 2017;44:4045-4055.

[15] Kipritidis J, Siva S, Hofman MS, Callahan J, et al. Validating and improving CT ventilation imaging by correlating with ventilation 4D-PET/CT using 68Ga-labeled nanoparticles. *Med Phys.* 2014;41:011910.DOI: 10.1118/1.4856055.

[16] Fraioli F, Serra G, Liberali S, Fiorelli A, et al. Clinical application of dual-source ct in the evaluation of patients with lung cancer: Correlation with perfusion scintigraphy and pulmonary function tests. *Radiologia Medica* 2011;116:842-857.DOI: 10.1007/s11547-011-0674-9.

[17] Bahig H, Campeau MP, Lapointe A, Bedwani S, et al. Phase 1-2 study of dual-energy computed tomography for assessment of pulmonary function in radiation therapy planning. *Int J Radiat Oncol Biol Phys.* 2017;99:334-343.DOI: 10.1016/j.ijrobp.2017.05.051.

[18] Lapointe A, Bahig H, Blais D, Bouchard H, et al. Assessing lung function using contrast-enhanced dual-energy computed tomography for potential applications in radiation therapy. *Med Phys.* 2017;18:18.DOI: 10.1002/mp.12475.

[19] Brower JV, Amini A, Chen S, Hullett CR, et al. Improved survival with dose-escalated radiotherapy in stage III non-small-cell lung cancer: Analysis of the national cancer database. *Annals of Oncology* 2016;27:1887-1894.DOI: 10.1093/annonc/mdw276.

[20] Hong J, Salama J. Dose escalation for unresectable locally advanced non-small cell lung cancer: End of the line? *Translational Lung Cancer Research* 2016;5:126-133.

[21] RTOG. RTOG 1106/ACRIN 6697, randomized phase II trial of individualized adaptive radiotherapy using during-treatment fdg-pet/ct and modern technology in locally advanced non-small cell lung cancer (NSCLC), 2016.

[22] Socinski MA, Blackstock AW, Bogart JA, Wang X, et al. Randomized phase II trial of induction chemotherapy followed by concurrent chemotherapy and dose-escalated thoracic conformal radiotherapy (74 gy) in stage III non–small-cell lung cancer: CALGB 30105. *Journal of Clinical Oncology* 2008;26:2457-2463.DOI: 10.1200/jco.2007.14.7371.

[23] Kong F-M, Zhao J, Wang J Faivre-Finn C. Radiation dose effect in locally advanced non-small cell lung cancer. *Journal of Thoracic Disease* 2014;6:336-347.DOI: 10.3978/j.issn.2072-1439.2014.01.23.

[24] Fowler JF. 21 years of biologically effective dose. *The British Journal of Radiology* 2010;83:554-568.DOI: 10.1259/bjr/31372149.

[25] Chapet O, Khodri M, Jalade P, N'guyen D, et al. Potential benefits of using non coplanar field and intensity modulated radiation therapy to preserve the heart in irradiation of lung tumors in the middle and lower lobes. *Radiotherapy and Oncology*;80:333-340.DOI: 10.1016/j.radonc.2006.07.009.

[26] Wang K, Pearlstein KA, Patchett ND, Deal AM, et al. Heart dosimetric analysis of three types of cardiac toxicity in patients treated on dose-escalation trials for stage III non-small-cell lung cancer. *Radiotherapy and Oncology* 2017;125:293-300.DOI: 10.1016/j.radonc.2017.10.001.

[27] Adams J, Hardenbergh PH, Constine LS Lipshultz SE. Radiation-associated cardiovascular disease. *Critical Reviews in Oncology/Hematology* 2003:55-75.

[28] Chello M, Mastroroberto P, Romano R, Zofrea S, et al. Changes in the proportion of types I and III collagen in the left ventricular wall of patients with post-irradiative pericarditis. . *Cardiovasc Surg* 1996;4.

[29] Jaworski C, Mariani JA, Wheeler G Kaye DM. Cardiac complications of thoracic irradiation. *Journal of the American College of Cardiology* 2013;61:2319-2328.

[30] CSANZ. Coronary artery calcium scoring – position statement 2017.

[31] Ren Y, Jiao Y, Zhang L Zheng X. Dual-energy ct-based iodine quantitation for response evaluation of lung cancers to chemoradiation therapy/radiation therapy: A comparison with 18fdg–pet/ct-based percist. *Int J Radiat Oncol Biol Phys.* 2017;99:S48-S49.DOI: https://doi.org/10.1016/j.ijrobp.2017.06.124.

[32] Lee E, Zeng J, Miyaoka RS, Saini J, et al. Functional lung avoidance and response-adaptive escalation (flare) rt: Multimodality plan dosimetry of a precision radiation oncology strategy. *Medical Physics* 2017;44:3418-3429.

[33] Hoover DA, Capaldi DPI, Sheikh K, Palma DA, et al. Functional lung avoidance for individualized radiotherapy (FLAIR): Study protocol for a randomized, double-blind clinical trial. *BMC Cancer* 2014;14 (1).

[34] Yaremko B, Hoover D, Capaldi D, Sheikh K, et al. Functional lung avoidance for individualized radiation therapy (FLAIR): Results of a randomized, double-blind clinical trial. *Int J Radiat Oncol Biol Phys.* 2017;99:E507.DOI: 10.1016/j.ijrobp.2017.06.1815.

[35] Yamamoto T, Kabus S, Bal M, Keall P, et al. The first patient treatment of computed tomography ventilation functional image-guided radiotherapy for lung cancer. *Radiotherapy & Oncology* 2016;118:227-231.

[36] Callahan J, Hofman MS, Siva S, Kron T, et al. High-resolution imaging of pulmonary ventilation and perfusion with 68Ga-VQ respiratory gated (4-d) pet/ct. *Eur J Nucl Med Mol Imaging* 2014;41:343-349.DOI: 10.1007/s00259-013-2607-4.

[37] Siva S, Hardcastle N, Kron T, Bressel M, et al. Ventilation/perfusion positron emission tomography-based assessment of radiation injury to lung. *Int J Radiat Oncol Biol Phys* 2015;93:408-417.DOI: 10.1016/j.ijrobp.2015.06.005.

[38] Macintyre N, Crapo R, Viegi G, Johnson D, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *European Respiratory Journal* 2005;26:720.

[39] Martinez FJ, Han MLK, Andrei AC, Wise R, et al. Longitudinal change in the bode index predicts mortality in severe emphysema. *American journal of respiratory and critical care medicine* 2008;178:491.

[40] Allan E, Williams T JC JG. Mortality, cardiac toxicity, and radiation dose to the heart in patients treated with curative intent fractionated radiation therapy for lung cancer. *Int J Radiat Oncol Biol Phys* 2015;93.

[41] Darby SC, Ewertz M, McGale P, Bennet AM, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. *NEJM* 2013;368:987-998.

[42] Ming X, Feng Y, Yang C, Wang W, et al. Radiation-induced heart disease in lung cancer radiotherapy. *Medicine* 2016;95.DOI: http://dx.doi.org/10.1097/MD.0000000000005051.

[43] Prescribing I. Recording and reporting photon beam therapy. ICRU report 50. *Bethesda, MD: International Commission on Radiation Units and Measurement* 1993.

[44] Prescribing I. Recording and reporting photon beam therapy (supplement to ICRU report 50). *ICRU report* 1999;62.

[45] Wambersie A Landberg T. Icru report 62: Prescribing, recording, and reporting photon beam therapy (supplement to icru report 50). *Bethesda, MD: International Commission on Radiation Units and Measurements* 1999.

[46] Kong F-M, Ritter T, Quint DJ, Senan S, et al. Consideration of dose limits for organs at risk of thoracic radiotherapy: Atlas for lung, proximal bronchial tree, esophagus, spinal cord, ribs, and brachial plexus. *Int J Radiat Oncol Biol Phys.* 81:1442-1457.DOI: 10.1016/j.ijrobp.2010.07.1977.

[47] Wang K, Eblan MJ, Deal AM, Lipner M, et al. Cardiac toxicity after radiotherapy for stage III non–small-cell lung cancer: Pooled analysis of dose-escalation trials delivering 70 to 90 gy. *Journal of Clinical Oncology* 2017;35:1387-1394.DOI: 10.1200/jco.2016.70.0229.

[48] Speirs CK, DeWees TA, Rehman S, Molotievschi A, et al. Heart dose is an independent dosimetric predictor of overall survival in locally advanced non–small cell lung cancer. *Journal of Thoracic Oncology* 2017;12:293-301.DOI: https://doi.org/10.1016/j.jtho.2016.09.134.

[49] RTOG. RTOG 1106/ACRIN 6697, Randomized phase II trial of individualized adaptive radiotherapy using during-treatment fdg-pet/ct and modern technology in locally advanced non-small cell lung cancer (NSCLC) trial protocol: RTOG, 2016.

[50] Tsoutsou PG Koukourakis MI. Radiation pneumonitis and fibrosis: Mechanisms underlying its pathogenesis and implications for future research. *I Int J Radiat Oncol Biol Phys.* 66:1281-1293.DOI: 10.1016/j.ijrobp.2006.08.058.

[51] Anscher MS, Murase T, Prescott DM, Marks LB, et al. Changes in plasma TGF β; levels during pulmonary radiotherapy as a predictor of the risk of developing radiation pneumonitis. *Int J Radiat Oncol Biol Phys.* 30:671-676.DOI: 10.1016/0360-3016(92)90954-G.

[52] Siva S, Lobachevsky P, MacManus MP, Kron T, et al. Radiotherapy for non–small cell lung

cancer induces DNA damage response in both irradiated and out-of-field normal tissues. *Clinical Cancer Research* 2016;22:4817.

[53] Siva S, MacManus M, Kron T, Best N, et al. A pattern of early radiation-induced inflammatory cytokine expression is associated with lung toxicity in patients with non-small cell lung cancer. *PLOS ONE* 2014;9:e109560.DOI: 10.1371/journal.pone.0109560.

[54] Conti I Rollins BJ. Ccl2 (monocyte chemoattractant protein-1) and cancer. Elsevier. 2004;14:149-154.DOI.

[55] Kim TD, Li G, Song KS, Kim JM, et al. Radiation-induced thymidine phosphorylase upregulation in rectal cancer is mediated by tumor-associated macrophages by monocyte chemoattractant protein–1 from cancer cells. *Int J Radiat Oncol Biol Phys.* 2009;73:853-860.

[56] Wolff HA, Rolke D, Rave-Fränk M, Schirmer M, et al. Analysis of chemokine and chemokine receptor expression in squamous cell carcinoma of the head and neck (scchn) cell lines. *Radiation and environmental biophysics* 2011:1-10.

[57] Little MP, Gola A Tzoulaki I. A model of cardiovascular disease giving a plausible mechanism for the effect of fractionated low-dose ionizing radiation exposure. *PLoS computational biology* 2009;5:e1000539.

[58] Newman AM, Bratman SV, To J, Wynne JF, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nature Medicine* 2014;20:548.DOI: 10.1038/nm.3519

[59] Nellessen U, Zingel M, Hecker H, Bahnsen J, et al. Effects of radiation therapy on myocardial cell integrity and pump function: Which role for cardiac biomarkers? *Chemotherapy* 2010;56:147–152.

[60] Korff S, Katus HA Giannitsis E. Differential diagnosis of elevated troponins. *Heart* 2006;92:987-993.DOI: 10.1136/hrt.2005.071282.

[61] Thygesen K, Alpert JS, Jaffe AS, Simoons ML, et al. Third universal definition of myocardial infarction. *European Heart Journal* 2012;33:2551-2567.DOI: 10.1093/eurheartj/ehs184.

[62] Jeremias A Gibson C. Narrative review: Alternative causes for elevated cardiac troponin levels when acute coronary syndromes are excluded. *Annals of Internal Medicine* 2005;142:786-791.DOI: 10.7326/0003-4819-142-9-200505030-00015.

[63] Zhang Junan, Ma J, Zhou S, Hubbs JL, et al. Radiation-induced reductions in regional lung perfusion: 0.1-12 year data from a prospective clinical study. *International Journal of Radiation Oncology, Biology and Physics* 2010.DOI: 10.1016/j.ijrobp.2009.02.005.

[64] Van Elmpt W, De Ruysscher D, van der Salm A, Lakeman A, et al. The PET-boost randomised phase II dose-escalation trial in non-small cell lung cancer. *Radiotherapy and Oncology* 2012;104:67-71.DOI: 10.1016/j.radonc.2012.03.005.

[65] Kotzerke J, Andreeff M Wunderlich G. PET aerosol lung scintigraphy using Galligas. *Eur J Nucl Med Mol Imaging* 2010;37:175-177.

[66] Mac Manus MP, Hicks RJ, Matthews JP, McKenzie A, et al. Positron emission tomography is superior to computed tomography scanning for response-assessment after radical radiotherapy or chemoradiotherapy in patients with non–small-cell lung cancer. *Journal of Clinical Oncology* 2003;21:1285.

[67] Schenzle JC, Sommer WH, Neumaier K, Michalski G, et al. Dual energy CT of the chest: How about the dose? *Investigative Radiology* 2010;45:347-353.DOI: 10.1097/RLI.0b013e3181df901d.

[68] UICC. Lung, pleural, and thymic tumours. In: Brierley JD, Gospodarowicz MK and Wittekind C, eds. TNM classification of malignant tumours, 8th edition: Wiley, 2016;pp. 106-113.

[69] Manus MPM, Hicks RJ, Matthews JP, McKenzie A, et al. Positron emission tomography is superior to computed tomography scanning for response-assessment after radical radiotherapy or chemoradiotherapy in patients with non–small-cell lung cancer. *Journal of Clinical Oncology* 2003;21:1285-1292.DOI: 10.1200/jco.2003.07.054.

[70] Cella D, Eton DT, Fairclough DL, Bonomi P, et al. What is a clinically meaningful change on the functional assessment of cancer therapy; lung (FACT-L) questionnaire? *Journal of Clinical*

Epidemiology;55:285-295.DOI: 10.1016/S0895-4356(01)00477-2.

APPENDIX 1 – TNM STAGING NSCLC

Staging
TNM system- tumour node metastases system

The TNM system is one of the most commonly used staging systems. This system has been accepted by the International Union against Cancer (UICC) and the American Joint Committee on Cancer (AJCC). Most medical facilities use the TNM system as their main method for cancer reporting. PDQ®, the NCI's comprehensive cancer database, also uses the TNM system.

The TNM system is based on the extent of the tumour (T), the extent of spread to the lymph nodes (N), and the presence of metastasis (M). A number is added to each letter to indicate the size or extent of the tumour and the extent of spread.

The 8th edition (2014) of the TNM classification of malignant tumours was released in 2016.[68]

T: primary tumour
Tx: primary tumour cannot be assessed or tumour proven by presence of malignant cells in sputum or bronchial washings but not visualised by imaging or bronchoscopy
T0: no evidence of primary tumour
Tis: carcinoma in situ
T1: tumour under 3 cm in greatest dimension surrounded by lung or visceral pleura without bronchoscopic evidence of invasion more proximal than the lobar bronchus (i.e. not in the main bronchus)
T1a(mi): minimally invasive adenocarcinoma
T1a ss: superficial spreading tumour in central airways (spreading tumour of any size but confined to the tracheal or bronchial wall)
T1a: tumour ≤1 cm in greatest dimension
T1b: tumour >1 cm but ≤2 cm in greatest dimension
T1c: tumour >2 cm but ≤3 cm in greatest dimension
T2: tumour >3 cm but ≤5 cm or tumour with any of the following features:
Involves main bronchus regardless of distance from the carina but without involvement of the carina
Invades visceral pleura
Associated with atelectasis or obstructive pneumonitis that extends to the hilar region
Involving part or all of the lung
T2a: tumour >3 cm but ≤4 cm in greatest dimension
T2b: tumour >4 cm but ≤5 cm in greatest dimension
T3: tumour >5 cm but ≤7 cm in greatest dimension or associated with separate tumour nodule(s) in the same lobe as the primary tumour or directly invades any of the following structures:

- Chest wall (including the parietal pleura and superior sulcus)
- Phrenic nerve
- Parietal pericardium

T4: tumour >7 cm in greatest dimension or associated with separate tumour nodule(s) in a different ipsilateral lobe than that of the primary tumour or invades any of the following structures

- Diaphragm
- Mediastinum
- Heart
- Great vessels
- Trachea
- Recurrent laryngeal nerve

- Oesophagus
- Vertebral body
- Carina

N: regional lymph node involvement
Nx: regional lymph nodes cannot be assessed
N0: no regional lymph node metastasis
N1: metastasis in ipsilateral peri bronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2: metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
N3: metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

M: distant metastasis
M0: no distant metastasis
M1: distant metastasis present
M1a: separate tumour nodule(s) in a contralateral lobe; tumour with pleural or pericardial nodule(s) or malignant pleural or pericardial effusions
M1b: single extrathoracic metastasis
M1c: multiple extrathoracic metastases in one or more organs

Stage groupings
Stage IIIa
TNM equivalent: T1/T2, N2, M0 *or* T3/T4, N1, M0 *or* T4, N0, M0
5-year survival: 36%
Stage IIIb
TNM equivalent: T1/T2 , N3, M0 *or* T3/T4, N2, M0
5-year survival: 26%
Stage IIIc
TNM equivalent: T3/T4, N3, M0
5-year survival: 13%

APPENDIX 2 - ECOG PERFORMANCE STATUS CRITERIA

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self care. Totally confined to bed or chair.
5	Dead

As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

APPENDIX 3 - ASSESSMENT OF RESPONSE

The date of definitive response assessment will be at 90 days post treatment (+/- 10 days).

Participant Assessments: Assessments must include physical examination and participant interviews to evaluate signs and symptoms, particularly those required to be reported on the Case Report Forms (CRFs). The results of clinical assessments must be fully documented in the medical records (source documents)

Imaging is to be done at time points specified in the protocol, or at any time at which there is clinical suspicion of recurrence or progression.

CT Assessment of Response

Baseline tumour measurements must be undertaken prior to any protocol treatment commencing. All tumour measurements must be recorded using measurable disease provided in RECIST (Response Evaluation Criteria in Solid Tumours) version 1.1.

Definition of progressive disease will be used to assess the presence of local failure. The initial assessment of disease progression based on RECIST criteria will be determined at the 3 month follow up after all protocol treatment has been completed, whether or not all the planned treatment was received.

Definition of Local Failure: The definition of local failure is based on the RECIST 1.1 criteria definition of progressive disease.

Definitions of Regional and Distant Failure

- Regional Failure: The presence of positive radiological evidence of recurrent disease adjacent to the high dose region or in the draining hilar or mediastinal lymph nodes.
- Distant Failure: The presence of positive radiological evidence of recurrent disease at any site of the body with the exception of those classified as local or regional; this may require confirmation by FDG PET scan or positive pathology.

Dating Time of Relapse / Failure

- Time of relapse is defined as when the first radiological suspicion of failure is observed provided it is subsequently confirmed by further imaging (which may include FDG-PET scan) or positive pathology.

PET Assessment of Response

FDG-PET/CT treatment response at the definitive treatment response assessment time point will occur at 3 months post treatment. This will be assessed by the PERCIST and Peter Mac PET response criteria.[69]

PERCIST Response Criteria

Complete Metabolic Response (CMR)

- Complete resolution of 18F-FDG uptake within the measurable target lesion so that it is less than mean liver activity and at the level of surrounding background blood pool activity.
- Disappearance of all other lesions to background blood pool levels.
- No new suspicious 18F-FDG avid lesions.
- If progression by RESIST must verify with follow up

Partial Metabolic Response (PMR)

- Reduction of a minimum of 30% in target measurable tumor 18F-FDG SUL peak, with absolute drop in SUL of at least 0.8 SUL units.
- No increase >30% of SUL or size in all other lesions
- No new lesions

Stable Metabolic Disease (SMD)

- Not CMR, PMR, or Progressive metabolic disease (PMD)
- No new lesions

Progressive Metabolic Disease (PMD)

- >30% increase in 18F-FDG SUL peak, with >0.8 SUL units increase in tumor SUV peak from the baseline scan in pattern typical of tumour and not of infection/treatment effect.

OR

- Visible increase in the extent of 18F-FDG tumour uptake.

OR

- New 18F-FDG avid lesions, which are typical of cancer and not related to treatment effect or infection.

Peter Mac Response Criteria

Complete Metabolic Response (CMR)

No abnormal tumour FDG uptake; activity in the tumour absent or similar to mediastinum.

Partial Metabolic Response (PMR)

Any appreciable reduction in intensity of tumour FDG uptake or tumour volume. No disease progression at other sites.

Stable Metabolic Disease (SMD)

No appreciable change in intensity of tumour FDG uptake or tumour volume: no new sites of disease.

Progressive Metabolic Disease (PMD)

Appreciable increase in tumour FDG uptake or volume of known tumour sites and/or evidence of disease progression at other intrathoracic or distant metastatic sites. Radiation-induced inflammatory changes in the lungs and pleura with different distribution from tumour uptake are not scored as persistent or progressive disease. Inflammatory changes conforming to the volume of irradiated lung, readily distinguished from persistent tumour uptake by their location and pattern of uptake are not scored as persistent or progressive disease.

Managing Treatment of Relapse/Progression

Treatment at relapse is at the discretion of the treating clinician if appropriate the case should be discussed at the Lung MDT for further management options which may include surgery, chemotherapy, targeted therapies, radiation or palliation

APPENDIX 4 – CTCAE

CTCAE (Common Terminology Criteria for Adverse Events, V4.03)

CTCAE v4.0 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
Cough	Mild symptoms; nonprescription intervention indicated	Moderate symptoms, medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL	-	-
Dyspnoea	Shortness of breath with moderate exertion	Shortness of breath with minimal exertion; limiting instrumental ADL	Shortness of breath at rest; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Hypoxia	-	Decreased oxygen saturation with exercise (e.g., pulse oximeter <88%); intermittent supplemental oxygen	Decreased oxygen saturation at rest (e.g., pulse oximeter <88% or PaO2 <=55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Pulmonary hypertension	Minimal dyspnoea; findings on physical exam or other evaluation	Moderate dyspnoea, cough; requiring evaluation by cardiac catheterization and medical intervention	Severe symptoms, associated with hypoxemia, right heart failure; oxygen indicated	Life-threatening airway consequences; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Acute coronary syndrome	-	Symptomatic, progressive angina; cardiac enzymes normal; hemodynamically stable	Symptomatic, unstable angina and/or acute myocardial infarction, cardiac enzymes	Symptomatic, unstable angina and/or acute myocardial infarction, cardiac enzymes	Death

			abnormal, hemodynamically stable	abnormal, hemodynamically unstable	
Conduction disorder	Mild symptoms; intervention not indicated	Moderate symptoms	Severe symptoms; intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Pericardial effusion	-	Asymptomatic effusion size small to moderate	Effusion with physiologic consequences	Life-threatening consequences; urgent intervention indicated	Death
Cardiac disorders - Other, specify	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Anaphylaxis	-	-	Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related oedema/angioedema; hypotension	Life-threatening consequences; urgent intervention indicated	Death
Lung infection	-	Moderate symptoms; oral intervention indicated (e.g., antibiotic, antifungal, antiviral)	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Bronchial fistula	Asymptomatic; clinical or diagnostic observations only;	Symptomatic; tube thoracostomy or medical management	Severe symptoms; limiting self care ADL; endoscopic or	Life-threatening consequences; urgent operative	Death

	intervention not indicated	indicated; limiting instrumental ADL	operative intervention indicated (e.g., stent or primary closure)	intervention with thoracoplasty, chronic open drainage or multiple thoracotomies indicated	
Bronchial stricture	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic (e.g., rhonchi or wheezing) but without respiratory distress; medical intervention indicated (e.g., steroids, bronchodilators)	Shortness of breath with stridor; endoscopic intervention indicated (e.g., laser, stent placement)	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Bronchopleural fistula	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; tube thoracostomy or medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL; endoscopic or operative intervention indicated (e.g., stent or primary closure)	Life-threatening consequences; urgent operative intervention with thoracoplasty, chronic open drainage or multiple thoracotomies indicated	Death
Bronchopulmonary hemorrhage	Mild symptoms; intervention not indicated	Moderate symptoms; medical intervention indicated	Transfusion, radiologic, endoscopic, or operative intervention indicated (e.g., hemostasis of bleeding site)	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Respiratory, thoracic and mediastinal disorders - Other, specify	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death

Available at:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_4.03.xlsx

As published by the U.S Department of Health and Human Services, National Institutes of Health National Cancer Institute: April 28, 2010

APPENDIX 5 - QUALITY OF LIFE SCORING

FACT-L Questionnaire [70]

Below is a list of statements that other people with your illness have said are important**. Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4

.....

Q1 *Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box and go to the next section.*

GS7	I am satisfied with my sex life	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	EMOTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4

GF 5	I am sleeping well	0	1	2	3	4
GF 6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF 7	I am content with the quality of my life right now	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	ADDITIONAL CONCERNS	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
B1	I have been short of breath	0	1	2	3	4
C2	I am losing weight	0	1	2	3	4
L1	My thinking is clear	0	1	2	3	4
L2	I have been coughing	0	1	2	3	4
B5	I am bothered by hair loss	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
L3	I feel tightness in my chest	0	1	2	3	4
L4	Breathing is easy for me	0	1	2	3	4
Q3	Have you ever smoked? No ___ Yes ___ If yes:					
L5	I regret my smoking	0	1	2	3	4

Participant Information Sheet/Consent Form

Interventional Study - *Adult providing own consent*

Title	**H**igh **I**ntensity **F**unctional **I**mage guided **V**mat lung **E**vasion
Short Title	HI FIVE
Project Sponsor	Peter MacCallum Cancer Centre
Principal Investigator	Dr Nicholas Bucknell
Supervisor	Associate Professor Shankar Siva
Location	Sunshine Radiation Therapy Centre & Sunshine Hospital

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project. This is because you have non-small cell lung cancer (NSCLC) and are to receive radiotherapy to treat this cancer. Your doctors including surgeons, lung specialists and cancer specialists and have discussed your case and have recommended radiotherapy. In most cases chemotherapy will be recommended at the same time as the radiotherapy.

This research project is testing a specialised type of PET scan to give your doctors pictures of your lungs. These pictures will show areas of air-flow (called ventilation) and blood flow (called perfusion). The pictures will be used to find areas of lung that are working well (called functional lung). Identifying the functional lung will assist your doctors to use advanced radiotherapy planning techniques to minimise radiation dose to the functioning areas of lung, minimise radiation dose to the heart and give a higher dose of radiation to the main tumour. The higher dose of radiation given to the main tumour may increase the risk of side effects.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand

or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.
If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read.
- Consent to take part in the research project.
- Consent to have the tests and treatments that are described.
- Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

The maximum dose of radiation that can be safely delivered to lung cancer is limited by the side effects to the surrounding normal lung. These side effects are related to changes in blood flow and air-flow in the lung after radiotherapy. Many patients with your type of cancer have these changes in the lung function before radiotherapy starts. These changes can be detected using a special type of PET scan called a Ventilation and Perfusion PET scan. A PET (Positron Emission Tomography) scan is an advanced type of scan that is able to develop a map of function of various parts of the body. You may have already had different type of PET scan – a glucose PET scan which is used to find the lung tumour and to identify any areas that it could have spread.

Our previous study (the GalliPET study) has shown that conventional radiotherapy results in decreased blood-flow (Perfusion) and air-flow (Ventilation) in healthy lung. Functional lung information from these PET scans can be now combined with advanced computerised planning techniques to identify areas of lung with high amounts of blood flow and air-flow (called functioning lung) that need to be preserved. The computerised planning technique we will use in this study is called VMAT (Volumetric Modulated Arc Therapy). VMAT is an advanced technology that enables your radiation oncologist to avoid normal tissues such as the functional lung and heart. This treatment will be given over the same time period as standard radiotherapy treatment.

If you participate in this study a higher radiation dose than standard of care dose will be given per day to the area the tumour has developed (this is called a boost). The dose given to the primary tumour will be 15% higher than the usual prescribed dose. This has the potential to increase side effects from treatment. These side effects are discussed in further detail in **section 9**.

Your blood samples collected for research will be used to investigate ways to improve radiotherapy for future patients. The body responds to radiotherapy by releasing chemicals that circulate in the bloodstream. We will measure these chemicals and also test how the blood cells react to radiotherapy and how well they are able to repair DNA damage. Blood samples may also predict the success of treatment by measuring the amount of tumour DNA in the bloodstream. We aim to measure this and compare the levels of this DNA to treatment outcomes.

Heart damage is a risk of radiation treatment to the chest area. Recent studies have shown that 1 in 5 patients experience radiation heart related side effects following lung cancer radiotherapy. This can impact on a patient's quality of life and survival. This study is investigating how to reduce these risks as much as possible. This project will involve additional heart tests that will aim to identify a way that patients at increased risk of heart

side effects will be able to be identified in future. This study will involve ultrasound heart scans (echocardiogram) before and after radiotherapy as well as the blood tests discussed above to assess any possible heart damage.

3 What does participation in this research involve?

You will not be paid for your participation in this research. Participation in this study is entirely voluntary.

If you choose to participate in this study;

1. You will have a Ventilation and Perfusion PET scan before treatment and 3 and 12 months after treatment. This will be on the same day as a CT scan of the lung.
2. You will have a glucose PET scan before treatment (this is a standard of care test to diagnose the extent of your cancer). Standard of care means the usual medical care (including medical treatment and tests) that your doctor would recommend if you did not participate in this trial. As part of this study you will have another one of these at 3 months after treatment.
3. You will also undergo 5 additional blood tests in total, 1 before treatment, 3 during the course of treatment and 1 at 3 months after the radiotherapy treatment finishes. These can be done while you are attending for scans or treatment. Where possible, blood will be drawn at the time of the scans or during normal blood tests to avoid the discomfort of an additional blood draw.
4. When you have these additional blood tests, the nurse will ask you about any heart symptoms you may be having.
5. Lung function testing including a breathing and 6 minute walk test will be performed before treatment and 3 and 12 months after treatment.
6. An ultrasound and electrical tracing of the heart will be performed before treatment, 3 and 12 months after treatment.
7. You will be asked to complete a quality of life questionnaire at one time before and four times after radiotherapy treatment. This consists of 37 questions and takes most patients approximately half an hour to complete. This survey is provided in multiple other languages if you do not speak English. If the survey is not available in your primary language you may either choose to complete the questionnaire with an interpreter present or not do the survey at all.

Your follow-up visits to clinic will be at the same intervals as if you were not participating in this study. Your symptoms will be recorded and a physical examination will be performed at 3, 6, 9 and 12 months after treatment. Your total participation in the study will last 12 months after completing radiotherapy.

Before starting the study

We ask that you carefully read this consent form, and discuss the trial in detail with your study doctor. When you are satisfied that you fully understand the purpose and nature of this research, you will sign the consent form with your study doctor. After signing the consent form, you will need to have the following procedures (called “screening tests”) to find out if this study is suitable for you. Many of these procedures may be part of your regular cancer care and may be done even if you do not join the study. If you have had some of them recently, they may not need to be repeated. Depending on the outcome of these tests, you may or may not be able to take part in the study.

Screening

Your doctor has given you this consent form because based on your current results you are eligible for this trial. You will undergo standard screening tests to check your cancer is confined to the chest and to test your general health.

- A medical history and physical examination, including measurements of height, weight and vital signs (blood pressure, pulse rate, temperature and breathing rate).
- You will also be asked questions about the medicines you currently take, and your general wellbeing and ability to manage everyday tasks.

- Scans will be performed to assess the size and location of your cancer if they haven't been done already:
  - A glucose PET scan (called an FDG-PET scan) will be performed as standard of care
  - A brain scan will be performed as standard of care – this will either be a CT or MRI. Your treating clinician will determine which scan is best for you.

- Laboratory assessments
  - Blood will be collected to check your blood cell counts, liver and kidney function to assess your ability to tolerate chemotherapy. These will be performed as standard of care.
  - If you are a woman who is able to have children, you will have a blood or urine pregnancy test

Study Treatment Phase
You will be able to participate in the study if the results of all the screening assessments show that the research project is suitable for you.

During treatment you will have weekly review by your treating radiation oncologist. If receiving chemotherapy you will also see a medical oncologist. At each visit any side effects will be documented and any medications you are taking will be recorded.

End of Treatment Visit
An End of Treatment Visit will occur 1-4 weeks after your last radiotherapy treatment. The purpose of this visit is to determine if your health was impacted by the treatment (including any side-effects) and to check any side effects are resolving. You will be asked how you are feeling and any medications you have been taking will be documented. You will also be asked to fill out a quality of life questionnaire.

Follow-up
After your End of Treatment Visit, you will be asked to return to the clinic for Follow-up Visits every 3 months until the study ends 1 year after you finish treatment. Your follow-up visits to clinic will be at the same intervals as if you were not participating in this study. At the Follow-up Visits you will have the following assessments:

- You will be asked about any changes to your health since your last visit, and whether you have commenced any treatments (new medications and/or new anti-cancer therapies)
- You will be asked about your ability to manage your daily activities
- Your weight will be recorded and you will have your vital signs measured
- You will have scans and lung function tests that are described above
- You will be asked to fill out a quality of life questionnaire

Blood collection for research tests:
Researchers would also like to collect some blood for the reasons described in **section 2.**. Less than 2 tablespoons of blood will be collected for these tests at the following time points:

- Before you start radiotherapy treatment (at the time of your CT scan while the cannula is inserted for the scan)
- During radiotherapy treatment; within 1 hour after your first treatment, before your second treatment and within 1 hour before your 20th radiotherapy treatment.

- 3 months after you finish radiotherapy treatment (at the time of your CT scan while the cannula is inserted for the scan)

You will be asked if you would like to provide consent for the storage of your blood for future research on the consent form at the end of this information sheet. Please read Section 10 "what will happen to my test samples" for more detailed information. Storage of these samples for future research is optional; you do not have to consent to this in order to participate in this research project.

4 What do I have to do?

If you decide to participate in this study it will be your responsibility to tell your study doctor about any other medical conditions you have, any other medications you are taking (including non-prescription medications, vitamins or herbal remedies) and/or if you have experienced any previous reactions to a medicine. You must also inform your study doctor of any changes to these medications during your participation in the study.

It is important that you do not take any other additional medications, including over the counter medications, immunisations and vaccinations during this study without talking to your study doctor.

If, at any time, you have any symptom, side effect or injury affecting you physically or mentally during the study, **you should tell your study doctor or a member of the research team**, even if you do not think it was caused by the study treatment.

5 Other relevant information about the research project

It is planned that 20 participants with stage III non-small cell lung cancer will be entered into this study at the Peter MacCallum Cancer Centre (at both the Sunshine and Parkville locations).

There are no additional costs associated with participating in this research project, nor will you be paid. All medication, tests and medical care required as part of the research project will be provided to you free of charge. This will be the same as if you were not participating in a research project.

You may have to pay for some medicines according to hospital policy. For example dispensing fees for PBS-listed drugs. This cost would be the same as the expected costs if you weren't participating in the study.

If you decide to participate in this research project, the study doctor will inform your local doctor (GP).

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with Western Health or Peter MacCallum Cancer Centre.

7 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at this hospital. If you do not participate in this research, conventional radiotherapy will be offered to you (with or without chemotherapy) as standard of care.

Participation in this study is optional you do not have to participate.

Please talk to your study doctor about your options before you decide if you will take part in this study.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research. The post treatment PET scans can indicate how your tumour has responded to treatment better than standard CT scans. This additional glucose PET scan is not currently standard of care at all treatment centers and may provide your doctors with additional information about your response to treatment compared to a standard CT scan. Even if you do not receive a benefit, this project and the information we collect may reveal important information that may benefit future patients.

9 What are the possible risks and disadvantages of taking part?

The major risk of participation in this study is that the study treatment uses a higher than usual radiotherapy dose which may increase the risk of side effects. The trial dose to the primary tumour is 69 Gy in 30 treatments whereas the standard of care dose is 60Gy in 30 treatments. This dose is therefore significantly higher than the usual standard of care radiation dose.

Previous large trials using higher doses in lung cancer have shown that patients experience worse outcomes with higher doses of radiotherapy. The causes of this are currently not fully understood. These previous trials gave the higher dose to a large area involving the primary tumour and lymph nodes with an additional margin.

The investigational treatment (VMAT radiotherapy) in this study aims to give increased dose to a smaller area (the primary tumour) provided it is far enough away from the heart and the central airways. Your study team will aim to develop a radiation treatment plan using strict guidelines and use advanced radiotherapy techniques to ensure dose to the normal tissues around the cancer are within safe limits. Although these extra steps will be taken to try and this treatment is safe, the exact risks of giving the higher doses of radiation used within this trial is unknown and these doses are not given in any standard of care radiation.

The potential risks with the increased dose delivered in the trial radiotherapy could include an increased risk (compared to standard of care radiotherapy) of; radiation pneumonitis, damage to the heart, damage to blood vessels in the chest or an area called the central airways or proximal bronchial tree (which could cause coughing up blood), oesophagitis (inflammation of the gullet), dermatitis (redness of the skin), dyspnoea (shortness of breath), cough, brachial plexopathy (damage to arm nerves if the tumour is high in the lung) and myelopathy (damage to the spinal cord).

Other Possible Side Effects Associated with Study Procedures

There are other risks and possible discomforts you might experience from the study procedures these include:

- **Blood tests:** A blood test may cause inflammation of the vein, pain, bruising and discomfort, redness, burning, or bleeding at the site where the needle is placed to draw the blood. You may feel dizzy or you may faint. There is a slight chance of infection.
- **CT scans:** You may experience fear of being in a narrow or enclosed space while having a CT scan. Contrast dye is usually injected into your vein when you get a CT scan. The contrast dye may cause pain or burning when it is injected, and may

worsen kidney function in people who already have kidney disease or who are dehydrated (have not had enough liquids that day). The contrast dye may also cause an allergic reaction, which could be severe and life threatening.

- **PET scan:** You will be asked not to move during the test and to relax and breathe normally. Severe allergic reactions to the PET tracers have not been reported, however there is a small risk of a mild allergic reaction.
- **Echocardiogram:** this is a non-invasive ultrasound test of the heart. This does not involve any radiation. The test may cause slight discomfort from the application of cool ultrasound jelly on the skin.

A possible risk of participating in this trial is the extra PET and CT scans causing increased exposure to ionizing radiation. This exposure is due to the additional PET and CT scans performed before and after radiotherapy treatment. The doses expected from the scans performed as part of the study are tiny in comparison to the radiation dose you will receive during your course of radiotherapy.

This research study involves exposure to a moderate amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisievert (mSv) each year. The effective dose from this study is approximately 92 mSv.

Attending the extra scans may also result in inconvenience due to the additional time required from you for each scan (approximately 20-40 minutes per scan). The scan itself can occasionally cause possible mild discomfort from lying still or from the injections you will receive.

Risks Associated with Pregnancy

There are potential significant side effects of any radiation exposure whilst pregnant. This includes during radiotherapy treatment and for CT and PET scans. Because of this, it is important that research project participants are not pregnant or breast-feeding and do not become pregnant during the course of the research project. You must not participate in the research if you are pregnant or trying to become pregnant, or breast-feeding. If childbearing is a possibility, you will be required to undergo a pregnancy test at the screening phase.

For female participants: You should not become pregnant for a minimum of 9 months after completing your last 'Gallium PET' scan (approximately 2 years after enrolling in the trial). If you do become pregnant whilst undergoing radiotherapy or following your radiotherapy, you should advise your study doctor immediately. Your study doctor will withdraw you from the research project and advise on further medical attention should this be necessary. You must not continue in the research if you become pregnant.

If you are male, you should not father a child for a minimum of 9 months after completing your last 'Gallium PET' scan (approximately 2 years after enrolling in the trial).

Both male and female participants are strongly advised to use effective contraception during the course of the research. You should discuss methods of effective contraception with your doctor.

10 What will happen to my test samples?

Routine blood samples

The blood samples you provide for routine testing (e.g. to check for side effects, kidney, liver, thyroid function) will be analysed at the local pathology department at the hospital and will be destroyed according to the local guidelines after these tests have been done.

Blood samples collected for research

Blood samples will be collected (if feasible and safe) for research with your permission.

The researchers are asking permission to store these blood samples for future research. Analysis of these blood samples will be conducted at the Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia.

Your de-identified blood samples will be stored at the Peter MacCallum Cancer Centre Research Laboratory. Any future research undertaken using your stored blood will first be reviewed and approved by an accredited Human Research Ethics Committee.

Your blood samples will be de-identified and will be linked to your study identification number, initials and date of birth. No-one will be able to identify you personally from your samples, except your study doctors and co-ordinators, who will be able to match the identification number with your name, if necessary.

The research will not have an effect on your medical care. We will not examine if cancer is hereditary in your family. In the unlikely case that information relevant to you comes up in the future, we will contact your doctor. Your information will not be released for other uses without your prior consent.

Your blood samples will be very helpful for future research. The research that may be done with your blood samples is not designed specifically to benefit you. It may help develop new treatments for this type of lung cancer for others. By broadening the knowledge about lung cancer, it could help other patients.

The choice to let us keep this blood for future research is up to you. **No matter what you decide to do, your choice will in no way affect the quality of care you receive.**

If you decide now to consent for your blood being used for future research, you can change your mind at any time; just contact your doctor and let him/her know that you do not want to use your blood any longer.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your study doctor about any changes to these during your participation in the research project. Your study doctor should also explain to you which treatments or medications need to be stopped for the time you are involved in the research project.

It may also be necessary for you to take medication during or after the research project to address side effects or symptoms that you may have. You may need to pay for these

medications and so it is important that you ask your doctor about this possibility.

13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project. If you withdraw from the treatment itself you may be asked to attend follow-up visits to allow collection of information regarding your health status. Alternatively, a member of the research team may contact me to request your permission to obtain access to your medical records for collection of follow-up information for the purposes of research and analysis. You also have a right this.

14 Could this research project be stopped unexpectedly?

We do not expect the research project to be stopped prior to completion

You might stop receiving study treatment without your consent for the following reasons:

- If the doctors treating you detect side effects that they consider dangerous.
- If you refuse to have the treatments, follow-up examinations and/or tests needed to determine whether the treatment is safe and effective.
- If the early analyses of the study data shows insufficient benefit or a significant potential harm of the treatment. In these circumstances, the research team will fully disclose to you the reasons why this has occurred.
- If the study sponsor (Peter MacCallum Cancer Centre) decides to stop the study or your treatment.

15 What happens when the research project ends?

Once you have completed study treatment, your study doctor will discuss your future treatment options and ongoing longer term care with you.

The results of this research project will be published in medical journals that are available to the public. Please ask your doctor if you want to know more about this. The study results are expected to be published approximately 3 years after treatment of the last patient. In the unexpected case that this research is not published, then all participants will receive a written report.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

The researchers will need to collect personal information from you such as your age, gender and relevant health information. By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential and securely stored. All hard copies of study data will be kept, as electronic data will be stored in password-protected databases. Only authorized research personnel will have access to study related data. All of your identifying information will be kept by the hospital for at least 15 years following the completion of the study. After this time, all identifying information at the hospital will be permanently deleted from the computer database and hard copies will be securely destroyed.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

Your health records and any information obtained during the research project are subject to inspection (for the purpose of verifying the procedures and the data) by the relevant authorities and the institution relevant to this Participant Information Sheet, or as required by law. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

All information about you provided for this research project is coded (de-identified) in a way that without a password it will not be possible to link the information to you. This information will be stored securely and only authorised research personnel, who understand that data must be kept confidential, will be able to get access to this coded information. The coded data will be stored in a database within the Peter MacCallum Cancer Centre, Melbourne, Australia. The coded data may be used in additional research or publications.

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission.

Information about your participation in this research project may be recorded in your health records. And you can access these with a freedom of information request.

In accordance with relevant Australian and Victorian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Any information obtained for the purpose of this research project and for the future research described in Section 10 that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Compensation for injury resulting from the study

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

18 Who is organising and funding the research?

This clinical research study has been initiated by Dr Nicholas Bucknell and Associate Professor Shankar Siva and is being co-ordinated and sponsored by the Peter MacCallum Cancer Centre. This research has been funded by Peter MacCallum Foundation and the Royal Australian and New Zealand College of Radiologists.

You will not benefit financially from your involvement in this research project even if knowledge gained from analysis of your samples prove to be of commercial value to Western Health or Peter MacCallum Cancer Centre. No investigator or member of research staff will receive a personal financial benefit from your involvement in this research project.

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people

called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the Peter MacCallum Cancer Centre HREC.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query. If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact any of the following people:

Clinical contact persons

Name	Nicholas Bucknell
Position	Radiation Oncology Research Fellow
Telephone	03 8559 5000 (please ask to be transferred to mobile)
Email	nick.bucknell@petermac.org

Western Health Investigator

Name	Shankar Siva
Position	Radiation Oncologist
Telephone	03 8559 5000 (please ask to be transferred to mobile)
Email	shankar.siva@petermac.org

If you require assistance after hours, please call (03) 8559 5000 and ask for the Radiation Oncologist on call. In the event of an emergency dial 000 immediately.

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Local HREC Office contact (Single Site - Research Governance Officer)

Name	Mr Bill Karanatsios
Position	Manager, Western Health Office for Research
Telephone	(03) 8395 8073
Email	ethics@wh.org.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	Peter MacCallum Cancer Centre Ethics Committee
HREC Executive Officer	Ethics Co-ordinator
Telephone	03 8559 7540
Email	ethics@petermac.org

Participant Consent Form

Title	**H**igh **I**ntensity **F**unctional **I**mage guided **V**mat lung **E**vasion
Short Title	HI-FIVE
Project Sponsor	Peter MacCallum Cancer Centre
Principal Investigator	Dr Nicholas Bucknell
Associate Investigator	Associate Professor Shankar Siva
Location	Sunshine Radiation Therapy Centre & Sunshine Hospital

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to the Peter MacCallum Cancer Centre concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that, if I decide to discontinue the study treatment, I may be asked to attend follow-up visits to allow collection of information regarding my health status. Alternatively, a member of the research team may contact me to request my permission to obtain access to my medical records for collection of follow-up information for the purposes of research and analysis.

I consent to the storage and use of coded study data, blood and tissue samples taken from me for use in research, as described in the relevant sections of the Participant Information Sheet.

Additionally, I consent to the storage and use of coded study data, blood and tissue samples taken from me for: *(please tick and initial all options that apply below)*

Initials ____ Yes ☐ No ☐ Other research that is closely related to this research project

and/or

Initials ____ Yes ☐ No ☐ Any future unspecified research.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Name of Witness* to Participant's Signature (please print) _____

Signature _____ Date _____

Name of Interpreter (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.
Note: All parties signing the consent section must date their own signature.

Bucknell N, *et al. BMJ Open* 2020; 10:e042465. doi: 10.1136/bmjopen-2020-042465

Form of Withdrawal of Participation

Title	**H**igh **I**ntensity **F**unctional **I**mage guided **V**mat lung **E**vasion
Short Title	HI-FIVE
Project Sponsor	Peter MacCallum Cancer Centre
Principal Investigator	Dr Nicholas Bucknell
Associate Investigator	Associate Professor Shankar Siva
Location	Sunshine Radiation Therapy Centre & Sunshine Hospital

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with the Peter MacCallum Cancer Centre.

☐ **Option 1: Withdrawal from treatment only:**
I wish to discontinue protocol treatment only but continue study follow-up procedures/ assessments via:
- ☐ Hospital clinic visits.
- ☐ Telephone follow-up with me or my GP (which may include collection of my medical information from my GP).

☐ **Option 2: Withdrawal of protocol treatment and follow-up**
- ☐ You **may** contact me or my GP at study closure to determine my health status only.
- ☐ You **may not** contact me or my GP at study closure to determine my health status.

Name of Participant (please print) _____

Signature _____ Date _____

Name of Interpreter (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project. Note: All parties signing the consent section must date their own signature

Form of Withdrawal of Consent to Storage of Samples for Future Research

Title	**H**igh **I**ntensity **F**unctional **I**mage guided **V**mat lung **E**vasion
Short Title	HI-FIVE
Project Sponsor	Peter MacCallum Cancer Centre
Principal Investigator	Dr Nicholas Bucknell
Associate Investigator	Associate Professor Shankar Siva
Location	Peter MacCallum Cancer Centre, Sunshine

Declaration by Participant

I wish to withdraw my consent to storage of my blood and/or tissue samples collected in the above research project for use in future research, and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with the Peter MacCallum Cancer Centre

Name of Participant (please print) _____

Signature _____ Date _____

Name of Interpreter (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project. Note: All parties signing the consent section must date their own signature

Bucknell N, *et al. BMJ Open* 2020; 10:e042465. doi: 10.1136/bmjopen-2020-042465