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Title:

ImmunoPET: IMaging of cancer imMUNOtherapy targets with positron Emission Tomography: a phase 0/1 study characterising PD-L1 with 89Zr-durvalumab (MEDI4736) PET/CT in stage III NSCLC patients receiving chemoradiation study protocol.

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BMJ Open ImmunoPET: IMaging of cancer imMUNOtherapy targets with positron Emission Tomography: a phase 0/1 study characterising PD-L1 with ⁸⁹Zr-durvalumab (MEDI4736) PET/CT in stage III NSCLC patients receiving chemoradiation study protocol

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ABSTRACT

Background ImmunoPET is a multicentre, single arm, phase 0–1 study that aims to establish if ⁸⁹Zr-durvalumab PET/CT can be used to interrogate the expression of PD-L1 in larger, multicentre clinical trials.

Methods The phase 0 study recruited 5 PD-L1+ patients with metastatic non-small cell lung cancer (NSCLC). Patients received 60MBq/70 kg ⁸⁹Zr-durva up to a maximum of 74 MBq, with scan acquisition at days 0, 1, 3 or 5±1 day. Data on (1) Percentage of injected ⁸⁹Zr-durva dose found in organs of interest (2) Absorbed organ doses (μSv/MBq of administered ⁸⁹Zr-durva) and (3) whole-body dose expressed as mSv/100MBq of administered dose was collected to characterise biodistribution.

The phase 1 study will recruit 20 patients undergoing concurrent chemoradiotherapy for stage III NSCLC. Patients will have ⁸⁹Zr-durva and FDG-PET/CT before, during and after chemoradiation. In order to establish the feasibility of ⁸⁹Zr-durva PET/CT for larger multicentre trials, we will collect both imaging and toxicity data. Feasibility will be deemed to have been met if more than 80% of patients are able complete all trial requirements with no significant toxicity.

Ethics and dissemination This phase 0 study has ethics approval (HREC/65450/PMCC 20/100) and is registered on the Australian Clinical Trials Network (ACTRN12621000171819). The protocol, technical and clinical data will be disseminated by conference presentations and publications. Any modifications to the protocol will be formally documented by administrative letters and must be submitted to the approving HREC for review and approval.

Trial registration number Australian Clinical Trials Network ACTRN12621000171819.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ Prospective, multicentre trial evaluating novel tracer PET tracers to image immunity.
- ⇒ Automated manufacturing of tracer to ensure consistent and uniform quality of tracer across all trial sites.
- ⇒ National-level imaging credentialing programme for ⁸⁹Zr PET/CT to ensure consistent imaging acquisition and reading of PET scans.
- ⇒ Small, feasibility study and technology will require roll-out in a larger multicentre trial to establish clinical efficacy.

INTRODUCTION

Background and rationale

Immunotherapy has revolutionised cancer care, providing clinically meaningful survival benefits even in patients with previously dire prognoses such as locally advanced and metastatic lung carcinomas,^{1,2} head and neck³ and skin cancers.⁴ However, the introduction of immunotherapies has exacted a significant cost in toxicity and futile treatment; a reflection of the limitations of standard biopsy approaches to patient selection.⁵ PET tracers to characterise the uptake of immune checkpoint inhibitors (ICIs) for both programmed death ligand-1 (PD-L1)⁶ and programmed cell death protein-1 (PD-1)⁷ have been tested in small clinical trials. However, the clinical implementation of Immuno-PET as a biomarker has been hampered by the

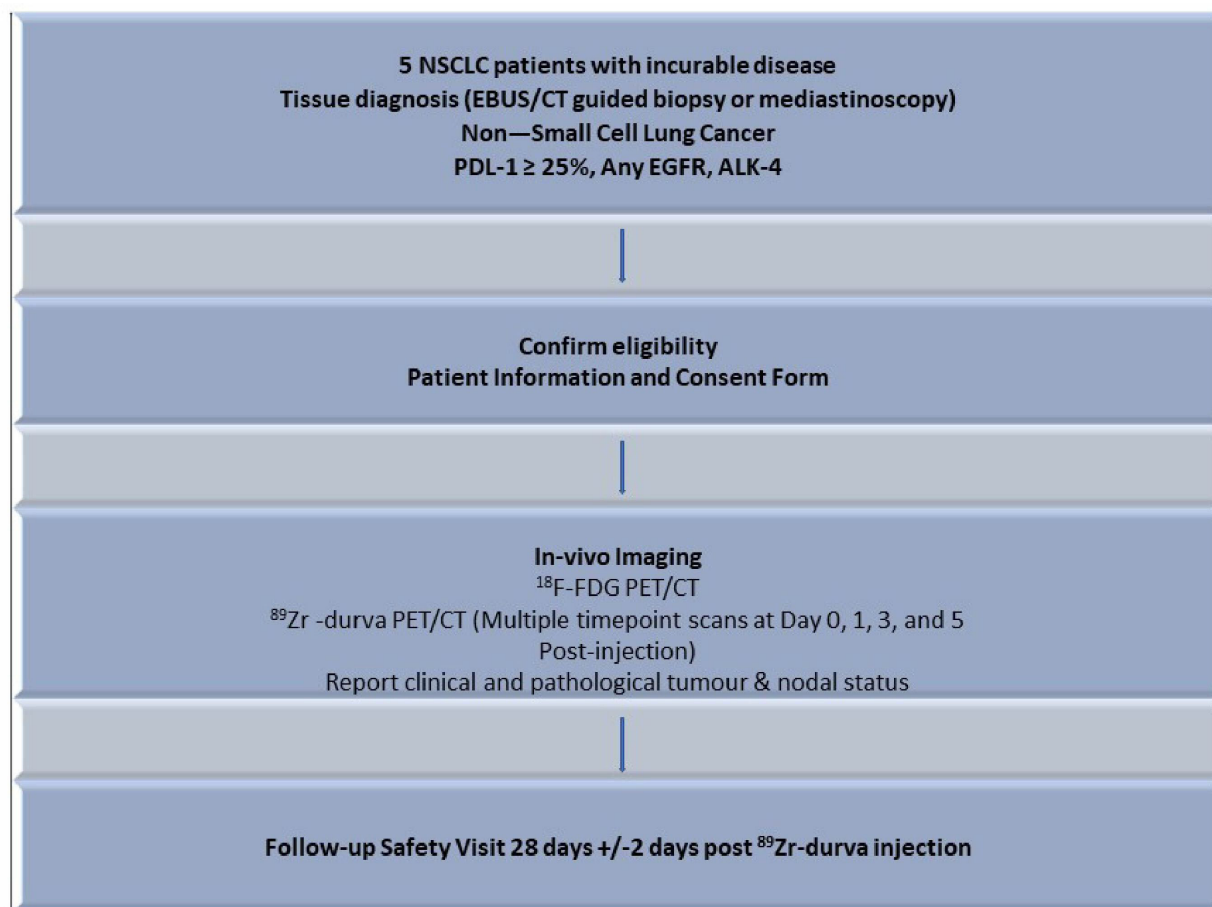


Figure 1 Phase 0 biodistribution study (five patients) to establish biodistribution of the ^{89}Zr -durva tracer and time point for ^{89}Zr -durva PET/CT image acquisition. FDG-PET fluorodeoxyglucose (FDG)-positron emission tomography (PET); EBUS, endoscopic bronchoscopic ultrasound; NSCLC, non-small cell lung cancer.

technical difficulties of performing the high-quality, large-scale, multicentre trials required for robust validation.

In this proposal, we describe our processes for automated production of zirconium-labelled Immune-PET tracers, validation processes and imaging credentialing for a multicentre trial of ^{89}Zr -durvalumab (^{89}Zr -durva) to characterise PD-L1 upregulation during the treatment of locally advanced non-small cell lung cancer (NSCLC) patients receiving radical chemoradiotherapy. We hope that this small, pilot study will establish a model for the larger multicentre trial required to develop this as a validated biomarker in patients with lung cancer, and improve the efficacy and safety of immunotherapy treatment.

Automation of tracer production

Production of clinical quality ^{89}Zr -durva was achieved via a novel procedure using an iPhase MultiSyn automated synthesiser and disposable cassette kits. Standard kits were customised to suit the requirements of ^{89}Zr -labelling of durvalumab and are now commercially available gamma-sterilised for clinical use. Batches of clinical grade desferrioxamine-squaramide (DFOSq)-durvalumab conjugate and buffer reagent kits were prepared centrally, validated and distributed to participating sites under controlled conditions. Quality control of ^{89}Zr -durva

included assessment of specific activity (273–357 MBq/mg), radiochemical purity (>99%), protein integrity (>96%), immunoreactive fraction (>75%), pH, sterility and endotoxin levels as well as preclinical imaging and biodistribution studies in PD-L1 positive models to confirm tumour targeting. This fully automated synthesis approach and centralised manufacturing of reagents, ensures standardised and consistent production of ^{89}Zr -when the final radiolabelling is performed at different sites.³

Imaging credentialing

All participating sites are certified for PET scanner validation for imaging of ^{89}Zr -durvalumab by the Australian Radiopharmaceutical Trials Network (ARTnet).

HYPOTHESES AND OBJECTIVES

Study hypotheses

1. We hypothesise that imaging with ^{89}Zr -durva PET/CT can identify NSCLC lesions with PD-L1 expression.
2. We hypothesise that the changes we see in host and tumour immunity in tissue will correlate with what we see in ^{89}Zr -durva PET/CT scans.

3. We hypothesise that the PD-L1 expression changes during treatment of NSCLC.

Objectives

Phase 0 study

The primary objective is to investigate the biodistribution and dosimetry of ^{89}Zr -durva and define the optimal imaging time-point for qualitative assessment of ^{89}Zr -durva.

Secondary objective

1. To investigate the interaction of ^{89}Zr -durva with malignant tissues.
2. To record any toxicity potentially associated with the ^{89}Zr -durva PET tracer.

Phase 1 study

The primary objective of this study is to demonstrate the feasibility ^{89}Zr -durva PET/CT imaging in patients with NSCLC in order to report tumour uptake prechemoradiotherapy, during and postchemoradiotherapy.

► Feasibility would be achieved if:

A total of >80% of patients can undergo the steps of recruitment, informed consent, enrolment and complete the trial within the time frame of interest. With no significant toxicity related to ^{89}Zr -durva PET tracer, which is defined as:

Any patient experiences grade 4/5 toxicity, grade 3 toxicity in $\geq 10\%$ of patients.

The secondary objective is to describe the distribution of ^{89}Zr -durva within tumours at baseline, during chemoradiation and after chemoradiation.

Trial design

ImmunoPET is an unblinded, observational single arm study. The study consists of two parts:

- Phase 0 Initial Biodistribution study (five patients): Initial clinical study to develop and investigate the biodistribution and dosimetry of ^{89}Zr -durva and to define the optimal imaging time point for ^{89}Zr -durva in five patients with incurable high PD-L1 (>25%) NSCLC (figure 1).
- Phase I Clinical Study of Sequential Imaging in stage III NSCLC (20 patients) to characterise PD-L1 dynamics during radical chemoradiotherapy in 20 stage III NSCLC patients (figure 2).

Patients in the phase 0 study will be closely monitored for adverse events, and the data reviewed by the Independent Data and Safety Monitoring Committee (IDSMC) prior to opening the phase 1 study.

METHODS: PARTICIPANTS, INTERVENTIONS AND OUTCOMES

Study setting

Patients will be recruited from the Lung Cancer Clinics at 3 Australia academic teaching hospitals, Peter MacCallum Cancer Centre and The Olivia Newton-John Cancer Research Institute (Austin Health) in Melbourne and the

Sir Charles Gardiner Hospital in Perth. The Phase 0 study opened in October 2021 and closed in June 2022, and the phase 1 study will open in September 2022 and close in May 2025.

Eligibility criteria

The initial phase 0 cohort of five high PD-L1 expressing NSCLC will be recruited to establish the initial feasibility of ^{89}Zr -durva administration. Twenty eligible stage III NSCLC patients will be prospectively recruited to the expansion phase study over a 3-year period. Patients with the following inclusion characteristics will be invited to participate.

Inclusion and exclusion criteria for phase 0 biodistribution study (five patients)

Key inclusion criteria

1. Written informed consent provided.
2. Life expectancy ≥ 12 weeks.
3. Patients with NSCLC and with advanced incurable disease, and with metastatic disease apparent on fluorodeoxyglucose positron emission tomography (FDG-PET).
4. Histopathology with PD-L1 positive tumour cells >25%. Although, in the metastatic setting >50% is the accepted cut-off, the threshold for stage III patients remains poorly defined. We use a cut-off of >25% to broaden the eligibility criteria for enrolment.
5. Subjects with an estimated glomerular filtration rate (eGFR) >50 mL/min as measured using the Modification of Diet in Renal Disease (MDRD) formula.
6. Eastern Cooperative Group Oncology Group (ECOG) performance score of 0–2.
7. Patient is willing and able to comply with the protocol for the duration of the study including undergoing treatment and scheduled visits and examinations including follow-up.
8. Evidence of postmenopausal status or negative urinary or serum pregnancy test for female premenopausal patients.

Exclusion criteria

- 1 Pregnant or breastfeeding females.
- 2 Known sensitivity or allergy to anti-PD-L1 agents.
- 3 Any serious medical condition which the investigator feels may interfere with the procedures or evaluation of the study.
- 4 Patients unwilling or unable to comply with protocol or with a history of non-compliance or inability to grant informed consent.

Inclusion and exclusion criteria for phase I observational clinical study of sequential imaging in stage III NSCLC cohort (20 patients)

Key inclusion criteria

1. Written informed consent provided.
2. Life expectancy ≥ 12 weeks.
3. Primary tumour characteristics: stage IIIA and IIIB NSCLC as defined by the American Joint Committee on Cancer version 8.0 staging (AJCC V.8).

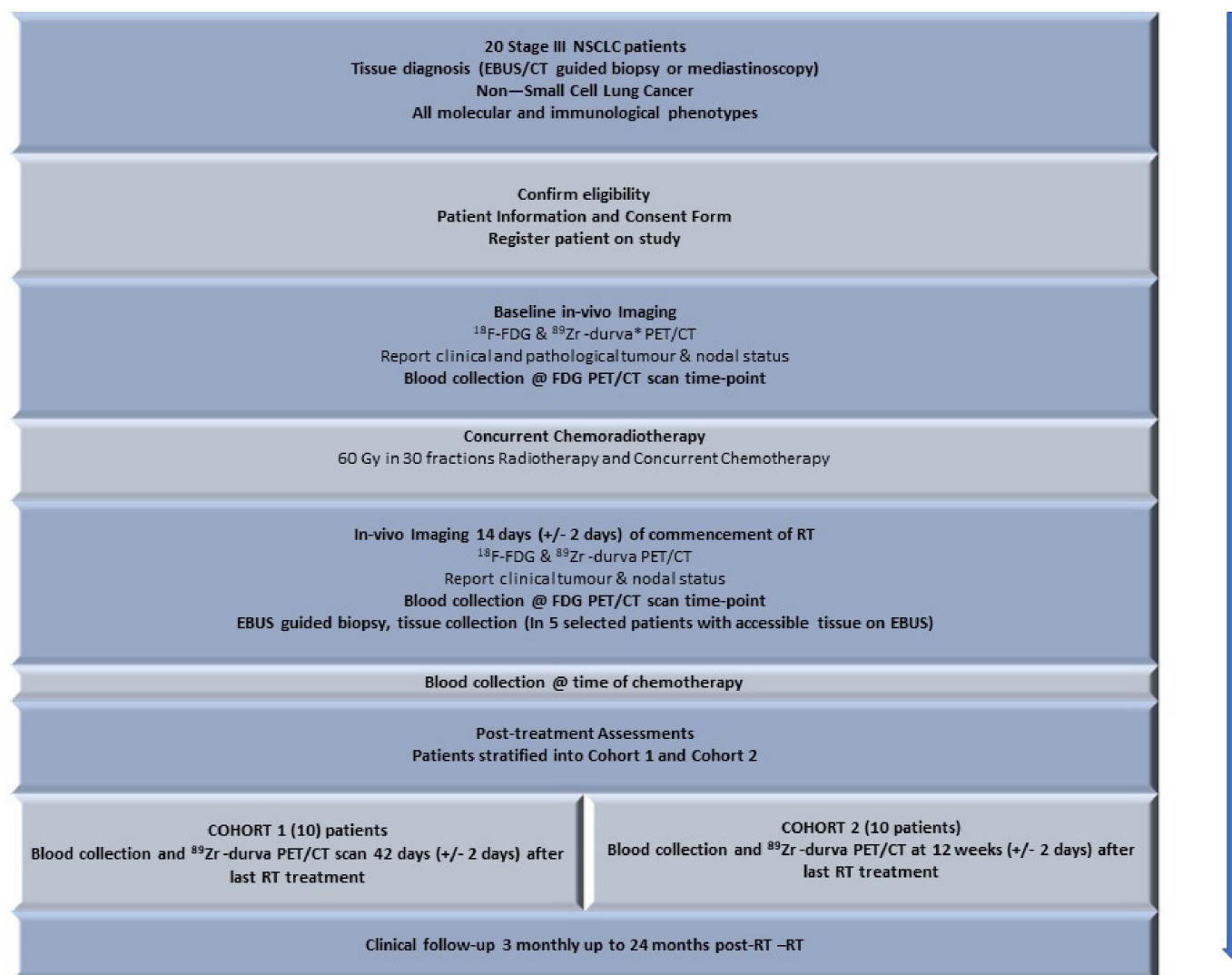


Figure 2 Phase I clinical study of sequential imaging in stage III NSCLC (20 patients). *Time point for scanning after ^{89}Zr injection to be determined after phase 0 initial biodistribution study. FDG-PET fluorodeoxyglucose (FDG) positron emission tomography (PET); EBUS, endoscopic bronchoscopic ultrasound; NSCLC, non-small cell lung cancer.

4. Any PD-L1 status.
5. Any epidermal growth factor receptor (EGFR) mutation status or anaplastic lymphoma kinase (ALK4) mutation status.
6. Candidate for radical radiotherapy, including concurrent chemoradiotherapy with radiotherapy dose of 60 Gy in 30 fractions and concurrent chemotherapy as per standard institutional protocol.
7. Willing and able to undergo additional imaging studies as required by the protocol.
8. Adequate organ and marrow function as defined below:
 - Serum creatinine $\text{CL} > 50 \text{ mL/min}$ by the Cockcroft-Gault formula.
 - Serum bilirubin $\leq 1.5 \times$ upper limit of normal (ULN).
9. ECOG performance status 0–1
10. Patient is willing and able to comply with the protocol for the duration of the study including undergoing treatment and scheduled visits and examinations including follow-up.
11. Evidence of postmenopausal status or negative urinary or serum pregnancy test for female premenopausal patients. Women will be considered post-menopausal if they have been amenorrhoeic for 12 months without an alternative medical cause.

Exclusion criteria

1. Any previous therapy that precludes radical treatment of stage III NSCLC with concurrent chemoradiation (radiotherapy, chemotherapy and immunotherapy).
2. Current or prior use of immunosuppressive medication within 28 days before the first dose of study drug, with the exceptions of intranasal and inhaled corticosteroids or systemic corticosteroids at physiological doses, which are not to exceed 10 mg/day of prednisone, or an equivalent corticosteroid. Systemic steroid administration required to manage toxicities arising from radiation therapy delivered as part of the chemoradiation therapy for locally advanced NSCLC is allowed.

3. Prior exposure to any anti-PD-1 or anti-PD-L1 antibody
4. Any prior grade ≥ 3 immune-related adverse event (irAE) while receiving any previous immunotherapy agent, or any unresolved irAE $\geq$ grade 1.
5. Recent major surgery within 4 weeks prior to entry into the study (excluding the placement of vascular access or endoscopic bronchoscopic ultrasound (EBUS) or mediastinal biopsy) that would prevent administration of study drug.
6. Active or prior documented autoimmune disease within the past 2 years. Note: Patients with vitiligo, Grave's disease or psoriasis not requiring systemic treatment (within the past 2 years) are not excluded.
7. Active or prior documented inflammatory bowel disease (eg, Crohn's disease, ulcerative colitis).
8. History of active primary immunodeficiency.
9. History of organ transplant that requires therapeutic immunosuppression.
10. Uncontrolled intercurrent illness including.
11. Active infection including tuberculosis (TB) (clinical evaluation that includes clinical history, physical examination and radiographic findings, and TB testing in line with local practice), hepatitis B (known positive HBV surface antigen result), hepatitis C.
12. Receipt of live attenuated vaccination within 30 days prior to study entry or within 30 days of receiving study drug.
13. History of another primary malignancy within 5 years prior to starting study drug, except for adequately treated basal or squamous cell carcinoma of the skin or cancer of the cervix in situ and the disease under study.
14. Female patients who are pregnant, breast-feeding or male or female patients of reproductive potential who are not employing an effective method of birth control.
15. PET contraindications, for example, total serum bilirubin >1.5 times ULN (abnormal hepatic metabolism may interfere with hepatic excretion).
16. Concurrent enrolment in another clinical study, unless it is an observational (non-interventional) clinical study or during the follow-up period of an interventional study.
17. Mean QT interval corrected for heart rate using Fridericia's formula (QTcF) ≥ 470 ms calculated from 3 ECGs (within 15 min at 5 min apart).
18. Known allergy or hypersensitivity to any of the study drugs or any of the study drug excipients.
19. Prior randomisation or treatment in a previous durvalumab clinical study regardless of treatment arm assignment.
20. Any condition that, in the opinion of the investigator, would interfere with evaluation of the study drug or interpretation of patient safety or study results.

Who will take informed consent?

Informed consent for the study will be performed by the clinical investigators at each participating site. Each investigator who is delegated to undertake consent procedures will have undergone appropriate training, participated in trial start-up initiation presentations, and be documented on the Study Delegation Log in accordance with GCP guidelines.

Additional consent provisions for collection and use of participant data and biological specimens

An optional translational substudy, in which patients undergo additional EBUS biopsies before and at 2 weeks after the commencement of radiotherapy will be discussed with selected, suitable patients. These patients will be given additional information about this procedure and a separate consent form will be signed.

Interventions

Concurrent chemoradiotherapy

Treatment will be performed according to current standard of care and treatment protocols of the participating institution. The study is observational and will not influence patient management unless a clinically significant finding is observed (eg, the detection of distant metastatic disease).

- a. Systemic therapies: Concurrent chemotherapy will be delivered with radiotherapy according to institutional policy treatment protocols.
 - a. Ideally patients should be able to receive platinum-based chemotherapy regimens according to the local standard of care regimens.
 - b. The final chemotherapy cycle must end prior to, or concurrently with, the final dose of radiation. Consolidation chemotherapy after radiation is not permitted but administration of chemotherapy prior to concurrent chemoradiation is acceptable.

Radiation therapy: In accordance with institutional guidelines, patients will be simulated with a radiotherapy (RT) CT scanner. RT plans will be calculated with 6 MV photons, AAA algorithm and 120 leaf multileaf collimation. Dose will be 60–66 Gy in 30–33 fractions.

Maintenance durvalumab: Patients who are deemed suitable for maintenance durvalumab by their treating medical oncologist will receive durvalumab 1500 mg by intravenous infusion every 4 weeks for 12 cycles or until disease progression or unacceptable toxicity develops.

⁸⁹Zr-durva PET/CT scan acquisition and analysis

All scans will be performed according to current departmental guidelines on an integrated PET/CT scanner and reported by a qualified PET physician. Baseline scans will be performed prior to the commencement of neoadjuvant systemic therapy and within 5 days of the baseline scan. Time point 3 scans will be performed following the completion of the final radiotherapy treatment.

DFOSq-durvalumab is prepared as 1.0 mg/vial, and the automated radiolabelling protocol specified above uses

a single vial for each patient infusion, with approximate specific activity achieved of 315 MBq/mg. Analysis of the biodistribution and radiosimetry data acquired in the phase 0 initial study demonstrates that the optimal time point for scan acquisition is between days 4 and 6 post-injection of tracer. This time point has been specified in the final protocol submitted for ethical and sponsorship review prior to recruitment to the phase 1 study.

Zr-89-DFOSq-durvalumab is infused over 30 min and scans acquired at 120 min postinfusion as follows: 10 mg unlabeled durvalumab is first infused over 30 min in accordance with investigator brochure guidance. The patient is monitored for any adverse reaction. After 60 min of observation the patient is then infused over 30 min with ⁸⁹Zr-durva (60 mBq/70 kg up to no more than 74 MBq with a concentration of 4 µg/MBq).

The whole-body PET/CT scan for the Phase 0 initial cohort was acquired at days 0, 1, 3 or 5±1 day after injection of ⁸⁹Zr-durva, and the precise timing of scan initiation for the day 0 measurement was recorded, enabling subsequent scan time points to be matched within 10 min. For each imaging time point, a scout CT scan was performed from vertex to mid thighs, followed by PET emission acquisition at 3 min per bed position on day 0, and 5 min per bed position for subsequent days. For PET/CT images with a 70 cm field of view (FOV), the following reconstruction parameters were used; 28 subsets, 2 iterations in a 192×192 matrix, the following filters were applied, a view point filter 6.4 mm and a light Z axis filter. Serum samples for measuring ⁸⁹Zr-durva pharmacokinetics were collected immediately postinfusion, and at 30 min, 1 hour, 1.5 hours, 2 hours, 4 hours, 24 hours and at each subsequent PET/CT imaging time point.

Based on analysis of biodistribution data (normal organ and tumour uptake) from the phase 0 part of the study by experienced nuclear medicine physicians, we have shown that the optimal imaging time point is day 5, and accordingly have specified that PET imaging acquisition take place between days 4–6 for the phase 1 study. This will be performed using the same PET/CT acquisition protocol as for the phase 0 part of the study, with documentation of scan parameters and timing to ensure subsequent biodistribution and dosimetry analysis is standardised.

For the phase I study, scans will be evaluated by experienced nuclear medicine physicians at each trial site. Biodistribution data will be analysed qualitatively for normal organ and tumour uptake and clearance, and the same image datasets will be used for dosimetry analysis. MIM (Cleveland, Ohio, USA) is being used for PET image analysis, and OLINDA/EXM V.1.1 software (Vanderbilt University, Nashville, Tennessee, USA) for dosimetry analyses. Aortic blood pool will be used to normalise the SUV to allow interpatient comparisons.

Criteria for discontinuing or modifying allocated interventions

Patients will be monitored before, during and after the infusion.

In the event of a ≤ grade 2 infusion-related reaction, the infusion rate of study drug may be decreased by 50% or interrupted until resolution of the event (up to 4 hours) and reinitiated at 50% of the initial rate until completion of the infusion. If the infusion related reaction is grade 3 or higher in severity, study drug will be discontinued.

OUTCOMES

Phase 0 study endpoints

Primary endpoints

1. Percentage of injected ⁸⁹Zr-durva dose found in organs of interest at days 0, 1, 3 and 5 days postinjection (± 1 day) of ⁸⁹Zr-durva.
2. Absorbed organ doses expressed as micro Sv/MBq of administered ⁸⁹Zr-durva, and whole-body dose expressed as milliSv/100 MBq of administered dose
3. The optimal imaging time point will be defined by identifying the time point at which the percentage of injected ⁸⁹Zr-durva dose in tumour reaches maximal accumulation. This time point will be used in the phase 1 study schema for all ⁸⁹Zr-durva scans.

Secondary endpoints

1. Whether ⁸⁹Zr-durva PET/CT scans demonstrate uptake in known sites of FDG-avid malignancy.
2. Whether ⁸⁹Zr-durva PET/CT scans demonstrate any non-physiological, non-tumour containing tissues with uptake greater than 1.5× that of the background.
3. Number of patients experiencing toxicity after tracer administration.

Phase 1 study

The primary endpoints are:

- ▶ Number of patients successfully completing the trial.
- ▶ Number of patients experiencing significant toxicity during scans after injection of PET tracer. These toxicities would be related to ⁸⁹Zr-durva PET/CT and defined as having any of the following:
Anaphylaxis.
Grade 4/5 toxicity.
Grade 3 toxicity.

Secondary endpoints include

- ▶ Quantitative measures of ⁸⁹Zr-durva PET/CT of PD-L1 expression level within NSCLC tumour at baseline, during and after treatment.
- ▶ Quantitative measures on FDG-PET/CT within NSCLC tumour at baseline, during and after treatment.
- ▶ Quantitative assessment of PD-L1 immunohistochemistry at baseline.

Data collection and management

Plans for assessment and collection of outcomes

Baseline demographic data will be collected on signing of the Consent Form, including patient and tumour characteristics and radiotherapy and chemotherapy treatment plans.

Imaging evaluation

1. Qualitative evaluation of imaging biodistribution will be compared with contemporaneous FDG PET/CT.
2. At sites of identified disease, quantitative measures, including maximum standardised uptake value (SUV_{max}), mean standardised uptake value (SUV_{mean}), peak standardised uptake value (SUV_{peak}) & percentage injected dose (%ID) ⁸⁹Zr-durva will be compared pre-RT and post-RT.
3. Comparison of baseline ⁸⁹Zr-durva PET with PD-L1 IHC as assessed by baseline tissue biopsy.

MIM (MIM Software, Beachwood, California, USA) is being for PET image analysis and OLINDA/EXM V.1.1 software for dosimetry. SUV is normalised to aortic blood pool to allow inter-patient comparisons.

Data management and confidentiality and dissemination policy

The Participant Information and Consent Form (PICF) explains that study data will be stored in a computer database, maintaining confidentiality in accordance with national privacy principles. The PICF also explains that for data verification purposes, a regulatory authority or a Human Research Ethics Committee (HREC) may require direct access to parts of the hospital or practice records relevant to the study, including patients' medical histories. Deidentified patient outcome data will be disseminated via publication in peer-reviewed journals.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use

Blood is being collected from all patients in the phase 1 study at the time of the ⁸⁹Zr-durvalumab PET/CT. Circulating tumour DNA (ctDNA) is being extracted and stored in Anatomical Pathology at Peter Mac for further analyses. EBUS biopsies from patients consenting to this substudy will be processed by Peter MacCallum Anatomical pathology, who will extract RNA and perform RNA sequencing.

STATISTICAL METHODS

Statistical methods for primary and secondary outcomes

In the feasibility phase (phase 0 study), 5 patients were recruited in order to characterise tracer biodistribution and determine the optimal imaging time points post-trace administration. This data informed the imaging protocol for the expansion phase of the study (phase I study).

We are primarily interested in precise estimates of feasibility and toxicity that will aid in the planning of a larger, sufficiently powered efficacy trial. A sample size of 20 will allow us to be relatively precise in our conclusions regarding feasibility outcomes. For example, if we observe an 80% feasibility rate out of the 20 enrolled, the 95% confidence interval (CI) for that feasibility rate would be (56.3% to 94.3%). If we observe a 10% toxicity rate out of the 20 enrolled, the 95% CI for toxicity rate

would be (1.2% to 31.7%). Thus, in the expansion phase observational study, 20 patients will provide rough estimates of patients who can undergo the steps of recruitment, informed consent, enrolment, and complete the trial within the time frame of interest and the toxicity rate of patients experiencing grade 4/5 toxicity, grade 3 toxicity. Although there is no guideline to suggest the appropriate sample size for a pilot study with PET, we postulate that 20 patients are likely to allow sample size estimation for larger subsequent confirmatory studies and feasible within the timescale of this study.

General considerations for sample size

This is an exploratory, feasibility study that serves to provide base case statistics for further prospective research. The PET/CT results will be both qualitatively and quantitatively assessed in 20 patients. Primarily descriptive statistics will be used. Demographic and baseline characteristics will be summarised using mean, standard deviation (SD), median, and range for continuous variables, and proportion for categorical variables. The proportions of patients with adverse events and serious adverse events (SAEs) will be reported. No hypothesis testing will be performed. No multivariable analyses are planned for this study.

Primary analysis

Number of patients successfully completing the trial and number of patients experiencing significant toxicity during scans after injection of PET tracer will be recorded.

The completion rate of will be estimated as the number of patients who complete study procedures, divided by the number of respective patients identified as eligible and recruited into the study.

The rate of significant toxicity will be estimated as the number of patients who experience significant toxicity procedures, divided by the number of respective patients identified as eligible and recruited into the study. A 95% exact CI will also be calculated for the primary endpoint.

Secondary analysis

The secondary objective is to describe the distribution of ⁸⁹Zr-durva within tumours at baseline, during chemoradiation and after chemoradiation. Quantitative measures on ⁸⁹Zr-durva PET/CT, FDG PET/CT of PD-L1 expression level and PD-L1 immunohistochemistry will be summarised. Correlations between Quantitative measures will be expressed as a Pearson/Spearman correlation coefficient depending on distribution of data.

Exploratory analysis

- ⁸⁹Zr-durva PET/CT and FDG PET/CT precurrent, during and postconcurrent chemoradiation in the following absolute measures of metabolic tumour volume (MTV), SUV_{max}, SUV_{mean}, SUV_{peak} and %ID will be summarised. Results will be displayed in graphs.

- Concordance between FDG and ^{89}Zr -durva PET/CT alterations over time will be assessed using a weighted kappa coefficient with weights based on quadratic differences.
- Associations between intratreatment response and overall survival/disease progression-free survival will be described graphically using Kaplan-Meier product limit curves.
- Proportion of patients in which ^{89}Zr -durva PET/CT provides information not seen on FDG-PET or PD-L1 immunohistochemistry performed at baseline and proportion of patients in which additional information from ^{89}Zr -durva PET/CT with the potential to alter management will be described.

Oversight and monitoring

Composition of the coordinating centre, trial steering committee and data monitoring committee

The protocol study authors make up the trial steering committee. Trial coordination for this study is being performed through the Department of Radiation Oncology Clinical Trial Unit at Peter MacCallum. The independent data safety monitoring committee consists of four clinicians (Radiation and Medical Oncology, and Respiratory Medicine), a consumer and biostatistician.

Adverse event reporting and harms

All SAEs will be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). The reporting period for SAEs is the period immediately following the time that written informed consent is obtained through 90 days after the last dose of durvalumab or until the initiation of alternative anticancer therapy. Events must be reported to the Study Sponsor (Peter Mac) within 24 hours and to the Peter Mac Research Governance Office within 72 hours. The sponsor will then report the suspected unexpected serious adverse reactions (SUSAR) or significant safety issue (SSI) to the Peter Mac HREC, the Therapeutic Good Administration (TGA) and Astra Zeneca.

Plans for communicating important protocol amendments to relevant parties (eg, trial participants, ethical committees)

Ethics and dissemination

This phase 0 study has ethics approval (HREC/65450/PMCC 20/100) and is registered on the Australian Clinical Trials Network (ACTRN12621000171819). The protocol, technical and clinical data will be disseminated by conference presentations and publications.

Any modifications to the protocol will be formally documented by administrative letters and must be submitted to the approving HREC for review and approval.

DISCUSSION

There is a compelling unmet need to develop imaging techniques for immunotherapy targets in humans with cancer, especially the immune checkpoint programmed

cell death ligand (PD-L1) and its receptor (PD1). ICI's such as durvalumab, targeting PD-L1, and nivolumab/pembrolizumab (targeting PD1) are revolutionising the management of common cancers, including lung carcinomas,^{1 2 4} head and neck⁵ and skin cancers.⁶ In the management of often advanced, solid tumours and refractory haematological cancers, clinical trials have demonstrated that ICIs can enable durable responses and even potential cures in previously hopeless cases.

Characterising the dynamics of PD-L1 during chemoradiotherapy will facilitate new clinical trials to test patient-individualised ICI scheduling, and may open avenues to toxicity reduction through patient-appropriate de-escalation and/or localised escalation of radiotherapy with dose-painting approaches. Finally, there is already evidence that the concurrent administration of antibodies during PET-antibody imaging does not impact on image quality and may even improve imaging efficacy by reducing non-specific binding outside the tumour.⁷ This makes the development of a durvalumab-PET tracer of high relevance to the implementation of the PACIFIC I study, and to any other future studies using durvalumab.

Immuno-PET is undergoing rapid evolution, with tracers moving from the preclinical to the human clinical trial space within the last 5 years.⁸⁻¹¹ This clinical trial protocol has focused on the clinical Implementation of ^{89}Zr -durva, which is attractive because of its direct link to a therapeutic intervention. However, there is also increasing interest in evaluating the ability of PET/CT to dynamically characterise elements of the immune system in vivo. CD8 and activated CD8 PET tracers are potential biomarkers to define the immunophenotype of the tumour microenvironment, which are entering larger studies.¹²

Cost and the high level of radiochemistry expertise were and continue to be substantial barriers to the full development of this technology. Furthermore, in many jurisdictions, stringent regulation of PET tracer production significantly limits the ability to perform the large, multicentre trials required for clinical validation. In turn, the speed of development of novel immunotherapeutics, demands a more responsive and adaptive paradigm to rapidly develop, evaluate and implement new Immune-PET tracers.

In this protocol, we have invested heavily in automation of tracer production, and in developing robust processes for quality control and imaging credentialing to provide a practical model for these trials. We hope through this work to create a path that can be followed to allow immuno-PET tracers to take their place as a precision tool to guide patient management, resulting in safer, cheaper and more effective immunotherapy treatment.

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Competing interests FH-J has clinical trial funding, has received honoraria and participated in advisory boards for Astra Zeneca. She has received payments and honoraria from BeiGene and MSD for lectures and presentations. Her work is supported by the Peter Mac Foundation and the Victorian Cancer Agency. SER and PSD are inventors on intellectual property relating to the use of DFOSq that have been licensed from the University of Melbourne to Telix Pharmaceuticals. TJ has received payments and honoraria from BMS and Astra Zeneca for lectures and presentations, and sits on Data Safety and Monitoring Boards or Advisory boards for Roche, BMS, Astra Zeneca, Novartis, Amgen, Puma, MSD and Merck. SF has received payments and honoraria from Astra Zeneca, Roche, Amgen, Novartis, Bayer, GSK, BMS, MSD and Janssen for lectures and presentations. KB has received payments and honoraria from Bayer and Abbvie for lectures and presentations and participates on Data Safety and Monitoring boards for Novartis.

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