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Keywords:	Urological tumours < ONCOLOGY, Urological tumours < UROLOGY, Nuclear radiology < RADIOLOGY & IMAGING, NUCLEAR MEDICINE

^{89}Zirconium-labelled girentuximab (^{89}Zr-TLX250) PET in Urothelial Cancer Patients (ZiPUP) – A phase I trial of a novel staging modality for urothelial carcinoma.

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ABSTRACT

Introduction

Bladder cancer is a lethal disease with a rising incidence. The current standard imaging modalities for staging are either CT of the chest-abdomen-pelvis or FDG-PET/CT. However, there are issues with using these modalities. CT is known to have relatively low sensitivity for detecting low volume metastatic disease, while FDG is predominantly renally excreted and therefore has intense activity in the urinary tract, which limits its utility to detect bladder and upper tract lesions, or nodal metastases in close proximity to the urinary tract. ^{89}Zr-TLX250 may have utility in the accurate staging of bladder and urothelial cancer, with less renal excretion as compared to FDG, however this has not previously been investigated.

Methods and analysis:

A single-arm phase I trial examining the feasibility, safety, and utility of ^{89}Zr-TLX250-PET/CT in patients either undergoing pre-operative staging of bladder or other urothelial carcinoma for curative intent, or with known metastatic urothelial carcinoma. All participants will undergo ^{89}Zr-TLX250-PET/CT and will need to have undergone recent FDG-PET/CT for means of comparison. This trial aims to recruit 10 participants undergoing pre-operative staging and 10 participants with known metastatic disease. The primary endpoint is feasibility; secondary endpoints are safety, tolerability, and sensitivity and specificity in detecting lymph node metastases compared to FDG-PET/CT.

Ethics and dissemination:

Ethics approval has been obtained from the South Metropolitan Health Service Human Research Ethics Committee (*RGS0000003940*). ZiPUP study has been registered with the Australian and New Zealand

Clinical Trials Registry (Trial ID *NCT05046665*, registration number is *ACTRN12621000411842*). Also, it has been registered with the ClinicalTrials.gov (ClinicalTrials.gov identifier is *NCT05046665*, obsolete identifier *NCT05018442*). Eligible patients will only be enrolled after providing written informed consent. Patients will be given a full explanation, in lay terms, of the aims of the study and potential risks including as a written patient information sheet.

Strength and limitation of the study:

- ^{89}Zr-TLX250 is metabolised in the liver with less renal excretion as compared to FDG.
- If proven effective this may present a useful staging modality for urothelial cancer and bladder cancer detecting small tumours.
- If proven effective, then it has the potentiality to therapeutic or 'theranostic.
- The aimed recruited patients' number is small.
- Unknown quality of images will be obtained.

Keywords:

Zirconium, Girentuximab, bladder cancer, urinary tract cancer, urothelial cancer, Positron Emission Tomography/Computed Tomography (PET/CT), Carbonic anhydrase IX, theranostic

ABBREVIATIONS

^{89}Zr-TLX250: ^{89}Zirconium-labelled girentuximab
CAIX: Carbonic anhydrase IX
FDG PET: Fluorodeoxyglucose positron emitting tomography
CT: Computed tomography
177Lu-PSMA-617: Lutetium-177 prostate-specific membrane antigen
ccRCC: Clear Cell Renal Cell Carcinoma
T-DM1: Ado-trastuzumab emtansine
HER2: human epidermal growth factor receptor 2
HSP90: Heat Shock Protein 90
NVP-AUY922: Luminespib
mBq: Megabecquerels
ECOG: Eastern Cooperative Oncology Group
ECG: Electrocardiogram
β-HCG: Beta-human chorionic gonadotropin

INTRODUCTION

Urothelial cancer:

Bladder cancer is a lethal disease with a rising incidence worldwide [1]. It is the most common malignancy involving the urinary system, and the tenth most common malignancy overall [2]. Transitional cell carcinoma is the predominant histologic type, accounting for approximately 90 percent of all bladder cancers. Urothelial cancers can also arise in the renal pelvis, ureter, or urethra. The diagnosis is usually confirmed histologically based on a transurethral resection or biopsy specimen, percutaneous biopsy, or urine cytology. The spectrum of urothelial cancer at presentation includes non-muscle-invasive, muscle-invasive, and metastatic disease, as determined by histopathology and staging investigations.

Current staging modalities:

The following modalities are currently utilised to detect the location and extent of urothelial tumours:

Computed Tomography (CT) of the chest, abdomen and pelvis along with delayed-phase images are used to identify urothelial tumours, which may appear as filling defects on delayed-phase imaging with enhancing soft tissue on the nephrographic phase. CT may demonstrate extravesical extension, nodal involvement in the pelvis or retroperitoneum, visceral or osseous metastasis, and tumour involvement or obstruction of the upper urinary tract. CT may miss tumours <1 cm in size, particularly those in the bladder trigone or dome, and it cannot accurately categorize depth of bladder wall invasion. The sensitivity of CT for identification of nodal involvement is relatively low (false-negative rate 68%, false-positive rate 16%) and may require a needle or excisional biopsy for confirmation [3]. Approximately 50% of patients with a filling defect in the renal pelvis or ureter will have associated hydronephrosis, hydroureter, or a delayed nephrogram secondary to obstruction [4].

^{18}F-fluorodeoxyglucose (FDG) **positron emitting tomography (PET)/CT** has limited value in the local staging of bladder cancer, largely due to urinary excretion of FDG affecting image interpretation of the bladder and any nodal disease in close proximity to the ureters [5]. However, FDG PET/CT is often useful in the distant staging of urothelial cancer, especially in high-risk disease with sensitivity of 78% in detecting locoregional lymph node metastasis as compared to 44% with CT only [6].

Carbonic anhydrase (CAIX)

CAIX is an enzyme that functions as a regulator of intracellular pH, cell proliferation, and cell adhesion in response to hypoxia [7]. CAIX is expressed abundantly in response to hypoxia in a wide range of cancer cell lines including bladder, renal, head and neck, lung, and colon cancer [7].

Previous data has demonstrated sensitivity and specificity of urinary CAIX of 86.2% and 95.1% respectively, for detection of urothelial bladder cancer (area under the curve 90.5%) [8]. A significant association between CAIX expression in paired urine and tumour specimens has also been established [7]. Notably, CAIX was shown to have significantly higher predictive accuracy compared to urinary cytology (90.5% vs 71.7%), especially in low-grade tumours (90% vs 61.8%).

CAIX appears to be expressed differently in non-muscle-invasive versus muscle-invasive bladder tumours, and in low-grade versus high-grade bladder cancers [9]. Importantly, in one study CAIX was distinctly expressed in >70% of urothelial carcinomas but was not expressed in normal urothelial tissue [10]. CAIX was also found to perform well as a prognostic marker, expression predicting for invasive recurrence of superficial disease and being the strongest independent predictor of worse recurrence-free and overall survival in invasive disease [10]. These findings provide strong rationale for

investigating the potential use of CAIX as a targeted imaging agent for the identification and diagnosis of bladder cancer. By the same token, the utility of CAIX as a therapeutic target also merits future investigation.

Positron Emission Tomography / Computed Tomography (PET/CT) and Zirconium-89-girentuximab (^{89}Zr-TLX250)

Theranostic PET is a novel modality for both imaging and treatment as it enables the tracking of targeted vehicles and carriers using isotope-labelled monoclonal antibodies. Many ligand-target combinations have been studied for both diagnostic and therapeutic use. A notable example in the field of theranostics with an established therapeutic role is 177Lu-PSMA-617, a radionuclide agent that has garnered success in the treatment of castrate-resistant prostate cancer [11].

Due to its intrinsic chemical properties, ^{89}Zr has been identified as a suitable ligand candidate for this approach [9]. Girentuximab (initially designated as TLX-250) is an antibody directed against CAIX that has been widely studied in the setting of renal cell carcinoma. For example, ^{89}Zr-TLX250 PET/CT has been shown to have a significant impact on clinical decision making in patients with an indeterminate renal mass [12]. Studies have also explored the use of ^{89}Zr-immuno-PET with other conjugated antibodies as a potential tool in staging breast cancer, and it has also shown utility for monitoring treatment in animal models [13].

Since its introduction in the 1980s, over 2000 girentuximab injections have been administered worldwide across multiple clinical trials. To date, there have been no reports of serious side effects or allergic reactions to girentuximab. There are a wide range of potential clinical applications for ^{89}Zr-TLX250 PET/CT including diagnosis and staging, patient stratification, monitoring of treatment response, and planning of radio-immunotherapy [14].

Urinary excretion of FDG PET/CT is intrinsically problematic when imaging urinary tract malignancies. Hepatic clearance of ^{89}Zr-TLX250 with low urinary excretion is therefore expected to be advantageous for the local and regional staging of bladder and urothelial carcinomas.

Collectively these studies highlight the potential diagnostic and therapeutic applications of ^{89}Zr-TLX250. This Phase I study aims to investigate the extension of its application into the staging of urothelial carcinoma or bladder cancer.

METHODS AND ANALYSIS

Protocol overview

This is a non-randomised, non-blinded, single centre, phase I trial comparing ^{89}Zr-TLX250 PET/CT with FDG PET/CT in patients with urothelial carcinoma or bladder cancer. The phase I study duration will be 18 months (Started May 2021 with anticipated date of last data collection will be December 31st, 2022). It is being conducted at a single tertiary centre in Western Australia. It will include two cohorts of adult patients: ten patients undergoing preoperative primary staging for recently diagnosed bladder cancer or urothelial carcinoma for curative intent, and ten patients with known metastatic urothelial carcinoma or bladder cancer.

The primary objective of this study is to evaluate the feasibility of using ^{89}Zr-TLX250 PET/CT as a staging modality for urothelial carcinoma or bladder cancer. The secondary objectives are to evaluate the safety and tolerability of ^{89}Zr-TLX250 PET/CT, as well as its effectiveness as compared to FDG PET/CT. The eligibility criteria are listed in Table 1 and the trial schema is outlined in Figure 1. Eligible patients are also required to have had an FDG PET/CT (as part of standard of care) within the last 28 days to allow accurate comparison between the two modalities. All participants will provide written informed consent.

TABLE 1: Key inclusion and exclusion criteria

Inclusion criteria
1. Aged ≥18 2. Able to provide informed consent 3. Histologically diagnosed with urothelial carcinoma or bladder cancer (or upper tract urothelial carcinoma diagnosed based on standard imaging and malignant urine cytology or direct visualisation on ureteroscopy) or known metastatic bladder or other urothelial carcinoma (based on previous imaging and /or histopathology) 4. Negative serum pregnancy test in female patients of childbearing potential at screening. Confirmation of negative pregnancy test result from urine within 24 hours prior to receiving investigational product. 5. Consent to practise double-barrier contraception until a minimum of 42 days after ^{89}Zr-TLX250 administration.
Exclusion criteria
1. Active malignancy other than urothelial carcinoma or bladder cancer 2. Administration of a radioisotope within 10 physical half-lives of ^{89}Zr prior to study enrolment. 3. Administration of chemotherapy, radiotherapy, or immunotherapy within 4 weeks prior to planned administration of ^{89}Zr-TLX250 or continuing adverse effects from such therapy 4. Planned antineoplastic therapies for the period between administration of ^{89}Zr-TLX250 and imaging 5. Serious non-malignant disease that may interfere with the objectives of the study 6. Renal insufficiency with glomerular filtration rate ≤45 mL/min/1.73m^2 7. Pregnancy or lactation 8. Exposure to murine or chimeric antibodies within the last 5 years 9. Known hypersensitivity or human anti-chimeric antibodies against girentuximab. 10. Exposure to any experimental diagnostic or therapeutic drug 30 days prior to the date of planned administration of ^{89}Zr-TLX250 11. Contraindications to FDG PET/CT

Screening:

Procedures performed during the screening visit include a review of patient eligibility (Table 1) and the obtaining of informed consent for trial enrolment. Participants will then undergo screening assessments including physical examination, recording of Eastern Cooperative Oncology Group (ECOG) Performance Status, vital signs, 12-lead electrocardiogram (ECG), review of prior/concomitant medications, and clinical laboratory tests (full blood count, urea and electrolytes, liver function test,

serum β-HCG if applicable and urine analysis) as summarized in the schedule of study assessments (Table 2).

Table 2: The schedule of study assessments is set out as follows:

Visit Name	Screening	IMP Administration	Imaging	Follow Up
Time point	**Day -28 to -1**	**Day 0**	**Day 5 ± 2**	**Day 14 (or before starting chemotherapy or undergoing surgery)**
Informed consent	**X**			
Eligibility criteria	**X**			
^{18}F-FDG-PET/CT	**X**			
Physical exam	**X**			
ECOG status	**X**			
Vital signs	**X**	**X Pre and post injection**	**X**	
12 lead ECG	**X**	**X Post injection**		
Haematology Biochemistry	**X**			
Liver function tests	**X**			
Serum β-HCG	**X**			
Urine analysis	**X**			
Urine pregnancy test		**X**		
PET/CT			**X**	
Adverse events		**X**	**X**	**X**
Concomitant Medications	**X**	**X**	**X**	**X**

^{89}Zirconium labelled girentuximab administration (Day 0)

On the day of injection, a urine pregnancy test will be performed to confirm ongoing non-pregnant status in all pre-menopausal women. A slow intravenous administration of 37 mBq (+/- 10%) ^{89}Zr-TLX250, containing a mass dose of 10mg of girentixumab, will be delivered over 3 minutes. Vital signs and a 12 lead ECG will be performed before and after the intravenous injection. Adverse event recording according to the NCI-CTCAE v 5.0 will be performed following administration of the agent.

Imaging (Day 5 +/- 2)

As part of PET/CT hybrid acquisition, whole body PET static and low-dose CT including brain to mid-thigh will be performed. Vital signs will be recorded. Those who have potentially significant lesions with increased uptake on ^{89}Zr-TLX250 PET/CT but not on FDG PET/CT will have their imaging discussed at the next available Uro-Oncology Multi-disciplinary Team (MDT) meeting to determine if further investigation or a deviation in management plan is required. Adverse event recording will be performed as previously discussed.

Follow up (Day 14)

Participants will receive a phone consultation two weeks following the administration of the ^{89}Zr-TLX250 (or before commencement of treatment) that will include a symptom enquiry, recording of medications, and adverse event recording (NCI-CTC v 5.0).

Endpoints:

The primary endpoint is the feasibility of using ^{89}Zr-TLX250 PET/CT as a staging modality for urothelial carcinoma or bladder cancer. The feasibility will be ascertained by the ability to recruit to the target sample size and deliver the ^{89}Zr-TLX250 PET/CT.

Secondary endpoints are the safety, tolerability, effectiveness, sensitivity, and specificity of ^{89}Zr-TLX250 PET/CT as compared to FDG PET/CT. Safety and tolerability will be assessed according to vital signs, 12-lead ECGs, adverse event records, and the requirement for new medications. As part of the effectiveness analysis, tumour versus mediastinal uptake ratios will be calculated and compared for both the primary tumour and any other lesions identified for both modalities. The sensitivity and specificity of ^{89}Zr-TLX250 PET/CT for detecting lymph node metastases will be calculated as compared to both FDG PET/CT and pathological lymph node status, where the patient proceeds to radical cystectomy and pelvic lymph node dissection.

A pragmatic target sample size of 20 patients has been chosen for this phase I feasibility study, based on clinical context and logistical factors. Descriptive statistics will be used in the reporting of the primary and secondary endpoints for this pilot study using appropriate parametric and non-parametric tests.

Patient and Public Involvement:

Patients or the public are not involved in the design, or conduct, or reporting, or dissemination plans of our research.

ETHICS AND DISSEMINATION

Ethics approval has been obtained from the South Metropolitan Health Service Human Research Ethics Committee (*RGS0000003940*). ZiPUP study has been registered with the Australian and New Zealand Clinical Trials Registry (Trial ID *NCT05046665*, registration number is *ACTRN12621000411842*). Also, it has been registered with the ClinicalTrials.gov (ClinicalTrials.gov identifier is *NCT05046665*, obsolete identifier *NCT05018442*).

Eligible patients will only be enrolled, and study-related procedures carried out after providing written informed consent. Patients will be given a full explanation, in lay terms, of the aims of the study and potential risks including as a written patient information sheet. It will be explained that they may refuse to take part in or withdraw from the study without prejudice to their future care and treatment at any time. In any case where the patient is not fluent in English an interpreter will be present during the consenting process. Participants will be issued with a copy of the information provided and their signed consent to participate in the study

DISCUSSION

^{89}Zr-immuno-PET/CT has proven utility as a diagnostic modality in both renal cell cancer and breast cancer. In this trial, we are evaluating the utility of ^{89}Zr-TLX250 PET/CT in staging urothelial cancer and bladder cancer, as compared with FDG PET/CT. Of note, ^{89}Zr-TLX250 is metabolised in the liver with minimal renal excretion, which may be advantageous when staging tumours of the urinary tract. If proven effective this may present a useful staging modality for urothelial cancer and bladder cancer, with the potential to be extended as a therapeutic or 'theranostic' in patients with these cancers.

CONTRIBUTIONSHIP STATEMENT

Al-Zubaidi M: Write and reviewing the protocol as per BMJ Open requirement.
Viswambaram P: Write the initial protocol draft and review the final draft.
McCombie Steve: Review and edit the final draft.
Elizabeth Liow: Participated in protocol draft writing.
Nat Lenzo: Review and edit the final draft.
Thomas Ferguson: Participated in protocol draft writing.
Andy Redfern: Review and edit the final draft
Richard Gauci: Write the protocol draft.
Dickon Hayne: Review the protocol draft, main supervisor of the study.

COMPETING INTERESTS

There is no competing interests

FUNDING

The study is sponsored by the South Metropolitan Health Service, Western Australia and is funded by Telix pharmaceuticals®, who will also supply the ^{89}Zr-TLX250 at no cost.

DATA SHARING STATEMENT

Data collected from participants during the trial will be available by the end of it after deidentification. Study protocol, statistical analysis plan, informed consent form and clinical study report will be available after publication indefinitely, and available to be accesses by any individual.

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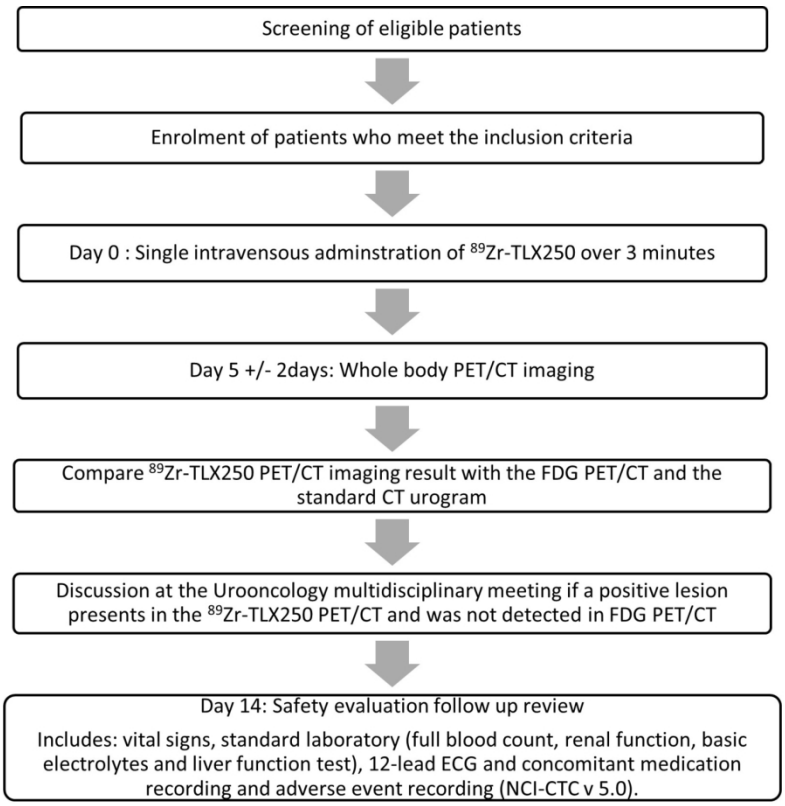
FIGURES

Figure 1: Trial Schema



Trial schema

159x128mm (300 x 300 DPI)

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Primary Subject Heading:	Urology
Secondary Subject Heading:	Radiology and imaging, Oncology
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ABSTRACT

Introduction

Bladder cancer is a lethal disease with a rising incidence on a background of limited conventional imaging modalities for staging (either CT of the chest-abdomen-pelvis or FDG-PET/CT). CT is known to have relatively low sensitivity for detecting low volume metastatic disease, an important goal when considering surgical interventions entailing significant potential morbidity. FDG is also limited, being predominantly renally excreted and therefore producing intense non-specific activity in the urinary tract, which limits its utility to detect bladder and upper tract lesions, or nodal metastases in close proximity to the urinary tract. ^{89}Zr-TLX250 may have utility in the accurate staging of bladder and urothelial carcinomas, with less renal excretion as compared to FDG, however this has not previously been investigated.

Methods and analysis:

ZiPUP is a single-arm phase I trial examining the feasibility of using ^{89}Zr-TLX250-PET/CT as a staging modality for urothelial carcinomas or bladder cancer by examining isotope uptake by the cancer. This trial will also examine the safety and utility of ^{89}Zr-TLX250-PET/CT in patients either undergoing pre-operative staging of bladder or other urothelial carcinomas for curative intent, or with known metastatic urothelial carcinomas. All participants will undergo ^{89}Zr-TLX250-PET/CT and will need to have undergone recent FDG-PET/CT for comparison. This trial aims to recruit 10 participants undergoing pre-operative staging and 10 participants with known metastatic disease. The primary endpoint is feasibility; secondary endpoints are safety, tolerability, sensitivity and specificity in detecting lymph node metastases compared to FDG-PET/CT.

Ethics and dissemination:

Ethics approval has been obtained from the South Metropolitan Health Service Human Research Ethics Committee (*RGS0000003940*). ZiPUP study has been registered with the Australian and New Zealand Clinical Trials Registry (registration number *ACTRN12621000411842*) and with the ClinicalTrials.gov (ClinicalTrials.gov identifier *NCT05046665*). Eligible patients will only be enrolled after providing written informed consent. Patients will be given a full explanation, in lay terms, of the aims of the study and potential risks including as a written patient information sheet.

Strengths and limitations of the study:

- ^{89}Zr-TLX250 is metabolised in the liver with less renal excretion as compared to FDG.
- The targeted recruitment is small and so power to detect modest differences limited.
- The resolution quality of images that will be obtained and the optimum imaging timing have yet to be determined.

Keywords:

Zirconium, Girentuximab, bladder cancer, urinary tract cancer, urothelial carcinoma, 18F-fluorodeoxyglucose (FDG), Positron Emission Tomography/Computed Tomography (PET/CT), Carbonic anhydrase IX.

ABBREVIATIONS

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ccRCC: Clear Cell Renal Cell Carcinoma
T-DM1: Ado-trastuzumab emtansine
HER2: human epidermal growth factor receptor 2
HSP90: Heat Shock Protein 90
NVP-AUY922: Luminespib
MBq: Mega-Becquerel
ECOG: Eastern Cooperative Oncology Group
ECG: Electrocardiogram
β-HCG: Beta-human chorionic gonadotropin
NCI-CTCAE v 5.0: National Cancer Institute – Common Terminology Criteria for Adverse Effects version 5.0

INTRODUCTION

Urothelial cancer:

Bladder cancer is the most common malignancy involving the urinary system, and the tenth most common malignancy overall [1] with a rising incidence worldwide [2]. Transitional cell carcinoma is the predominant histologic type, accounting for approximately 90% of all bladder cancers. Transitional cell carcinoma also affects the renal pelvis, ureter or urethra as all are lined with transitional cell urothelium. Histology is the usual confirmatory diagnostic test, tissue can be obtained as a transurethral resection, biopsy, or urine cytology. As with all malignancies, the prognosis and treatment of the disease is determined by the histopathology and staging investigations.

Current staging modalities:

The following modalities are currently utilised to detect the distribution and extent of urothelial tumours:

Computed Tomography (CT) of the chest, abdomen and pelvis including delayed-phase images are used to identify urothelial tumours, which may appear as filling defects on delayed-phase imaging or as enhancing soft tissue on the nephrographic phase. CT may demonstrate extravesical extension, tumour involvement or obstruction of the upper urinary tract nodal, involvement in the pelvis or retroperitoneum, and visceral or osseous metastasis. CT may miss tumours <1 cm in size, particularly those in the bladder trigone or dome, and it cannot accurately categorize depth of bladder wall invasion. The sensitivity of CT for identification of nodal involvement is relatively low (false-negative rate 68%, false-positive rate 16%) and may require a needle or excisional biopsy for confirmation [3]. Approximately 50% of patients with a filling defect in the renal pelvis or ureter will have associated hydronephrosis, hydroureter, or a delayed nephrogram secondary to obstruction [4].

^{18}F-fluorodeoxyglucose (FDG) positron emitting tomography (PET)/CT has limited value in the local staging of bladder cancer, largely due to urinary excretion of FDG affecting image interpretation of the bladder and any nodal disease in close proximity to the ureters [5]. However, FDG PET/CT is often useful in the distant staging of urothelial cancer, especially in high-risk disease with sensitivity of 78% in detecting locoregional lymph node metastasis as compared to 44% with CT alone [6].

Carbonic anhydrase IX (CAIX)

CAIX is an enzyme that functions as a regulator of intracellular pH, cell proliferation, and cell adhesion in response to hypoxia [7]. CAIX is expressed abundantly in response to hypoxia in a wide range of cancer cell lines including bladder, renal, head and neck, lung, and colon cancers [7].

CAIX was distinctly expressed in >70% of urothelial carcinomas but was not expressed in normal urothelial tissue [8]. Previous data has demonstrated sensitivity and specificity of urinary CAIX of 86.2% and 95.1% respectively, for detection of urothelial bladder cancer (area under the curve 90.5%) [9]. A significant association between CAIX expression in paired urine and tumour specimens has been established. Notably, CAIX was shown to have significantly higher predictive accuracy for urothelial cancer compared to urinary cytology (90.5% vs 71.7%), especially in low-grade tumours (90% vs 61.8%) [9].

These findings provide strong rationale for investigating the potential use of CAIX as a targeted imaging agent for the identification and diagnosis of bladder cancer. By the same token, the utility of CAIX as a therapeutic target also merits future investigation.

Positron Emission Tomography / Computed Tomography (PET/CT) and ^{89}Zirconium-girentuximab (^{89}Zr-TLX250)

Theranostic PET is a novel modality, combining the potential for both imaging and treatment as it enables the tracking of targeted vehicles and carriers using, for instance, isotope-labelled monoclonal antibodies. A notable example in the field of theranostics with an established therapeutic role is ^{177}Lu-PSMA-617, a radionuclide agent that has garnered success in the treatment of castrate-resistant prostate cancer [10].

Due to the intrinsic chemical properties of the relatively low energy positrons which provide high resolution PET images [11], ^{89}Zr has been identified as a suitable ligand candidate for this approach [12]. Girentuximab (TLX-250) is an antibody directed against CAIX that has been widely studied in the setting of renal cell carcinoma. In this context, ^{89}Zr-TLX250 PET/CT has been shown to have a significant impact on clinical decision making in patients with an indeterminate renal mass [13]. Studies have also explored the use of ^{89}Zr-immuno-PET with other conjugated antibodies, demonstrating utility for monitoring treatment in animal models as well as potential as a tool in the clinical staging of breast cancer[14]. Collectively these studies highlight the potential diagnostic and therapeutic applications of ^{89}Zr-TLX250.

Urinary excretion of FDG PET/CT is intrinsically problematic when imaging urinary tract malignancies as outlined above with low sensitivity for low volume or urinary tract associated disease. The hepatic clearance of ^{89}Zr-TLX250 with low urinary excretion is therefore anticipated to be advantageous for the local and regional staging of bladder and other urothelial carcinomas. This Phase I study aims to investigate ^{89}Zr-TLX250 utilization in the staging of urothelial carcinoma or bladder cancer exploiting the low urinary excretion.

METHODS AND ANALYSIS

Protocol overview

This is a non-randomised, non-blinded, single centre, phase I trial comparing ^{89}Zr-TLX250 PET/CT with FDG PET/CT in patients with urothelial carcinoma or bladder cancer. Study duration will be 18 months, having commenced in May 2021 with the anticipated date of last data collection being December 31st, 2022. It is being conducted at a single tertiary centre in Western Australia and will include two cohorts of adult patients; ten patients undergoing preoperative primary staging for recently diagnosed bladder cancer or urothelial carcinoma for consideration of treatment with curative intent, and ten patients with known metastatic urothelial carcinoma or bladder cancer.

The primary objective of this study is to evaluate the feasibility of using ^{89}Zr-TLX250 PET/CT as a staging modality for urothelial carcinoma or bladder cancer. The secondary objectives are to evaluate the safety and tolerability of ^{89}Zr-TLX250 PET/CT, as well as its effectiveness as compared to FDG PET/CT. The eligibility criteria are listed in Table 1 and the trial schema is outlined in Figure 1. Eligible patients are also required to have undergone FDG PET/CT scanning (a part of standard of care) within the

proceeding 28 days to allow accurate comparison between the two modalities. All participants will provide written informed consent.

TABLE 1: Key inclusion and exclusion criteria

Inclusion criteria
1. Age ≥18 years old 2. Able to provide informed consent 3. Histologically diagnosed with urothelial carcinoma or bladder cancer (or upper tract urothelial carcinoma diagnosed based on standard imaging and malignant urine cytology or direct visualisation on ureteroscopy) or known metastatic bladder or other urothelial carcinoma (based on previous imaging and /or histopathology) 4. Negative serum pregnancy test in female patients of childbearing potential at screening. Confirmation of negative pregnancy test result from urine within 24 hours prior to receiving investigational product. 5. Consent to practise double-barrier contraception until a minimum of 42 days after ^{89}Zr-TLX250 administration.
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Screening:

Procedures performed during the screening visit include a review of patient eligibility criteria (Table 1) and the obtaining of informed consent for trial enrolment. Participants will then undergo screening assessments including physical examination, recording of Eastern Cooperative Oncology Group (ECOG) Performance Status, vital signs, 12-lead electrocardiogram (ECG), review of prior/concomitant medications, and clinical laboratory tests (full blood count, urea and electrolytes, liver function test, serum β-HCG if applicable and urine analysis) as summarized in the schedule of study assessments (Table 2).

Table 2: The schedule of study assessments is set out as follows:

Visit Name	Screening	IMP Administration	Imaging	Follow Up
Time point	**Day -28 to -1**	**Day 0**	**Day 5 ± 2**	**Day 14 (or before starting chemotherapy or undergoing surgery)**
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Eligibility criteria	**X**			
^{18}F-FDG-PET/CT	**X**			
Physical exam	**X**			
ECOG status	**X**			
Vital signs	**X**	**X Pre and post injection**	**X**	
12 lead ECG	**X**	**X Post injection**		
Haematology Biochemistry	**X**			
Liver function tests	**X**			
Serum β-HCG	**X**			
Urine analysis	**X**			
Urine pregnancy test		**X**		
PET/CT			**X**	
Adverse events		**X**	**X**	**X**
Concomitant Medications	**X**	**X**	**X**	**X**

^{89}Zr labelled girentuximab administration (Day 0)

On the day of injection, a urine pregnancy test will be performed to confirm ongoing non-pregnant status in all pre-menopausal women. A slow intravenous administration of 37 MBq (+/- 10%) ^{89}Zr-TLX250, containing a mass dose of 10mg of girentixumab (this dosage has been arrived at based on a previous trial [15]), will be delivered over 3 minutes. Vital signs and a 12 lead ECG will be performed before and after the intravenous injection. Adverse event recording according to the NCI-CTCAE v 5.0 will be performed following administration of the investigational agent.

Imaging (Day 5 ± 2)

As part of PET/CT hybrid acquisition, whole body PET static and low-dose CT including brain to mid-thigh will be performed over a maximum of 45 minutes in 4 bed positions at a single time point on Day 5 ± 2 post administration of ^{89}Zr-TLX250. Vital signs will be recorded. Those who have potentially significant lesions with increased uptake on ^{89}Zr-TLX250 PET/CT but not on FDG PET/CT will have their imaging discussed at the next available Uro-Oncology multi-disciplinary team meeting to determine if further investigation or a deviation in management plan is indicated. Adverse event recording will be performed as previously discussed.

Follow up (Day 14)

Participants will receive a phone consultation two weeks following the administration of the ^{89}Zr-TLX250 (or before commencement of treatment) that will include a symptom enquiry, recording of concomitant medications, and adverse event recording (NCI-CTC v 5.0).

Endpoints:

The primary endpoint is the feasibility of using ^{89}Zr-TLX250 PET/CT as a staging modality for urothelial carcinoma or bladder cancer. The feasibility will be ascertained by the ability to recruit to the target sample size, deliver the ^{89}Zr-TLX250 PET/CT and generate diagnostic grade images.

Secondary endpoints are the safety, tolerability, sensitivity, and specificity of ^{89}Zr-TLX250 PET/CT as compared to FDG PET/CT. Safety and tolerability will be assessed according to vital signs, 12-lead ECGs, adverse event records, and the requirement for new medications. As part of the effectiveness analysis, tumour versus mediastinal uptake ratios will be calculated and compared for both the primary tumour and any other lesions identified for both modalities. The sensitivity and specificity of ^{89}Zr-TLX250 PET/CT for detecting lymph node metastases will be calculated as compared to both FDG PET/CT and pathological lymph node status, where the patient proceeds to radical cystectomy and pelvic lymph node dissection.

A pragmatic target sample size of 20 patients has been chosen for this phase I feasibility study, based on the target population and other logistical factors. Descriptive statistics will be used in the reporting of the primary and secondary endpoints for this pilot study using appropriate parametric and non-parametric tests.

Patient and Public Involvement:

Patients or the public are not involved in the design, conduct, reporting, or dissemination plans of our research.

ETHICS AND DISSEMINATION

Ethics approval has been obtained from the South Metropolitan Health Service Human Research Ethics Committee (*RGS0000003940*). The ZiPUP study has been registered with the Australian and New Zealand Clinical Trials Registry (registration number is *ACTRN12621000411842*). Also, it has been registered with the ClinicalTrials.gov (ClinicalTrials.gov identifier is *NCT05046665)*.

Eligible patients will only be enrolled, and study-related procedures carried out after providing written informed consent. Patients will be given a full explanation, in lay terms, of the aims of the study and potential risks including as a written patient information sheet. It will be explained that they may refuse to take part in or withdraw from the study without prejudice to their future care and treatment at any time. In any case where the patient is not fluent in English, an interpreter will be present during the consenting process. Participants will be issued with a copy of the information provided and their signed consent to participate in the study.

CONTRIBUTIONSHIP STATEMENT

Al-Zubaidi M: Write and reviewing the protocol as per BMJ Open requirement.

Viswambaram P: Write the initial protocol draft and review the final draft.
McCombie Steve: Review and edit the final draft.
Elizabeth Liow: Participated in protocol draft writing.
Nat Lenzo: Review and edit the final draft.
Thomas Ferguson: Participated in protocol draft writing.
Andy Redfern: Review and edit the final draft
Richard Gauci: Write the protocol draft.
Dickon Hayne: Review the protocol draft, main supervisor of the study.

COMPETING INTERESTS

There is no competing interests

FUNDING

The study is sponsored by the South Metropolitan Health Service, Western Australia and is funded by Telix pharmaceuticals®, who will also supply the ^{89}Zr-TLX250 at no cost.

DATA SHARING STATEMENT

Data collected from participants during the trial will be available by the end of it after deidentification. Study protocol, statistical analysis plan, informed consent form and clinical study report will be available after publication indefinitely, and available to be accesses by any individual.

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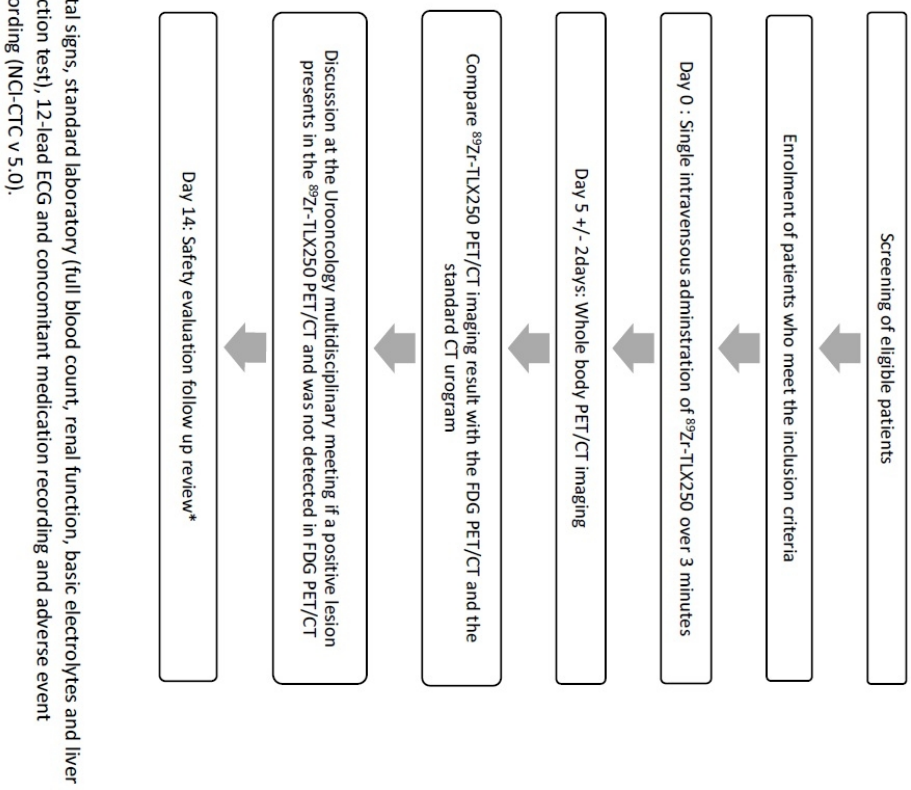
15. Evaluation of Safety, Biodistibution and Sensitivity/Specificity of PET/CT Imaging With 89Zr-TLX250 in Subjects With RCC - Full Text View - ClinicalTrials.gov

FIGURES

Figure 1: Trial Schema, Schema showing the pathway for patients recruited into the phase I trial of ^{89}Zirconium-labelled girentuximab (^{89}Zr-TLX250) PET in Urothelial Cancer Patients (ZiPUP).



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81x67mm (300 x 300 DPI)

BMJ Open

89Zirconium-labelled girentuximab (89Zr-TLX250) PET in Urothelial Cancer Patients (ZiPUP) – Protocol for a phase I trial of a novel staging modality for urothelial carcinoma.

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Primary Subject Heading:	Urology
Secondary Subject Heading:	Radiology and imaging, Oncology
Keywords:	Urological tumours < ONCOLOGY, Urological tumours < UROLOGY, Nuclear radiology < RADIOLOGY & IMAGING, NUCLEAR MEDICINE

^{89}Zirconium-labelled girentuximab (^{89}Zr-TLX250) PET in Urothelial Cancer Patients (ZiPUP) – Protocol for a phase I trial of a novel staging modality for urothelial carcinoma.

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ABSTRACT

Introduction

Bladder cancer is a lethal disease with a rising incidence on a background of limited conventional imaging modalities for staging (either Computed Tomography (CT) of the chest-abdomen-pelvis or ^{18}Fluorodeoxyglucose positron emitting tomography (FDG-PET/CT)). CT is known to have relatively low sensitivity for detecting low volume metastatic disease, an important goal when considering surgical interventions entailing significant potential morbidity. FDG is also limited, being predominantly renally excreted and therefore producing intense non-specific activity in the urinary tract, which limits its utility to detect bladder and upper tract lesions, or nodal metastases in close proximity to the urinary tract. ^{89}Zirconium-labelled girentuximab (^{89}Zr-TLX250) may have utility in the accurate staging of bladder and urothelial carcinomas, with less renal excretion as compared to FDG, however this has not previously been investigated.

Methods and analysis:

^{89}Zirconium-labelled girentuximab PET in Urothelial Cancer Patients (ZiPUP) is a single-arm phase I trial examining the feasibility of using ^{89}Zr-TLX250-PET/CT as a staging modality for urothelial and bladder carcinomas by examining isotope uptake by the cancer. This trial will also examine the safety and utility of ^{89}Zr-TLX250-PET/CT in patients either undergoing pre-operative staging of bladder or other urothelial carcinomas for curative intent, or with known metastatic urothelial carcinomas. All participants will undergo ^{89}Zr-TLX250-PET/CT and will need to have undergone recent FDG-PET/CT for comparison. This trial aims to recruit 10 participants undergoing pre-operative staging and 10 participants with known metastatic disease. The primary endpoint is feasibility defined by the ability

to recruit to the target sample size within the study duration; secondary endpoints are safety, tolerability, sensitivity, and specificity in detecting lymph node metastases compared to FDG-PET/CT.

Ethics and dissemination:

Ethics approval has been obtained from the South Metropolitan Health Service Human Research Ethics Committee (*RGS0000003940*). ZiPUP study has been registered with the Australian and New Zealand Clinical Trials Registry (registration number *ACTRN12621000411842*) and with ClinicalTrials.gov (ClinicalTrials.gov identifier *NCT05046665*). Eligible patients will only be enrolled after providing written informed consent. Patients will be given a full explanation, in lay terms, of the aims of the study and potential risks including as a written patient information sheet.

Strengths and limitations of the study:

- This will be the first study to generate data assessing the role of ^{89}Zr-TLX250 in the imaging of urothelial carcinoma patients.
- As a high-volume quaternary centre, there is capacity to recruit suitable trial subjects within a realistic timeframe.
- As a small study, the ability to detect modest differences between the imaging modalities is limited.
- The resolution quality of images that will be obtained and the optimum imaging timing have yet to be determined.

Keywords:

Zirconium, Girentuximab, bladder cancer, urinary tract cancer, urothelial carcinoma, 18F-fluorodeoxyglucose (FDG), Positron Emission Tomography/Computed Tomography (PET/CT), Carbonic anhydrase IX.

ABBREVIATIONS

^{89}Zr-TLX250: ^{89}Zirconium-labelled girentuximab
CAIX: Carbonic anhydrase IX
FDG PET: Fluorodeoxyglucose positron emitting tomography
CT: Computed tomography
177Lu-PSMA-617: Lutetium-177 prostate-specific membrane antigen
MBq: Mega-Becquerel
ECOG: Eastern Cooperative Oncology Group
ECG: Electrocardiogram
β-HCG: Beta-human chorionic gonadotropin
NCI-CTCAE v 5.0: National Cancer Institute – Common Terminology Criteria for Adverse Effects version 5.0

INTRODUCTION

Urothelial cancer:

Bladder cancer is the most common malignancy involving the urinary system, and the tenth most common malignancy overall [1] with a rising incidence worldwide [2]. Transitional cell carcinoma is the predominant histologic type, accounting for approximately 90% of all bladder cancers. Transitional cell carcinoma also affects the renal pelvis, ureter, or urethra as all are lined with transitional cell urothelium. Diagnosis is usually made histologically with tissue obtained via transurethral resection, biopsy, or from urine cytology. As with all malignancies, the prognosis and treatment of the disease is determined by the histopathology and staging investigations.

Current staging modalities:

The following modalities are currently utilised to detect the distribution and extent of urothelial tumours:

Computed Tomography (CT) of the chest, abdomen and pelvis including delayed-phase images are used to identify urothelial tumours, which may appear as filling defects on delayed-phase imaging or as enhancing soft tissue on the nephrographic phase. CT may demonstrate extravesical extension, tumour involvement or obstruction of the upper urinary tract nodal, involvement in the pelvis or retroperitoneum, and visceral or osseous metastasis. CT may miss tumours <1 cm in size, particularly those in the bladder trigone or dome, and it cannot accurately categorize depth of bladder wall invasion. The sensitivity of CT for identification of nodal involvement is relatively low (false-negative rate 68%, false-positive rate 16%) and may require biopsy for confirmation [3]. Approximately 50% of patients with a filling defect in the renal pelvis or ureter will have associated hydronephrosis, hydroureter, or a delayed nephrogram secondary to obstruction [4].

^{18}F-fluorodeoxyglucose (FDG) positron emitting tomography (PET)/CT has limited value in the local staging of bladder cancer, largely due to urinary excretion of FDG affecting image interpretation of the bladder and any nodal disease in close proximity to the ureters [5]. However, FDG PET/CT is often useful in the distant staging of urothelial cancer, especially in high-risk disease with sensitivity of 78% in detecting locoregional lymph node metastasis as compared to 44% with CT alone [6].

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These findings provide strong rationale for investigating the potential use of CAIX as a targeted imaging agent for the identification and diagnosis of bladder cancer. By the same token, the utility of CAIX as a therapeutic target also merits future investigation.

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^{18}F-FDG-PET/CT	**X**			
Physical exam	**X**			
ECOG status	**X**			
Vital signs	**X**	**X Pre and post injection**	**X**	
12 lead ECG	**X**	**X Post injection**		
Haematology Biochemistry	**X**			
Liver function tests	**X**			
Serum β-HCG	**X**			
Urine analysis	**X**			
Urine pregnancy test		**X**		
PET/CT			**X**	
Adverse events		**X**	**X**	**X**
Concomitant Medications	**X**	**X**	**X**	**X**

^{89}Zr labelled girentuximab administration (Day 0)

On the day of injection, a urine pregnancy test will be performed to confirm ongoing non-pregnant status in all pre-menopausal women. A slow intravenous administration of 37 Mega-Becquerel (MBq) (+/- 10%) ^{89}Zr-TLX250, containing a mass dose of 10mg of TLX250 (this dosage has been arrived at based on a previous trial [15]), will be delivered over 3 minutes. Vital signs and a 12 lead ECG will be performed before and after the intravenous injection. Adverse event recording according to the National Cancer Institute – Common Terminology Criteria for Adverse Effects version 5.0 (NCI-CTCAE v 5.0) will be performed following administration of the investigational agent.

Imaging (Day 5 ± 2)

As part of PET/CT hybrid acquisition, whole body PET static and low-dose CT including brain to mid-thigh will be performed over a maximum of 45 minutes in 4 bed positions at a single time point 5 ± 2 days post administration of ^{89}Zr-TLX250. Vital signs will be recorded. Those who have potentially

significant lesions with increased uptake on ^{89}Zr-TLX250 PET/CT but not on FDG PET/CT will have their imaging discussed at the next available Uro-Oncology multi-disciplinary team meeting to determine if further investigation or a deviation in management plan is indicated. Adverse event recording will be performed as previously discussed.

Follow up (Day 14)

Participants will receive a phone consultation two weeks following the administration of the ^{89}Zr-TLX250 (or before commencement of treatment) that will include a symptom enquiry, recording of concomitant medications, and adverse event recording (NCI-CTC v 5.0).

Endpoints:

The primary endpoint is the feasibility of using ^{89}Zr-TLX250 PET/CT as a staging modality for urothelial carcinoma or bladder cancer. The feasibility will be ascertained by the ability to recruit to the target sample size, deliver the ^{89}Zr-TLX250 PET/CT and generate diagnostic grade images.

Secondary endpoints are the safety, tolerability, sensitivity, and specificity of ^{89}Zr-TLX250 PET/CT as compared to FDG PET/CT. Safety and tolerability will be assessed according to vital signs, 12-lead ECGs, adverse event records, and the requirement for new medications. As part of the effectiveness analysis, tumour versus mediastinal uptake ratios will be calculated and compared for both the primary tumour and any other lesions identified for both modalities. The sensitivity and specificity of ^{89}Zr-TLX250 PET/CT for detecting lymph node metastases will be calculated as compared to both FDG PET/CT and pathological lymph node status, where the patient proceeds to radical cystectomy and pelvic lymph node dissection.

A pragmatic target sample size of 20 patients has been chosen for this phase I feasibility study, based on the target population and other logistical factors. Descriptive statistics will be used in the reporting of the primary and secondary endpoints for this pilot study using appropriate parametric and non-parametric tests.

Patient and Public Involvement:

Patients or the public are not involved in the design, conduct, reporting, or dissemination plans of our research.

ETHICS AND DISSEMINATION

Ethics approval has been obtained from the South Metropolitan Health Service Human Research Ethics Committee (*RGS0000003940*). The ZiPUP study has been registered with the Australian and New Zealand Clinical Trials Registry (registration number is *ACTRN12621000411842*). Also, it has been registered with ClinicalTrials.gov (ClinicalTrials.gov identifier is *NCT05046665)*.

Eligible patients will only be enrolled, and study-related procedures carried out after providing written informed consent. Patients will be given a full explanation, in lay terms, of the aims of the study and potential risks including as a written patient information sheet. It will be explained that they may refuse to take part in or withdraw from the study without prejudice to their future care and treatment at any time. In any case where the patient is not fluent in English, an interpreter will be present during the consenting process. Participants will be issued with a copy of the information provided and their signed consent to participate in the study.

CONTRIBUTIONSHIP STATEMENT

Al-Zubaidi M: Write and reviewing the protocol as per BMJ Open requirement.
Viswambaram P: Write the initial protocol draft and review the final draft.
McCombie Steve: Review and edit the final draft.
Elizabeth Liow: Participated in protocol draft writing.
Nat Lenzo: Review and edit the final draft.
Thomas Ferguson: Participated in protocol draft writing.
Andy Redfern: Review and edit the final draft
Richard Gauci: Write the protocol draft.
Dickon Hayne: Review the protocol draft, main supervisor of the study.

COMPETING INTERESTS

There is no competing interest.

FUNDING

The study is sponsored by the South Metropolitan Health Service, Western Australia and is funded by Telix pharmaceuticals®, who will also supply the ^{89}Zr-TLX250 at no cost.

DATA SHARING STATEMENT

Data collected from participants during the trial will be available by the end of it after deidentification. Study protocol, statistical analysis plan, informed consent form and clinical study report will be available after publication indefinitely, and available to be accesses by any individual.

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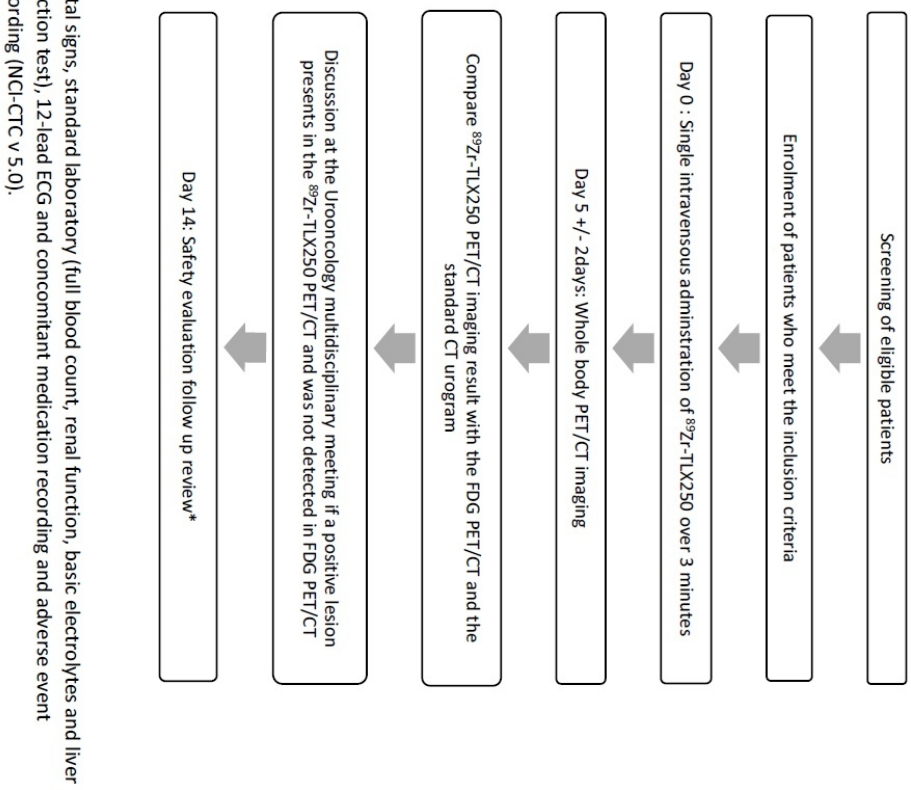
FIGURES

Figure 1: Trial Schema, Schema showing the pathway for patients recruited into the phase I trial of ^{89}Zirconium-labelled girentuximab (^{89}Zr-TLX250) PET in Urothelial Cancer Patients (ZiPUP).



Trial Schema, Schema showing the pathway for patients recruited into the phase I trial of 89Zirconium-labelled girentuximab (89Zr-TLX250) PET in Urothelial Cancer Patients (ZiPUP).

81x67mm (300 x 300 DPI)