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Safety and tolerability of Miltuximab® - a first in human study in patients with advanced solid cancers

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ABSTRACT

Objective(s): Miltuximab® is a chimeric antibody targeting Glypican-1 (GPC-1), a cell surface antigen which is overexpressed in solid cancers. Miltuximab® has shown promising safety and efficacy in radioimmunotherapy models of prostate cancer. This first in human study used Miltuximab® radiolabelled with Gallium-67 ([^{67}Ga]Ga-DOTA-Miltuximab®). The primary study endpoint was to establish safety and tolerability of Miltuximab®. Secondary endpoints were biodistribution, tumour targeting and pharmacokinetic analysis.

Methods: Four cohorts of three patients (9 with advanced prostate cancer, 2 with pancreatic and 1 with bladder cancer) were dosed with 1 mg, ~250 MBq of [^{67}Ga]Ga-DOTA-Miltuximab®. Cohort 1 received [^{67}Ga]Ga-DOTA-Miltuximab® alone, while cohorts 2-4 were pre-infused with increasing doses (3.5, 11.5 and 24 mg, respectively) of unlabelled Miltuximab®-DOTA 1 hour prior to [^{67}Ga]Ga-DOTA-Miltuximab®. Safety and tolerability were assessed by clinical and standard laboratory assessments. Patients underwent whole body gamma-camera scans and SPECT/CT scans up to 144 h post-infusion. Total organ radiation exposure was determined by dosimetry of whole-body gamma scans.

Results: The dosing regimen was well tolerated, with no drug-related adverse events observed. Liver and spleen uptake of [^{67}Ga]Ga-DOTA-Miltuximab® was observed. Liver uptake was reduced by pre-infusion of unlabelled Miltuximab®-DOTA. Dosimetry analysis showed a favorable exposure profile. [^{67}Ga]Ga-DOTA-Miltuximab® targeting to tumour sites was observed in two prostate cancer patients who had failed enzalutamide treatment. Higher doses of unlabelled antibody achieved lower liver uptake and increased antibody serum half life.

Conclusions: This study is the first in human for Miltuximab® a first in class antibody targeting GPC-1. The trial met its primary endpoint of safety, demonstrating its potential as a safe and tolerable monoclonal antibody. This safety data, together with targeting to tumour lesions and biodistribution information supports the further clinical development of Miltuximab® as a theranostic agent in a planned Phase I human trial.

Trial registration: ANZCTR, ACTRN12616000787482, https://www.anzctr.org.

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Introduction

Metastatic or recurrent cancer continues to be a challenge in patients with urogenital and pancreatic cancers. Despite recent improvements in therapeutic strategies and outcomes for clinically localized disease, overall survival in patients with the majority of metastatic and recurrent urothelial and pancreatic malignancies remains relatively unchanged (1-3). Modern advances in the field of immunotherapy have had promising results in multiple cancers including melanoma, non-small cell lung cancer, renal cell cancer, and urothelial cancer, however the results in prostate and pancreatic cancer have been disappointing and agents against novel targets are needed (4).

A theranostic strategy utilizes molecular targeted radiation to image and treat cancers. The use of a targeted radionuclide via an antibody or small molecule has been successful in treating hematological cancers (5–9) and more recently in solid tumours, including prostate and neuroendocrine cancers (10–12). This approach, which involves assessing tumour targeting prior to therapy, allows personalized therapy, including calculation of likely safe and therapeutic doses.

Miltuximab® (GlyTherix Ltd) is a chimeric antibody (human IgG1) targeting Glypican-1, a cell surface proteoglycan overexpressed in several solid tumours, including prostate, pancreatic and bladder cancers, which plays a critical role in tumour progression (13–15). High levels of GPC-1 expression have been associated with poor prognosis in solid tumours such as pancreatic, esophageal and glioblastoma (16–18). Importantly, GPC-1 is not expressed in normal adult tissue (19, 20), and targeting of the molecule in preclinical animal studies has demonstrated its safety (20, 21), suggesting that targeting of GPC-1 may hold therapeutic potential. Immunohistochemistry in prostate cancer tumour specimens using the parent antibody to Miltuximab® showed reactivity with 80% of prostate cancer specimens, but not with benign prostate or normal tissue (19). Targeting of prostate tumour xenografts with Zirconium-89 ([^{89}Zr]Zr) labeled Miltuximab® demonstrated specific targeting to tumour sites, and Miltuximab® radiolabelled with Lutetium-177 ([^{177}Lu]Lu) showed strong activity against DU-145 prostate cancer xenografts with no drug related adverse events reported (22). Previous studies using the murine parent antibody to Miltuximab® have demonstrated effective inhibition of prostate and bladder xenograft growth using both alpha and beta therapies (23–26). Biodistribution of Miltuximab® in xenograft models is comparable to J591 (27), which has established clinical activity as a radioimmunotherapy (RIT) for prostate cancer (28–30). Targeting of tumour xenografts and lack of adverse events in animal studies suggest Miltuximab® may be a good candidate for radioimmunotherapy (22, 25, 27).

No studies to date have examined the safety of any GPC-1-targeting agents in humans, therefore the first step in the development of Miltuximab® was a first in human safety trial to establish initial safety data for the molecule with secondary assessments of biodistribution and targeting to tumour lesions. For this initial study, radiolabeling with Gallium-67 (^{67}Ga) was chosen, given the half-life of ^{67}Ga is approximately 78 hours (h), matching the timing of optimal Miltuximab® tumour uptake in preclinical models, allowing assessment of the antibody distribution throughout the body. ^{67}Ga is relatively inexpensive and easily accessible and has been used safely for decades in diagnostic imaging for infectious and inflammatory conditions and has many desirable characteristics for a theranostic imaging radionuclide (31). The study began with delivery of [^{67}Ga]Ga-DOTA-Miltuximab® (1 mg mAb, ~250 MBq) for the first cohort, followed by cold antibody dosing Cohorts 2-4 (3.5–24 mg). The study's primary endpoint was safety with biodistribution, tumour targeting, dosimetry and pharmacokinetics as secondary aims.

Methods

Study design

This study was an open label, first in human, single center, phase I trial conducted at Macquarie University Hospital (Sydney, Australia) and Macquarie Medical Imaging (located within Macquarie University Hospital). This study was approved by the Macquarie University Human Research Ethics Committee Ref: 5201600149 and was registered with the Australian Clinical Trials Registry ACTRN 12616000787482.

Miltuximab® was conjugated to the chelating agent 1, 4, 7, 10 tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA) then radiolabelled with [^{67}Ga]Ga ([^{67}Ga]Ga-DOTA-Miltuximab®). A radio-active dose of MBq (include space) of ^{67}Ga was chosen as this was consistent with standard ^{67}Ga imaging procedures .

Cohort 1 received only radiolabelled [^{67}Ga]Ga-DOTA-Miltuximab® (1 mg mAb, ~250 MBq), while cohorts 2-4 were pre-infused with 3.5 mg,

11.5 mg and 24 mg respectively of non-radiolabelled antibody (Supplementary Figure 1).

The primary study endpoint was the safety and tolerability of Miltuximab® in humans with advanced solid tumours. The secondary aims were to assess the utility of [^{67}Ga]Ga-DOTA-Miltuximab® to target prostate, pancreatic and urothelial cancers on imaging, to assess the biodistribution of Miltuximab®, and to investigate pharmacokinetics. The study was divided into four cohorts with an independent safety monitoring committee reviewing the results of each cohort prior to progression to the next dose level. After the first cohort of patients were dosed and safety and imaging data reviewed, a protocol amendment was submitted to allow pre-infusion of DOTA-Miltuximab® for the remaining three cohorts. Increasing doses of the unlabelled, or "cold" antibody, were injected one hour prior to the [^{67}Ga]Ga-DOTA-Miltuximab® infusion, with the aim of improving the biodistribution, pharmacokinetics and tumour targeting capacity of [^{67}Ga]Ga-DOTA-Miltuximab®. This also allowed for the assessment of safety and tolerability of increasing doses of the antibody. Pre-dosing was selected as the radiolabelled antibody was prepared off site and delivered on morning of infusion and the literature suggests that biodistribution is not affected by co- versus pre-dosing of cold antibody (32).

The protocol followed a classic 3+3 dose escalation model, defined as: If one drug-related grade 1 adverse event (AE) occurred in the first 3 patients at a dose level, a further 3 patients were to be recruited at that same dose level. If a further drug-related grade 1 AE occurred in any of the next 3 patients, then that dose was defined as the maximum tolerated dose, otherwise the following cohort proceeded to the next dose level. If a grade 2 drug-related AE occurred in any patient, this was defined as a dose limiting toxicity and the study was deemed to have not met the primary endpoint. Patient infusion within each cohort was staggered to monitor for AEs, with a two-week interval between each patient infusion. Before proceeding to the subsequent cohort, an independent safety monitoring committee reviewed the cohort data and agreed upon the escalation to the next dose level in the next cohort.

Study population

Patients included in the study were required to have metastatic or locally advanced, histologically confirmed, prostate adenocarcinoma, pancreatic adenocarcinoma or urothelial carcinoma. Patients had to have radiologic evidence of stable or slowly progressive disease. Treatment for the malignancy could not have been changed in the preceding four weeks prior to enrolment. Other key inclusion criteria included: at least two but no more than 15 lesions on standard of care imaging, Eastern Cooperative Oncology Group Performance Status (ECOG PS) <2, adequate hepatic and renal function, and left ventricular ejection fraction >50%.

Study drug

Miltuximab® was produced for GlyTherix Ltd by Catalent LLC (USA). Miltuximab® was conjugated with the chelating agent DOTA to allow labeling with the ^{67}Ga radionuclide. The unlabelled Miltuximab®-DOTA antibody was manufactured by Auspep Pty Ltd (Australia). Conjugation conditions were optimized so that the chelation had minimal effect on the ability of Miltuximab®-DOTA to bind the GPC-1 target, as assessed by cell based assays via flow cytometry and a GPC-1 antigen-binding ELISA .

Miltuximab®-DOTA was radiolabelled with ^{67}Ga at the Australian Nuclear Science and Technology Organisation (ANSTO, NSW, Australia). The radiolabeling process was developed to maintain the antigen binding ability with optimal radiostability. The drug was supplied at a specific activity of ~250 MBq per mg protein in 250 mM ammonium acetate buffer, pH 7.

At the infusion center, 1 mg of [^{67}Ga] Ga-DOTA-Miltuximab® or unlabelled antibody was drawn up into a syringe and the volume expanded to 8 mL with sterile PBS. The drug was administered via slow intravenous (IV) push through a cannula followed by a normal saline flush. Vital signs including blood pressure, pulse rate, temperature and oxygen saturation were monitored at the end of infusion, every 15 min for the first two h, hourly for the next 4 h, then at 12, 24, 48, 72 and 144 h post infusion and then weekly for four weeks.

Clinical assessments

Safety assessments were performed from screening up to four weeks post infusion. These included complete medical history, physical examination, ECOG PS, 12-lead electro-cardiogram (ECG), and standard laboratory measurements including complete blood count with differential, electrolyte panel, blood sugar level, liver function tests, amylase, lipase and tumour markers (prostate cancer – prostate specific antigen (PSA); pancreatic cancer - Ca 19.9, carcinoembryonic antigen (CEA). Additional investigations included an echocardiogram to assess left ventricular

ejection fraction at baseline and four weeks post infusion. Toxicity and adverse events were evaluated on clinical examination and laboratory assessments performed for a total of 11 timepoints. Assessments were held at screening (up to 14 days prior to infusion), pre-infusion, 6, 12, 24, 48, 72, 144 h, 2, 3 and 4 weeks post infusion. All adverse events were graded according to the National Cancer Institute-Common Terminology Criteria Version 4.03. Concomitant medications were recorded at each visit post infusion. Standard tumour imaging was undertaken at screening and then again at the final assessment timepoint at four weeks post infusion.

Imaging

The [^{67}Ga]Ga imaging protocol included a baseline transmission scan with Cobalt-57 with and without the patient, followed by step-and-shoot, tri-windows acquisition for ^{67}Ga of 93, 184, and 300 keV, whole body anterior-posterior (A/P) planar gamma camera scans and single-photon emission computed tomography and low dose X-ray CT (SPECT/CT) over the abdomen at 30 min, 6 h, 24 h, 48 h, 72 h and 144 h with the potential for further scans if required. A calibrated standard was included in the field of view of all scans. The nuclear medicine physician was blinded to clinical history and standard of care imaging at first image reading and then unblinded for the second reading.

Standard of care whole body bone scans were performed using ^{99m}Tc-HDP and collected on a SPECT/CT gamma camera.

Pharmacokinetic (PK) sampling

Blood sample collection for PK analysis was performed at baseline, pre-infusion, then 1, 6, 12, 24, 48, 72 and 144 h post infusion and then weekly for four weeks.

Two separate methods were utilized to assess PK of Miltuximab®. Firstly, a 'hot PK' method was developed using a gamma counter to determine the radioactivity of patient whole blood samples compared to a standard of ^{67}Ga. Blood clearance rate half-life (BCR $t_{1/2}$) calculations for [^{67}Ga]Ga-DOTA-Miltuximab® were calculated based on decay-corrected gamma (γ) counter measurements of blood samples taken 2 h pre-infusion (baseline) and at post-infusion timepoints. The measurements were then calculated against the maximal gamma count signal (C_{max}) observed at 1 h post infusion and the values were fitted into a one-phase decay equation to calculate the BCR $t_{1/2}$ of [^{67}Ga]Ga-DOTA-Miltuximab®. The terminal phase rate-constant (λ_z) was estimated from the log-linear plot of the concentration-time curve, and the BCR $t_{1/2}$ was then calculated as $0.693/\lambda_z$. Secondly, a GPC-1 direct binding ELISA was developed and optimized to analyze the levels of total Miltuximab® in all samples which were derived from the 450 nm wavelength signal of serum antibody bound to GPC-1 in the ELISA. For each cohort, the values were then fitted into a one-phase decay equation to derive its BCR $t_{1/2}$ from the estimated terminal phase rate-constant (λ_z) as the formula: BCR $t_{1/2} = 0.693/\lambda_z$. The ELISA assay development and results will be reported elsewhere (manuscript in preparation).

Normal Organ Absorbed Dose

Organ and whole body effective doses were calculated using whole body gamma camera scans using the Organ Level Internal Dose Assessment/Exponential Modelling (OLINDA/EXM) (33) software package together with injection and calibration controls. The A/P planar whole-body images were combined to give a geometric mean (GM) image for each time point. The GM image was corrected for photon attenuation using a single "lumped" attenuation coefficient averaged for the three energy photopeaks of [^{67}Ga]Ga. The accuracy of this quantitative image was checked using the total rate activity in the body at the first time point, prior to avoiding, as well as using the calibration standard. Each attenuation-corrected GM image was then aligned to the first time point image and regions of interest (ROIs) drawn over the following organs on the image: heart contents, liver, lung (left), spleen and urinary bladder. Total radioactivity remaining in the body was also determined from the images and used for the determination of the dose to the remainder of the body. Bone marrow dosimetry was not estimated due to the difficulty in extracting radioactivity uptake in this compartment on planar images. The data from each time point, expressed as the percentage of injected dose in the organ, was decay-corrected and imported into the OLINDA/EXM package for the appropriate adult model (male/female). These data were then used to determine the absorbed doses from the ^{67}Ga radiotracer. Further, the selection of a different radionuclide in OLINDA (e.g., Zirconium-89, Lutetium-177) permitted dosimetry to be calculated from the decay-corrected biodistribution that had been recorded. The abdominal SPECT images were not used for dosimetry calculations due to the limited axial field view that was imaged.

Statistics

The primary focus of this study was to determine the safety and tolerability of [^{67}Ga] Ga-DOTA-Miltuximab® alone and in combination with unlabelled DOTA-Miltuximab® in patients with advanced prostate, pancreatic and urothelial cancers. Due to the nature and design of the study, the statistical analyses were descriptive or exploratory. Categorical data were described using contingency tables with frequencies and percentages. Quantitative data were presented using number of observations, mean, median, maximum and minimum values. The sample size planned for this study was not driven by statistical considerations.

All patients receiving a dose of [^{67}Ga]Ga-DOTA-Miltuximab® were included in the population for safety and efficacy analysis. All available data including adverse events, changes in laboratory values and other safety and efficacy parameters were evaluated in each patient.

To investigate uptake of [^{67}Ga]Ga-DOTA-Miltuximab® in the liver, remainder of body and whole body over time between cohorts, we used a mixed-effects model for repeated measures approach. The model included fixed effects for cohort (i.e. dose group), assessment hours relative to the first dose of study medication (i.e. time) and treatment-by-time interaction. Time was treated as the repeated variable within a subject. The response variable was the percentage of injected dose of [^{67}Ga] Ga-DOTA-Miltuximab®. The differences in uptake across the time course, between each pair of cohorts were then assessed using Tukey's honest significance test. To test differences in uptake between cohorts at individual time points, we used ANOVA test with Bonferroni correction.

The correlation between the increase in % of Injected Dose in remainder of body compared with that in liver and the amount of cold dose was evaluated using Pearson correlation and linear regression analysis. Statistical significance was considered at the level of 5% (p value ≤0.05). All statistical analyses were performed using GraphPad Prism 8.0.2 and R (The R Foundation), version 4.0.0.

Results

Patient characteristics and consort diagram

The study was conducted from August 2016 to July 2018. Fifteen patients were screened and 12 patients with metastatic or locally advanced pancreatic, prostate or urothelial cancer were enrolled into the study (Consort diagram Supplementary Figure 1). Nine patients had prostate adenocarcinoma, two had pancreatic adenocarcinoma and one had urothelial carcinoma. All patients in Cohorts 2 and 4 had prostate cancer, while Cohort 1 had prostate cancer or pancreatic cancer, and Cohort 3 had prostate cancer or bladder cancer (Table 1).

Clinical characteristics are described in Table 1. All patients were male. The median age was 66.5 years (range 53-79) and the median number of lines of treatment for metastatic disease was two. Both patients with pancreatic cancer were refractory to standard of care treatment and all patients had slowly progressive disease. Eight out of nine patients with prostate cancer had been treated with or were currently being treated with one of the novel androgen receptor targeted therapies, enzalutamide or abiraterone. All patients had evidence of metastatic disease of at least two to fifteen lesions on standard imaging.

Table 1. Patient characteristics

		Cohort 1	Cohort 2	Cohort 3	Cohort 4
Age in years	Median	63	63	65	72
	Range	61-78	53-69	53-75	68-79
Gender (%)	Male	100	100	100	100
Cancer (n)	Prostate	1	3	2	3
	Pancreas	2	0	0	0
	Bladder	0	0	1	0
Metastatic sites (%)	Lymph nodes	0	100	33	33
	Liver	67	0	33	100
	Bone	33	67	67	67
ECOG	Median	1	1	1	1
Lines of treatment for advanced disease	Median	1	2	2	2
Radiotherapy (%)		33	0	67	33

Safety

All laboratory abnormalities reported in the trial (recorded as adverse events) were present at baseline prior to [^{67}Ga]Ga-DOTA-Miltuximab® drug administration. The most common laboratory abnormalities were hematological (grade 1 anemia and leukopenia) followed by elevated alkaline phosphatase (ALP) and were assessed as being related to the metastatic cancer and/or previous anti-cancer therapy. All adverse events are shown in Table 2a. Eleven out of 12 patients experienced an abnormal laboratory finding of any grade. Grade 1 elevation of ALP in patients 7, 8, 9 was determined to be related to pre-existing bone metastases from prostate adenocarcinoma. Grade 2 elevation of ALP in Patient 2, which was present at baseline was also determined to be related to pre-existing bone metastases secondary to prostate cancer. No laboratory measures changed grade during or after infusion. There were no grade 3 or 4 or severe adverse events noted during the study. No significant changes in vital signs, deterioration of ECOG PS or electrocardiogram changes were observed.

No drug related adverse events were identified in the intention to treat analysis of all twelve patients that received study drug (Table 2b). No infusion reactions were observed during the infusion of either the cold antibody or [^{67}Ga]Ga-DOTA-Miltuximab®.

Imaging and biodistribution

Biodistribution of [^{67}Ga]Ga-DOTA-Miltuximab® was assessed using planar gamma scans and SPECT-CT imaging. At 30 min post infusion, whole body planar images demonstrated physiological radionuclide blood pooling in the vasculature and the mediastinum. Reticulo-endothelial uptake, particularly hepatic and splenic uptake, was seen 30 min after end of infusion and demonstrated consistently high uptake until 144 h. At 48 h post infusion, low intensity uptake was seen in the large bowel and this was further established at 72 h, consistent with liver processing and excretion via the bile (Figure 1A).

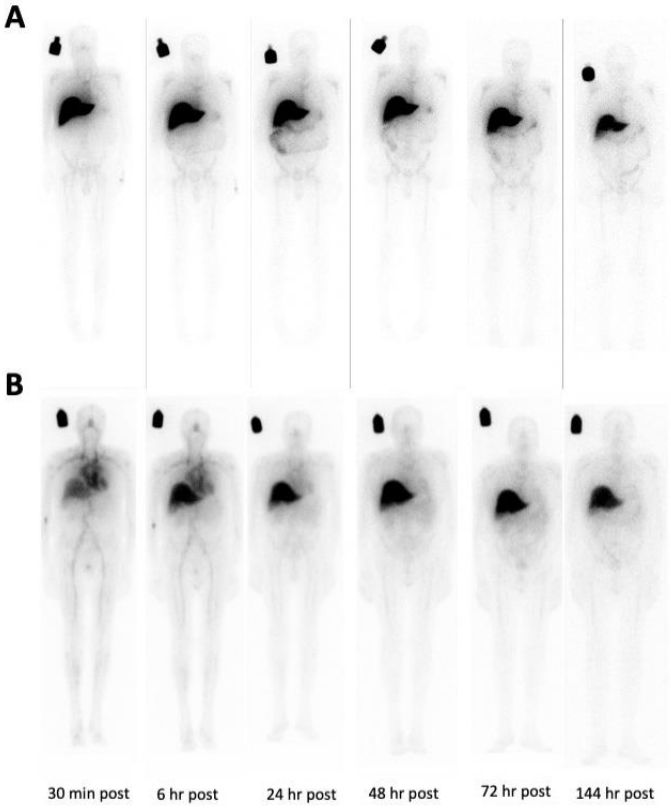

Figure 1. Distribution in major organs over all time points and effect of pre-infusion of cold dose. (a) Patient 3 (cohort 1, no pre-infusion). (b) Patient 12 (cohort 4, 24 mg pre-infusion). Whole body gamma scans were performed across time points 30 min – 144 h has indicated

Increasing cold mAb dose of patients in cohorts two, three and four resulted in differences in liver uptake (p=0.002), with a progressive decline in the liver uptake observed with increasing cold dose (Figure 1B). There was a corresponding increase in the amount of radionuclide seen in the remainder of the body - whole body excluding liver, lungs, red marrow, bladder, spleen, and heart (p=0.039; Figures 2A-2C). Across time, this trend was significant for Cohorts 3 and 4 as compared to Cohort 1 (p=0.036 and p=0.002, respectively). At 48 h, we saw significantly reduced liver uptake in Cohort 4 as compared to Cohort 3 (p=0.009), Cohort 2 (p=0.018) and Cohort 1 (p=0.007). The dose dependency observed between Cohort 3 and 4 suggests that increasing amount of unlabelled antibody had not saturated the effect on liver uptake. Statistical analyses are included as Supplementary Table 2.

The effect of increasing cold mAb dose was most noticeable at the 30 min timepoint, when [^{67}Ga]Ga-DOTA-Miltuximab® uptake in the rest of the body (whole body excluding liver, lungs, red marrow, bladder, spleen, and heart), as compared to liver uptake, was significantly increased by pre-infusion of unlabelled antibody, with a high correlation to dose (p<0.001; Supplementary Figure 2). No clear differences were noted with increasing unlabelled Miltuximab® dose in other organs.

Table 2a. All adverse events

			Patient ID											
			1	2	3	4	5	6	7	8	9	10	11	12
Adverse Event (AE)		**AE Grade**	**Number of assessments with AE**											
Hematology	Anemia	1	11	11	3	8	2	6	7	10	3	11	0	0
	Leukopenia	1	4	0	7	0	0	7	0	0	0	6	0	0
	Thrombocytopenia	1	0	0	0	0	0	0	0	0	0	6	6	0
Renal	Electrolyte levels	1	3	2	0	9	3	8	0	0	11	0	3	0
	Creatinine level	1	0	1	0	0	0	0	0	0	8	0	0	0
Hepatic	Elevated ALT/AST	1	2	0	8	0	0	0	7	0	0	0	0	0
	Elevated ALP	1	0	0	0	0	0	7	11	1	11	0	0	0
	Elevated ALP	2	0	11	0	0	0	0	0	0	0	0	0	0
Cardiac		1	0	1	0	0	0	0	0	1	0	0	0	0
Clinical AE		1	0	4	3	0	1	1	0	0	1	0	0	0

Patients were assessed for adverse events (clinical and hematological/biochemical) over 11 time points

Table 2b. Drug related adverse events

			Patient ID											
			1	2	3	4	5	6	7	8	9	10	11	12
Adverse Event (AE)		**AE Grade**	**Number of assessments with AE**											
Hematology	Anemia	1	0	0	0	0	0	0	0	0	0	0	0	0
	Leukopenia	1	0	0	0	0	0	0	0	0	0	0	0	0
	Thrombocytopenia	1	0	0	0	0	0	0	0	0	0	0	0	0
Renal	Electrolyte levels	1	0	0	0	0	0	0	0	0	0	0	0	0
	Creatinine level	1	0	0	0	0	0	0	0	0	0	0	0	0
Hepatic	Elevated ALT/AST	1	0	0	0	0	0	0	0	0	0	0	0	0
	Elevated ALP	1	0	0	0	0	0	0	0	0	0	0	0	0
	Elevated ALP	2	0	0	0	0	0	0	0	0	0	0	0	0
Cardiac		1	0	0	0	0	0	0	0	0	0	0	0	0
Clinical AE		1	0	0	0	0	0	0	0	0	0	0	0	0

Patients were assessed for adverse events (clinical and hematological/biochemical) over 11 time points

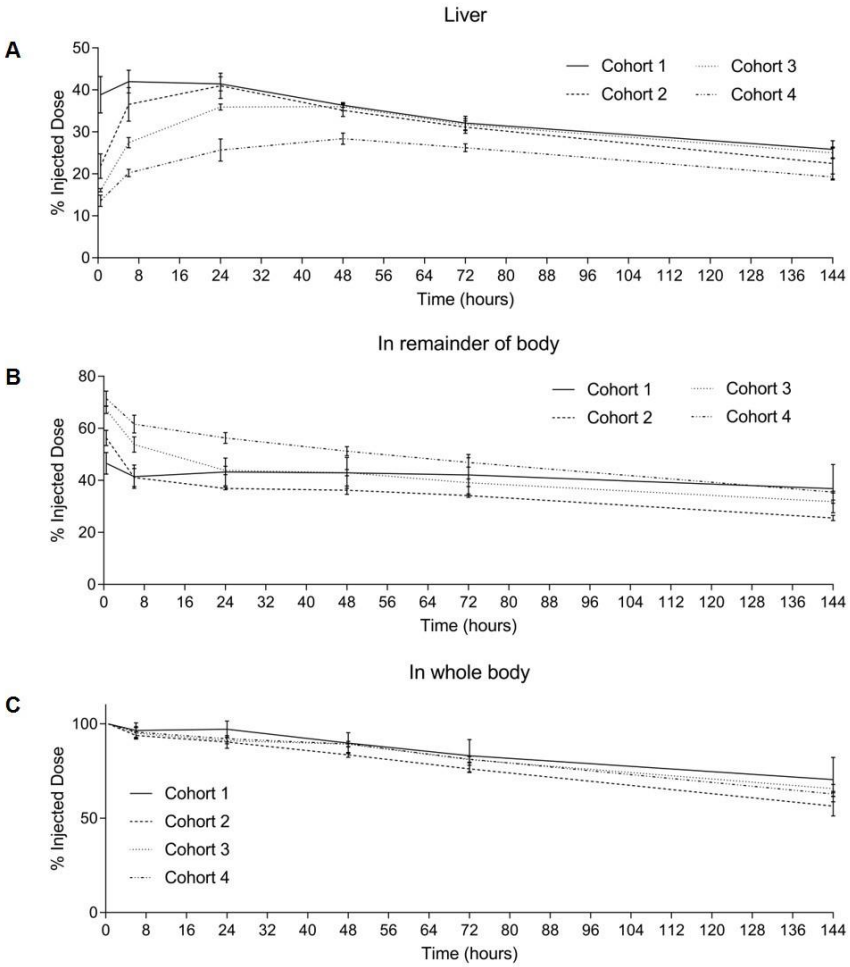

Figure 2. Percentage Injected Dose (ID) over time per cohort, in (a) Liver, (b) Remainder of body (whole body excluding liver, lungs, red marrow, bladder, spleen and heart) and (c) Whole body. The data at each time point are shown as mean and standard errors (error bars). A mixed effects model for repeated measure approach was used to investigate differences in % Injected Dose between Cohorts (varying dose of unlabelled Miltuximab-DOTA®: Cohort 1 =0 mg unlabeled; Cohort 2 =3.5 mg; Cohort 3 =11.5 mg; Cohort 4 =24 mg) across the time course: Liver: p=0.002; In whole body: p=0.815; In remainder of body: p=0.039. Further investigation using Tukey's honest significance test showed statistically significant decrease in Liver uptake over time course between Cohort 4 v Cohort 1: p=0.002; Cohort 3 v Cohort 1: 0.036. Comparison of % Injected Dose at 48 h in Liver was achieved using ANOVA test with Bonferroni's correction. Cohort 4 liver uptake was significantly lower than Cohorts 1 (p=0.007), 2 (p=0.018) and 3 (p=0.009) at this time point

Normal Organ Absorbed Dose

Dosimetry results averaged per cohort are shown in Figure 3. Dosimetry results averaged over all patients are shown in Supplementary Table 1a and individual patient results are shown in Supplementary Table 1b. The highest absorbed doses were seen in the liver and spleen, with mean values of 0.62 and 0.31 mSv/MBq respectively. The mean whole body effective dose across all cohorts was 0.09 mSv/MBq giving approximately 20 mSv ED for the study. This is comparable to other estimates of whole-body dosimetry from [^{67}Ga]Ga such as [^{67}Ga]Ga-citrate used for infection and tumour imaging in clinical nuclear medicine practice (34).

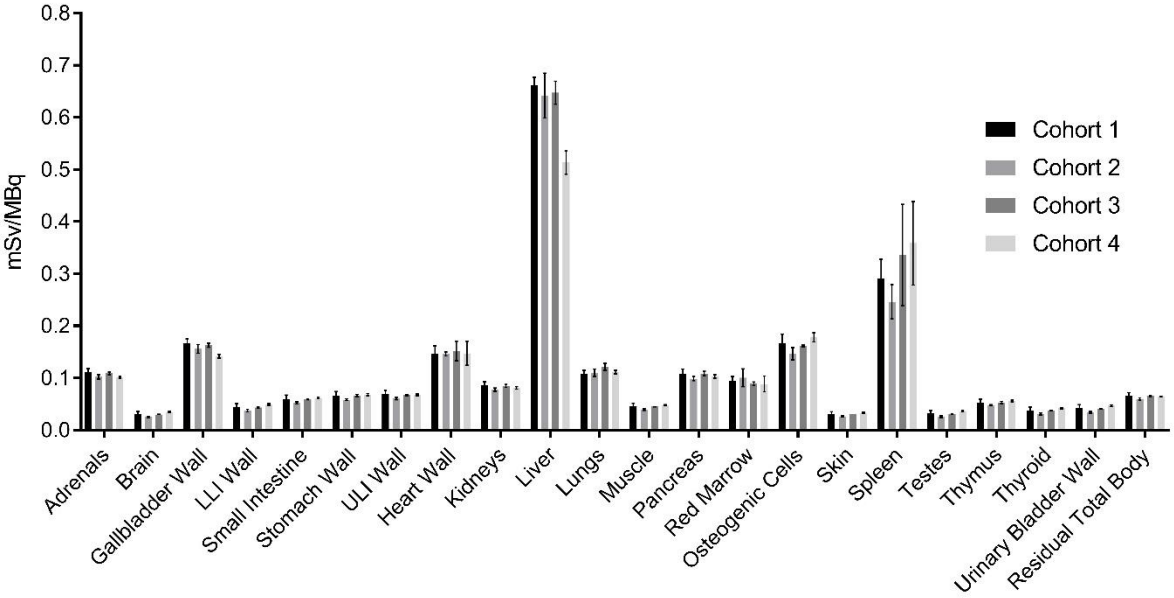

Figure 3. Bar chart of dosimetry (mSv/MBq) per cohort in all organs. Means are shown with error bars representing ± Standard Error

Pharmacokinetics

Blood samples from patients in cohorts 3 and 4 were available for assessment of post-infusion radiation counts. The blood clearance rate (BCR t1/2) of [^{67}Ga]Ga-DOTA-Miltuximab® was estimated to be 7.3 h for Cohort 3 and 12 h for Cohort 4. Blood clearance rates for Cohorts 3 and 4 for [^{67}Ga]Ga-DOTA-Miltuximab® are shown in Supplementary Figure 3A, while individual patient data is shown in Supplementary Table 3.

Pharmacokinetic assessment of Miltuximab® levels using the antigen-binding ELISA was performed. Miltuximab® levels could not be determined in Cohorts 1 and 2, due to low levels of antibody and/or interference in the assay. Data for Cohorts 3 and 4 allowed exploratory analysis to estimate half-life. In Cohorts 3 and 4 (excluding patient 11, whose sample was unevaluable due to high serum assay background), the half-life of total Miltuximab® was estimated to be 5 h in cohort 3 and 18.2 h in cohort 4. Blood clearance rates for cohorts 3 and 4 for Miltuximab® are available as Supplementary Figure 3B. These exploratory analyses indicated that the increasing dose of cold antibody over cohorts 3 and 4 contributed to a prolonged duration of antibody retention in the circulation.

Tumour targeting

Patients 2 (receiving 1 mg [^{67}Ga]Ga-DOTA-Miltuximab®) and 7 (receiving 11.5 mg Miltuximab®-DOTA prior to 1 mg [^{67}Ga]Ga-DOTA-Miltuximab®) demonstrated tumour uptake of [^{67}Ga]Ga-DOTA-Miltuximab® in sites of particularly active disease. Patient 2 had significant pain associated with cancer in the right lower limb, particularly in the right foot, which corresponded to sites of antibody uptake in SPECT-CT images in the [^{67}Ga]Ga-DOTA-Miltuximab® scans (Figure 4). Patient 7 demonstrated [^{67}Ga]Ga-DOTA-Miltuximab® uptake in the skull which corresponded to sites of active disease progression on magnetic resonance imaging (MRI) and identified as a dural based metastases (Figure 4).

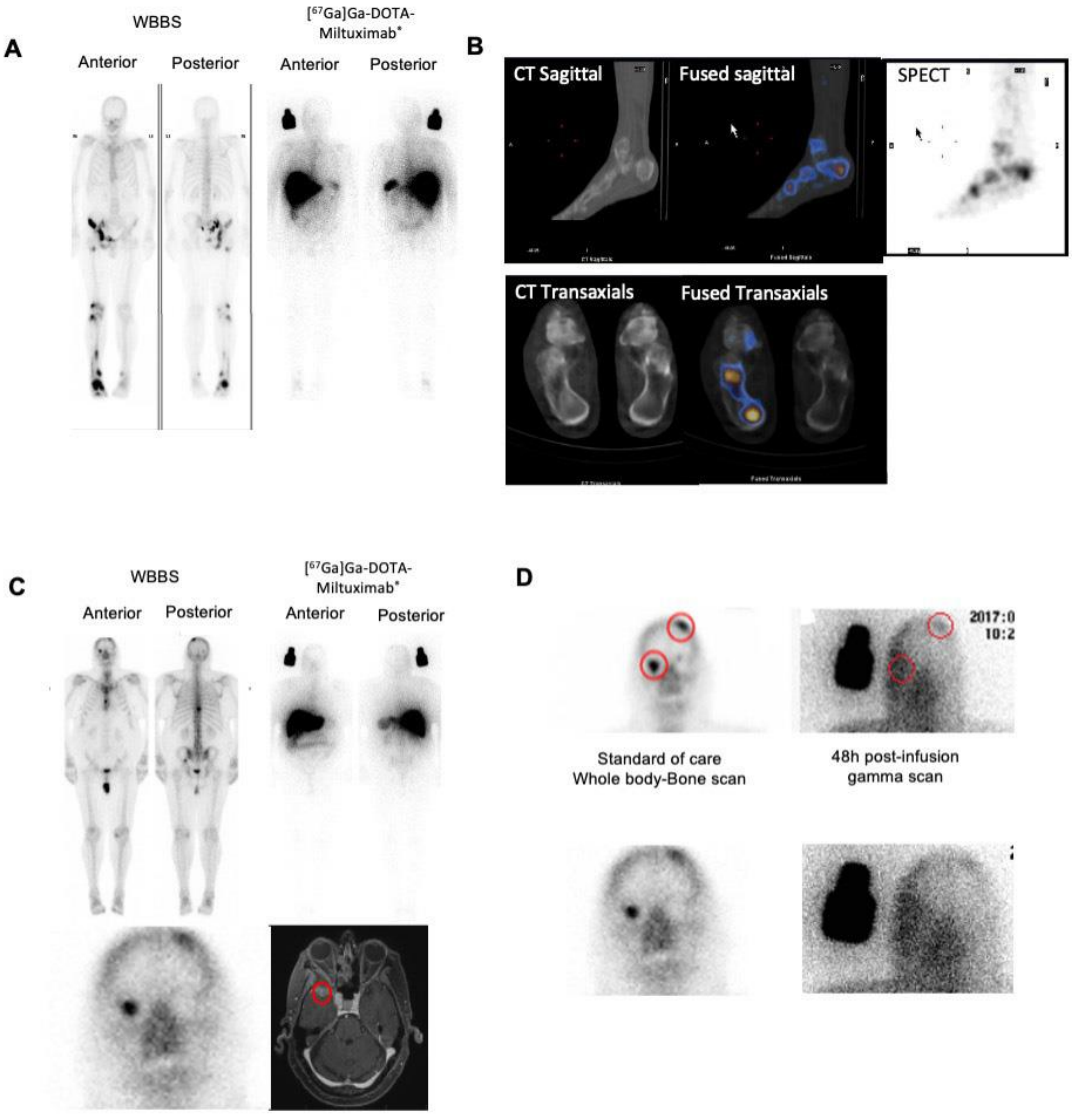

Figure 4. Targeting of [^{67}Ga]Ga-DOTA-Miltuximab® to patient tumours. Patient 2. (A) Whole-Body Bone Scan (WBBS) imaging taken during screening showing a dominant metastases on the right lower limb. Also shown is [^{67}Ga]Ga-DOTA-Miltuximab® whole body planar gamma scan taken at 24 h post infusion. (B) SPECT-CT images taken 24 h post-infusion showing [^{67}Ga]Ga-DOTA-Miltuximab®. Antibody uptake is shown in the metatarsals and calcaneus. Patient 7. (C). WBBS taken during screening. Two metastases are noted in the skull (see zoomed inlet). MRI scan of one lesion identified as being dural based. Also shown is [^{67}Ga]Ga-DOTA-Miltuximab® whole body planar gamma scan taken at 48 h post infusion. (D) Comparison of Patient 7's WBBS to the planar image taken 48 h-post infusion. Uptake evident at two sites of metastases

Discussion

GPC-1 is an attractive target for antibody mediated cancer therapy due to its over expression in many solid tumours and lack of expression in normal tissue (15, 19). Antibody based therapies targeting GPC-1 have proven safe and efficacious in preclinical studies conducted by us and other groups using a variety of mechanisms of action, including radioimmunotherapy, antibody drug conjugates and naked antibodies (21, 22, 35). In our hands, the most potent anti-tumour activity was achieved using [^{177}Lu]Lu-DOTA-Miltuximab®, hence we are developing Miltuximab® as a theranostic with ^{89}Zr and ^{177}Lu isotope pair.

There have been no previous human clinical trials with any agent targeting GPC-1, hence we set out to assess initial safety and biodistribution data using dosing strategies and antibody mass levels that have been widely used previously for other antibodies for RIT. Gallium-67 was chosen as an imaging isotope due to its safety profile, half life and availability together with other favorable characteristics (31).

Here we report the results of a Phase I, first-in-human study primarily assessing safety and

tolerability of [67Ga]Ga-DOTA-Miltuximab® in patients with advanced prostate, pancreatic or urothelial carcinoma. All patients enrolled had stable or slowly progressing disease. The primary study endpoint was to establish the safety of administering [67Ga]Ga-DOTA-Miltuximab® plus unlabelled antibody to a total antibody mass of 25 mg. The study met its primary endpoint, with no drug related adverse events observed in patients at all dose levels tested. This provides the first information that it is safe to target GPC-1 with antibodies in humans and establishes a baseline for further exploration of antibody mass dose escalation in future studies.

The radiation dose received by the major organs of liver and spleen ranged from 0.48 to 0.69 mSv/MBq and 0.20 to 0.53 mSv/MBq while the whole body effective dose ranged from 0.08 to 0.10 mSv/MBq. These values are below the doses seen in other similar studies of radiolabelled antibodies, albeit these studies used different radioisotopes (36, 37). The dosimetry analysis shows that the radiation exposure to all major organs in all patients was safe and well below the whole body radiation safety threshold of 50/250 mSv/MBq (38). This is an important result, as this information allowed extrapolation to calculate predicted exposures for [89Zr]Zr and [177Lu]Lu labeled Miltuximab®, informing our future clinical study design.

Biodistribution of the antibody was consistent with that observed for other radiolabelled antibodies. The first three patients demonstrated significant hepatic uptake immediately after infusion of [67Ga]Ga-DOTA-Miltuximab® which remained high for up to 144 h. This is a common phenomenon seen in the biodistribution assessment of many other mAbs (39–42). Solid tumours are relatively radio-resistant and require a high dose of radiation for a cytotoxic effect, so strategies are required to increase the amount of radiation dose delivered to the tumour while limiting systemic toxicities. Typically, pre- or co-dosing with unlabelled antibody to saturate sites of non-tumour target uptake, thereby enhancing uptake of the radionuclide in tumour, is used to achieve this goal. This strategy has proven effective in reducing hepatic uptake and increasing tumour targeting with the J591 anti-PSMA antibody in prostate cancer (42) amongst many other mAbs. The reported amount of cold antibody used to optimally reduce liver uptake while maximizing tumour uptake varies significantly in the literature, ranging from 25 mg to greater than 1000 mg (6, 37). This variation is expected, given the varying nature of each antigen-antibody interaction, as well as significant expected variance in different target antigen expression (in tumour and normal tissue) and heterogeneity (both within tumour lesions and individual patients). Furthermore, even with the same antibody (J591) different studies report different optimal unlabelled doses, with Morris et al reporting hepatic uptake saturation between 10 and 25 mg while in the study by Pandit-Taskar et al., hepatic uptake of the radiolabelled mAb progressively decreased when total antibody doses increased from 25 mg to 100 mg, with a simultaneous increase in the uptake of the radiolabelled mAb in the circulation and in tumour lesions (32, 42). Recent studies with this antibody use between 20–25 mg total antibody dose (43, 44).

As this was a first in human study primarily aimed at testing the safety of Miltuximab® as well as targeting of Glypican-1, the cold antibody dose chosen was conservative, limited at a maximum of 24 mg for a total of 25 mg antibody administered. Similar to published studies, we saw reduced hepatic uptake and increased circulatory levels of radiolabelled mAb detected with increasing levels of cold antibody. It is probable that the highest dose of 24 mg cold antibody may not have been sufficient to completely saturate non-tumour target uptake, supported by the continued reduction of liver uptake in Cohort 4. Moreover, the half-life of [67Ga]Ga-DOTA Miltuximab® in this study at the highest cold antibody dose of 24 mg was 12 h, markedly shorter than the half-life observed in the [111In]In-DOTA-J591 study which demonstrated a half-life of 1.9 days at a 25 mg cold antibody dose level, although, of course, these are different antibodies with different target antigens (42). Similarly, other 89Zr labelled mAbs have shown half-lives of 111 h and 70 h for trastuzumab and cetuximab respectively (36,41). The relatively short half-life for [67Ga]Ga-DOTA Miltuximab® which is improved with increased cold dosing in Cohort 4 compared to Cohort 3, further suggests that increased cold dosing may improve antibody retention in circulation.

The finding that 24 mg cold antibody may not saturate non-tumour sites informs planning of the next clinical study, a Phase I study of [89Zr]Zr and [177Lu]Lu labeled Miltuximab® (45). This study, supported by preclinical findings of specific tumour targeting and PET imaging using [89Zr]Zr DFO-Miltuximab®, and dose-dependent anti-prostate cancer efficacy in animal studies (22), aims to evaluate the imaging and therapeutic potential of [89Zr]Zr-DFO-Miltuximab® and [177Lu]Lu-DOTA-Miltuximab® in patients with GPC-1 positive cancers,

including advanced prostate, pancreatic and bladder cancer (45). The initial cohort of patients will commence with a total antibody mass of 25 mg, with the doses progressively increased to 150 mg total dose. The optimal antibody mass will be determined using a fixed dose of [^{89}Zr]Zr-DFO-Miltuximab® and determining the optimal lesion imaging and tumour to normal tissue exposure dosimetry calculations.

Imaging of tumours was a secondary endpoint of this phase I study. Significant inter-patient differences in radionuclide uptake were seen. Two patients demonstrated radionuclide uptake at highly active sites of disease (symptomatic, progressive disease on standard imaging). The remaining ten patients demonstrated low or no uptake in sites of disease. Although the sample size was sufficient to determine the primary endpoint of safety, the sample size and patient characteristics of stable or slowly progressive disease may have limited the ability to fully investigate targeting of Miltuximab® to tumours. A second consideration when interpreting the observed tumour targeting was that tumour biopsy or immunohistochemical staining for GPC-1 was not a requirement for entry. The majority of patients enrolled in the study had metastatic prostate cancer. Despite approximately 80% of prostate cancers being reported as positive for GPC-1 (19), the GPC-1 status for patients enrolled on this study is unknown, thus, it was unknown whether targeting could be expected. Finally, pharmacokinetic and imaging data suggests that the half-life of Miltuximab® may be sub-optimal at the maximum cold antibody dose tested of 24 mg. In pre-clinical subcutaneous tumour xenograft models, accumulation of Miltuximab® in tumour lesions began at 24 h and peaked at 96 h (unpublished data), suggesting that the current dose in this phase 1 study which resulted in rapid clearance of the mAb, may have been inadequate to allow tumour targeting. Higher unlabelled doses, as discussed above, may be required to optimize tumour accumulation.

Despite these considerations, tumour targeting in two prostate cancer patients was observed. Interestingly, both patients in whom tumour uptake was identified had more aggressive disease. Both had rapid progression of prostate cancer after completion of the trial despite novel androgen receptor targeted therapy, with the development of metastases in the dura and leptomeninges and then death thereafter. Published studies show that higher levels of GPC-1 are associated with poor prognostic disease including higher metastatic potential, stage of disease and reduced overall survival (16, 17, 46). Biologically, GPC-1 plays a role in tumour progression and is critically involved in tumour cell invasion and metastasis, both elements of tumour aggression (13–15). It is possible that GPC-1 is more highly expressed in patients with aggressive as opposed to indolent cancers and that Miltuximab® may identify those patients with poorer prognosis. Since this was a safety study with no therapeutic intervention, eligibility criteria focused on patients with stable or slowly progressive cancer, thus potentially posing a limit on the secondary imaging endpoint. Most patients on this study had relatively indolent disease and may not have been ideal candidates for Miltuximab® tumour targeting.

Conclusion

There is compelling evidence that GPC-1 plays a role in many solid tumours including prostate, pancreatic, bladder, gastroesophageal, pancreatic, ovarian and glioblastoma. A theranostic agent targeting GPC-1 therefore has substantial potential for clinical utility. The current study demonstrated the safety of Miltuximab® to 25 mg in patients with prostate, pancreatic and urothelial cancers. Expected uptake in the reticuloendothelial system, particularly the liver, was seen in all patients, which could be reduced by introduction of cold antibody dosing, and this reduction was dose dependent. Potentially, increased cold dosing amount could further saturate non-tumour targeting, allowing for increased serum antibody retention and access to tumour sites, supported by the relatively short blood half life of Miltuximab® observed with the highest cold dose tested. Despite this, targeting of antibody to lesions was observed for two patients who had especially active disease. Importantly, the current study has informed the clinical design of our next Phase I study of Miltuximab® as a theranostic agent, radiolabelled with [^{89}Zr]Zr for imaging and [^{177}Lu]Lu for therapy .

Abbreviations

AE: adverse events; ALT: alanine transaminase; A/P: anterior-posterior; AST: aspartate transaminase; ANSTO: Australian Nuclear Science Technology Organisation; $BCRt_{1/2}$: blood clearance rate; CEA: carcino-embryonic antigen; Cmax: maximal observed response; DOTA: 1,4,7,10 tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid; ECG: electrocardiogram; ECOG PS: Eastern Cooperative Oncology Group Performance Status; ED: effective dose; IV: intravenous; ^{67}Ga: Gallium-67; GPC-1: Glypican-1; h: hours; ^{177}Lu:

Lutetium-177; MAb: monoclonal antibody; MBq: megabecquerel; mg: milligrams; MRI: magnetic resonance imaging; mSv: millisievert; OLINDA/ EXM: organ level internal dose assessment/ exponential modelling; PK: pharmacokinetic; PSA: prostate specific antigen; SPECT/CT: Single-photon emission computed tomography; WBBS: whole body bone scan; ID injected dose; γ: gamma.

Declarations

Ethics approval and consent to participate

This study was approved by the Macquarie University Human Research Ethics Committee Ref: 5201600149. It was registered with the Australian Clinical Trials Registry ACTRN 12616000787482 on 16th June 2016, https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=370904.

Competing interests

DHC, TRM, SW, MEL, YL and BJW are employees and shareholders of GlyTherix Ltd. DG is Chair of the GlyTherix Ltd Clinical Advisory Board.

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Authors' contributions

Conception and design: DS, DC, DG, DS, BW, HG, VV, HM

Development of methodology: DS, DC, SW, MT, YL, DB

Acquisition of data: DS, DC, TS, SW, MT, KHS, TM, YL, PR, DB, PP, VV

Analysis and interpretation of data: DS, DC, DG, BW, SW, YL, TS, ML, HG, PR and DB

Writing, review and/or revision of the manuscript: DS, DC, ML, BW, HG, DB

Administrative, technical, or material support: N/A

Study supervision: DS, HG, DG

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References

1. Loehrer PJ, Einhorn LH, Elson PJ, Crawford ED, Kuebler P, Tannock I, et al. A randomized comparison of cisplatin alone or in combination with methotrexate, vinblastine, and doxorubicin in patients with metastatic urothelial carcinoma: A cooperative group study. J Clin Oncol. 1992; 10(7):1066–73.
2. Bellmunt J, De Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med. 2017; 376(11): 1015–26.
3. Sabanathan D, Nagrial AM, Chin VT. The changing landscape of systemic therapy in advanced pancreatic cancer. Cancer Forum. 2016; 40:53–8.
4. Nixon NA, Blais N, Ernst S, Kollmannsberger C, Bebb G, Butler M, et al. Current landscape of immunotherapy in the treatment of solid tumours, with future opportunities and challenges. Curr Oncol. 2018; 25(5):e373–84 .
5. Cheson BD. Radioimmunotherapy of non-Hodgkin lymphomas. Blood. 2003; 101(2): 391–8.
6. Press OW, Eary JF, Appelbaum FR, Martin PJ, Badger CC, Nelp WB, et al. Radiolabeled-Antibody Therapy of B-Cell Lymphoma with Autologous Bone Marrow Support. N Engl J Med. 1993; 329(17):1219–24.
7. Massicano A, Pujatti P, Alcarde L, Suzuki M, Spencer P, Araújo E. Development and biological studies of ^{177}Lu-DOTA-rituximab for the treatment of Non-Hodgkin's lymphoma. Curr Radiopharm. 2015; 9(1): 54–63.
8. Forrer F, Oechslin-Oberholzer C, Campana B, Maecke HR, Mueller-Brand J, Lohri A. Is there need for radioimmunotherapy? Results of a phase I/II study in patients with indolent B-cell lymphomas using lutetium-177-DOTA-rituximab. Q J Nucl Med Mol Imaging. 2012; 56(6):544–50.
9. Forrer F, Chen J, Fani M, Powell P, Lohri A, Müller-Brand J, et al. In vitro characterization of ^{177}Lu-radiolabelled chimeric anti-CD20 monoclonal antibody and a preliminary dosimetry study. Eur J Nucl Med Mol Imaging. 2009; 36(9):1443–52.
10. Vallabhajosula S, Goldsmith SJ, Hamacher KA, Kostakoglu L, Konishi S, Milowski MI, et al. Prediction of myelotoxicity based on bone marrow radiation-absorbed dose: radioimmunotherapy studies using 90Y- and ^{177}Lu-labeled J591 antibodies specific for prostate-specific membrane antigen. J Nucl Med. 2005; 46(5):850–8.
11. A.P. Breeman W, Sze Chan H, M.S. de Zanger R, K. Konijnenberg M, de Blois E. Overview of Development and Formulation of ^{177}Lu-DOTA-TATE for PRRT. Curr Radiopharm. 2015; 9(1):8–18.
12. Strosberg J, El-Haddad G, Wolin E, Hendifar A, Yao J, Chasen B, et al. Phase 3 Trial of ^{177}Lu-Dotatate for Midgut Neuroendocrine Tumors. N Engl J Med. 2017; 376(2):125–35.

13. Kleeff J, Ishiwata T, Kumbasar A, Friess H, Büchler MW, Lander AD, et al. The cell-surface heparan sulfate proteoglycan glypican-1 regulates growth factor action in pancreatic carcinoma cells and is overexpressed in human pancreatic cancer. J Clin Invest. 1998 1; 102(9):1662-73.
14. Matsuda K, Maruyama H, Guo F, Kleeff J, Itakura J, Matsumoto Y, et al. Glypican-1 is overexpressed in human breast cancer and modulates the mitogenic effects of multiple heparin-binding growth factors in breast cancer cells. Cancer Res. 2001; 61(14): 5562–9.
15. Lund ME, Campbell DH, Walsh BJ. The role of glypican-1 in the tumour micro-environment. In: Advances in Experimental Medicine and Biology. 2020. p. 163–76.
16. Lu H, Niu F, Liu F, Gao J, Sun Y, Zhao X. Elevated glypican-1 expression is associated with an unfavorable prognosis in pancreatic ductal adenocarcinoma. Cancer Med. 2017; 6(6):1181–91.
17. Hara H, Takahashi T, Serada S, Fujimoto M, Ohkawara T, Nakatsuka R, et al. Overexpression of glypican-1 implicates poor prognosis and their chemoresistance in oesophageal squamous cell carcinoma. Br J Cancer. 2016; 115(1):66–75.
18. Saito T, Sugiyama K, Hama S, Yamasaki F, Takayasu T, Nosaka R, et al. High Expression of Glypican-1 Predicts Dis-semination and Poor Prognosis in Glioblastomas. World Neurosurg.2017; 105: 282–8.
19. Russell PJ, Ow KT, Tam PN, Juarez J, Kingsley EA, Qu CF, et al. Immuno-histochemical characterisation of the monoclonal antibody BLCA-38 for the detection of prostate cancer. Cancer Immunol Immunother. 2004; 53(11):995–1004.
20. Kato D, Yaguchi T, Iwata T, Katoh Y, Morii K. GPC1 specific CAR-T cells eradicate established solid tumor without adverse effects and synergize with anti-PD-1 Ab. Elife. 2020; 9: e49392.
21. Harada E, Serada S, Fujimoto M, Takahashi Y, Takahashi T, Hara H, et al. Glypican-1 targeted antibody-based therapy induces preclinical antitumor activity against esophageal squamous cell carcinoma. Oncotarget. 2017; 8(15):24741–52.
22. Yeh MC, Tse BWC, Fletcher NL, Houston ZH, Lund M, Volpert M, et al. Targeted beta therapy of prostate cancer with ^{177}Lu-labelled Miltuximab® antibody against glypican-1 (GPC-1). EJNMMI Res. 2020; 10(1):46.
23. Russell PJ, Davis K, Kingsley E, Humphreys J, Hanley J, O'Grady H, et al. Preclinical studies of monoclonal antibodies for intravesical radioimmunotherapy of human bladder cancer. Cell Biophys. 1994; 24–25(1–3):155–61.
24. Li Y, Song E, Rizvi SMA, Power CA, Beretov J, Raja C, et al. Inhibition of micrometastatic prostate cancer cell spread in animal models by 213Bi-labeled multiple targeted α radioimmunoconjugates. Clin Cancer Res. 2009; 15(3):865–75.
25. Lightfoot D V., Walker KK, Boniface GR, Hetherington EL, Izard ME, Russell PJ. Dosimetric and therapeutic studies in nude mice xenograft models with 153Samarium-labelled monoclonal antibody, BLCA-38. Antib Immunoconjugates Radio-pharm. 1991; 4:319–30.
26. Walker KZ, Russell PJ. WO 90/14433 Monoclonal Antibodies Which Recognise Malignant Cells from Bladder Carcinomas. European Patent; WO 90/14433, 1990. p. 1–76.
27. Carter T, Sterling-Levis K, Ow K, Doughty L, Hattarki M, Shapira D, et al. Biodistributions of intact monoclonal antibodies and fragments of BLCA-38, a new prostate cancer directed antibody. Cancer Immunol Immunother. 2004; 53(6):533–42.
28. Bander NH, Milowsky MI, Nanus DM, Kostakoglu L, Vallabhajosula S, Goldsmith SJ. PhaseI Trial of 177 Lutetium-Labeled J591, a Monoclonal Antibody to Prostate-Specific Membrane Antigen, in Patients with Androgen-Independent Prostate Cancer. J Clin Oncol. 2005; 23(21):4591–601.
29. Tagawa ST, Milowsky MI, Morris M, Vallab-hajosula S, Christos P, Akhtar NH, et al. Phase II study of lutetium-177-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for metastatic castration-resistant prostate cancer. Clin Cancer Res. 2013; 19(18):5182–91.
30. Vallabhajosula S, Nikolopoulou A, Jhanwar YS, Kaur G, Tagawa ST, Nanus DM, et al. Radioimmunotherapy of Metastatic Prostate Cancer with ^{177} Lu-DOTA- huJ591 Anti Prostate Specific Membrane Antigen Specific Monoclonal Antibody. Curr Radio-pharm. 2016; 9(1):44–53.
31. Bailey DL, Sabanathan D, Aslani A, Campbell DH, Walsh BJ, Lengkeek NiA. RetroSPECT: Gallium-67 as a Long-Lived Imaging Agent for Theranostics. Asia Ocean J Nuc Med Biol. 2021; 9(1): 1-8.
32. Morris MJ, Divgi CR, Pandit-Taskar N, Batraki M, Warren N, Nacca A, et al. Pilot trial of unlabeled and indium-111-labeled anti-prostate-specific membrane antigen

antibody J591 for castrate metastatic prostate cancer. Clin Cancer Res. 2005; 11(20):7454–61.
33. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: The second-generation personal computer software for internal dose assessment in nuclear medicine. J Nucl Med. 2005; 46(6):1023–7.
34. ARSAC. Notes for Guidance on the Clinical Administration of Radiopharmaceuticals and Use of Sealed Radioactive Sources. Public Heal Engl. 2018.
35. Matsuzaki S, Serada S, Hiramatsu K, Nojima S, Matsuzaki S, Ueda Y, et al. Anti-glypican-1 antibody-drug conjugate exhibits potent preclinical antitumor activity against glypican-1 positive uterine cervical cancer. Int J Cancer. 2018; 142(5):1056–66.
36. O'Donoghue JA, Lewis JS, Pandit-Taskar N, Fleming SE, Schöder H, Larson SM, et al. Pharmacokinetics, biodistribution, and radiation dosimetry for ^{89}Zr-trastuzumab in patients with esophagogastric cancer. J Nucl Med. 2018; 59(1):161–6.
37. Pandit-taskar N, O'Donoghue JA, Beylergil V, Lyashchenko S, Ruan S, Solomon SB, et al. ^{89}Zr-huJ591 immuno-PET imaging in patients with advanced metastatic prostate cancer. Eur J Nucl Med Mol Imaging. 2014; 41(11):2093–105.
38. Information for workers .https://www.arpansa.gov.au/our-services/monitoring/australian-national-radiation-dose-register/information-for-workers
39. Baum RP, Hertel A, Lorenz M, Schwarz A, Encke A, Hör G. 99Tcm-labelled anti-CEA monoclonal antibody for tumour immune-scintigraphy: First clinical results. Nucl Med Commun. 1989; 10(5):345–52.
40. Wong JYC, Chu DZ, Williams LE, Liu A, Zhan J, Yamauchi DM, et al. A phase I trial of 90Y-DOTA-anti-CEA chimeric T84.66 (cT84.66) radioimmunotherapy in patients with metastatic CEA-producing malignancies. Cancer Biother Radiopharm. 2006; 21(2): 88–100.
41. Makris NE, Boellaard R, Van Lingen A, Lammertsma AA, Van Dongen GAMS, Verheul HM, et al. PET/CT-derived whole-body and bone marrow dosimetry of ^{89}Zr-Cetuximab. J Nucl Med. 2015; 56(2):249–54 .
42. Pandit-Taskar N, O'Donoghue JA, Morris MJ, Wills EA, Schwartz LH, Gonen M, et al. Antibody Mass Escalation Study in Patients with Castration-Resistant Prostate Cancer Using 111In-J591: Lesion Detectability and Dosimetric Projections for 90Y Radioimmunotherapy. J Nucl Med. 2008; 49(7):1066–74.
43. Tagawa ST, Vallabhajosula S, Christos PJ, Jhanwar YS, Batra JS, Lam L, et al. Phase 1/2 study of fractionated dose lutetium-177–labeled anti–prostate-specific membrane antigen monoclonal antibody J591 (^{177}Lu-J591) for metastatic castration-resistant prostate cancer. Cancer. 2019; 125(15): 2561–2569.
44. Pandit-Taskar N, O'Donoghue JA, Durack JC, Lyashchenko SK, Cheal SM, Beylergil V, et al. A Phase I/II Study for Analytic Validation of ^{89}Zr-J591 ImmunoPET as a Molecular Imaging Agent for Metastatic Prostate Cancer. Clin Cancer Res. 2015; 21(23): 5277–85.
45. Campbell D, Sabanathan D, Gurney H, Gillatt D, Trifunovic M, Poursoultan P, et al. Outcomes of the Miltuximab first in human trial and proposed study design for a Phase I trial ^{89}Zr/ ^{177}Lu theranostic trial. J Clin Oncol. 2019; 37: 261–261.
46. Saito T, Sugiyama K, Hama S, Yamasaki F, Takayasu T, Nosaka R, et al. High Expression of Glypican-1 Predicts Dissemination and Poor Prognosis in Glioblastomas. World Neurosurg. 2017; 105: 282–8.