STATISTICAL ANALYSIS PLAN

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A confirmatory, prospective, open-label, multi-centre phase 3 study to evaluate diagnostic performance of ^{89}Zirconium-labelled girentuximab(^{89}Zr-TLX250) to non-invasively detect clear cell renal cell carcinoma (ccRCC) by positron emission tomography/CT (PET/CT) imaging in patients with indeterminate renal masses (ZIRCON study)

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Sponsor (for full data extraction and analysis)

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Table of Contents

1 LIST OF ABBREVIATIONS

Abbreviations	Description of abbreviations
^{89}Zr	Zirconium-89
AE	Adverse Event
CAIX	Carbonic Anhydrase IX
ccRCC	Clear Cell Renal cell carcinoma
CT	Computed tomography
DFO	Desferoxamine
DMP	Data Management Plan
ECG	Electrocardiogram
FAS	Full Analysis Set
FDA	US Food and Drug Administration
FN	False Negative
FP	False Positive
GFR	Glomerular filtration rate
HACA	Human anti-chimeric antibodies
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee
IRM	Indeterminate renal masses
MRI	Magnetic resonance imaging
MedDRA	Medical Dictionary for Regulatory Activities
NPV	Negative predictive value
PET	Positron-emission tomography
PPV	Positive predictive value
PT	Preferred term
ROC	Receiver operating characteristics

SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SOC	System Organ Classification
STD	Standard Deviation
SUV	Standard Uptake Value
TN	True negative
TP	True positive
WHO-ATC	World Health Organization ATC Hierarchy

2 INTRODUCTION

This Statistical Analysis Plan (SAP) is a detailed and technical description of statistical procedures to be employed in the analysis of ^{89}Zr-TLX250 for PET/CT imaging of ccRCC study. The SAP has its origins well-grounded in the detailed study protocol and is produced in line with ICH-E9 guidelines. This document shall describe the study variables, outcomes, hypotheses and statistical methodology to be used for analysis (ICH,1998).

Data analysis and statistical computing will be conducted using the statistical software SAS version 9.4 or higher.

3 OBJECTIVES OF THE STUDY

3.1 Primary Objective

The primary objective of this study will be:

To evaluate sensitivity and specificity of qualitative assessment of PET/CT imaging with ^{89}Zr-TLX250 to non-invasively detect ccRCC in patients with indeterminate renal masses, using histology as standard of truth.

3.2 Secondary Objectives

Key secondary objectives of this study will be:

1. To determine sensitivity of ^{89}Zr-TLX250 PET/CT imaging to detect ccRCC in the subgroup of patients with indeterminate renal masses of ≤ 4 cm in largest diameter (cT1a)
2. To determine specificity of ^{89}Zr-TLX250 PET/CT imaging to detect ccRCC in the subgroup of patients with indeterminate renal masses of ≤ 4 cm in largest diameter (cT1a)
3. Other Secondary objectives of this study will be:To determine positive predictive value (PPV), negative predictive value (NPV), and accuracy of ^{89}Zr-TLX250 PET/CT imaging to detect ccRCC in patients with indeterminate solid renal masses, and in patients with indeterminate renal masses of ≤ 4 cm (cT1a)
4. To identify a standardized uptake value (SUV) cut-off for ^{89}ZrTLX250, suitable to discriminate ccRCC from non-ccRCC
5. To determine inter-reader variability of diagnostic assessments of ^{89}Zr-TLX250 PET/CT images, when performed by multiple readers
6. To determine intra-reader variability of diagnostic assessment of ^{89}Zr-TLX250 PET/CT images
7. To establish safety and tolerability of ^{89}Zr-TLX250 in patients with indeterminate renal masses.
8. To evaluate sensitivity and specificity of ^{89}Zr-TLX250 PET/CT imaging to detect ccRCC in patients with IRMs ≤ 3 cm, IRMs ≤ 2 cm, and Bosniak 3 and 4 lesions.

3.3 Exploratory Objectives

Exploratory objectives will be:

- To quantitatively estimate achievable tumour uptake, retention and radiation absorbed doses (Gy) from therapeutic ^{177}Lu-girentuximab, based on single time point ^{89}Zr-TLX250 PET/CT images, a general model of average girentuximab tumour kinetics, derived from serial ^{89}Zr-girentuximab imaging, and an assumed specific activity of ^{177}Lu-girentuximab.

- To evaluate the correlation between ^{89}Zr-TLX250 SUVs and degree of histological CAIX expression

- To evaluate the distant masses outside the kidney identified on 89Zr-TLX250 whole body PET/CT in patients who present with unexpected evidence of disseminated disease

4 ENDPOINTS OF THE STUDY

4.1 Co-Primary Endpoint

The co-primary endpoints of this study are the sensitivity and specificity of ^{89}Zr-TLX250 PET/CT imaging to detect ccRCC in patients with IRM of tumour stage cT1, i.e. ≤ 7 cm in largest diameter and scheduled nephrectomy. PET/CT scans will be determined as true positive (TP) and true negative (TN) by visual reading among 3 independent central readers and comparison to histopathological diagnosis for ccRCC. Image data analyses will be performed by a central image core lab. Qualitative visual analysis (presence or absence of localised ^{89}Zr-TLX250 uptake inside or in vicinity of lesions, as seen on contrast-enhanced pre-baseline (pre-BL) imaging), will be used to assess test performance of ^{89}Zr-TLX250 PET/CT imaging to non-invasively detect ccRCC, using histological results from the central histological reference laboratory as standard of truth.

4.2 Secondary Endpoints

Key secondary endpoints include the determination of the study drug's potential to detect ccRCC in IRMs ≤ 4 cm in largest diameter (tumour stage cT1a), and secondary endpoints include determination of predictive values (standard uptake values (SUVs)) of PET signals in the kidney in CT1 and cT1a patients. Moreover, the reproducibility of the qualitative visual assessment of tumour targeting will be determined and safety and tolerability will be assessed.

Reading for the co-primary endpoints (sensitivity and specificity) will be conducted independently by three trained central readers, blind to history, and histology results. Inter-reader concordance will be assessed using kappa statistics. Similarly, kappa statistics will be used to assess intra-reader variability, using a subset of 10% of randomly selected cases read twice by individual readers.

^{89}Zr-TLX250 SUVs will be determined for each tumour lesion. Receiver operating characteristics (ROC) will be analysed to identify a SUV cut-off value most appropriate to discriminate between ccRCC or non-ccRCC as evidenced by central histology results.

^{89}Zr-TLX250 activity and antibody mass concentrations in tumour at the time of imaging (MBq/cm³, mg/cm³) will be determined, considering the absolute activity bound to the tumour, lesion volume as determined by CT/MRI, and the decay-corrected specific activity, using a general model of average girentuximab kinetics in tumour over time (derived from sequential ^{89}ZrTLX250 biodistribution data from earlier studies).

A sub-group analysis will evaluate sensitivity and specificity of ^{89}Zr-TLX250 PET/CT imaging to detect ccRCC in patients with IRMs ≤ 3 cm, IRMs ≤ 2 cm, and Bosniak 3 and 4 lesions.

5 STUDY DESIGN AND SCHEDULE OF ASSESSMENTS

5.1 Study Design

This will be a confirmatory, prospective, open-label, multi-centre phase 3 study (ZIRCON) in adult patients with pre-study imaging evidence of indeterminate renal masses (IRM) of ≤ 7 cm in largest diameter, and scheduled for partial or total nephrectomy as part of their standard of care. The study is designed to evaluate sensitivity and specificity of ^{89}Zr-TLX250 PET/CT imaging to non-invasively detect clear cell renal cell cancer (ccRCC).

Histological confirmation will serve as standard of truth, to assess sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), as well as accuracy of ^{89}Zr-TLX250 PET/CT imaging. Consequently, this study will be conducted in patients with IRM of stage cT1, in which histological material is obtained as part of routine nephrectomy, circumventing the need for study-specific invasive procedures. Given a reported high rate of non-diagnostic kidney biopsies, patients scheduled to undergo kidney biopsy only, rather than nephrectomy are not eligible for this study.

Approximately 252 adult patients were originally planned to be recruited in 35 – 40 renal cancer care specialist centres in Europe (Belgium, France, Spain, the Netherlands), UK, Turkey, Australia, Canada and USA, who have access to state-of-the-art PET/CT imaging. The number of enrolled patients was increased to 300 by a protocol version 8 amendment 7 to ensure adequate precision in measuring both sensitivity and specificity of ^{89}Zr-TLX250PET/CT imaging.

5.2 Schedule of Assessments

The schedule of assessments for the study comprised eight study visits as shown in Table 1:.

Table 1: Schedule of Assessments

^{89}Zr-girentuximab (^{89}Zr-TLX-250) for PET/CT Imaging of ccRCC (ZIRCON Study)

	Pre-screening	Screening	Study Period								Final Study Visit	
Examination/Evaluation			Treatment					Imaging	Surgery	Post-imaging Study Visit	If surgery within 28 days p.a.	If surgery 28 to 90 days p.a.
Time point	Pre-BL	BL	0									
Days	-90 to -1	-30 to -1	0					5±2**	90***	42±7	42±7	35±7 post surgery
Hours*			Pre-dose	dosing	0.5	1	2					
Abdo MRI with contrast or equivalent imaging[A]	x											
Informed consent		x										
Review Inclusion/Exclusion criteria		x										
General												
Medical history / Interim history		x	x									
Physical Exam		x									x	x
Vital Signs		x	x		x	x	x			x		x
Haematology & Serum Chemistry[H]		x	x					x			x	x
Urinalysis (Dipstick)[H]		x	x					x			x	x
Pregnancy Test[B]		x	x							x		
12 lead ECG		x	x				x					
^{89}Zr - girentuximab PET/CT imaging												
^{89}Zr girentuximab administration				x								
Abdominal PET/CT[C]								x				
Whole Body PET/CT[D]								x[D]				
Standard of truth determination												
Pre-scheduled nephrectomy (SoC)[E]								←	→ x			
Central histology								←	→ x			
Safety												
Concomitant medications		x	x ←					→ x	x	x		x
Baseline findings / adverse events		x	x ←					→ x	x	x		x
Anti-drug antibody / HACA blood draw[G]		x								x		x

*) hours post administration (p.a.)
**) not sooner than 72 hours p.a.
***) any time after imaging visit, but no later than 90 days p.a.

(A) Evidence of an indeterminate renal mass (IRM). MRI or equivalent standard of care imaging with contrast agent from within 3 months (90 days) of D0 is acceptable; ultrasound is not acceptable.
(B) Pregnancy test for pre-menopausal female patients at baseline, and re-test with urine pregnancy test pre-dose (within 24 hours before dosing) and on day 42 ±7.
(C) Abdominal PET + low dose CT to be conducted for all patients
(D) In case of unexpected evidence of disseminated disease (N1, M1) on imaging, patients may receive an additional whole body PET/CT from skull base to mid-thigh for complete staging at the descretion of the investigator.
(E) Standard of care (SoC): partial or total nephrectomy, open or laparoscopic, as locally established
(F) Histology to characterise ccRCC and degree of CAIX expression
(G) HACA: Human anti-chimeric antibody
(H) Safety laboratory up to 10 days before surgery including day of surgery

6 PATIENT SELECTION

It is planned to have 300 adult patients with an indeterminate renal mass (IRM) on standard of care imaging, obtained at pre-study, and who are clinically suspicious for renal cell carcinoma and scheduled for partial or total nephrectomy as part of their regular diagnostic work-up/clinical care.

6.1 Eligibility Criteria

Inclusion Criteria:

All patients must meet all of the following criteria:

1. Written and voluntarily given informed consent
2. Male or female ≥ 18 years of age
3. Imaging evidence of a single indeterminate renal mass of ≤ 7 cm in largest diameter (tumour stage cT1), on standard of care imaging based on national standards, not older than 90 days on Day 0, but performed before any screening procedure.
4. Scheduled for lesion resection as part of regular diagnostic work-up within 90 days from planned IV ^{89}Zr-TLX250 administration.
5. Negative serum pregnancy tests in female patients of childbearing potential at screening. Confirmation of negative pregnancy test result from urine within 24 hours prior to receiving investigational product.
6. For patients included in France only, verification and confirmation of their affiliation with a social security.
7. Sufficient life expectancy to justify nephrectomy.
8. Consent to practise highly effective contraception until a minimum of 42 days after IV ^{89}Zr-TLX250 administration.

Exclusion Criteria:

A patient will be excluded from participation in the trial if one or more of the following criteria are met:

1. A biopsy procedure only (rather than partial or total nephrectomy) planned for histological species delineation of IRM.
2. Renal mass known to be a metastasis of another primary tumour.
3. Active non-renal malignancy requiring therapy during the time frame of the study participation.

4. Chemotherapy, radiotherapy, or immunotherapy within 4 weeks prior to the planned administration of ^{89}Zr -TLX250 or continuing adverse effects (> grade 1) from such therapy (Common Terminology Criteria for Adverse Events [CTCAE] version 5.0).

5. Planned antineoplastic therapies (for the period between IV administration of ^{89}Zr-TLX250 and imaging).

6. Exposure to murine or chimeric antibodies within the last 5 years.

7. Previous administration of any radionuclide within 10 half-lives of the same.

8. Serious non-malignant disease (e.g. psychiatric, infectious, autoimmune or metabolic), that may interfere with the objectives of the study or with the safety or compliance of the subject, as judged by the investigator

9. Mental impairment that may compromise the ability to give informed consent and comply with the requirements of the study

10. Exposure to any experimental diagnostic or therapeutic drug within 30 days from the date of planned administration of ^{89}Zr-TLX250

11. Women who are pregnant or breastfeeding.

12. Known hypersensitivity to girentuximab or DFO (desferoxamine)

13. Renal insufficiency with GFR ≤ 45 mL/min/ 1.73 m²

14. Vulnerable patients (e.g. being in detention)

6.2 Treatment Groups

All patients who successfully meet the inclusion criteria will be given the study drug ^{89}Zr-TLX250 followed by PET/CT imaging to noninvasively detect clear cell renal cell cancer (ccRCC). Histological confirmation will be done on all patients and serve as standard of truth, to assess sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), as well as accuracy of ^{89}Zr-TLX250 PET/CT imaging.

Patients who do not have an evaluable PET/CT imaging post study drug administration and do not have a confirmed histopathology diagnosis are defined as drop-outs. To ensure credibility in the assessment of sensitivity and specificity, drop-out patients will be replaced to achieve the desired sample size and power for the primary analysis.

7 SAMPLE SIZE ESTIMATION

The study sample size for the population of patients with indeterminate renal mass (i.e., patients with cT1 tumors) has 2 components: Sensitivity and Specificity. Sample size is estimated for Sensitivity and Specificity, respectively, and the larger of the two estimates will represent the final study sample size. The basic assumptions underlying sample size estimation are the following:

- In the population of patients with indeterminate renal mass (i.e. cT1 patient population), approximately 70% are cT1a and 30% are cT1b patients.
- In the cT1a subpopulation, approximately a third (34%) are expected to show no signs of renal cell carcinoma following histopathology assessment (i.e. ccRCC negative).
- It is expected from a clinical standpoint that the subpopulation of cT1b patients would show a much lower rate of negative histopathologies than the cT1a subpopulation. For the purpose of powering this trial, it is assumed that the rate of non-ccRCC histopathologies in the cT1b subpopulation would be at least 17%, half the rate in the cT1a subpopulation.

Sensitivity: to ensure the study has 90% power to show that the lower limit of the 2-sided 95% confidence interval (Wilson) for sensitivity is above the critical limit (or non-inferiority limit) of 70%, the minimum sample size required for the population of cT1 patients under above assumptions is 125, when assuming a true sensitivity of 83%.

Specificity: to ensure the study has 90% power to show that the lower limit of the 2-sided 95% confidence interval (Wilson) for the specificity is above the critical limit (or non-inferiority limit) of 68% the minimum sample size required for the population of cT1 patients under above assumptions is 252, when assuming a true specificity of 83%.

An estimated total of 252 patients with an IRM of ≤ 7 cm and scheduled partial or radical nephrectomy will be included in the study. However, in order to maintain adequate study power, this number may be increased depending on the proportion of non-ccRCC patients after surgery and histological verification.

Following comments from FDA and considering the actual numbers of patients with cT1a tumours enrolled into the trial either, the total sample size was increased to allow a minimum number of ccRCC negative patients to ensure sufficient statistical power for the key secondary endpoint. The statistical sample size calculation triggered a slight change to the definition of the statistical power adaptation of patient recruitment.

Table 2 provides an example of the expected sample size required with different observed non-ccRCC rates.

Table 2: Planned sample size estimates*

	Outcome	Null	Alt.	Non-ccRCC Rate in CT1a Group	Non-ccRCC Rate in CT1b Group	Alpha	Power	N Total
Scenario (1)	Specificity	0.68	0.83	0.34	0.17	0.05	0.90	**252**
	Sensitivity	0.70	0.83	0.34	0.17	0.05	0.90	125
Scenario (2)	Specificity	0.68	0.83	0.25	0.20	0.05	0.90	**284**
	Sensitivity	0.70	0.83	0.25	0.20	0.05	0.90	116
Scenario (3)	Specificity	0.68	0.83	0.25	0.15	0.05	0.90	**317**
	Sensitivity	0.70	0.83	0.25	0.15	0.05	0.90	114

* Sample size estimates to achieve 90% power based on rates of non-ccRCC in both CT1a and CT1b groups. Scenario (1) represents the originally planned sample size; while Scenario (2) and Scenario (3) provide potential sample size updates based on different non-ccRCC rates.

Note that both sensitivity and specificity will be determined for each of three independent readers. The study will be successful if if the lower bounds of the 95% confidence intervals (CIs) for sensitivity (>70%) and specificity (>68%) in at least the same 2 out of 3 independent readers.

7.1 Independent Data Monitoring Committee

An independent data monitoring committee (IDMC) will be established by the sponsor and will monitor the number of evaluable patients cT1a and cT1b tumors, and with ccRCC^{positive} ccRCC^{negative} tumors and give feedback to the sponsor on whether the planned sample size is appropriate or not.

The IDMC will evaluate patient accrual and histological results at 50% (126) and 75% (189) of evaluable cases. Counts of evaluable cases (excluding cases where ^{89}Zr-TLX250 PET/CT imaging and/or tissue collection were either not performed or are pending) in 4 categories based on initial tumour size (cT1a, cT1b) and IRM histology (ccRCC, non-ccRCC). The IDMC will provide feedback to the sponsor of whether the proposed sample size will be likely to be sufficient or not. Based upon this feedback the sponsor may consider an amendment to the protocol that could modify inclusion criteria to include only cT1a patients subsequently or to increase the sample size to ensure sufficient power.

Enrolment will not be halted during the data review procedure.

The IDMC members will not review ^{89}Zr-TLX250 PET/CT images or imaging data, nor will they have access to the results generated by either the independent image readers or the local reader. The committee will be composed of at least three members, specialised in biostatistics, pathology and/or oncology. The committee will have the above described responsibility only that will be detailed in an IDMC charter.

8 ANALYSIS SETS

The following analysis sets will be used for the **^{89}Zr-TLX250-003** data analyses. All data to be analysed must be from patients who have provided informed consent.

8.1 Full Analysis Set

The Full Analysis Set (FAS) will consist of all enrolled patients who have evaluable PET/CT imaging and have a confirmed histopathology diagnosis.

8.2 Safety Analysis Set

The Safety Analysis Set (SAF) will consist of all patients who received ^{89}Zr-TLX250.

8.3 Screening Analysis Set

The Screening Analysis Set (for CONSORT-style accounting of subject disposition) will consist of all patients assigned a Patient Number.

All primary and secondary efficacy analyses will be carried out using the FAS.

8.4 Intent to Treat Analysis Set

The set of entered subjects includes all subjects who were enrolled, regardless of whether or not the subject took any study drug (intention to treat (ITT) population).

9 ANALYSIS VARIABLES

9.1 Co-primary Variables

The primary study objective is to evaluate the diagnostic efficacy in all patients by comparing the sensitivity and specificity of 89Zr-TLX250 PET/CT imaging to thresholds of 0.70 (sensitivity) and 0.68

(specificity), using histology as standard of truth. The co-primary variables of this study will be the sensitivity and specificity of ^{89}Zr-TLX250 PET/CT imaging to detect ccRCC.

a) Sensitivity is defined as the proportion of study patients with a true positive (TP) ^{89}Zr-TLX250 PET/CT scan (detection of clear cell carcinoma), relative to those with positive histopathological diagnosis for ccRCC:

Sensitivity (%) = [TP / (TP + FN)] x 100

b) Specificity is defined as the proportion of study patients with a true negative (TN) ^{89}Zr-TLX250 PET/CT imaging result (no ccRCC), relative to those with a negative histopathological diagnosis of non-ccRCC (histological result:= TN + FP):

Specificity (%) = [TN / (TN + FP)] x 100

The sensitivity and specificity of ^{89}Zr-TLX250 PET/CT imaging will be compared separately to their respective prespecified thresholds. The comparison of sensitivity/specificity data will be based on data from the following 2x2 tables (Table 3), which summarises the results of the qualitative ^{89}Zr-TLX250 tumour targeting (tracer uptake in target lesion: yes/no) as determined by visual reading and the central study pathology as standard of truth.

Table 3: Probability Calculated on All Cases with Positive Histopathology

		Reference Standard Histopathology (standard of Truth)		
		positive (ccRCC)	**negative (no ccRCC)**	**Total**
Outcome ^{89}Zr-TLX250 PET/CT scan	**positive (ccRCC)**	TP	FP	# of cases with positive PET/CT: TP+FP
	negative (no ccRCC)	FN	TN	# of cases with negative PET/CT:FN+TN
total		# of cases with ccRCC: TP+FN	# of cases without ccRCC: FP+TN	N

Abbreviations: FN = false negative; FP = false positive; TN = true negative; TP = true positive;

The point estimate of sensitivity of PET/CT is TP/(TP+FN) and the point estimate of specificity is TN/(TN+FP). Wilson's binomial (score) confidence intervals will be used to compare the 95% confidence interval lower boundary of each quantity to the threshold of 0.70 (sensitivity) and 0.68 (specificity).

Note that both sensitivity and specificity will be determined for each of three independent readers. The study will be successful if the lower bounds of the 95% confidence intervals (CIs) for sensitivity (>70%) and specificity (>68%) in at least the same 2 out of 3 independent readers

9.2 Secondary Variables and Exploratory Analyses

To investigate diagnostic performance in the subgroup of patients with small lesions (cT1a, dimension ≤ 4 cm in longest diameter), sensitivity and specificity with corresponding Wilson (score) 95% CIs will be calculated for such patients.

In addition to the primary efficacy parameters (sensitivity, specificity), the positive predictive value, the negative predictive value and the accuracy will also be calculated as secondary efficacy parameters using data from patients in the subgroup of tumours ≤ 4 cm in largest diameter. Sensitivity and specificity in the cT1a group will be the key secondary endpoints; while positive predictive value, the negative predictive value and the accuracy in the cT1a group with be additional secondary endpoints.

9.2.1 Predictive Values and Accuracy

a) Positive predictive value (PPV)
The positive predictive value is defined as the probability that a positive histopathology diagnosis was obtained given that the result of the ^{89}Zr-TLX PET/CT scan is positive (detection of ccRCC).

$$PPV\ (\%) = [TP / (TP + FP)] \times 100$$

b) Negative predictive value (PPV)
The negative predictive value is defined as the probability that a negative histopathology diagnosis was obtained given that the result of the ^{89}Zr-TLX250 PET/CT scan is negative (no detection of ccRCC).

$$NPV(\%) = [TN / (TN + FN)] \times 100$$

c) Accuracy
The accuracy or probability of a correct test result is defined as the probability that the ^{89}Zr-TLX250 PET/CT scan result is correct, i.e., the probability that the PET/CT scan is positive if the histopathological diagnosis for ccRCC is positive or that the PET/CT scan is negative if the histopathological diagnosis for ccRCC is negative.

$$Accuracy\ (\%) = [TP + TN / (TP + FP + TN + FN)] \times 100$$

9.2.2 Standardised Uptake Value (SUV)

^{89}Zr-TLX250 SUVs will be determined for each tumour lesion. Receiver operating characteristics (ROC) will be analysed, to identify a SUV cut-off value, most appropriate to discriminate between ccRCC or non-ccRCC as evidenced by central histology results.

$$SUV = C_{image} / (\text{injected dose} / \text{body weight})$$

9.2.3 Inter-reader Variability

Fleiss´ kappa statistics will be used to determine the agreement between the qualitative visual assessment of ^{89}Zr-TLX250 tumour targeting (tracer uptake in target lesion: yes/no), as assessed by three independent blinded readers. An intra-class kappa of 0.70 or higher will be considered as an acceptable value. Note that both sensitivity and specificity will be determined for each of three independent readers. The study will be successful if the lower bounds of the 95% confidence intervals (CIs) for sensitivity (>70%) and specificity (>68%) in at least the same 2 out of 3 independent readers

9.2.4 Intra-reader Variability

Intra-reader variability will be assessed by having each of the 3 independent blinded readers perform 2 image evaluations on a randomly selected set of 10% cases. A washout period will be introduced between the two interpretations to minimise recall bias. The percent of agreement between the two interpretations will be computed for each reader. Cohen´s kappa statistics will be used to determine the reproducibility of the qualitative visual assessment by individual readers when analysing the same data repeatedly.

9.2.5 Sub group analysis

To evaluate sensitivity and specificity of ^{89}Zr-TLX250 PET/CT imaging to detect ccRCC in patients with IRMs ≤ 3 cm, IRMs ≤ 2 cm, and Bosniak 3 and 4 lesions.

1. Bosniak 3 and 4 lesions (as determined by the independent central readers)

2. Indeterminate renal masses ≤ 3 cm in largest diameter (as determined by the independent central readers)

3. Indeterminate renal masses ≤ 2 cm in largest diameter (as determined by the independent central readers)

9.2.6 Safety

a) General safety parameters:

Frequency of occurrence and severity of abnormal findings in safety investigations (physical examination, vital signs, 12-lead ECG, clinical laboratory, urinalysis, adverse events, concomitant medication)

b) HACA testing

9.2.7 Exploratory Variables

In situ biological half-life of girentuximab protein in tumour lesions will be estimated, using a general model of average girentuximab kinetics in tumour tissue, derived from serial ^{89}Zr-TLX250 imaging. Based on (a) protein residence times in the lesions, (b) an assumed specific activity of therapeutic labelling with Lutetium-177 (^{177}Lu), and (c) the measured tumour lesion volume (CT dimensions), an achievable therapeutic absorbed dose to tumour (Gy), will be determined for several specific activities and hypothetical activity doses of ^{177}Lu.

Exploratory analyses will include Extra-renal lesions used as a histology standard of truth (when N1 or M1 disseminated disease is suspected) along with investigations correlating the ^{89}Zr-TLX250 tumour lesion SUVs, semi-quantitation of CAIX expression as determined by immunohistochemisty, and evaluation of the distant masses outside the kidney identified on ^{89}Zr-TLX250 whole body PET/CT in patients with unexpected evidence of disseminated disease will be conducted.

9.3 Population Characteristics

Population characteristics will include:

- Clinical suspicion of ccRCC due to imaging evidence of indeterminate renal mass of ≤ 7 cm (stage cT1)
- Scheduled for total or partial nephrectomy as part of regular diagnostic work-up at a given date

Summary tables and listings will be provided for demographics and other baseline characteristics, including:

- Age, ethnic origin, sex, physical exam
- Medical/surgical history and baseline findings
- Prior and concomitant medication

10 STATISTICAL METHODOLOGY

10.1 General Considerations

The aim of this study is to evaluate sensitivity, specificity, PPV, NPV, as well as accuracy of ^{89}Zr-TLX250 PET/CT imaging using a 37 MBq (1 mCi) activity dose to detect ccRCC in patients with IRM of stage cT1, and who are scheduled for partial or total nephrectomy.

Histological confirmation from nephrectomy (or biopsies of extrarenal lesions from patients in whom unexpected evidence of disseminated disease is observed) will serve as standard of truth. The qualitative assessment of the 3 independent readers will be the primary imaging result.

All data will be listed and trial summary tables will be provided. Descriptive statistics will be presented.

For continuous variables, descriptive statistics will include the number of patients (N), mean, standard deviation (STD), median, first and third quartiles, minimum (MIN) and maximum (MAX) by analysis group and, if applicable, by activity administration and/or time.

Frequencies and percentages will be displayed for categorical data by analysis group and, if applicable, by dose administration and/or time. Percentages by categories will be based on the number of patients with no missing data. Percentages will be rounded off to one decimal place.

The statistical evaluation will be performed by using the SAS software package (version 9.4, or higher).

10.2 Study Population

10.2.1 Disposition of Patients

The following patient data will be summarised:

- Number and percentage of patients enrolled in each analysis set;
- Number and percentage of patients who completed the study or prematurely discontinued from the investigational period by reasons for discontinuation, to be tabulated for each analysis set.
- Patients who are enrolled, but are then either not administered with study drug, or who do not undergo PET/CT imaging after administration of study drug, in whom PET/CT images can not be analysed due to technical failure, or in whom surgical material can not be assessed for presence or absence of ccRCC, are defined as drop-outs. Drop-out patients will be replaced for the primary analysis and study sample size. Missing data is not expected for efficacy outcomes which involve the standard of truth (i.e., sensitivity, specificity, PPV, NPV, accuracy, inter-reader and intra-reader variability

10.2.2 Protocol Deviations

Major protocol deviations include, but are not limited to the following:

- Wrong study medication administered (e.g., wrong activity, etc.);
- Deviation from any inclusion criteria having an influence on the efficacy outcomes;
- Missing ^{89}Zr-TLX250 PET/CT imaging or histopathological diagnosis;
- Administration of prohibited concomitant medication as described in the study protocol

All major protocol deviations will be provided in a listing.

10.2.3 Demographic and Other Baseline Characteristics

Descriptive statistics for age, gender and race at study entry will be presented. Basic statistics for age will be calculated, tabulated and presented by gender. Frequency tabulations for gender and race will be presented.

10.2.4 Previous and Concomitant Medications

Previous and concomitant medications will be coded with World Health Organization ATC Hierarchy list (WHO-ATC). Concomitant medications are those medications or therapies taken after starting study drug administration during investigational period. Patients taking the same medication multiple times will be counted once within “previous” and once within “concomitant”.

All previous and concomitant medications data will be provided in a listing.

10.2.5 Medical, Surgical and Oncology History

All medical, surgical and oncology history data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). It will be used to summarise disease terms /surgical events by primary system organ class (SOC) and preferred term (PT) and provided in a listing. More details on MedDRA coding are found in the Data Management Plan (DMP).

10.2.6 PET/CT Scan

All image evaluations performed on PET/CT will be provided in tables and listings per reader.

10.3 Study Drugs

Drug exposure and administration data will be provided in a table and listing.

10.4 Analysis of Safety and Tolerability

10.4.1 Adverse Events

The coding dictionary for this study will be Medical Dictionary for Regulatory Activities (MedDRA). It will be used to summarise AEs by primary system organ class (SOC) and preferred term (PT).

A summary table will be provided for the overview of AEs by grade (severity), and overall. The number and percentage of patients with AEs, as classified by primary SOC and PT, as well as the number and percentage of patients with at least one AE, will be summarised by analysis group and overall. Similar summaries will also be provided for AEs related to study drug, serious AEs, AEs leading to study discontinuation and for expected and unexpected AEs. A drug-related AE is defined as any AE with a possible or related to study treatment as assessed by the investigator or with missing assessment of the causal relationship. AEs will be presented as baseline findings and Treatment emergent AEs.

All AEs will also be displayed in listings including their onset and duration.

10.4.2 Laboratory results

Haematology, Biochemistry, Clotting parameters, Urinalysis and Immunogenicity data will be provided in a listing and summarised in tables. Clinical significance will be presented as a flag on the laboratory listings. Change from baseline will be calculated and statistical significance will be tested using Wilcoxon signed-rank test. Figures to show laboratory results trend over time will be provided.

10.4.3 ECG results

ECG results data will be provided in a listing and summarized in a table by time point. Clinical significance will be presented as a flag on the ECG listings and summary table will be provided.

10.5 Analysis of Efficacy

10.5.1 Primary and Key Secondary Variables (Sensitivity and Specificity)

Within this trial, two co-primary endpoints (sensitivity and specificity) shall be analysed for each reader.

The analysis for sensitivity uses only those patients with a positive histopathological diagnosis of clear cell renal cell carcinoma. These are expected to be about 70% of the included patient population. Sensitivity of ^{89}Zr-TLX250 will be considered adequate if the **lower boundary of a 95% Wilson (score) confidence interval for sensitivity is higher than 0.7** for at least the same two of three independent readers.

The analysis for specificity uses only those patients with a negative histopathological diagnosis of clear cell carcinoma. These are expected to be about 30% of the included patient population. Specificity of ^{89}Zr-TLX250 will be considered adequate if **the lower boundary of a 95% Wilson (score) confidence interval for specificity is higher than 0.68** for at least the same two of three independent readers. Two-sided tests will be consistently used in the evaluation.

To account for multiplicity and to control Type I error under the paradigm of two co-primary endpoints sensitivity and specificity will each be estimated at the 5% significance level. For the study to be deemed successful, the lower limit of the 95% Wilson confidence interval for both sensitivity and specifity must exceed their respective pre-specified thresholds.

Assuming the null hypothesis is rejected for both co-primary endpoints, formal statistical testing will proceed to the family of Key Secondary Endpoints (i.e., sensitivity and specificity in the cT1a group). Within this family, the fixed-sequence method will first assess sensitivity, followed by specificity. Results pertaining to specificity will only be considered formal evidence if the null hypothesis pertaining to sensitivity is rejected.

10.5.2 Standard Uptake value (SUV)

Standard uptake value will be analysed as described in section 9.2.2 for each reader to determine 3 cut off values and corresponding sensitivities and specificities. SUV data will be provided in tables and listings.

10.5.3 Predictive values and accuracy

Secondary efficacy will be analysed using positive predictive values, negative predictive values and accuracy for each of the three readers. Computation of the values is shown in the section 9.2.1

10.5.4 Imaging analysis

Imaging will be analysed in terms of inter reader variability and intra reader variability based on Fleiss´ kappa statistics and Cohen’s kappa statistics respectively. If normality assumption is not met or there is zero agreement in either negative or positive result by a reader or readers, Brennan-Prediger adjustment will be applied.

10.6 Interim Analysis

No interim analysis will be conducted for this study.

10.7 Handling of Missing Data, Outliers and Visit Windows

10.7.1 Missing Data

Missing test results or assessments will not be imputed because missing data is not expected for efficacy outcomes which involve the standard of truth (Section 5.2).

If the start or stop date (or time) for AEs and concomitant medication is incomplete or missing, then a worst-case scenario will be applied for the allocation of an AE or medication. The corresponding listings will always show the original date and time information.

10.7.2 Outliers

In general, outliers should not be excluded purely on the basis of their extreme values, but rather for specified reasons. Such data may be excluded from the analysis at the discretion of the sponsor.

10.7.3 Visit Windows

In general, analyses will not exclude patient data simply due to the patient's failure to comply with the scheduled time/visit. The responsible investigators will decide whether, and to which nominal time points, to include off-window measures, if any, for descriptive summaries of the data.

11 TABLES, LISTINGS AND FIGURES

11.1 Tables

Tables will be produced for all analyses carried out on variables of the study. Names of tables will be provided above the table. Tables will be named according to the contents as prescribed by ICH guidelines.

11.2 Listings

Data listings will be provided and numbered in accordance to appropriate regulations.

11.3 Figures

Figures will be provided where it is necessary.

12 REFERENCES

ICH Harmonised Tripartite Guideline E 3. Structure and Content of Clinical Study Reports, November 1995. (www.ich.org; Guidelines; "Efficacy" Topics)

ICH Harmonised Tripartite Guideline E 9. Statistical Principles for Clinical Trials, February 1998. (www.ich.org; Guidelines; "Efficacy" Topics)

13 SIGNATURES

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