Institut de Cancérologie de l'Ouest
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Short Title :	**OPALESCENCE**
Ref ICO :	ICO-2020-25
N° EudraCT :	2020-003805-71

OPALESCENCE

zircOn PET-CT imAging TLX mEtaStatiC triplE Negative CancEr

"Prospective feasibility pilot study, assessing imaging performance of ^{89}Zirconium-labelled Girentuximab (^{89}Zr-TLX250) PET-CT in metastatic triple negative breast cancer patients"

N° EUDRACT:	2020-003805-71
Ref ICO:	ICO-2020-25
Study type:	RIPH – cat.1 (Médicament)

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APPROVAL AND PROTOCOL SIGNATURE

SPONSOR REPRESENTATIVE

The sponsor undertakes to ensure that this study is conducted in accordance with this protocol, and all applicable legislation.		
SPONSOR ***Raphaëlle Charron-Bighetti*** *Deputy Director of Research* Institut de Cancérologie de l'Ouest (ICO) Institut de Cancérologie de l'Ouest unicancer PAYS DE LA LOIRE	**Date:**	**Signature:**

COORDINATING INVESTIGATOR FOR STUDY

I have read all the pages of the clinical trial protocol of which Institut de Cancérologie de l'Ouest is the sponsor. I confirm that it contains all the information required to conduct the trial. I undertake to carry out the study in accordance with the protocol and the terms and conditions defined therein. . I undertake to conduct the trial in accordance with :

- La Déclaration d'Helsinki de l'AMM,
- Les ICH Guidelines for Good Clinical Practices (E6)
- Le Code de la Santé Publique,
- La Loi n°2004-806 du 9 août 2004 relative à la politique de santé publique portant notamment transposition de la directive européenne n°2001/20/CE du 4 avril 2001 et la loi n°2006-450 du 18 avril 2006
- La Loi Jardé n°2012-300 du 12 mars 2012 (décret d'application du 16 novembre 2016) concernant la recherche sur la personne humaine)
- La Loi n°2011-814 du 7 juillet 2011 relative à la bioéthique,
- La Loi n° 78-17 du 6 janvier 1978 relative à l'informatique aux fichiers et aux libertés, modifiée notamment par la Loi n° 204-801 du 6 août 2004 et la loi n°2018-493 du 20 juin 2018, dans sa version en vigueur au moment de la réalisation de l'Essai.
- Le Règlement (UE) n°2016/679 du 27 avril 2016 relatif à la protection des données personnelles (RGPD).

I also undertake to ensure that investigators and other qualified members of my team have access to copies of this protocol and the trial conduct documents to enable them to work in accordance with the protocol guidelines.

Investigator Coordinator	**Name:** **Dr Caroline ROUSSEAU**	**Date:**	**Signature:**
Principal Investigator	**Name and centre:**	**Date:**	**Signature:**

CLINICAL TRIAL AUTHORIZATION

PROTOCOL OPALESCENCE

"Prospective feasibility pilot study, assessing imaging performance of *^{89}Zirconium-labelled Girentuximab (^{89}Zr-TLX250)* PET-CT in metastatic triple negative breast cancer patients"

CLINICAL TRIAL AUTHORIZATION	
Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM):	**Date of authorization: 12/03/2021**
	Ref. ANSM (IDRCB) : MEDAECPP-2021-01-00009_2020-003805-71
Ethics committee : **CPP SUD EST VI (CLERMONT-FERRAND)**	**Date of approval: 23/04/2021**
	Ref. CPP: Ref. CPP: 21.01.13.57453-AU_1682

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Enrolment	Enrolment is made online, directly via the eCRF. In case of problem, please write to the following email addresses, indicating the protocol name in the subject line of the email: promotionRC@ico.unicancer.fr and datamanagement@ico.unicancer.fr
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PROTOCOL SYNOPSIS

The purpose of this study is to evaluate the use of ^{89}Zr-labeled girentuximab (^{89}Zr-TLX250) as a novel, carbonic anhydrase IX (CAIX) targeted PET/CT tracer for the imaging of metastatic triple negative breast cancer (TNBC) patients.

TNBC patients are known to be rapidly progressive and have a poor prognosis. This poor prognosis is due to the lack of common breast cancer targets in TNBC. As TNBC expresses CAIX, we plan to evaluate CAIX targeting by using a radiolabeled monoclonal antibody that recognizes carbonic anhydrase IX (CAIX) (^{89}Zr-girentuximab otherwise known as ^{89}Zr-TLX250). Previous and ongoing studies have demonstrated the potential application of ^{89}Zr-TLX250 as a new PET/CT imaging tracer for the detection of renal cancer.

After establishing the TNBC targeting properties of the ^{89}Zr-TLX250 PET/CT imaging tracer, it should be interesting to develop a new targeted therapy using TLX250- radiolabeled with a therapeutic radionuclide such as 177Lutétium.

ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
^{18}F	Fluor-18
^{124}I	Iodine-124
^{111}In	Indium-111
^{177}Lu	Lutetium-177
^{89}Zr	Zirconium-89
ADL	Activities of Daily Living
AE	Adverse event
ALAT	Alanine aminotransferase
ANSM	French Health Authority
ASAT	Aspartate aminotransferase
ATZ	Acetazolamide
B-HCG	Beta human chorionic gonadotropin
BP	Blood Pressure
CA IX	Carbonic anhydrase IX
CA 15-3	Cancer antigen 15-3
CEA	carcinoembryonic antigen
CPP	French Ethics Committee
CT	Computed tomography
DSUR	Development Safety Update Reports
eCRF	Electronic case report form
EMA	European Medicines Agency
ER	Estrogen Receptor
EOS	End Of Study
EVCTM	EudraVigilance Clinical Trial Module
FDG	Fluorodésoxyglucose
FU	Follow up
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GLP	Good Laboratory Practice
HACA	Human Anti-Chimeric Antibodies
HER 2	human Epidermal growth factor Receptor 2
HIF1α	Hypoxia-Inducible Factor 1-alpha
HSA	Human Serum Albumin
ICF	Informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IHC	ImmunoHistoChemistry
IV	Intravenous(ly)
mAb	Monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
MBq	Mega Becquerel
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events

Abbreviation or special term	Explanation
PD-L1	Programmed death-ligand 1
PET	Positron-emission tomography
PK	Pharmacokinetic(s)
PR	Progesterone Receptor
PTX	Paclitaxel
SAE	Serious adverse event
SID	Subject identification
SUSAR	Suspected unexpected serious adverse reaction
SUV	Standardized uptake value
TEAE	Treatment-emergent adverse event
TNBC	Triple Negative Breast Cancer
UAE	Unexpected Adverse Event
WB	Whole Body
WHO	World Health Organization

HISTORY OF PROTOCOLE AMENDMENTS

	Version number / Date (after amendment)	**Justifications of amendment**
Initial authorisation	V1.2 du 29/03/2021	Initial authorized version
Substantial Amendment 1	V2 du 21/06/2022	18-month extension of the recruitment period Inclusion criteria 4 modified

TRIAL CALENDAR

NUMBER OF EXPECTED PATIENTS	**12 patients**
DURATION OF ENROLMENT PERIOD :	**30 months**
DURATION OF PROCEDURE / DURATION OF FOLLOW-UP :	**5 days / 3 months**
MAXIMUM TIME OF PARTICIPATION PER PATIENT :	**4 months (including approximately 1 month for the inclusion assessment)**
GLOBAL DURATION OF THE TRIAL :	**33 months**
END OF THE CLINICAL TRIAL :	**Last visit of the last enrolled patient**

CONSIDERED DURATION UNTIL THE ANALYSIS OF THE PRIMARY OBJECTIVE :	**45 months (33 + 12 months data analysis)**
INTERIM ANALYSIS :	**NA**

SCHEDULE OF STUDY ASSESSMENTS

Visit	Screening	^{89}Zr- Girentuximab PET/CT					FU # 1	End of Study
Day	**-30 to -1**	**0**	**1**	**3**	**5[f]**	**7**	**30**	**90**
Written informed consent	X							
Formal verification of eligibility criteria	X							
Medical and disease history	X							
Enrolment	X							
Physical Exam								
Physical examination, Weight	X							
WHO performance status	X						X	
Vital signs (temperature, respiratory rate, blood pressure, pulse)	X							
Laboratory Exam								
Haematology and Serum chemistry [a]	X				X			
Tumor Biomarkers: CEA, CA 15-3	X							
Pregnancy test [e]	X							
Imaging								
FDG PET/CT scan with contrast agent injection	X							X [g]
CT scan								X[g]
^{89}Zr-Girentuximab PET/CT Imaging								
^{89}Zr-Girentuximab administration		X						
Whole body PET/CT imaging					X			
Ancillary study : ^{89}Zr-Girentuximab biodistribution								
^{89}Zr-Girentuximab imaging Biodistribution [c]		X	X	X	X	X		
^{89}Zr-Girentuximab Blood pharmacokinetics [d]		X	X	X	X	X		
Histological Analysis								
New or archival Tumor Histology	X							
Safety								
Baseline findings / AE / SAE assessments	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X
Antidrug Antibody HACA blood sample	X							X
Vital signs [b]		X						

(a) Blood count, creatinine clearance, glucose, bilirubin, ALAT, ASAT, Alkaline Phosphatases, Gamma GT.

(b) Vital signs to be measured and monitored before ^{89}Zr-Girentuximab administration and within 2 hours after administration (0,5 ; 1 and 2 hours)

(c) whole body PET-CT imaging at D0 (dynamic imaging during ^{89}Zr-Girentuximab administration), D1, D3 and D7

(d) Pharmacokinetic blood samples to be taken before injection and at D0 after dynamic imaging, D1, D3, D5 and D7

(e) A serum β-HCG pregnancy test will be performed only in premenopausal women at screening. Pregnancy test from urine (for premenopausal female patients) will be performed within 72 hours before ^{89}Zr-Girentuximab administration

(f) The Whole body PET/CT imaging could be done at D5 ± 2

(g) CT scan or FDG PET/CT according to standard of care (with or without contrast agent injection)

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The study sponsor, the Institut de Cancérologie de l'Ouest (ICO), declares that the "**OPALESCENCE**" clinical trial will be conducted in accordance with the protocol, the French Public Health Code (articles 1121-1 *et seq)*, and the document Good Clinical Practice of November 24, 2006.

1. INTRODUCTION

1.1 Disease background

Triple-negative breast cancer (TNBC) is associated with a high risk of recurrence and generally a bad prognosis. More than one-third of patients with TNBC will present distant metastases during the course of their disease. Although chemotherapy has been the main treatment option for metastatic TNBC for a long time, this scenario has changed recently with the results of immunotherapy in patients with PD-L1-positive tumors. In patients with PD-L1-positive or negative tumors, a single agent chemotherapy metastatic first-line treatment regimen containing taxanes with or without bevacizumab could be used.

The treatment of TNBC is constantly evolving. To improve metastatic TNBC treatment outcomes, patients are encouraged to enroll in ongoing trials which are evaluating new targeted agents, immunotherapy and predictive biomarkers (1,2).

Since Warburg introduced the so-called Warburg effect, characterized by unregulated glucose uptake and disruption of glycolytic metabolism in cancer cells, dysregulated energy metabolism has become known as an important part of the pathogenesis of uncontrolled growth in cancer cells (3). TNBC have generally been shown to have an exacerbated glycolytic metabolism when compared to non-TNBC (4,5).

Recent insight on regulation of glycolysis suggests hypoxia-inducible factor 1-alpha (HIF1α) as a key regulator that enables tumor cells to survive by altering glucose metabolism (6). HIF1α is controlled by oxygen levels and is stabilized under hypoxic conditions. Stabilized HIF1α activates transcription of several genes implicated in glycolytic cancer metabolism, by increasing glucose transporter expression. This accelerates influx of glucose into tumor cells, and subsequent glycolysis results in tumor microenvironment acidosis (7). In order to overcome acidity, these microenvironmental stresses activate transcription of compensatory genes including the gene encoding carbonic anhydrase IX (CAIX), a member of metalloenzyme family that is a major downstream target of HIF1α (3,8,9).
Based on these well characterized metabolic pathways in cancer cells and the tumor microenvironment, we propose to focus on the targeting of CAIX in TNBC.

1.2 Procedure background/non-clinical and clinical experience

CAIX is normally expressed in intestinal and stomach mucosa, gallbladder and testis (10), but is upregulated in several forms of cancer, including breast cancer. Indeed, CAIX is a marker for hypoxic

regions of breast tumors (9,11) and is associated with poor prognosis (12–14) and high-grade, ER-negative breast tumors (15). Its role in tumor growth and disease progression has been attributed to its ability to reduce pericellular pH in response to hypoxia (15–17), creating a toxic environment for normal cells (18). Acidic environments also facilitate the breakdown of the tumoral extracellular matrix (19) which can enhance metastatic activity (20,21).

Several studies have identified altered HIF1α and CAIX protein expression levels to be related to poor prognosis in breast cancer (14,22–26). CAIX is expressed in 7 % of an unselected breast cancer cohort and in 25 % of TNBCs (27). CAIX expression rates increased with tumor grade ($p < 0.001$) and associations with tumor size were significant for CAIX ($p < 0.001$; positive association). Although based on only a few studies, CAIX showed profound lower expression rates in normal breast tissue and benign breast disease ($p < 0.001$), and high rates in carcinoma in situ (28). The expression levels of HIF1α and CAIX vary depending on the intrinsic subtype, and they are increased in TNBC compared to other subtypes (22).

Three studies have investigated HIF1α and glucose metabolism for alternative therapeutic options to overcome breast cancer resistance to adjuvant therapeutic modalities (24,29,30). More recently, in early TNBC, combined HIF1α and CAIX protein expression may serve as an unfavorable prognostic indicator, particularly in patients receiving a cyclophosphamide-based chemotherapy or radiotherapy as well as those with basal phenotype of breast cancer (31).

CAIX has recently emerged as a very attractive therapeutic target due to the following features:

1. CAIX shows a tissue-restricted pattern and low expression levels in normal tissues, while it is induced in a broad range of cancers (32);

2. It is located on the surface of cells (33);

3. Its overexpression in cancers confers a survival advantage in hypoxia and facilitates cell migration and metastasis (34).

Depletion of CAIX in cancer cell lines has been shown to attenuate the growth of tumor xenografts and inhibit the formation of spontaneous metastasis (35). Interestingly, CAIX was found to contribute to cell migration and invasion by facilitating formation of focal adhesions, ion-transport and pH control at the leading edge of lamellipodia of moving cells (36). Currently, substantial effort is devoted to the development of potent, cancer-selective CAIX inhibitors, including monoclonal antibodies (37). Moreover, recently, Chafe et al., suggested that targeting CAIX in the tumor microenvironment in combination with immune-checkpoint blockade is a potential therapeutic strategy for enhancing response and survival in patients with hypoxic solid malignancies (38). Another team developed a CAIX-targeted, human serum albumin (HSA) drug delivery vehicle which contained the potent anticancer drug, Paclitaxel (PTX). They used Acetazolamide (ATZ), a small molecule ligand of CAIX to selectively deliver HSA-PTX to TNBC cells (39).

1.3 Rationale for conducting this study

Metastatic TNBC, due to its lack of usual BC target expression, its poor prognosis and the expression of CAIX in 25% of cases, seems to be an interesting clinical indication for studying CAIX targeting

using a radiolabeled a monoclonal antibody that recognizes carbonic anhydrase IX (^{89}Zr-Girentuximab or otherwise known as ^{89}Zr-TLX250).

^{89}Zr-Girentuximab is under clinical development as a PET/CT imaging tracer for the detection of renal tumor masses and metastases (which overexpress CAIX). When the anti-CAIX antibody, girentuximab is labelled with a PET radionuclide such as ^{89}Zirconium, it can be used as an imaging probe for PET. After intravenous administration of this imaging agent, the tumor, lymph nodes and distant metastases can be non-invasively assessed using whole-body PET imaging. There is extensive clinical evidence demonstrating the ability of girentuximab-radiolabeled with PET radionuclides (Immuno-PET) to target CAIX lesions in patients with different type of solid tumors (37,40–42)

While there is a lack of expression of common BC targets in TNBC, it is well known that 25% of TNBCs express CAIX, therefore there is a strong rationale for using a radiolabeled CAIX-targeting monoclonal antibody to detect and image TNBCs. At present, FDG PET is routinely used to image breast cancer and we propose to compare the detection sensitivity of ^{89}Zr-Girentuximab with FDG PET. In addition, we would like to assess the biodistribution and dosimetry of the radiopharmaceutical in metastatic TNBC patients with the objective of defining any possible future theranostic applications.

1.4 Benefit/risk and ethical assessment

1.4.1 Individual benefit

There is no individual benefit to the patients included in the study.

1.4.2 Collective benefit

It is therefore important to identify specific molecular targets that can be used for targeted cancer therapy, thereby limiting the progression and metastasis of this invasive tumor and improving patient outcomes.

Although a challenge, the development of a targeted therapy for the treatment of TNBC is greatly needed and as it is essential that these patients gain access improved treatment regimens.

CAIX, a cell surface antigen expressed in TNBC with a low expression level in normal tissues seems, thereby highlighting its potential value as a therapeutic target. girentuximab is a monoclonal antibody that recognizes CAIX. The extensive clinical experience with radiolabeled girentuximab has provided strong clinical evidence to support its use as a targeted PET imaging agent.

The initial objective of this study is to evaluate the biodistribution of ^{89}Zr-girentuximab using PET-CT (immuno-PET) imaging, which will allow us to show that CAIX could be a promising target and that the immuno-PET imaging tracer could act as an effective companion imaging diagnostic when screening patients for future therapies.

A secondary objective, is to provide preliminary clinical data that after screening patients with CAIX immuno-PET, the girentuximab has the potential to be radiolabeled with a therapeutic radionuclide such as Lutetium-177 (^{177}Lu) to treat TNBC metastases in future clinical trials.

1.4.3 Overall risks

In previous clinical studies, administration of Zirconium-89 (^{89}Zr) labeled monoclonal antibodies was proven to be safe.

Although never observed in previous trials, allergic-type reactions are possible during and immediately following the administration of girentuximab. In a total of more than 2000 administrations of radiolabeled murine and chimeric girentuximab to humans, no allergic-type reaction was observed.

In clinical studies administration of (^{89}Zr) labelled monoclonal antibodies were proven to be safe (40,43).

Patients will be carefully monitored after ^{89}Zr-Girentuximab administration.

1.4.4 Benefit / risk balance

There is more than 20 years of experience in humans with SPECT/CT imaging using girentuximab labelled with various radioligands including ^{124}I and ^{111}In. These studies investigated the pharmacokinetics, toxicity, immunogenicity, and imaging characteristics of radiolabelled girentuximab in kidney cancer patients.

In a clinical phase 1 study investigating safety, tolerability, imaging characteristics, biodistribution and dosimetry, ^{89}Zr-TLX250 was found to be safe, and to provide improved imaging characteristics, compared to ^{124}I-girentuximab, in line with preclinical findings (44). The clinical development of ^{89}Zr-Girentuximab has found the optimal dose of radioactivity to 37 MBq ± 10% of ^{89}Zr with a total dose of girentuximab of 10 mg.

Preliminary safety data in the ZIRDOSE study using ^{89}Zr-girentuximab, administered ^{89}Zr-TLX250 to 5 patients at 5 mg and 10 mg girentuximab mass dose, each, did not prompt any safety concern. In the course of the study, no treatment-related SAEs or SUSARs were reported from any of the 10 patients. A total of 7 AEs were experienced in 4 patients. An unlikely AE or no relationship to treatment was assessed by the investigator for 6 AEs. Mild nausea in one patient was considered by the investigator as possibly related to the study treatment.

In previous ^{89}Zr-TLX250 clinical studies, all patients had a whole body PET/CT scan. The CT scans were a low-dose CT for the purpose of attenuation correction of the PET data. Because acquisition parameters for the low-dose CT were restricted, the radiation dose from the low-dose CT was approx. 3 mSv.

Radiation dosimetry was evaluated in a bridging dosimetry study (ZIRDOSE study) investigating the safety and tolerability of ^{89}Zr-girentuximab. The whole body effective dose for administration of ^{89}Zr-girentuximab in this phase 1 study was 0.487 ± 0.014 mSv/MBq using the most widely

established dose calculation tool OLINDA 1.1 and the ICRP 60 standard (45). The absorbed/effective dose was calculated accordingly and resulted in a total whole body effective dose of 18.0 ± 0.5 mSv for 37 MBq. When a newer software IDAC-Dose 2.1 and the ICRP 103 standard (46) is used, the resulting effective dose will be 0.551 ± 0.030 mSv/MBq or 20.5 ± 1.1 mSv for 37 MBq.

The combined whole body effective dose of ^{89}Zr-girentuximab administration and low-dose CT is expected to be below 20 mSv based on the accepted standard OLINDA 1.1 and ICRP 60, or below 23 mSv based on IDAC-Dose 2.1 and ICRP 103, for all patients. The lifetime attributable risk for incidence of radiation-induced cancer for an effective dose of 23 mSv is 0.4% (ICRP 103, 2007), which is comparable to the risk from a diagnostic CT of chest, abdomen and pelvis (47).

Given the well-known safety and tolerability of radiolabelled girentuximab, confirmed for ^{89}Zr-TLX250 in the context of a phase I safety study, and further considering the low antibody doses used in this study, no harmful effects exceeding the risk of established diagnostic imaging procedures are to be expected. The results of this study may contribute to the development of a new targeted therapy for TNBC, it appears that the benefit/risk ratio will be clearly in favour of the benefit to the patient. Indeed, if the imaging can clinically substantiate a potential new imaging and therapeutic target, this imaging agent could become a "companion imaging diagnostic" used to screen patients that have a higher likelihood of responding to a CAIX-targeted therapeutic radiolabeleld with ^{177}Lu. Although, highly unlikely, ^{89}Zr-TLX250 may cause allergic-type reactions. The study sites are well equipped to treat allergic/anaphylactic reactions.

2. OBJECTIVES

2.1 Primary objective(s) and endpoint

2.1.1 Primary objective

To assess the concordance for tumor lesion detection using ^{89}Zr-TLX250 PET/CT versus a conventional ^{18}FDG PET/CT scan where comparison will be made on a per lesion analysis basis.

2.1.2 Endpoints of primary objective

Concordance study of metastatic uptake seen in ^{18}FDG PET/CT scan and ^{89}Zr-TLX250 PET/CT scan per "lesion" by comparing for each lesion the CT scan, ^{18}FDG PET scan and ^{89}Zr-TLX250 PET/CT scan by assessing a ratio "Number of positive or negative ^{89}Zr-TLX250 lesions / Number of positive or negative FDG lesions" and "Number of positive or negative ^{89}Zr-TLX250 lesions / Number of CT scan lesions".

2.2 Secondary objective(s) and endpoint

2.2.1 Secondary objectives

1. To determine the percent of total tumor burden (whole body) detected on ^{89}Zr-TLX250 PET/CT scan compared to that defined on ^{18}FDG PET/CT used as the reference.
2. To assess the correlation between the normalized uptake values (SUV) of ^{89}Zr-TLX250 and CAIX histological expression if a biopsy is done.
3. To confirm the perfect safety and tolerability of ^{89}Zr-TLX250 and assess the generation of human anti-chimeric antibodies in response to the girentuximab.

2.2.2 Endpoints of secondary objectives

1. Percent of positive CA IX metastatic tumor burden compared to total metastatic tumor burden by ^{18}FDG (ratio "Number of positive ^{89}Zr-TLX250 lesions / Number of positive FDG lesions")
2. If a metastasis biopsy is conducted, assessment of the correlation between the normalized uptake values (SUV) of ^{89}Zr-TLX250 positive lesions and CAIX histological expression will be done by comparing the ^{89}Zr-TLX250 semi-quantitative data with the immunohistochemical results (IHC) of biopsied metastases.
3. The safety and tolerability of ^{89}Zr-TLX250 will be evaluated by measuring and monitoring vital signs within 2 hours after injection of the radiopharmaceutical. The patient will be informed that in the event of abnormal physical signs, occurring within 24 hours after ^{89}Zr-TLX250 administration, he must report them to the investigator for

registration. The reporting period of AEs (related to ^{89}Zr-TLX250) and SAEs cover a period of 30 days after ^{89}Zr-TLX250 administration.

⇒ The NCI Common Toxicity Criteria, version 5.0 reference will be used.

⇒ HACA (Human anti-chimeric antibody) will be assessed.

2.3 Exploratory objective(s)

2.3.1 Exploratory objectives

For 3 to 5 patients, assessment of the absorbed doses received by organ and in whole body after ^{89}Zr-TLX250 administration for imaging purpose in metastatic TNBC patients.

2.3.2 Endpoints of Exploratory objectives

For 3 to 5 patients, quantitative biodistribution of ^{89}Zr-TLX250 will be evaluated from sequential **whole body PET-CT imaging** and **pharmacokinetic data** (blood samples) taken at **D0** (before and after ^{89}Zr-TLX250 administration), **D1**, **D3**, **D5** and **D7**.

3. STUDY DESIGN

3.1 Overview of study design

The research presents the following characteristics:

- feasibility study
- Imaging Study
- Pilot trial
- Monocentric
- Non randomized
- Uncontrolled
- Open-label
- Prospective

3.2 Study design schedule

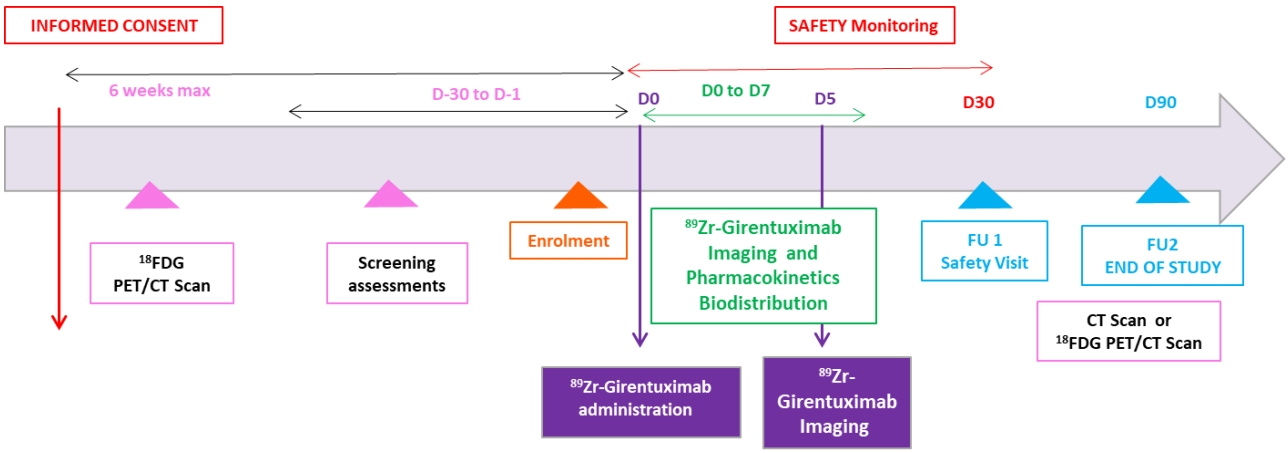

Figure 1: Study design schedule

3.3 Study discontinuation

The study could be interrupted or terminated by the sponsor in agreement with the coordinator and with the competent authorities for the following reason:

- frequency and/or unexpected severity of the toxicity,
- recruitment of patients too low,
- poor quality of the data collected,

4. PATIENT SELECTION

Each patient should meet all of the inclusion criteria and none of the non-inclusion criteria for this study. Under no circumstances can there be exceptions to this rule.

If they meet the inclusion and non-inclusion criteria, patients will be offered the research after discussion with a nuclear physician in charge of the study. The latter will present the study as well as the benefits and risks of the trial procedure. The patient will be given the necessary reflection time before signing the consent form.

4.1 Population description

Patients with Triple Negative Breast Cancer recurrence.

4.2 Inclusion criteria

Patients must meet all of the following inclusion criteria to be eligible for enrolment into the study:

1. Written informed consent obtained from the patient prior to performing any protocol-related procedures, including screening evaluations.
2. Female or male, Age ≥ 18 years at time of study entry.
3. Primitive triple negative breast cancer proven histologically, defined according to the following criteria:
   - ⇒ Estrogen receptors ≤10%.
   - ⇒ **And** progesterone receptors ≤10%.
   - ⇒ **And** HER2 not amplified or not overexpressed.
4. Metastatic Breast Cancer documented by conventional imaging and/or FDG PET/CT with at least one measurable metastatic lesion according to RECIST and/or PERCIST.
5. Consent to use a contraception method for at least 30 days after administration of ^{89}Zr-TLX250.
6. ECOG Performance Status 0 or 1.
7. Life expectancy at least 6 months.
8. Patient has valid health insurance.
9. Patient is willing and able to comply with the protocol for the duration of the study including undergoing treatment and scheduled visits and examinations including follow up.

4.3 Non-inclusion criteria

Patients should not enter the study if any of the following non-inclusion criteria are fulfilled:

1. History of another primary malignancy except for basal cell carcinoma within the last 5 years.
2. 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).

3. Planned antineoplastic therapies (for the period between IV administration of ^{89}Zr-TLX250 and imaging).
4. Exposure to murine or chimeric antibodies within the last 5 years.
5. Previous administration of any radionuclide within 10 half-lives of the same.
6. Impossibility to hold lying motionless at least 1 hour, or known claustrophobia.
7. 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.
8. Mental impairment that may compromise the ability to give informed consent and comply with the requirements of the study.
9. Pregnant or likely to be pregnant or nursing patient.
10. Known hypersensitivity to girentuximab or desferoxamine.
11. Renal insufficiency with GFR ≤ 45 mL/min/ 1.73 m².
12. Persons deprived of their liberty, under a measure of safeguard of justice, under guardianship or placed under the authority of a guardian.
13. Disorder precluding understanding of trial information or informed consent.

Participation to another interventional therapeutic clinical trial is not authorized.

Procedures for withdrawal of incorrectly enrolled patients are presented in Section 4.4.

If a patient withdraws from participation in the study, then his or her enrolment code cannot be reused.

4.4 Withdrawal of patients from study

4.4.1 Permanent discontinuation of procedure

Patients may be discontinued from study in the following situations:

- Patient decision.
- Investigator decision.
- Patient did not undergo ^{89}Zr-TLX250 PET/CT imaging study procedure.

4.4.2 Withdrawal of consent

Patients are free to withdraw from the study at any time (study procedure and assessments) without prejudice to their further medical care.

In case of withdrawal of consent, the patient's study generated data may be used until withdrawal of consent date, unless the patient has objected.

4.5 Patients replacement

A patient who is included into the study, but then fulfils any one of the following, will be considered a drop-out:

- Has not received IV ^{89}Zr-TLX250;
- Did not undergo ^{89}Zr-TLX250 PET/CT imaging after IV administration of study drug;
- Whose PET/CT images cannot be analysed due to technical failure;

Patients who dropped out of the study will be replaced. All patients who dropped-out after receiving IV ^{89}Zr-TLX250 will need to get all the examinations and blood draws as for the final study visit.

5. STUDY PROCEDURE ^{89}Zr-TLX250 PET/CT SCAN

5.1 Study drug ^{89}Zr-TLX250

5.1.1 Chemical Properties

^{89}Zr-TLX250, is a chimeric monoclonal antibody (INN name: Girentuximab) with specificity for the CAIX (carbonic anhydrase 9) antigen, radiolabelled with the positron emitting radio-metal zirconium-89. Girentuximab has a CAS number of 916138-87-9.
The chemical formula, without the $_{89}$Zr, is $C_{6460}H_{1006}N_{1718}O_{2018}S_{48}$ with a molecular mass of 146.5 kg/mol.

5.1.2 Pharmaceutical Properties

^{89}Zr-TLX250 is formulated as a solution for intravenous administration in glass vials at the nominal dosage strength 37 MBq (±10%) for single intravenous use. The ^{89}Zr-TLX250 drug product is manufactured as "ready-to-use". The composition of ^{89}Zr-TLX250 solution for IV administration includes the active pharmaceutical ingredient in a buffered solution without other excipients, as follows:

Active component:	37 MBq ^{89}Zr-TLX250
Batch volume:	≤ 10 mL
Mass dose girentuximab	10 mg
Other ingredients:	Girentuximab, ^{89}Zr-DFO-girentuximab
Solvent:	NaCl 0.9%
Appearance:	Clear, colourless to light yellow and free from visible particles
pH:	5.0 – 8.0
Radiochemical purity: (ITLC after purification)	^{89}Zr-TLX250 ≥ 90%
Radiochemical purity (SE-HPLC after purification)	^{89}Zr-DFO-girentuximab ≥ 90% ^{89}Zr / ^{89}Zr-DFO ≤ 10% Aggregates ≤ 5%
Identity:	Rtsample = Rtreference ± 10%
Sterility	No growth
Endotoxins	≤ 17.5 EU/mL
Expiry time	96 hours at 15°C - 30°C in bulk vial
Primary packaging	Type 1 glass vials

A complete record of batch numbers and expiry dates of all study medication will be maintained in the trial master file (TMF).

5.1.3 Storage and Handling

The product is to be shipped and stored at room temperature (15°C to 30°C) inside the lead-shielded container provided and protected from light.

The product must be handled within a hospital environment only, by an accredited radiopharmacist and/or nuclear medicine physician according to international and local radiation protection guidelines.

5.1.4 Packaging and Labelling

^{89}Zr-TLX250 solution for IV administration will be supplied in glass vials in appropriate packaging (lead-shielded containers bearing a radioactive warning symbol in accordance with radioactive pharmaceutical requirements). The labels of the packaging supplied by the sponsor will include the following information as a minimum:

- Name and address of sponsor
- Study number
- Name of study drug and formulation
- Dose strength
- Batch number
- Expiry date
- Storage instructions
- Radioactive warning symbol
- “For Clinical Trial Use only”.

All manufacturing, formulation and labelling will be done in accordance with applicable current GMP and local guidelines and laws.

Medication Numbering:

A system of medication numbering in accordance with all requirements of Good Manufacturing Practice (GMP) and any applicable regulatory requirement will be used for all study drugs. This will ensure that, for each patient, any dosing of study drug can be identified and traced back to the original bulk ware of the active ingredients.
Lists linking all numbering levels will be maintained by the institutions in charge of study drug packaging.

5.1.5 Drug Logistics and Accountability

5.1.5.1 Supply, Storage, Dispensation and Return

^{89}Zr-TLX250 solution for IV administration will be manufactured, handled and stored in accordance with GMP. ^{89}Zr-TLX250 contains radioactive material and should only be handled by personnel trained in the use of radioactive isotopes with proper shielding and monitoring. Receipt and use of ^{89}Zr-TLX250 is limited to institutions holding an appropriate handling permit by their competent national or regional authority.

All required documentation, e.g. written approval from the independent ethics committee (IEC)/institutional review board (IRB) and regulatory authority, as appropriate, needs to be provided before ordering for a site can take place. The dose order will be a direct order from the study site to the Sponsor who shall arrange appropriate supply of IMP (please refer to the IMP Handling Manual

for details). Upon establishment of patient eligibility (see Section 4), the clinical site manager will order individualized doses of ^{89}Zr-TLX250 solution for IV administration, via the Sponsor for direct delivery to the study site. A dose can be cancelled at any time however if the cancellation is less than 2 days prior to administration date then the site will need to follow IMP handling manual for appropriate disposal of product. ^{89}Zr-TLX250 for IV administration will be provided by Telix International Pty Ltd and used unchanged from the original state. The treating investigator at the site will delegate ordering of ^{89}Zr-TLX250 solution for IV administration to the clinical site manager, overseeing eligibility and planned treatment dates, for direct delivery to the site to the attention of the radiopharmacist.

The IMP will be shipped at room temperature (15°C to 30°C) inside an appropriately shielded container.

Upon receipt at site, ^{89}Zr-TLX250 solution for IV administration will be kept in a secure, temperature-controlled, restricted-access location and in accordance with applicable regulatory requirements at the radiopharmacy of the site. The IMP should be stored at ambient temperature (15°C to 30°C) without freezing, and should be used by the expiration date and time printed on the label.

^{89}Zr-TLX250 doses will be accompanied by an individual certificate of analysis for each batch. Upon verification of the correct radioactive dose, as specified by the study protocol, the radiopharmacist will hand over the investigational product in a syringe, kept in a lead-shielded container, to the nuclear medicine investigator, or a designated and suitably qualified deputy for administration. This syringe will be labelled by the radiopharmacist according to institutional standards.

Storage, handling and destruction must be performed according to local guidelines regarding radioactive waste management. Details are outlined in the IMP Handling Manual.

5.1.5.2 Drug Accountability

The radiopharmacist will confirm receipt of the study drug by e-mail or the method given in the IMP Handling Manual and will use the study drug only within the scope of this clinical study and in accordance with this study protocol. He / she will keep a record of the dispensed study drug.

Receipt, distribution and return of the study drug must be properly documented on the forms provided by the sponsor giving the following information: study protocol number, sender, receiver, date, mode of transport, quantity, batch number, expiration date and retest date, if applicable.

The sponsor will monitor the drug accountability records at regular points during the study and will perform drug reconciliation at the end of the study.

5.2 Study procedure: ^{89}Zr-TLX250 PET/CT Imaging

All imaging sequences are done on an outpatient basis. The patient does not have to be fasting. There is no premedication or other treatment before and after the ^{89}Zr-TLX250 administration.

5.2.1 Dosage and Administration

The mass dose of ^{89}Zr-TLX250 to be used in this study will be 10 mg, labelled with 37 MBq (±10%) ^{89}Zr per dose.

Each patient will receive a single slow intravenous (IV) administration over a minimum of 3 minutes on Day 0 (after pre-dose assessments), at the nuclear medicine service of the respective study site.
No dietary constrictions prior to dosing are necessary.
Prior to administration, an indwelling intravenous catheter has to be placed into the antecubital vein or an equivalent venous access. The radiopharmaceutical will be slowly administered through the indwelling catheter and followed with a saline flush. The syringe will be assayed prior and after administration to assess injected radioactive dose (MBq).
The dose that will be administered to the individual patient will be assessed using a dosing calibrator as outlined in the IMP Handling Manual.

5.2.2 ^{89}Zr-TLX250 PET/CT Imaging

On D5 (± 2) days post administration of ^{89}Zr-TLX250, a whole body PET/CT scan will be acquired from skull to mid-thigh using 6-8 bed positions with 10 minute acquisition time per bed position.

Ancillary imaging study:

For 5 patients, imaging quantitative biodistribution of ^{89}Zr-TLX250 will be evaluated from sequential whole body PET-CT imaging and pharmacokinetic blood samples at D0, D1, D3, D5 and D7 (see section 6.6).

5.2.3 Imaging Analysis

5.2.3.1 Qualitative ^{89}Zr-TLX250 Tumor Targeting

^{89}Zr-TLX250 tumor uptake will qualitatively be assessed, considering whether or not ^{89}Zr-TLX250 binding the target lesions determined on FDG PET-CT as metastatic lesions.

The interpretation of the ^{89}Zr-TLX250 PET/CT images as positive or negative for metastatic TNBC will be made on the basis of visual examination only.

The ^{89}Zr-TLX250 PET/CT images will be evaluated for evidence of radioactive uptake in the metastatic TNBC lesions. The local and central reviewers will designate the lesions as having a status of positive or negative.

The ^{89}Zr-girentuximab PET/CTs will be assessed in a central reviewing system to ensure true lesion detection and reproducible inter-observer agreement. All ^{89}Zr-girentuximab PET/CTs would be assessed by two expert nuclear physicians independently.

The first evaluation will be done by the nuclear physician, in charge of the patient during the ^{89}Zr-girentuximab PET/CT exam.

The second evaluation will be done by a second nuclear physician who should be blinded for evaluation of the first nuclear physician. However, for patient safety reasons, the nuclear physician should be allowed to communicate findings that required (local) interventions (e.g. brain metastases).

The two reports would be harmonized to one final report by the first reviewer. In case of different findings, a third nuclear physician should evaluate the ^{89}Zr- girentuximab PET/CT exam to reach a consensus.

A tumor lesion will be defined visually positive based on anatomical substrate on low-dose CT in combination with ^{18}FDG and/or ^{89}Zr-girentuximab-uptake.Quantification of positive lesions as defined by evaluation reports for ^{18}FDG and ^{89}Zr-girentuximab PET/CT will be performed by drawing regions-of-interest using dosisoft (PLANET Edition 3 – PLANET Onco Dose, Version 3.1.1.50 – 2021-01-03).

The maximum and mean standardized uptake values (SUV) will be calculated. SUVmax will be used for tumor tracer-uptake; SUVmean for measuring uptake in healthy organs and blood pool.

The lesion will be classified as PET-positive if:

- Radioactivity in the lesion is clearly visible (as described above), **AND**
- With a same location as FDG positive lesions described previously.

5.2.3.2 Semiquantitative ^{89}Zr-TLX250 Tumor Targeting

SUVs will be calculated for each tumor lesion, considering the measured tumor-bound activity, the injected activity dose, and the body weight and height of each patient. SUV values will be compared with the degree of CAIX expression determined by immunohistochemistry, using a 5-point scale.

5.3 Treatment Assignment

Once full establishment of eligibility by the site, the physician will confirm patient eligibility, the patient number will be allocated and ^{89}Zr-TLX250 imaging should be scheduled before the administration of the study drug.

Then, the authorized site representative for the study can order the study drug through the "^{89}Zr-TLX250 Order Form".

5.4 Blinding

Not applicable, this is an open-label study.

5.5 Treatment Compliance

^{89}Zr-TLX250 will be administered by study personnel at the site. Details of each administration will be recorded in the eCRF.

5.6 Radiation Precautions

Medical administration of radioactive diagnostic tracers such as ^{89}Zr-TLX250 is guided by national radiation safety regulations, differing extensively between countries.

Excretion limits acceptable for discharge will be defined by the investigators in compliance with the local regulations. Commonly, patients will be discharged from the hospital 2 hours p.a., unless the investigator decides otherwise.

Patients will be encouraged to increase fluid intake and to void frequently through the first day after administration.

The following safety precautions apply for patients:

- Patients should be advised to observe rigorous hygiene in order to avoid risk of contamination of others using the same toilet facility.
- A double toilet flush is recommended.
- Patients should wash their hands thoroughly every time after using the toilet.
- During the first week after treatment, patients should follow detailed instructions, as given in the trial informed consent form, regarding their distance from and contact to other persons (especially pregnant women and children)..

The following precautions apply for health care workers and for laboratory assessments:

Healthcare personnel are advised to limit the time of close contact with patients injected with (89Zr)-labelled radiopharmaceuticals. Use appropriate shielding on the day of administration. Laboratory assessments will be performed by the central laboratory. Because of the potential for radioactivity in some blood and urine samples, the site personnel must adhere to their SOPs and/or any guidance and regulations for handling radioactive substances. It is mandatory to use protective high quality (latex/nitrile) gloves in any direct contact with the radiopharmaceutical (vial/syringe) and with the patient.

5.7 Total Radiation Exposure

Radiation dosimetry was evaluated in a bridging dosimetry study (ZIRDOSE study) investigating the safety and tolerability of ^{89}Zr-girentuximab. The whole body effective dose for administration of ^{89}Zr-girentuximab in this phase 1 study was 0.487 ± 0.014 mSv/MBq using the most widely established dose calculation tool OLINDA 1.1 and the ICRP 60 standard (45). The absorbed/effective dose was calculated accordingly and resulted in a total whole body effective dose of 18.0 ± 0.5 mSv for 37 MBq. When a newer software IDAC-Dose 2.1 and the ICRP 103 standard (46) is used, the resulting effective dose will be 0.551 ± 0.030 mSv/MBq or 20.5 ± 1.1 mSv for 37 MBq.

5.8 Prior and Concomitant therapy

At baseline screening, all prior cancer-related treatments are to be recorded.
All other medications taken within 30 days before ^{89}Zr- Girentuximab PET/CT exam, and any medications prescribed for chronic or intermittent use during the study or dose adjustments of these medications must be recorded on the case report form.

5.8.1 Permitted concomitant therapy

Not applicable.

5.8.2 Prohibited concomitant therapy

Not applicable.

6. STUDY PROCEDURES: DESCRIPTION AND SCHEDULE

Before study entry, throughout the study, and following study drug discontinuation, various clinical and diagnostic laboratory evaluations are outlined. The purpose of obtaining these detailed measurements is to ensure adequate safety and tolerability assessments. Clinical evaluations and laboratory studies may be repeated more frequently if clinically indicated. The Schedules of Assessments during the screening and treatment period is provided following the Protocol.

6.1 Screening visit

Screening procedures will be performed up to 30 days before ^{89}Zr- Girentuximab PET/CT exam. All patients must first read, understand, and sign the ICF.

No trial-related procedures will be initiated before the signed consent has been obtained.

After signing the ICF, completing all screening procedures, and all eligibility criteria confirmed, patient will be enrolled in the study.

All procedures performed prior to the signing of the ICF and considered as standard of care may be used as screening assessments if they are performed within the 30 days prior to ^{89}Zr-TLX250 PET scan.

Except for radiological assessments which could be done within 6 weeks prior to ^{89}Zr-TLX250 PET scan and Tumor Biomarkers within 3 months prior to ^{89}Zr-TLX250 PET scan).

The following procedures will be performed during the Screening Visit:

- Informed Consent
- Subject registration and subject number assignment
- Review of eligibility criteria and confirm eligibility
- Demographics
- Record Medical History
- Record Disease History: Biopsy and surgery histopathological reports
- Record Breast Cancer-related treatment
- Perform full physical examination with ECOG Performance Status and Vital signs
- Review of prior/concomitant medications
- Imaging exams: ^{18}FDG PET/CT scan with contrast agent injection (Within 6 weeks prior to ^{89}Zr-TLX250 PET/CT scan)
- Tumor Biomarkers: ACE, CA 15-3 (within 3 months prior to ^{89}Zr-TLX250.
- Standard laboratory samples for Haematology and serum Chemistry.
- Serum β-HCG pregnancy test in women of childbearing potential and Urine Pregnancy test within 72 hours before ^{89}Zr-TLX250 PET/CT scan.
- Blood draw for HACA testing.

- **<u>Medical history</u>**

Breast Cancer Disease history will be reported, including date of diagnosis, histological results and previous Breast cancer treatments.

Relevant findings from medical history (obtained at screening) and physical examination will be reported.

Relevant concomitant medications will be reported.

- **Physical examination and vital signs**

A complete physical examination will be performed within 30 days before patient enrolment.

The performance status will be assessed.

Vital signs (blood pressure [BP], pulse, temperature, and respiration rate) will be evaluated according to the assessment schedules Body weight and height.

- **Imaging assessments**

^{18}FDG PET/CT scan with contrast agent injection should be performed within 30 days prior to ^{89}Zr-TLX250 PET/CT scan.

^{18}FDG-PET/CT scans are performed according to standard clinical practice by an experienced local nuclear physician.

- **Laboratory evaluations**

Clinical laboratory analyses (Haematology: blood count, and Chemistry: Creatinine clearance, fasting Glucose, Bilirubin, ALAT, ASAT, Alkaline Phosphatases, Gamma GT) are to be performed by the local laboratory within 30 days prior to ^{89}Zr-TLX250 PET/CT scan.

Tumor Biomarkers: CEA, CA 15-3 will be performed (accepted if done within 3 months prior to ^{89}Zr-TLX250).

All female patients of childbearing potential (premenopausal women) must undergo a serum β-HCG pregnancy test at screening.
Pregnancy test from urine must be performed (for patients of childbearing potential) within 72 hours before ^{89}Zr-TLX250 PET/CT scan.

- **Immunogenicity evaluation**

A blood sample will be drawn in order to evaluate the immunogenicity (possible formation of antibodies) against the Girentuximab antibody (human anti-chimeric antibodies, HACA).

An approximately 4 mL blood sample will be collected, before ^{89}Zr-TLX250 administration, in an red top SST tube, clotted 30 minutes (max.60 min) and centrifuged, within 1 hour of collection, at 1800 g for 10-15 minutes. Three serum aliquots will be transferred into three separately labelled 2 mL cryo-vial and stored at –20°C. Please refer to the central laboratory manual for details on shipping.
The presence of HACA in sera of patients will be tested quantitatively and qualitatively using validated ELISAs in the Bioanalytics Department of Agilex Biolabs Pty Ltd, South Australia, Australia.

Instructions for collection, preparation and shipment can be found in the laboratory manual. Required sample collection information must be entered on the appropriate eCRF pages and requisition forms.

6.2 Enrolment

Patient Enrolment may occur only after the completion of the screening evaluations and confirmation of subject eligibility.

Enrolment will be centralized and performed via the eCRF.

How to connect to the eCRF:

- Go to website: https://www.cancero-go-online.org/CSOnline/
- Study code: **CRGA0065**
- Study Sponsor number : ICO-2020-25
- Username and password: Nominative and personal, they will be given to each participant after investigation site participation setup visit opening of the site.

If the software is unavailable, the enrolment registration request will be centralized at the sponsor ICO "Service Promotion de la Recherche Clinique". A registration Form is provided for this purpose.

It will be sent by e-mail to the sponsor (between 9am and 5pm). An enrolment number will be assigned to the patient and e-mailed to the site.

Sponsor Contact Information : DRC de l'ICO / Cellule de Promotion
Tél. : +33 (0)2 40 67 98 26
Chef de Projet : Nadia ALLAM
E-mail : promotionrc@ico.unicancer.fr

After the enrolment procedure is completed, the investigator will receive by email a confirmation of the patient enrolment, the centre identification number, the patient identification number.

After the enrolment, each patient will receive a "**patient card**" (Appendix 6) specifying the procedure received in the trial. The patient may present this card to any health professional encountered during the trial.

6.3 ^{89}Zr-TLX250 PET/CT Imaging

6.3.1 ^{89}Zr-TLX250 administration

The following examinations and procedures will be performed on Day 0 before ^{89}Zr-TLX250 administration:

- Physical exam.
- Pre-dose Vital signs.
- Pregnancy test from urine (for female patients of childbearing potential) within 72 hours before ^{89}Zr-TLX250 administration.

- Baseline findings.
- Concomitant medication.

Administration of ^{89}Zr-TLX250 will be slowly conducted over a minimum of 3 minutes via the IV route (see Section 5.2.1). The intravenous line should be flushed slowly with normal saline and the end of administration time recorded.

The dose syringe should be assayed for residual radioactivity and the net activity administered to the patient recorded.

Vital signs will be assessed immediately after the slow injection of ^{89}Zr-TLX250 dose, 30 minutes, 1 hour and 2 hours after ^{89}Zr-TLX250 administration.

Adverse event monitoring will be assessed within 2 hours post administration.

If adverse events occur within 30 days post administration, the patient must inform the investigator. These adverse events will be reported in the e-CRF.

6.3.2 ^{89}Zr-TLX250 Imaging

On D5 ± 2 days post administration of ^{89}Zr-TLX250, a whole body PET/CT imaging will be performed from skull to mid-thigh using 6-8 bed positions with 10 minute acquisition time per bed position.

Laboratory assessments will be performed: Blood count, creatinine clearance, glucose, bilirubin, ALAT, ASAT, Alkaline Phosphatases, and Gamma GT.

6.4 Follow-up visit: Day 30 following ^{89}Zr-TLX250 PET scan

30 days after ^{89}Zr-TLX250 PET scan, a visit by the investigator will be performed to assess:

- Performance Status (WHO)
- The clinical status of the patient and review of AE and SAE.

6.5 End of study visit: 3 months (day 90) following ^{89}Zr-TLX250 PET scan

End of study visit will be performed 3 months after ^{89}Zr-TLX250 PET / CT scan.

All required procedures may be completed within 3 months ± 15 days of the end of study visit.

- Complete physical exam
- Evaluation of Performance Status (WHO)
- Review of AE and SAE + prior/concomitant medications
- Blood draw for HACA testing.
- Imaging exams: CT scan or ^{18}FDG PET/CT scan with or without contrast agent injection.

6.6 Ancillary studies

The ancillary imaging and biological studies are optional. All patients enrolled in this trial will be asked to consent to these ancillary studies.

6.6.1 Ancillary Imaging study

For 3 to 5 patients, imaging quantitative biodistribution of ^{89}Zr-TLX250 will be evaluated from sequential whole body PET-CT imaging at:

- **D0**, **dynamic data acquisition during** ^{89}Zr-TLX250 administration.
- **D1 after** ^{89}Zr-TLX250 administration.
- **D3 after** ^{89}Zr-TLX250 administration.
- **D5 after** ^{89}Zr-TLX250 administration.
- **D7 after** ^{89}Zr-TLX250 administration.

6.6.2 Ancillary Biological study

For these 3 to 5 patients, quantitative biodistribution of ^{89}Zr-TLX250 will be evaluated in serum. Blood samples (one heparin tube : 4 ml) will be drawn at:

- **D0 before** ^{89}Zr-TLX250 administration.
- **D0**, **after dynamic acquisition**, after ^{89}Zr-TLX250 administration.
- **D1 after** ^{89}Zr-TLX250 administration.
- **D3 after** ^{89}Zr-TLX250 administration.
- **D5 after** ^{89}Zr-TLX250 administration.
- **D7 after** ^{89}Zr-TLX250 administration.

Each blood sample will be analyzed for serum ^{89}Zr radioactivity levels in the local nuclear medicine department.

6.7 Independent Central Histological analysis

An independent central histology analysis will be carried out at ICO Saint Herblain. The purpose of this analysis is the determination of the degree of CAIX expression from the biopsy tumor sample.

The degree of CAIX expression will be investigated and classified.

7. SAFETY

ICH requires that both investigators and sponsor follow specific procedures when notifying and reporting adverse events/reactions in clinical trials. These procedures are described in this section of the protocol.

7.1 Definitions

7.1.1 Adverse event Definition

An adverse event (AE) is defined as any untoward medical occurrence, in a patient or clinical trial subject treated by a medicinal product and which **does not necessarily have a causal relationship with this treatment**.

Intensity (severity):

Intensity of adverse events is assessed by the investigator. The intensity criterion should not be confused with the seriousness criterion, which is the guide for defining the reporting requirements.

The intensity of adverse events will be classified according to the NCI-CTCAE Version 5.0.

For any event not listed in this classification, the classification will be assessed as follows:

- **Mild** (grade 1): does not affect the patient's Activities of Daily Living (ADL)
- **Moderate** (grade 2): disrupts the patient's ADL
- **Severe** (grade 3): disabling; limiting self-care ADL
- **Life-threatening** (grade 4): urgent intervention indicated.
- **Death** (Grade 5)

Causal relationship to study drug/procedure:

Relationship between the AE and the study drug /procedure must be assessed following EVCTM (EudraVigilance Clinical Trial Module) criteria: reasonable possibility / no reasonable possibility. Important factors to be considered in assessing the relationship of the AE to study treatment include:

- The temporal sequence from drug/procedure administration
- Recovery on drug discontinuation (de-challenge), recurrence on drug re-introduction (re-challenge).
- Underlying, concomitant, intercurrent diseases.

7.1.2 Definition of serious adverse events

A serious adverse event (SAE) is an AE occurring during any study phase (i.e., screening, run-in, treatment, wash-out, follow-up), at any dose of the study drugs that fulfils one or more of the following criteria:

- **Results in death**

- **Is life-threatening**
- **Requires in-patient hospitalization or prolongation of existing hospitalization**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital abnormality or birth defect in offspring of the patient**
- **Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above.**

The terms disability and incapacity correspond to any temporary or permanent physical or mental handicap that is clinically significant and affects the patient's physical activity and/or quality of life.

Hospitalisations already scheduled before the start of the trial are not considered a serious adverse event.

7.1.3 Expected adverse events

An Expected adverse event (EAE) is an effect already mentioned in the most current version of the applicable document used in this trial.

Reminder: Expected serious adverse events will be submitted by the sponsor to the competent authorities.

Clinical experience in 69 patients dosed with a single administration of ^{89}Zr-girentuximab exhibited that less than 10% of patients experienced a mild nausea, which was considered related to the study medication. Therefore, mild nausea is an expected side effect of girentuximab.

7.1.4 Unexpected adverse events

An unexpected adverse event (UAE) is an event whose nature, severity, frequency, or course is not consistent with the information about the procedures and methods used in the trial, as defined in the Investigator's Brochure or trial documents.

Reminder: Suspected Unexpected Serious Adverse Reaction (SUSAR) should be reported without delay in the event of death or life-threatening events, or otherwise within 15 days of the sponsor's notification to the competent authorities.

For all SUSARs, the information of follow-up must be submitted in a new delay of 8 days to the Competent Authorities.

7.1.5 New Safety Issue

Any new data that may lead to a reassessment of the benefit/risk ratio of the trial, or that may be sufficiently important to consider changes in the conduct of the study, or trial-related documents, or that may lead to the suspension, termination or modification of the study protocol or similar trial.

7.2 Investigator obligations

7.2.1 Adverse Events reporting

Adverse Events will be recorded in the corresponding section of the e-CRF.

For this study, all adverse events related to ^{89}Zr- Girentuximab PET/CT should be recorded in the e-CRF **from the date of administration of ^{89}Zr- Girentuximab until 30 days after administration**.

7.2.2 Serious Adverse Events (SAEs) Reporting

7.2.2.1 Reporting information to Sponsor

Any expected or unexpected Serious Adverse Event (SAE) requires the completion of SAE form. The investigator must verify that the information provided on this form is accurate and clear (e.g., do not use abbreviations).

For each reported event, the investigator will complete the SAE Form with at least:

- The patient identification,
- The description of the event, as clearly as possible and in medical terminology,
- The intensity of the event,
- The start and end date of the event,
- The measures taken and whether or not corrective treatment is required,
- If the trial treatment has been interrupted,
- The evolution of the event. In case of a non-fatal event, the evolution have to be followed until the healing or the return to the previous state or the stabilization of possible after-effects,
- The causal relationship between this event and the treatment being tested or a research-related constraint,
- The causal relationship of the event with the treated pathology, another pathology or another treatment.
- The investigator should also attach to the SAE report, whenever possible :
  - ⇒ A copy of the hospitalization report or extension of hospitalization,
  - ⇒ A copy of the autopsy report if necessary,
  - ⇒ A copy of all additional test results performed, including any relevant negative results with the laboratory's normal values,
  - ⇒ Any other document it deems useful and relevant.

All these documents must be anonymized.

All SAE will be followed by the investigator until complete resolution or stabilization (at a level acceptable to the investigator or return to the previous state) even if the patient is out of the trial. A final assessment will be sent to the sponsor.

7.2.2.2 SAE reporting procedure

All SAEs, whether or not related to the study drug/procedure (with the exception of those identified in the protocol as not requiring immediate reporting, see section 7.2.2.4), must be documented and reported to the Sponsor and the study vigilance contact (contact information below).

Completion and reporting procedure:

1. A SAE Form will be completed as precisely as possible, dated and signed by the investigator.
2. This SAE Form must be sent via e-mail to both e-mail addresses bellow:

   anne.millaret@ctvigilance.fr **AND** **drci.pv@ico.unicancer.fr**

   E-mail title should be completed as follows:

SAE_STUDY name_ddmmyyyy_site number-patient number_short SAE title with grade

   The competed, signed and dated SAE Form should be attached to this e-mail (in pdf format)

3. An acknowledgment of receipt will be kept in the investigator study master file.

7.2.2.3 Notification to the sponsor

The investigator must **immediately** (**without delay**) and **no later than 24 hours** following knowledge of the event, notify the sponsor pharmacovigilance unit of **any SAE or any new issue** defined in section 7.1.2**:**

- from informed consent signature up to ^{89}Zr-TLX250 administration: only SAE related to the study procedures must be reported to the sponsor.

- from ^{89}Zr-TLX250 administration up to 30 days post-^{89}Zr-TLX250 administration: all SAEs, whether or not related to the study drug/procedure, must be reported to the sponsor.

All delayed Serious Adverse Events (occurring after this period) considered reasonably related to ^{89}Zr-TLX250 must be reported without time limit.

Except those that are identified in the protocol as not requiring immediate reporting.

This initial SAE notification shall be followed promptly by a detailed written follow-up report(s) until event resolution.

7.2.2.4 Protocol specifications

Some events should not be considered as SAE.

In this protocol, the following events **are not to be considered as SAE**:

- All hospitalizations for medical or surgical treatment scheduled before study start.

- All hospitalizations for routine treatment or monitoring of the studied pathology not associated with a deterioration of the patient's condition.

- Admission for social or administrative reasons.

- Outpatient hospitalization,

- Progressions related to the disease under study.
- Deaths related to disease under study progression.

7.3 Sponsor obligations

7.3.1 SAE Analysis

The sponsor should assess:

- Causality of serious adverse events (all adverse events, for which there is a reasonable possibility that there is a causal relationship between the study drug/procedure and the event, should be qualified by the investigator or sponsor as reasonably suspected adverse events. If the assessment by the sponsor and the investigator are different, both opinions are mentioned in the report to the competent authority if required).
- The sponsor should qualify the SAE in suspected expected serious adverse reaction or in Suspected Unexpected Serious Adverse Reaction (SUSAR). The evaluation of expectedness is based on knowledge of the adverse reaction and the study drug/procedures. The sponsor should maintain detailed records of all serious adverse events reported by the investigators. These records will be forwarded to the National Health Authority (ANSM), upon request.

7.3.2 Relationship Scoring

In accordance with the ICH Recommendations on the Management of Adverse Events in Clinical Trials, ICH E2B(R3), version of May 12, 2005, a relationship assessment should be performed for any reported SAE.

EVCTM (EudraVigilance Clinical Trial Module) criteria should be used: reasonable possibility / no reasonable possibility.

7.3.3 Notification of SUSARs to competent authorities

The sponsor should report all Suspected Unexpected Serious Adverse Reaction (SUSAR) to the EMA (European Medicines Agency - Eudravigilance database), the French Health Authorities (ANSM), and the principal investigators.

In accordance with article R1123-53 of the Public Health Code, Suspected Unexpected Serious Adverse Reaction (SUSAR) should be reported to the competent authorities from the day the sponsor becomes aware of them:

- Without delay for death and life-threatening SUSAR,
- Until 15 calendar days for all other SUSAR.

Relevant additional Follow-up information regarding SUSARs should be reported to the EMA, ANSM and the investigators within eight days of the initial report.

7.3.4 Notification of New Safety Issue

The sponsor is responsible for alerting the principal investigator in case of identification of a New Safety Issue and should propose urgent measures to be implemented.

The sponsor is responsible for reporting to the competent authorities, **without delay**, any New Safety Issue and the proposed measures.

Relevant follow-up information regarding any New Safety Issue should be reported to the competent authorities within a new delay of 8 days.

7.3.5 Reporting of DSURs (Development Safety Update Report)

On the anniversary date of the Health Authorities trial authorization, the sponsor should report an Annual Safety Report (ASR) consisting of three parts:

- Part 1: Analysis of patient safety.
- Part 2: the list of all suspected serious adverse events (including unexpected serious adverse events) that occurred in the trial in France (and abroad, including in third countries) during the period covered by the report.
- Part 3: summary tables of all serious adverse events and serious adverse reactions that have occurred in the trial since the study start.

This report is sent to the competent authorities (ANSM), Ethics Committee (CPP) and principal investigators within 60 days of the anniversary date of the trial authorization.

7.3.6 Reporting of other safety data

The sponsor should report any safety data or new safety event to the competent authorities (ANSM and CPP) **as soon as possible and no later than 15 calendar days** from the first time the sponsor became aware of the minimum information for immediate reporting.

Relevant additional information must be transmitted within 8 days and 15 days new delay.

7.3.7 In utero exposure

If a patient becomes pregnant during the study, the pregnancy must be reported to the sponsor within a time frame defined by the sponsor.
The investigator informs the sponsor using a standard "Initial Pregnancy Data Collection" sheet. This form should include the expected date of delivery, the contact details of the obstetrician and of the planned maternity hospital if the pregnancy is ongoing.

The investigator should follow the patient until completion of the pregnancy and should notify the sponsor of the outcome using a standard pregnancy outcome form.

If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the investigator should follow the procedures for reporting an SAE.

8. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

The analysis will cover 12 patients.

8.1 Brief reminder of study objectives

8.1.1 Primary objective

To assess the concordance for tumor lesion detection using ^{89}Zr-TLX250 PET/CT versus a conventional ^{18}FDG PET/CT scan where comparison will be made on a per lesion analysis basis.

8.1.2 Endpoints of primary objective

Concordance study of metastatic uptake seen in ^{18}FDG PET/CT scan and ^{89}Zr-TLX250 PET/CT scan per "lesion" by comparing for each lesion the CT scan, ^{18}FDG PET scan and ^{89}Zr-TLX250 PET/CT scan by assessing a ratio "Number of positive or negative ^{89}Zr-TLX250 lesions / Number of positive or negative FDG lesions" and "Number of positive or negative ^{89}Zr-TLX250 lesions / Number of CT scan lesions".

8.1.3 Secondary objectives

4. To determine the percent of total tumor burden (whole body) detected on ^{89}Zr-TLX250 PET/CT scan compared to that defined on ^{18}FDG PET/CT used as the reference.
5. To assess the correlation between the normalized uptake values (SUV) of ^{89}Zr-TLX250 and CAIX histological expression if a biopsy is done.
6. To confirm the perfect safety and tolerability of ^{89}Zr-TLX250 and assess the generation of human anti-chimeric antibodies in response to the girentuximab.

8.1.4 Endpoints of secondary objectives

4. Percent of positive CA IX metastatic tumor burden compared to total metastatic tumor burden by ^{18}FDG (ratio "Number of positive ^{89}Zr-TLX250 lesions / Number of positive FDG lesions")
5. If a metastasis biopsy is conducted, assessment of the correlation between the normalized uptake values (SUV) of ^{89}Zr-TLX250 positive lesions and CAIX histological expression will be done by comparing the ^{89}Zr-TLX250 semi-quantitative data with the immunohistochemical results (IHC) of biopsied metastases.
6. The safety and tolerability of ^{89}Zr-TLX250 will be evaluated by measuring and monitoring vital signs within 2 hours after injection of the radiopharmaceutical. The patient will be informed that in the event of abnormal physical signs, occurring within 24 hours after ^{89}Zr-TLX250 administration, he must report them to the investigator for registration. The reporting period of AEs (related to ^{89}Zr-TLX250) and SAEs cover a period of 30 days after ^{89}Zr-TLX250 administration.

⇒ The NCI Common Toxicity Criteria, version 5.0 reference will be used.

⇒ HACA (Human anti-chimeric antibody) will be assessed.

8.1.5 Exploratory objectives

For 5-6 patients, assessment of the absorbed doses received by organ and in whole body after ^{89}Zr-TLX250 administration for imaging purpose in metastatic TNBC patients.

For these patients, blood samples will be drawn at D0 (before ^{89}Zr-TLX250 administration), D1, D3, D5 and D7.

Each sample will be analyzed for serum ^{89}Zr radioactivity levels in the local nuclear medicine department.

8.1.6 Endpoints of Exploratory objectives

For 5-6 patients, quantitative biodistribution of ^{89}Zr-TLX250 evaluated from sequential whole body PET-CT imaging D0, D1, D3, D5 and D7 and pharmacokinetic data (blood samples taken before injection and at the same time of imaging).

8.2 Determination of sample size

As this study is a proof-of-concept, exploratory and descriptive pilot study, the sample size determination is not based on statistical considerations, it is planned to enrol 12 evaluable patients.

8.3 Selection of patients to be included in the statistical analysis

All included patients will be analysed.

8.4 Study feasibility

The estimated duration of study recruitment is approximately 12 months.

8.5 Interim analyses

Not applicable.

8.6 Statistical criteria for study termination

Not applicable.

8.7 Handling of missing, unused or invalid data

Special care will be taken to minimize any missing data for the 12 patients enrolled.

8.8 Description of analysis sets

8.8.1 For Safety Analysis Set:

The frequency and severity of the clinical and biological effects collected over a period of 15 days then at 3 months following the completion of the ^{89}Zr-TLX250 PET/CT will be described by means of frequency of their respective modalities. HACA test at the visit at 3 months post ^{89}Zr-TLX250 PET/CT.

8.8.2 For the determination of ^{89}Zr-TLX250 PET-CT preliminary analysis of diagnostic performance:

From the reference ("gold standard") determined for each patient from all imaging and histology screening data and/or 3-months imaging follow-up (RECIST 1.1 criteria, or PERSIST criteria). The reporting qualitative and semi-quantitative parameters of the lesions will be established at D5 post-injection. The diagnostic performance of ^{89}Zr-TLX250 PET/CT obtained at the post-injection delay considered optimal related, ^{18}FDG PET/CT scan and CT scan compared to gold standard will be described by the sensitivity, specificity, positive and negative predictive values (with their 95% CI) and compared by means of Mc Nemar test. Concordance between techniques will be described by means of Kappa.

Total number of lesions (gold-standard) will be the number of lesions identified by at least one modality.

Lesion detection rates per imaging modality and combined imaging modalities (CT combined with PET) will be estimated with their 95% CI and compared by Wald test, overall and according to organ sites.

Agreement in detecting lesions between modalities will be assessed using kappa’s (95%CI).

Furthermore, we will compare the median number of affected organ sites across patients assessed by ^{18}FDG-PET/CT or ^{89}Zr-girentuximab-PET/CT using Wilcoxon signed rank tests.

The physiological biodistribution of ^{89}Zr-girentuximab will be described in normal tissues (lung, liver) using geometric mean SUVmean.

The overall geometric mean ^{89}Zr-girentuximab or ^{18}FDG SUVmax will be used to quantify uptake in lesions. Link between uptake and lesions location and lesions diameter on CT will be tested.

Correlation between ^{18}FDG -uptake and ^{89}Zr-girentuximab-uptake will be calculated by Rho's coefficient (Spearman test).

8.8.3 For ancillary study set:

The dose received for healthy tissues, the effective dose received for the whole body from sequential whole-body imaging and pharmacokinetic data (at D0, D1, D3 and D7) will be described by means of mean, standard deviation, median, minimum, maximum and interquartile range.

8.9 Managing changes to the analysis sets

Not applicable.

9. CONTROL AND QUALITY ASSURANCE

9.1 Oversight Committees

9.1.1 Independent data monitoring committee

Not applicable.

9.1.2 Steering Executive Committee

Not applicable.

9.2 Quality assurance

9.2.1 Study monitoring

In order to guarantee the authenticity and credibility of the data in accordance with the GCP of 24 November 2006, the sponsor is setting up a quality assurance system which includes:

- The management and monitoring of the trial according to the procedures of the ICO, Clinical Research Department.
- The quality control of the data from the investigator site by the monitor whose role is:
  - ⇒ To check compliance with the protocol, GCP and the APPLICABLE laws and regulations,
  - ⇒ To check the consent and eligibility of the patients participating to the trial,
  - ⇒ To check the concordance and consistency of the CRF data compared to source documents,
  - ⇒ To check the notification of serious adverse events,
  - ⇒ To monitor the tracking of the study treatments (dispensing, storage and accountability of study treatments),
  - ⇒ To ensure that patients who may be suitable for research are not already participating in research that might make it impossible to include them in the proposed research. The monitor also ensures that patients have not participated in research for which an exclusion period is currently required.
- The audit of the participating investigational sites when deemed necessary,

The monitors in charge of quality control of this study are duly mandated for this purpose by the sponsor and must have access to the patients data, strictly required for this control. The monitors are bound by professional secrecy under the conditions defined by articles 226-13 and 226-14 of the penal code. The tracking of monitoring visits is ensured by a written monitoring report.

In order to ensure the optimal research quality control, the investigator commits to provide the monitor with direct access to all patient files.

The same applies to representatives of the health authorities.

9.2.2 Monitoring Plan

The monitoring plan is established by the study team and the responsible institution according to the study objectives.

The frequency of visits will depend on the number of patients enrolled, the rate of inclusion and the difficulties observed during the study.

Monitoring will be carried out by the Clinical Research Promotion Department of the "Institut de Cancérologie de l'Ouest". A Clinical Research Associate (CRA) will visit each site in order to carry out quality control of the data reported in the case report forms.

On-site monitoring visits will be organised after an appointment with the investigator. The CRAs must be able to consult:

- The eCRF of patients enrolled,
- Patients' medical and nursing files,
- The investigator file.

The protocol has been classified according to risk level D with a "LIR" score of 8: The level of monitoring required is High:

100 % of patient files will be monitored at 100%.

<u>Monitoring will be carried out as follows:</u>

Each enrolling site will be monitored at least once (on-site and/or remote visit), with the following data collected:

1) The existence of patients enrolled.

2) The collection and archiving of signed informed consents.

3) Compliance with the eligibility criteria (inclusion and non-inclusion).

4) The presence of the primary objective endpoint:

⇒ Concordance evaluation of metastatic uptake seen in CT scan, ^{18}FDG PET/CT scan and ^{89}Zr-TLX250 PET/CT scan per "lesion".

5) The presence of the major secondary objective endpoints:

⇒ Percent of positive CA IX metastatic tumor burden compared to total metastatic tumor burden by ^{18}FDG (ratio "Number of positive ^{89}Zr-TLX250 lesions / Number of positive FDG lesions")

⇒ If a metastasis biopsy is realized, assessment of the correlation between the normalized uptake values (SUV) of ^{89}Zr-TLX250 positive lesions and CAIX histological expression.

6) Reporting and monitoring of Adverse Events

a) Serious Adverse Events

b) New Safety Event

9.2.3 Inspection / Audits

As part of its audit program, the sponsor may need to audit some investigational sites. The site and the investigator agree that audits are carried out by Sponsor or any person duly authorised for at least ten years after the trial.

More generally, the investigator site and the investigator undertake to devote all necessary time to audit procedures, control and additional information requested by the sponsor or by a Concerned Competent Authority.

10. ETHICAL AND REGULATORY CONSIDERATIONS

The clinical trial must be conducted in accordance with:

The current Declaration of Helsinki. The ICH Guidelines for Good Clinical Practices (E6) and the French national regulatory requirements:

- Le Code de la Santé Publique.
- La Loi n°2004-806 du 9 août 2004 relative à la politique de santé publique portant notamment transposition de la directive européenne n°2001/20/CE du 4 avril 2001 et la loi n°2006-450 du 18 avril 2006.
- La Loi Jardé n°2012-300 du 12 mars 2012 (décret d'application du 16 novembre 2016) concernant la recherche sur la personne humaine).
- La Loi n°2011-814 du 7 juillet 2011 relative à la bioéthique.
- La Loi n° 78-17 du 6 janvier 1978 relative à l'informatique aux fichiers et aux libertés, modifiée notamment par la Loi n° 204-801 du 6 août 2004 et la loi n°2018-493 du 20 juin 2018, dans sa version en vigueur au moment de la réalisation de l'Essai.
- Le Règlement (UE) n°2016/679 du 27 avril 2016 relatif à la protection des données personnelles (RGPD).

10.1 Clinical trial authorisation

The protocol has been submitted to the Committee for the Independent Ethic Committee (IEC) *"CPP SUD EST VI (Clermont Ferrand)"*, and received a written approval.

The protocol has been submitted to the National health Authority (ANSM) and received a written approval.

10.2 Patient Information and Consent

Prior to the participation of a patient in the trial, this patient will be about the trial. The patient written consent must be obtained after the investigator has given him/her full information.

The consent form must be dated and signed personally by the patient and the investigator. The original will be filed in the investigator's binder and the duplicate will be given to the patient.

10.3 Sponsor responsibilities

The "Institut de Cancérologie de l'Ouest" (ICO), as sponsor and initiator of this trial, is accountable for trial management and for verifying that the financing schedule covers the anticipated expenses of the trial.

The main sponsor responsibilities are:

- The subscription of a civil-responsibility insurance;
- The obtaining of an ID RCB identification number and registration of the trial in the European Drug Regulatory Authorities Clinical Trials database.
- The obtaining of the authorization of Ethics committee (IEC) and ANSM on the initial protocol and possible amendments when applicable.
- The notification of any suspected (unexpected) serious adverse reactions (SUSAR), according to the local regulatory requirements, to the respective regulatory authorities, and the notification of this information to the IEC and the investigators of the study.
- The transmission of the Development Safety Update Reports (DSUR) to the respective regulatory authorities and to the IEC according to the local regulatory requirements.
- The information about the study to the investigational sites institutions, pharmacists and investigators, according to local regulatory requirements.
- The notification of the beginning and the end of the study to the respective competent authorities and to the IEC, according to the local regulatory requirements.
- Writing the final report of the trial and forwarding the summary to the ANSM.
- The information on the study's results, according to local regulatory requirements, to the respective Competent Authorities, to the IEC to the research participant.
- The archiving of the study's essential documents for a minimal duration of 15 years after the research has ended.

10.4 Investigator responsibilities

The principal investigator of each investigational site participating in the trial commits to conduct the trial as specified in this protocol and in accordance with the current regulatory requirements, and in particular the current Guideline for Good Clinical Practice of 24 November 2006.

<u>It is the responsibility of the principal investigator to:</u>

- Provide to the sponsor with her/his own curriculum vitae (CV) and those of his/her collaborators.
- Identify the members of his/her team who participate in the trial and to define their individual responsibilities.
- Start recruiting patients after approval of the sponsor.
- Be available for monitoring visits, audits and investigator meetings.

<u>It is the responsibility of each investigator to:</u>

- Ensure the confidentiality of all data recorded during the trial.
- Collect the informed consent, written, dated and signed personally by each individual research participant before any specific selection procedure to the trial (to be documented in the patient file).

- Regularly complete the case report form (CRF) for each of the patients included in the trial and allow the clinical research assistant (CRA) mandated by the sponsor to have direct access to the source documents in order to validated the data collected in the CRF.
- Declare to the sponsor as soon as he becomes aware of any serious adverse event occurring during the trial.
- Accept regular visits by the CRA and possibly those of auditors as mandated by the sponsor or the inspectors of the respective regulatory authorities.
- Date, correct and sign the corrections made in the CRF and the requests of the data correction forms (DCF).

10.5 Human biological samples collection

Non applicable.

10.6 Patient Committees

The sponsor has asked the Committee of the “Ligue contre le Cancer” to proof-read the information note.

The aim of the Ligue contre le Cancer's patient committee is to reread the clinical trial protocols in cancerology. Created in 1998, the Patients' Committee brings together patients from the League's departmental committees and cancer patient associations.

It can be consulted, in particular for the purpose of reviewing information notes intended for patients. These reviews help to improve the legibility, acceptability and clarity of the information provided to patients and to facilitate decision-making for the patients concerned, thanks to the quality of the information.

11. COLLECTION AND MANAGEMENT OF RESEARCH DATA

11.1 Collection of study data

11.1.1 Collection and conservation of study data

The data will be collected via an electronic Case Report Form (eCRF) accessible from a secure internet connection, allowing traceability of data access and modifications.

A Case Report Form (eCRF) will be created per patient. All the information required by the protocol must be provided in the CRF within 15 days following its availability. The CRF will include the data needed for statistical analyses based on the study objectives, as well as the data needed to detect major deviations from the protocol (inclusion and non-inclusion criteria, premature discontinuation and causes, follow-up of protocol visits and examinations, collection of adverse events and concomitant treatments).

Data entry will be performed online by Investigators and authorised staff identified in the Delegation of Responsibilities Form for each site. This form is kept in the investigator Master file.

The study data are under the responsibility of the Director of “Institut de Cancérologie de l’Ouest”.

Until the results are published, the study data will be hosted on a centralised server provided by the company ENNOV. An ISO 9001:2008 approved bi-site data hosting centre ensures data storage, protection and security (Data Centre in Poitiers and Roubaix, mirror sites). Exports are carried out in parallel in a secure area of the ICO's information system servers for carrying out analyses.

The study data and essential documents will then be archived **for a minimum period of 15 years**, either on the ICO's premises or with a service provider specialising in medical archiving.

11.1.2 Identification of all source data not contained in the patient medical file

Not applicable.

11.1.3 Data coding

By signing this protocol, the principal investigator and all co-investigators undertake to keep confidential the identities of the patients enrolled in the study.

Patients will be identified by an identification code assigned directly from the electronic CRF during the enrolment procedure. This identifier will be composed of the patient's initials, i.e. the first letter of the surname and first name, supplemented by a number assigned at the time of the patient's enrolment.
This unique identifier code will be the only information that will appear on the CRF and will allow the CRF to be linked to the patient's identity afterwards.

The sponsor is also required to provide the necessary elements for the de-identification of any documents in its possession (reports of imaging or biological reports, etc.) that may be attached to the CRF.

11.2 Study Data Processing

11.2.1 By the Sponsor

The processing of the study data is carried out by the Clinical Research department (DRCI) of the ICO, in the Data Factory Unit, under the responsibility of François Bocquet; the data being the property of the ICO, the sponsor of the research.

The data processing software is ENNOV CLINICAL, Excel, R, Stata and SAS.

In accordance with Deliberation No. 2018-153 of 3 May 2018 approving a reference methodology relating to the processing of personal data (implemented within the framework of research in the field of health with the consent of the person concerned), the ICO has undertaken to follow the **MR001** reference methodology of the National Commission for Information Technology and Civil Liberties. This processing has been registered in the register file held at the disposal of the "Commission Nationale de l'Informatique et des Libertés (CNIL)" under number 285.

11.2.2 By sites, in the case of a computerised medical file is used

If the data needed for research within the sites are processed or managed by computerised systems, each site must:

a) Ensure and document that the computerised systems used in the research comply with the requirements in terms of data integrity, accuracy, reliability and performance (i.e. validation);
b) Establish and monitor standard operating procedures for the use of such systems;
c) Ensure that these systems allow modifications of the collected data, that each modification is automatically authentified, and that the data cannot be removed (i.e. maintain an audit trail of data and changes);
d) Establish and maintain a security control system that prevents unauthorised access to data;
e) Maintains a list of persons authorised to modify the data;
f) Carry out appropriate backups of the data;
g) Preserves the blinding, where appropriate (e.g. when entering and processing data);
h) Ensures that the processing of personal data implemented in the research is carried out under the conditions defined by Law No. 78-17 of 6 January 1978 relating to information technology, files and liberties as amended and the regulatory texts adopted for its application, as well as by the General Regulations on data protection .

If the data are transformed while being processed, it should always be possible to compare them with the original observations/records.

The computerized system used to identify the patients participating in the trial must not be ambiguous and should allow the identification of all data collected for each patient while preserving their confidentiality in compliance with amended Law no. 78-17.

11.2.3 Retention of documents

All documentation relating to the trial (protocol, consents, case report forms, correction requests, investigator file, etc.), as well as the original documents (laboratory results, radiology, consultation reports, clinical examination reports, etc.) are considered confidential and must be kept in a secured place.
For each site and according to the national regulatory requirements (order of 08 November 2006), the Principal Investigator must keep the data as well as a patient identification list for a minimum of **15 years after the end of the study.** At the end of this period, the site may only destroy this documentation after the sponsor has given its written authorization.

12. CONFIDENTIALITY AND OWNERSHIP OF DATA

All the information communicated or obtained and all the data and results generated during the trial are the sole and exclusive property of the sponsor: “Institut de Cancérologie de l’Ouest”, which may freely dispose of them. No oral or written communication concerning the study data may be made without the agreement of the sponsor.

The investigator undertakes, for himself and for all persons involved in the trial, to guarantee the confidentiality of all information provided by the ICO until publication of the trial results. This obligation of confidentiality will not apply to any information that the investigator may have to communicate to patients concerning their participation in the trial, nor to information already published.

The investigator undertakes not to publish, disclose or use in any way, directly or indirectly, scientific or technical information related to the trial.

Nevertheless, in accordance with article R 5121-13 of the Public Health Code, the site and the investigator may provide information relating to the trial:

- To the Minister in charge of Health.
- Public health inspectors.
- Pharmacist’s public health inspectors.
- To the Director General and ANSM inspectors.

13. PUBLICATION AND VALORISATION RULES

The results of this study will be submitted for publication(s) and/or communication (s).

All information resulting from this research project is considered confidential, at least until appropriate analysis and control by the sponsor, the coordinating investigator, data manager and trial statistician is completed.
A clinical study report will be prepared by the sponsor and sent to the study participating investigators.

Any scientific communication (abstract/publication, poster, presentation/communication (oral or written), manuscript, intranet, extranet, etc.) including the results of the research project **should be submitted to the sponsor (ICO) for approval.**

For each publication/communication, the **ICO will be designated as Sponsor** and the **funder (SIRIC)** will be explicitly mentioned.

According to the international rules of authorship, (L'intégrité scientifique à l'INSERM, SIGNATURE DES PUBLICATIONS SCIENTIFIQUES, les bonnes pratiques, 2018, the authors who participated actively will be cited, i.e.:

- substantial contribution to the design of the project and the experimental protocol, to the conception of the results, and/or to the analysis and interpretation of the results;
- Drafting the work or revising it critically for important intellectual content;
- explicitly approve the final version of the manuscript, both the scientific content and the authors list, and thus directly engage their responsibility; this is also a requirement of the publishers.

Those who have contributed to the work without meeting the three criteria should be thanked at the end of the article, with their agreement.

Thus, for **every publication**, the order of authors will be defined by the sponsor (ICO) and the coordinating investigator.

	Rank among the authors
Project coordinator or sponsor representative	1st author or Last author
Investigators	2nd author / 3rd author and more (cited according to their rank of recruitment/inclusion in the study
Statistician	Penultimate or last author
A representative of Telix Pharmaceutical	

Any conflict regarding author's name appearance will be submitted to the sponsor (ICO) decision.

In the event of ancillary studies (biological or other): Specific publications may be produced; they should cite the publication of the main study as a reference. These publications should be submitted to the coordinating Investigator and the sponsor for approval.

They will include the name of the person who carried out the side job as well as the names of all other persons involved in the side job.

In addition, all communications, manuscripts or presentations must include a heading which imperatively mentions the ICO, all institutions, investigators and cooperating groups, the learned societies, the partners who contributed to the project, as well as the organisations that financially supported the research.

In addition, in order to be fully involved and to be able to consent to it, the sponsor will be notified in advance of any dissemination of data in Open Research Data, any commercial exploitation and/or patent filing procedures that are relevant to this research project.

14. FINANCIAL ASPECTS

The study is funded by SIRIC ILIAD.

TELIX Pharmaceutical support this study by providing the study drug: ^{89}Zr-TLX250 (carrier-free) for all 12 patients.

15. LIST OF REFERENCES

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LIST OF APPENDIX

Appendix 1: Synopsis

Appendix 2: Investigators list

Appendix 3: Informed consent form

Appendix 4: SAE Form

Appendix 5: NCI-CTCAE V5.0 form

Appendix 6: Patient Card