# PERTINENCE

***"A Pilot open-label, feasibility study to assess safEty, tolerability, radiation dosimetry, and imaging properties of $^{89}$Zr-labeled giRenTuximab ($^{89}$Zr-Girentuximab) in patIents with Non-musclE-iNvasive bladder CancEr (NMIBC)"***

**N° EUDRACT:** 2021-001709-61
**Ref ICO:** ICO-2021-03
**Study type:** RIPH – cat.1 (Médicament)



| | |
|---|---|
| **Investigator Coordinator** | **Dr Caroline ROUSSEAU, MD**<br>Nuclear Medicine Department<br>Institut de Cancérologie de l'Ouest<br>Phone: +33 2 40 67 99 31 ; Fax : +33 2 40 67 97 87<br>Email : caroline.rousseau@ico.unicancer.fr |
| **Scientific Coordinator** | **Dr Jean François CHATAL, MD**<br>Head of Medical Affairs<br>ATONCO<br>Email : jf.chatal@atonco-pharma.com |
| **Methodologist** | **Dr Loïc Campion, MD And Elodie Martin MSc**<br>Oncology Data Factory and Analytics<br>Institut de Cancérologie de l'Ouest (ICO)<br>Phone : +33 2 40 67 99 00 ; Fax : +33 2 40 67 97 87<br>Email : loic.campion@ico.unicancer.fr<br>and elodie.martin@ico.unicancer.fr |
| **Sponsor :** | **Institut de Cancérologie de l'Ouest,**<br>**Service Promotion de la Recherche Clinique (DRC)**<br>Boulevard Jacques Monod - 44805 SAINT HERBLAIN CEDEX<br><br>Contact : **Nadia ALLAM, PhD Project Manager**<br>Phone : +33 2 40 67 98 26 ; Fax : +33 2 40 67 97 87<br>Email : nadia.allam@ico.unicancer.fr |

# 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**<br>***Raphaëlle Charron-Bighetti***<br>*Deputy Director of Research*<br>Institut de Cancérologie de l'Ouest (ICO) | **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.

| | **Name:** | **Date:** | **Signature:** |
|---|---|---|---|
| **Investigator Coordinator** | **Dr Caroline ROUSSEAU** | | |

Institut de Cancérologie de l'Ouest
unicancer PAYS DE LA LOIRE

Short Title : **PERTINENCE**
Ref ICO : ICO-2021-03
N° EudraCT: 2021-001709-61

# CLINICAL TRIAL AUTHORIZATION

# PROTOCOL PERTINENCE

**"A pilot open-label, feasibility study to assess safety, tolerability, radiation dosimetry, and imaging properties of $^{89}$Zr-labeled girentuximab ($^{89}$Zr-Girentuximab) in patients with Non-muscle-invasive bladder cancer (NMIBC)"**

| **CLINICAL TRIAL AUTHORIZATION** | |
|---|---|
| **Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM):** | **Date of authorization: 29/06/2021** |
| | **Ref. ANSM (IDRCB) :** MEDAECNAT-2021-05-0022_2021-001709-61 |
| **Ethics committee :**<br>**COMITE DE PROTECTION DES PERSONNES ILE DE FRANCE III** | **Date of approval: 07/06/2021** |
| | **Ref. CPP :** 3917 I<br>**Réf CNRIPH** : 21.05.10.47712 |

| **SPONSOR REPRESENTATIVE** | |
|---|---|
| **Dr Caroline ROUSSEAU, MD**<br>***Coordinator*** | Nuclear Medicine Department<br>Institut de Cancérologie de l'Ouest<br>Phone: +33 2 40 67 99 31 ; Fax : +33 2 40 67 97 87<br>Email : caroline.rousseau@ico.unicancer.fr |
| **Dr Jean François CHATAL, MD**<br><br>**Sientific Coordinator** | Head of Medical Affairs<br>ATONCO<br>Email : jf.chatal@atonco-pharma.com |
| **Dr Loïc Campion, MD**<br>**And Elodie Martin MSc**<br>***Methodologist*** | Oncology Data Factory and Analytics<br>Institut de Cancérologie de l'Ouest (ICO)<br>Phone : +33 2 40 67 99 00 ; Fax : +33 2 40 67 97 87<br>Email : loic.campion@ico.unicancer.fr &<br>elodie.martin@ico.unicancer.fr |
| **Nadia ALLAM, PhD**<br>***Project Manager*** | Service Promotion de la Recherche Clinique (DRCI)<br>Phone : +33 2 40 67 98 26 ; Fax : +33 2 40 67 97 87<br>Email : nadia.allam@ico.unicancer.fr |

| SPONSOR REPRESENTATIVE | |
|---|---|
| **Enrolment** | Enrolment is made online, directly via the eCRF.<br>In case of problem, please write to the following email addresses, indicating the protocol name in the subject line of the email:<br>promotionRC@ico.unicancer.fr and datamanagement@ico.unicancer.fr |
| **Pharmacovigilance for ICO**<br>**Anne MILLARET**<br>**Sending of notifications** | **Reception and management of notifications :**<br>Phone : +33 9 83 77 53 07<br>Fax : +33 9 81 40 42 80<br>E-mail : anne.millaret@ctvigilance.fr<br>**Reception of notifications :**<br>Phone : +33 2 40 67 99 08<br>E-mail : drci.pv@ico.unicancer.fr |

# 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 radiopharmaceutical for the imaging of Non-muscle-invasive bladder cancer (NMIBC) patients.

One of the objectives of this study is to assess the biodistribution in whole body and the safety of $^{89}$Zr-girentuximab when it is injected in bladder.

After establishing the targeting properties of $^{89}$Zr-Girentuximab PET/CT imaging radiopharmaceutical in NMIBC, it should be interesting to develop a new targeted therapy using girentuximab- radiolabelled with a therapeutic radionuclide such as Astatine-211 ($^{211}$At-Girentuximab) to treat NMIBC.

An alpha-immunotherapy project of non-muscle-invasive bladder cancer was initiated by ATONCO with anti-CAIX girentuximab labeled with Astatine-211.

## ABBREVIATIONS AND DEFINITION OF TERMS

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

| | |
|---|---|
| $^{211}$At | Astatine-211 |
| $^{213}$Bi | Bismuth-213 |
| $^{18}$F | Fluor-18 |
| $^{111}$In | Indium-111 |
| $^{124}$In | Indium-124 |
| $^{89}$Zr | Zirconium-89 |
| AE | Adverse Event |
| ALT | Alanine Aminotransferase / Glutamic Pyruvic Transaminase (GPT) |
| ANSM | French Health Authority |
| AST | Aspartate Aminotransferase / Glutamic Oxaloacetic Transaminase (GOT) |
| B-HCG | Beta human chorionic gonadotropin |
| BP | Blood Pressure |
| bpm | beats per minute |
| CA IX | Carbonic anhydrase IX |
| $C_{max}$ | Maximum Concentration |
| CFR | Code of Federal Regulations |
| CPP | French Ethics Committee |
| CRF | Case Report Form |
| CT | Computed tomography |
| CV | Coefficient of Variation |
| CL | Clearance |
| DSUR | Development Safety Update Reports |
| EC | Ethics Committee |
| eCRF | Electronic case report form |
| EMA | European Medicines Agency |
| EOS | End Of Study |
| EVCTM | EudraVigilance Clinical Trial Module |
| FDG | Fluorodésoxyglucose |
| FU | Follow up |
| GCP | Good Clinical Practices |
| GMP | Good Manufacturing Practices |

| | |
|---|---|
| GLP | Good Laboratory Practice |
| HACA | Human Anti-Chimeric Antibodies |
| 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 |
| NMIBC | Non-muscle-invasive bladder cancer |
| PET/CT | Positron emission tomography-computed tomography |
| PSCA | Potential clinically significant abnormalities |
| PK | Pharmacokinetic(s) |
| SAE | Serious Adverse Event |
| SD | Standard Deviation |
| SE | Standard Error |
| SID | Subject identification |
| SOC | System Organ Class |
| SOP | Standard Operating Procedure |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| SUV | Standardized uptake value |
| TEAE | Treatment-emergent adverse event |
| $t_{1/2}$ | Terminal half-life |
| TURBT | TransUrethral Resection of Bladder Tumor |
| UAE | Unexpected Adverse Event |
| $V_D$ | Distribution volume |
| WB | Whole Body |
| WHO | World Health Organization |

# HISTORY OF PROTOCOLE AMENDMENTS

| | Version number / Date (after amendment) | Justifications of amendment |
|---|---|---|
| Initial authorisation | V1.2 25/06/2021 | Initial authorized version |
| Substantiel Amendment N°1 | V2 03/01/2022 | Intravesical instillation pocedure modified |
| | | |
| | | |
| | | |

# TRIAL CALENDAR

| | |
|---|---|
| **NUMBER OF EXPECTED PATIENTS** | **6 patients** |
| **DURATION OF ENROLMENT PERIOD :** | **12 months** |
| **DURATION OF PROCEDURE / DURATION OF FOLLOW-UP :** | **2 days / 30 days** |
| **MAXIMUM TIME OF PARTICIPATION PER PATIENT :** | **30 days** |
| **GLOBAL DURATION OF THE TRIAL :** | **13 months** |
| **END OF THE CLINICAL TRIAL :** | **Last visit of the last enrolled patient** |

| | |
|---|---|
| **CONSIDERED DURATION UNTIL THE ANALYSIS OF THE PRIMARY OBJECTIVE :** | **25 months (13 + 12 months data analysis)** |
| **INTERIM ANALYSIS :** | **NA** |

# SCHEDULE OF STUDY ASSESSMENTS

| **Period** | **Screening** | **Study procedure** | | | **Follow-up** | | |
|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | **Day 0** | | | **Day 1** | **Day 2** | **Day 30** |
| **Visit number** | **1** | **2** | **3** | **4** | **5** | **6** | **7[6]** |
| **Study day** | **-30 to 0** | **T0-2H** | **T0** | **T0+ 2H** | **T0+20H** | **T0+44H** | **Day 30 (±5)** |
| Written Informed consent | X | | | | | | |
| Medical and disease history | X | | | | | | |
| **Physical Exam** | | | | | | | |
| Physical exam, height, Weight, performance status | X | | | | | | X[7] |
| Vital signs (temperature, Blood Pressure/Pulse) | X | X | | X | | | |
| **Laboratory Exam** | | | | | | | |
| Haematology/ Biochemistry | X | | | | X | | |
| Serum or urine Pregnancy test (if applicable) | X | | | | | | |
| Urine cytobacteriological testing | X[1] | | | | | | X |
| Urine dipstick analysis | | X[8] | | | X | X | |
| **Imaging** | | | | | | | |
| Cystoscopy report | X | | | | | | |
| **$^{89}$Zr-Girentuximab PET/CT (Administration and Imaging)** | | | | | | | |
| $^{89}$Zr-girentuximab intravesical instillation | | X | | | | | |
| PET/CT imaging whole body | | | | X[3] | | X[4] | |
| Bladder PET/CT imaging | | | X[2] | | X | X[5] | |
| Blood sampling for $^{89}$Zr- Girentuximab dosing | | | | | X | | |
| **Immuno-Histological Analysis** | | | | | | | |
| Bladder tumor from TransUrethral Resection of Bladder Tumor (TURBT) or cystectomy | X | | | | | | X |
| **Safety** | | | | | | | |
| Concomitant medications | X | X | | X | X | X | X |
| Adverse Events | X | X | | X | X | X | X |
| Serious Adverse Events | X | X | | X | X | X | X |

1. Negative urine cytobacteriological testing or Sterile urine culture 10 days before intravesical instillation (as far as possible before $^{89}$Zr-girentuximab ordering)
2. At the end of $^{89}$Zr-girentuximab administration: 2 hours after intravesical instillation (see intravesical instillation procedure).
3. 2 hours after $^{89}$Zr-girentuximab administration: 4 hours after intravesical instillation.
4. A Whole body scan should be performed only if blood radioactivity testing on day 1 is positive.
5. A bladder PET/CT imaging should be performed only if blood radioactivity testing on day 1 is negative.
6. Visit on site or teleconsultation.
7. Performance Status should be assessed.
8. To be performed before $^{89}$Zr-girentuximab administration

## TABLE OF CONTENTS PAGE

*PROTOCOL SYNOPSIS* 5
*ABBREVIATIONS AND DEFINITION OF TERMS* 6
*HISTORY OF PROTOCOLE AMENDMENTS* 8
*TRIAL CALENDAR* 9
*SCHEDULE OF STUDY ASSESSMENTS* 10
*TABLE OF CONTENTS* 11
*1. INTRODUCTION* 15
**1.1 Disease background** 15
**1.2 Procedure Background** 16
1.2.1 Girentuximab 16
1.2.2 $^{89}$Zr-girentuximab 17
1.2.3 Effects in Humans 28
**1.3 Rationale for conducting this study** 43
**1.4 Benefit/risk and ethical assessment** 44
1.4.1 Individual benefit 44
1.4.2 Collective benefit 44
1.4.3 Overall risks 44
1.4.4 Benefit / risk balance 45
*2. OBJECTIVES* 47
**2.1 Primary objective(s) and endpoint** 47
2.1.1 Primary objective 47
2.1.2 Endpoints of primary objective 47
**2.2 Secondary objective(s) and endpoint** 47
2.2.1 Secondary objectives 47
2.2.2 Endpoints of secondary objectives 47
*3. PATIENT SELECTION* 49
**3.1 Population description** 49
**3.2 Inclusion criteria** 49
**3.3 Non-inclusion criteria** 49
**3.4 Withdrawal of patients from study** 50
3.4.1 Permanent discontinuation of procedure 50
3.4.2 Withdrawal of consent 51
**3.5 Patients replacement** 51

*4. STUDY PROCEDURE $^{89}$Zr-TLX250 PET/CT SCAN ____ 52*

**4.1 Study drug $^{89}$Zr-TLX250 ____ 52**

4.1.1 Chemical Properties ____ 52
4.1.2 Pharmaceutical Properties ____ 52
4.1.3 Storage and Handling ____ 52
4.1.4 Packaging and Labelling ____ 52
4.1.5 Drug Logistics and Accountability ____ 53

**4.2 Study procedure: $^{89}$Zr-TLX250 PET/CT Imaging ____ 54**

4.2.1 Dosage and Administration ____ 54
4.2.2 $^{89}$Zr-TLX250 PET/CT Imaging ____ 55
4.2.3 Imaging Analysis ____ 57

**4.3 Treatment Assignment ____ 57**

**4.4 Blinding ____ 58**

**4.5 Treatment Compliance ____ 58**

**4.6 Radiation Precautions ____ 58**

**4.7 Total Radiation Exposure ____ 59**

**4.8 Prior and Concomitant therapy ____ 59**

4.8.1 Permitted concomitant therapy ____ 59
4.8.2 Prohibited concomitant therapy ____ 60

*5. STUDY DESIGN ____ 60*

**5.1 Overview of study design ____ 60**

**5.2 Study design schedule ____ 60**

*6. STUDY PROCEDURES: DESCRIPTION AND SCHEDULE ____ 61*

**6.1 Screening visit ____ 61**

6.1.1 Medical history ____ 62
6.1.2 Physical examination and vital signs ____ 62
6.1.3 Urine cytobacteriological testing ____ 62
6.1.4 Laboratory evaluations ____ 62

**6.2 Enrolment ____ 63**

**6.3 $^{89}$Zr-TLX250 PET/CT Imaging ____ 64**

6.3.1 $^{89}$Zr-TLX250 administration ____ 64
6.3.2 $^{89}$Zr-TLX250 PET/CT Imaging and Dosimetry ____ 64

**6.4 Follow-up visit: Day 30 following $^{89}$Zr-TLX250 administration ____ 64**

**6.5 Study discontinuation ____ 65**

*7. SAFETY ____ 66*

**7.1 Definitions ____ 66**

7.1.1 Adverse event Definition ____ 66

7.1.2 Definition of serious adverse events 67
7.1.3 Expected adverse events 67
7.1.4 Unexpected adverse events 67
7.1.5 New Safety Issue 68

**7.2 Investigator obligations 68**
7.2.1 Adverse Events reporting 68
7.2.2 Serious Adverse Events (SAEs) Reporting 68

**7.3 Sponsor obligations 70**
7.3.1 SAE Analysis 70
7.3.2 Relationship Scoring 70
7.3.3 Notification of SUSARs to competent authorities 71
7.3.4 Notification of New Safety Issue 71
7.3.5 Reporting of DSURs (Development Safety Update Report) 71
7.3.6 Reporting of other safety data 71
7.3.7 In utero exposure 72

***8. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION 73***

**8.1 Determination of sample size 73**

**8.2 Selection of patients to be included in the statistical analysis 73**

**8.3 Handling of missing, unused or invalid data 73**

**8.4 Description of analysis sets 73**

***9. CONTROL AND QUALITY ASSURANCE 74***

**9.1 Oversight Committees 74**
9.1.1 Independent data monitoring committee 74
9.1.2 Steering Executive Committee 74

**9.2 Quality assurance 74**
9.2.1 Study monitoring 74
9.2.2 Monitoring Plan 75
9.2.3 Inspection / Audits 76

***10. ETHICAL AND REGULATORY CONSIDERATIONS 77***

**10.1 Clinical trial authorisation 77**

**10.2 Patient Information and Consent 77**

**10.3 Sponsor responsibilities 77**

**10.4 Investigator responsibilities 78**

**10.5 Human biological samples collection 79**

**10.6 Patient Committees 79**

***11. COLLECTION AND MANAGEMENT OF RESEARCH DATA 80***

**11.1 Collection of study data 80**

11.1.1 Collection and conservation of study data 80
11.1.2 Identification of all source data not contained in the patient medical file 80
11.1.3 Data coding 80
**11.2 Study Data Processing 81**
11.2.1 By the Sponsor 81
11.2.2 By sites, in the case of a computerised medical file is used 81
11.2.3 Retention of documents 82
***12. CONFIDENTIALITY AND OWNERSHIP OF DATA 83***
***13. PUBLICATION AND VALORISATION RULES 84***
***14. FINANCIAL ASPECTS 86***
***15. LIST OF REFERENCES 87***
***16. LIST OF APPENDIX 90***

The study sponsor, the Institut de Cancérologie de l'Ouest (ICO), declares that the "**PERTINENCE**" 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

There is an unmet need for treatment of NMIBC. Currently the state of the art therapeutic options include BCG therapy and chemotherapy with mitomycine C. These therapies are efficient for some time but relapses repeat them at more and more close intervals and finally patients are refractory to them and require a radical cystectomy.

Intravesical immunotherapy with BCG remains the standard of care for patients with high-risk and intermediate-risk non-muscle-invasive bladder cancer (NMIBC). Most patients initially respond, but recurrence is frequent and progression to invasive cancer is a concern. No established and effective intravesical therapies are available for patients whose tumors relapse after BCG, representing a clinically important unmet need. Development and discovery of treatment options for BCG-unresponsive NMIBC is a high priority in order to decrease the morbidity, burden of health-care expenditures, and mortality related to bladder cancer. Alpha-immunotherapy is such a treatment option (1–4).

Moreover these treatments have some uncomfortable side effects in a part of patients (flu-like symptoms, such as fever, achiness, chills, and fatigue. These can last for 2 to 3 days after treatment. It also commonly causes a burning feeling in the bladder, the need to urinate frequently, and even blood in the urine).

The use of alpha-emitting radionuclides is a topical issue and of great interest to big pharmaceutical companies because of the high cytotoxic effect of alpha particles on tumor cells due to their high linear energy transfer and of the recognized good tolerance for healthy tissues due to the very short path length of alpha particle allowing to relatively spare normal tissue cells close to tumor cell clusters (5).

The situation of non-muscle invasive bladder cancer corresponds well to radiophysical characteristics of alpha particles. Tumors have a small thickness corresponding well to the very short path length (<0.1 mm) of alpha particles and their superficial localization in the vesical cavity makes them accessible to a radiopharmaceutical intravesically instilled. Moreover the clinical situation of patients refractory to conventional treatment using BCG therapy or chemotherapy and for whom the only alternative is a radical cystectomy, justifies implementation of an innovative therapy like alpha- immunotherapy. The clinical proof of concept of such treatment has been provided by an academic German team at Munich University in 2018 (6).

The preclinical study has been carried out in a mouse model by intravesical instillation of an anti-EGFR (Epidermal Growth Factor Receptor) antibody labeled with bismuth-213, an alpha particle

emitting radionuclide with a half-life of 45 minutes (7). In this study, mice were intravesically inoculated with human bladder carcinoma cells after the induction of urothelial lesions by electrocautery. Mice were treated by intravesical instillation of non-labeled and labeled with bismuth-213 anti-EGFR Mab or of mitomycine C. Median survival of mice, not treated or treated with radiolabeled antibody was respectively of 41 and 89 days. In mice treated with 0.925 MBq of $^{213}$Bi-anti-EGFR 7 days after inoculation of tumor cells, survival was more than 300 days in 80% of mice. In mice treated with mitomycine C 7 days after tumor cell inoculation, survival was more than 300 days in 50% of mice but with a nephrotoxicity unlike treatment with $^{213}$Bi-anti-EGFR which showed no toxicity including the absence of macroscopic urothelial lesions. Finally this study documented the feasibility, efficacy and absence of toxicity of intravesical alpha-immunotherapy of bladder cancer.

This preclinical study was followed by a clinical pilot study in 12 patients refractory to standard treatment and planned to undergo a radical cystectomy (6). An intravesical instillation of an activity of 366 to 821 MBq (10 to 22 mCi) of the 213Bi-anti-EGFR antibody was carried out in an empty bladder followed by 30 mL of PBS. Bladder was washed 120 min later. An excellent tolerability without any clinical of biological sign was observed in the 12 treated patients. Among the 12 treated patients, 3 who should have undergone a radical cystectomy, had a complete response which continued 3, 30 and 44 months after treatment.

These two preclinical and clinical studies showed the feasibility, absence of toxicity and efficacy of intravesical alpha-immunotherapy.

Intravesical instillation route has been chosen to limit the circulation of the radiopharmaceutical agent in the blood and to limit potential allergic immunoreactions with girentuximab which might occur by intravenous route.

The main objective of this study is to prove that the $^{89}$Zr-Girentuximab is safe before developing a treatment for therapeutic purposes with girentuximab radiolabeled with a therapeutic radionuclide such as Astatine-211 ($^{211}$At-Girentuximab).

## 1.2 Procedure Background

### 1.2.1 Girentuximab

Immunohistochemical cross-reactivity studies were performed with girentuximab and a variety of normal human tissues (Internal report, Human tissue cross-reactivity study with human-mouse chimeric antibody cG250. Report dated 11.06.2001) and human tumor tissues (Internal Report Human tumor reactivity study with chimeric antibody cG250. Report dated 02.09.2002). The data confirm that the CAIX-antigen is widely expressed in ccRCC and absent from normal kidney tissue. Non-target tissues with significant CAIX-antigen expression were found to be foveolar parietal cells in the stomach, the epithelium of duodenum and the large bile ducts.

Alpha-immunotherapy requires an antibody, as a vector of a radionuclide through a coupling technology. This antibody is specific for an antigen which is expressed on the membrane of tumor cells. Among a substantial number of candidates the Carbonic Anhydrase IX (CAIX) has been shown to be expressed on the membrane of Non-Muscle-Invasive Bladder Cancer cells. CAIX expression was higher in noninvasive (Ta) versus invasive (T1-T4) tumors (P<0.001) in low-grade versus high-grade bladder cancer (P<0.001) and in metastases versus the corresponding primary tumor (P<0.001) (8). The expression of CAIX on the luminal surface justifies investigation of its utility as a diagnostic/ therapeutic target/prognostic indicator (9).

### 1.2.2 $^{89}$Zr-girentuximab

#### 1.2.2.1 Structure

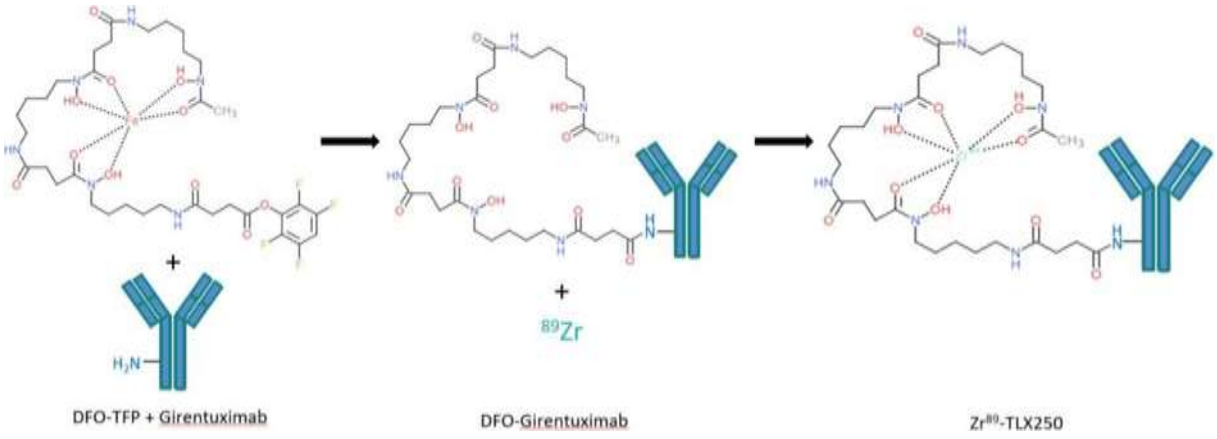



*Figure 1: Structure of $^{89}$Zr-girentuximab*

#### 1.2.2.2 Pharmaceutical development

The drug product $^{89}$Zr-DFO-girentuximab ($^{89}$Zr-TLX250 for intravenous administration), contains the radiometal $^{89}$Zr as a PET tracer agent at a dose of 37 MBq and is adjusted to a total antibody dose of 10 mg with unconjugated girentuximab in 0.9% NaCl.

Girentuximab is a chimeric lgG1 monoclonal antibody specific for CAIX. CAIX is expressed abundantly in clear cell kidney carcinoma. The radiolabeled antibody ($^{89}$Zr-girentuximab or $^{89}$Zr-TLX250) is developed for molecular imaging with PET. With this PET technique, it becomes possible to non-invasively image and quantify the uptake of girentuximab in kidney carcinomas.

$^{89}$Zr-TLX250 solution for intravenous administration consists of three major components (raw materials):

A) No carrier added $^{89}$Zr, is a synthetic, metastable isotope of the radio metal Zirconium(atomic number 40) which decays first via positron emission and electron capture to 89mY (half-life of 15.7s) which in turn decays via gamma ray emission (909 keV) to the stable 89Y (10). It emits

positrons with a main energy of 897 keV and an abundance of about 23%, thus providing high resolution PET imaging and good image quality. In addition, non-prompt 909 keV photons are emitted at an abundance of 99.9%. Nonclinical studies have shown that imaging and quantification of $^{89}$Zr by using a dedicated PET system is comparable with that of Indium-111 ($^{111}$In), which is a widely used tracer (11).

B) Bifunctional chelate (DFP-TFP) The bifunctional chelator N-sucDf-TFP is the active TFP ester of the succinylated form of desferrioxamine (DFO), which is conjugated to girentuximab.

C) girentuximab (INN), or cG250. Girentuximab is an IgG1 kappa light chain chimeric version of the original murine monoclonal antibody in which human constant regions for the heavy and light chains have been linked by molecular DNA techniques to the corresponding murine variable heavy and light chain regions derived from the murine G250 antibody. The antibody is produced in a mouse myeloma cell line. It has a molecular weight of approximately 150 kD and is composed of two heavy and two light chains of relative molecular weight of 47 kD and 24 kD, respectively. girentuximab recognizes the surface antigen CAIX (alternative names: MN, CAIX-antigen) which is predominantly expressed on ccRCC. The unconjugated antibody is supplied as a clear, colourless to slightly brownish liquid.

Girentuximab has been registered under the brand Rencarex® but has no marketing approval yet. $^{124}$I-radiolabelled girentuximab has been investigated in several nonclinical and clinical studies.

#### 1.2.2.3 Non clinical data

##### 1.3.2.3.1 Unlabelled girentuximab

Immunohistochemical cross-reactivity studies were performed with girentuximab and a variety of normal human tissues (Internal report, Human tissue cross-reactivity study with human-mouse chimeric antibody cG250. Report dated 11.06.2001) and human tumor tissues (Internal Report Human tumor reactivity study with chimeric antibody cG250. Report dated 02.09.2002). The data confirm that the CAIX-antigen is widely expressed in ccRCC and absent from normal kidney tissue. Non-target tissues with significant CAIX-antigen expression were found to be foveolar parietal cells in the stomach, the epithelium of duodenum and the large bile ducts.

The anti-tumor effects of the murine or chimeric G250 antibody were studied in several experiments with nude mice xenografted with RCC (12) ; Internal Report Study P- 1103-008NIJ: Study for the demonstration of a protective and therapeutic effect of chimeric G250 (IgG1, cG250, synonym: girentuximab) and murine G250 (IgG2a, mG250) in NU/NU mice challenged with SK-RC-52 cells; Report dated 29.03.2004). The murine antibody was used in these experiments due to poor interaction of girentuximab with murine effector cells. Repeated injections of the antibody significantly delayed the outgrowth of established tumors. This effect is assumed to be attributable to the induction of ADCC. In an in vitro experiment, girentuximab mediated potent ADCC against several RCC lines especially when effector cells had been stimulated with interleukin- 2 (13).

##### 1.3.2.3.2 Radiolabelled $^{89}$Zr-girentuximab

89Zr-girentuximab imaging was investigated in three nonclinical studies, using rodent xenograft tumor models of ccRCC.

Brouwers and colleagues (11) showed that conjugating girentuximab with the radionuclides $^{89}$Zr or $^{111}$In allows high-contrast imaging of RCC tumors in RNU nude rats, already after 2 days. 3D PET imaging was performed at 5 minutes, 24, 48 and 72 hours after IV injection of 20 MBq $^{89}$Zr-girentuximab, or of 4 MBq $^{111}$In-girentuximab. Comparative PET images after injection of 4 MBq [18F]FDG were recorded at 1 hour post-injection.

Both, $^{89}$Zr-girentuximab and $^{111}$In-girentuximab, were stable during 4 days of incubation in serum and immunoreactivity was preserved. Biodistribution in normal tissues, uptake in tumor at 3 days post injection were almost identical between both radiolabelled girentuximab preparations (tumor uptake 5.0 ± 2.4 and 4.9 ± 2.9 %ID/g, respectively) (Figure 3).

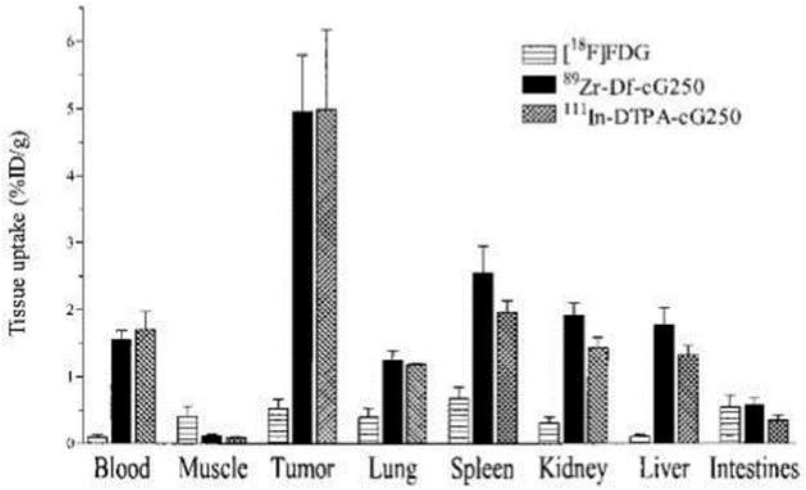



*Figure 2: Biodistribution of [18F]FDG (n =7), 89Zr-girentuximab (n =8), and 111In- girentuximab (n =6) in nude rats with subcutaneous SK-RC-52 RCC tumors at 2 hours post injection [18F]FDG, or 72 hours post injection (89Zr- girentuximab and 111In-girentuximab).*

Relatively small tumors of about 100 mg could be visualised from 24 hours post injection of 89Zr-girentuximab. The quality of tumor imaging improved with time. In contrast, tumors were not visualized with [18F]FDG (uptake in tumor of 0.5 ± 0.1 %ID/g, 1 hour after injection).

Blood levels at 3 days after injection were also identical (1.4 ± 0.4 versus 1.7 ± 0.7 %ID/g), and no significant differences were found in the biodistribution of normal tissues between the two radiolabelled girentuximab preparations.

Both radiolabelled girentuximab preparations showed a relatively high uptake in liver, spleen and heart, resembling frequent sites of RCC metastases.

Stillebroer et al. (14) recently reported a preclinical study in nude mice bearing cRCC tumors directly comparing 89Zr-girentuximab and 124I-girentuximab, showing differences in tracer uptake depending on the tumor model (SK-RC-52 or NU-12). Mice were injected with 89Zr-girentuximab (0.41 MBq/µg or 0.54 MBq/µg), or 124I-girentuximab (0.24 MBq/µg or 1.7 MBq/µg).

The group could show differences in tracer uptake depending on the tumor model. They observed significantly higher uptake of $^{89}$Zr-girentuximab compared with $^{124}$I-girentuximab.

12 tumors (114.7%–25.2% injected dose per gram (%ID/g) vs. 38.2–18.3%ID/g, p=0.029), while there were insignificant differences in uptake between tracers in the SK-RC-52 tumor model (48.7–15.2%ID/g vs. 32.0–22.9%ID/g, p=0.26) (Figure 4). The higher specific uptake of $^{89}$Zr- girentuximab produced higher PET image contrast, despite higher uptake in various normal tissues, such as the liver and spleen.

Intraperitoneal SK-RC-52 lesions as small as 7 mm3 were visualized with 89Zr-girentuximab PET. No information was reported on the size of lesions detected with $^{124}$I-girentuximab PET imaging.

The authors hypothesized that labelling girentuximab with the residualizing positron emitter $^{89}$Zr would lead to higher tumor uptake and more sensitive detection of ccRCC lesions compared with $^{124}$I. They concluded that small intraperitoneal ccRCC lesions could be visualized and easily discriminated from the liver and the spleen with girentuximab-based PET.

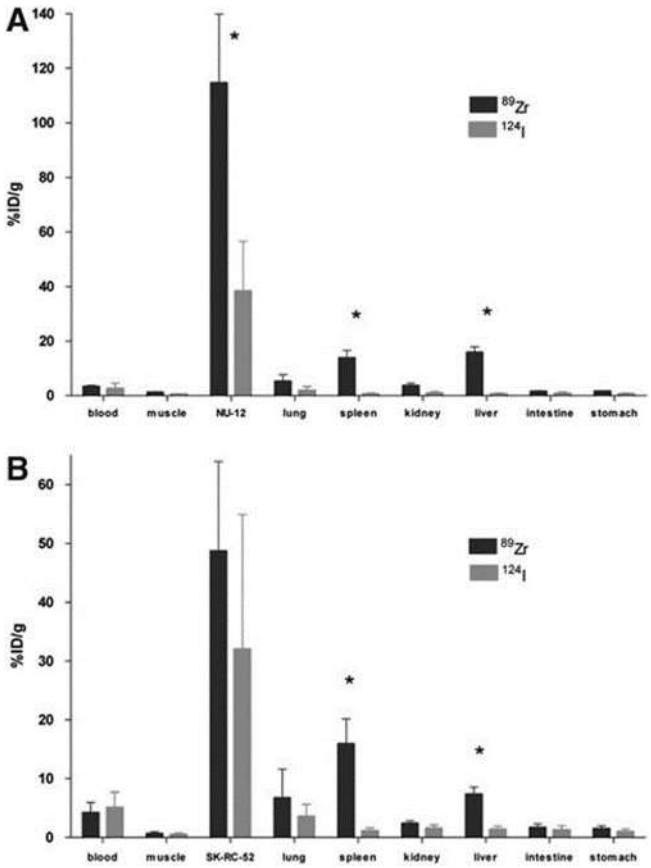



*Figure 3: Biodistribution of 89Zr-girentuximab and 124I-girentuximab in mice with a s.c. NU-12 tumor (A) or a s.c. SK-RC-52 tumor (B), 7 days after injection. Values are expressed as mean–SD. Antibody protein dose was 3 µg girentuximab/mouse in NU-12 and 30 µg girentuximab/mouse in*

*SK-RC-52 tumors. Significant differences (p<0.05) in uptake between 89Zr- girentuximab and 124I-girentuximab are marked with an asterisk**

Cheal and colleagues (15) conducted a quantitative comparison of absolute uptake and antibody turnover between $^{124}$I-girentuximab and $^{89}$Zr-girentuximab using a human cRCC xenograft tumor model (SK-RC-38) in female nude mice. The mice were injected IV with $^{89}$Zr-girentuximab (14.6 to 15.0 MBq, 40 µg, 267 pmol) or $^{124}$I-girentuximab (11.8–12.6 MBq, 100 µg, 667 pmol) and were serially imaged by PET over the course of 10-11 days to determine the kinetics of the tracer uptake in the tumor and the rate of clearance from the vascular department.
The stability of $^{89}$Zr-girentuximab in human serum was evaluated in vitro over 11 days at 37°C by ITLC, showing that ≥ 97.8% of the total $^{89}$Zr activity remained in a form consistent with 89Zr-girentuximab over the course of the study. For comparison, the stability of 124I-girentuximab in human serum was > 99% of the total $^{124}$I-activity, consistent with intact protein (i.e. $^{124}$I-girentuximab).
Tumor activity concentrations were expressed as the maximum %ID/g in manually drawn two-dimensional tumor-contouring regions of interest (ROIs). While both tracers allowed for high-contrast imaging of RCC, the comparative intra-tumoral concentrations varied significantly from 5 hours to 10 days after injection. This observation could not be explained by differences in clearance, as the biological half-lives as determined by quantitation of activity in circulation were not significantly different (Figure 5).

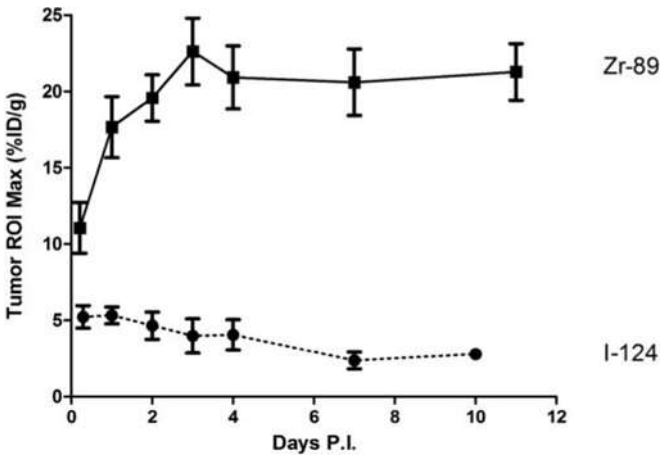



*Figure 4: Time-activity curves for 124I- or 89Zr-girentuximab uptake (over tumor ROIs) in SK-RC-38 xenografts (n = 5 for each cohort) up to 10 and 11 days post injection, respectively. Data are expressed as (mean ± standard error of the mean (SEM)).*

Biodistribution studies for 124I-girentuximab and 89Zr-girentuximab were performed 10 and 11 days after the injection, respectively (Figure 6).

The concentration of the tracer in the target tumor tissue was by far highest for $^{89}$Zr-girentuximab compared with the tumor activity of the non-residualizing $^{124}$I-girentuximab (40 %ID/g for $^{89}$Zr and 5 %ID/g for $^{124}$I).

Higher concentrations of $^{89}$Zr-girentuximab than $^{124}$I-girentuximab were found in liver ($p < 0.05$), spleen, kidney, and bone (all $p < 0.005$), presumably due to the metabolism and hepatobiliary clearance of radiometal complexes in catabolic organs such as liver and kidneys, and the affinity of the phosphate-rich bone for free $^{89}$Zr (16).
While non-specific tracer uptake in normal tissue, especially in the liver and bone, potentially might mask metastatic disease in these organs, the observed tumor-to-background ratio, relative to these organs would still allow identification of such lesions.

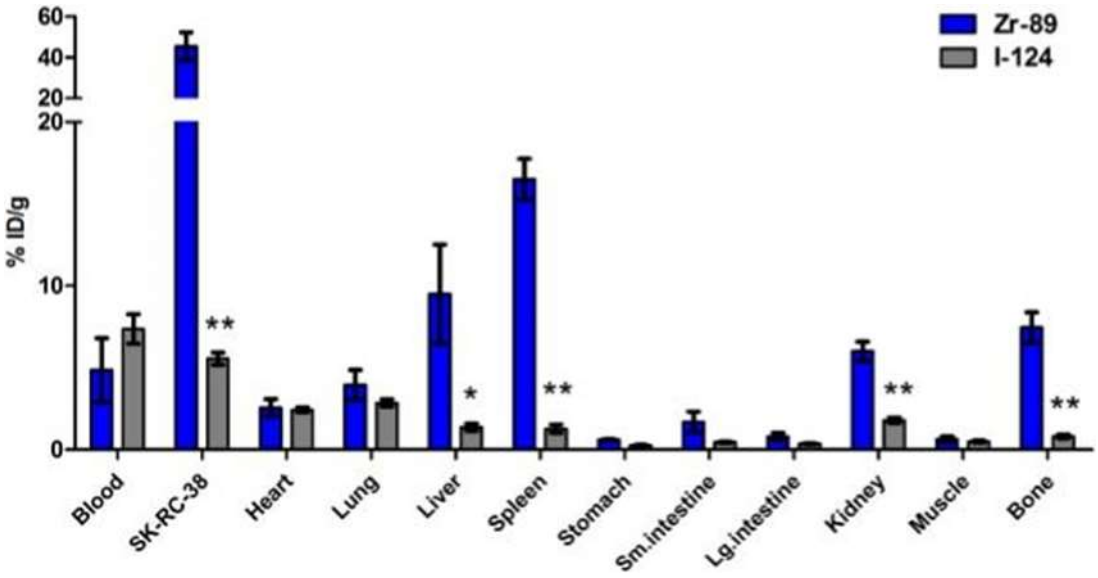



*Figure 5: Comparative biodistribution data for $^{89}$Zr-girentuximab and $^{124}$I- girentuximab following serial PET imaging (11 days for $^{89}$Zr-girentuximab and 10 days for $^{124}$I-girentuximab). Groups consisted of 4 or 5 mice each. Data for $^{124}$I- girentuximab is from group of animals with normal thyroid attime of tracer injection. The data are expressed as mean %ID/g ± SEM. (*) $p < 0.05$, (**) $p < 0.005$.*

$^{89}$Zr- and $^{124}$I-girentuximab were virtually identical in terms of clearance from the blood, Initial tumor binding and uptake within 5 hours, and rate of internalization of the girentuximab-CAIX antibody- receptor complex. However, notable differences in tumor uptake over time and retention of the tracer existed. Uptake of $^{89}$Zr-girentuximab further increased from 48 hours post injection to a plateau of about 20 %ID/g, which persisted to the last imaging time point at 11 days post injection. The peak uptake of $^{124}$I-girentuximab was much lower (at about 5 %ID/g) and was observed already at 5–24 hours, with subsequent washout to about 2 %ID/g at 10 days after injection (Figure 7).

In vivo kinetic models yielded estimates of internalization of the receptor-bound antibody of 20 %ID/g/h, with minimal release of $^{89}$Zr but with comparable uptake of $^{124}$I-girentuximab leading to release of considerable amounts of $^{124}$I. The $^{124}$I atoms were cleared from tumor more than 17.5 times more rapidly than $^{89}$Zr.

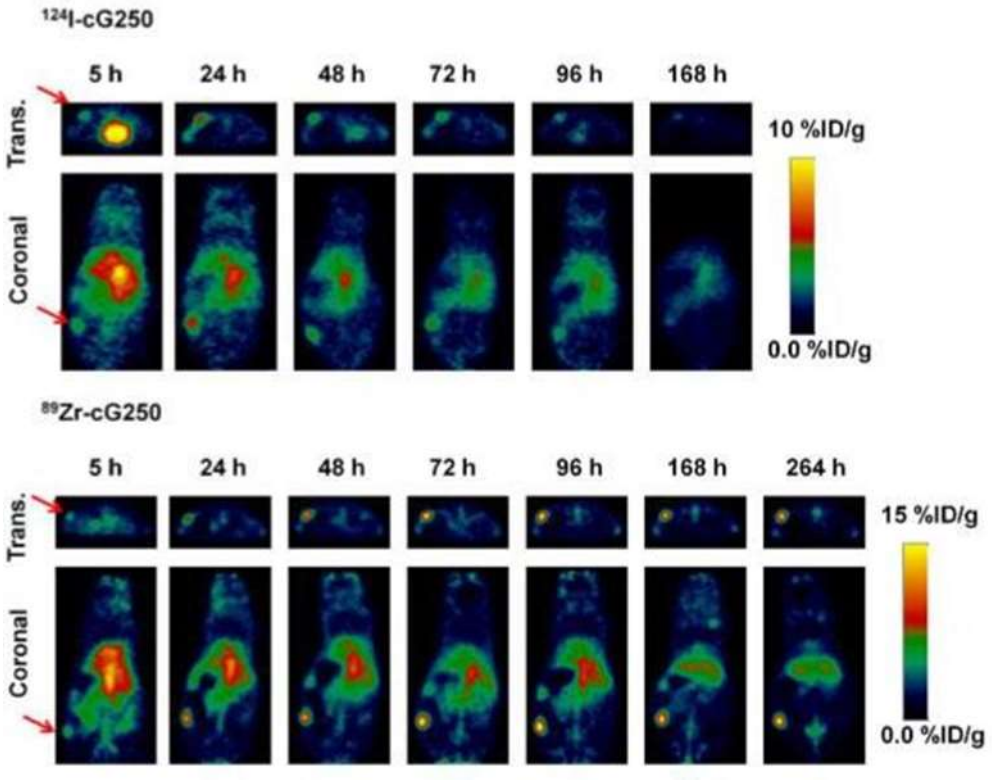



*Figure 6: Comparative serial-PET imaging in tumor-bearing mice using 89Zr- girentuximab and 124I-girentuximab at various time points up to 11 days post injection. Representative transverse (trans.) and coronal PET images of nude mice bearing subcutaneous SK-RC-38 xenografts in the lower hind limb imaged with 124I- or 89Zr-girentuximab at various time points up to 11 days post injection. Arrows indicate position of tumor. Images are parameterized as %ID/g.*

#### 1.2.2.4 Conclusions

The long-lived radiometal $^{89}$Zr has emerged as an attractive alternative to $^{124}$I, owing to its residualizing properties in vivo. The above described studies demonstrate, that the residualizing radionuclide $^{89}$Zr is well suited to image the girentuximab/CAIX biology. In all nonclinical studies, $^{89}$Zr-girentuximab PET imaging was superior compared to $^{124}$I-girentuximab PET imaging, because of prolonged trapping of the radiolabel in the tumor, while at the same time showing washout from normal tissues. Antibody-based molecular imaging with internalizing and residualizing radionuclides such as $^{89}$Zr PET or $^{111}$In SPECT imaging in rodent studies, demonstrated that the quality of tumor imaging improved with time, due to the washout of the background. This is a clear advantage over non-internalizing and non-residualizing tracers such as $^{124}$I, which has a > 17 times higher leakage rate form tumor after internalization.

#### 1.2.2.5 Pharmacokinetics and Product Metabolism in Animals

Binding characteristics of girentuximab are of primary relevance for its pharmacokinetic characteristics as a potential medical drug. Girentuximab binds to the extracellular domain of human CAIX and is cross-reactive with the primate and rabbit homologues of CAIX.

Pharmacokinetic studies were conducted in rabbit and Rhesus monkey (Macaca mulatta). Studies in rabbits were conducted with $^{131}$I- and $^{125}$I-labelled girentuximab. For pharmacokinetics studies antigen cross-reactivity and similarity of tissue-specific antigen expression pattern (as is given in both species), but not necessarily Fc receptor interaction, are prerequisite to qualify an animal model for this type of studies.

The immunogenicity of girentuximab (frequency and degree of induction of antibodies to girentuximab, i.e. Rhesus anti-chimeric antibody, RhACA) was characterized in the course of toxicity studies in Rhesus monkey.

#### 1.2.2.5.1 Single-Dose Pharmacokinetics and Elimination of Girentuximab in Rabbits

The pharmacokinetics and elimination of girentuximab following single intravenous administration were studied using $^{125}$I or $^{131}$I-labelled girentuximab. A non-primate animal model i.e. rabbit was chosen for the single-dose pharmacokinetic studies of radiolabelled girentuximab. The approach in rabbit was justified by the cross-reactivity of girentuximab shown with the rabbit CAIX-antigen (G250 binding site) and a similar tissue-specific antigen expression pattern in rabbit compared to man.

In three pharmacokinetic rabbit studies (Internal Reports 01-50015, 01-50022, and 01-50031) two girentuximab lots radiolabelled with $^{125}$I or $^{131}$I were mixed and simultaneously administered intravenously at two different dose levels of 50 and 500 µg/kg per lot. The combined total girentuximab dose was therefore 100 µg/kg and 1000 µg/kg. The elimination of the radiolabels was differentially tracked in whole blood samples taken at various time points after injection over 48 hours. The rabbits were sacrificed 48 hours after injection and tissue samples of liver, kidney, spleen, pancreas, duodenum, stomach, muscle, and colon were collected, weighed and the amount of radioactivity of each isotope was determined in a gamma counter for comparative girentuximab tissue distribution analysis.

Terminal half-life, t1/2ß was apparently dose-dependent with 17 to 19 hours for the low and 25 to 32 hours for the high antibody dose. At 48 hours after administration to the rabbits, highest levels of radioactivity were evident in the duodenum, followed by colon, liver, and stomach. This ranking of girentuximab binding to organs was consistent in all three rabbit studies and is in line with the binding to human nontarget tissues. Girentuximab specifically binds to CAIX expressed on the surface of these tissues.

A pharmacokinetic two compartment model was found to be optimal to describe the blood elimination curve of girentuximab in rabbit (Table 2). The same model is optimal in humans and monkeys.

Table 2: Elimination of girentuximab Following a Single Intravenous Administration in Rabbits

| **Study No** | **01-50015** | | **01-50022** | | **01-50031** | |
|---|---|---|---|---|---|---|
| **Sex / Number of animals** | F / 18 | | F / 18 | | F / 18 | |
| **Analyte** | cG250-CEN and cG250-AVID, both $^{125}$I- and $^{131}$I-radiolabelled | | cG250-WI and cG250-FLA, both $^{125}$I- and $^{131}$I-radiolabelled | | cG250-AVID-WI and cG250-AVID-FLA, both $^{125}$I- and $^{131}$I- radiolabelled | |
| **Assay** | gamma counting of $^{125}$I- and $^{131}$I- radiolabelled compound | | gamma counting of $^{125}$I- and $^{131}$I- radiolabelled compound | | gamma counting of $^{125}$I- and $^{131}$I- radiolabelled compound | |
| **PK parameters in (whole blood):** | 50 µg/kg | 500 µg/kg | 50 µg/kg | 500 µg/kg | 50 µg/kg | 500 µg/kg |
| $T_{1/2\alpha}$ [h] (lot A; lot B) | 2.2; 2.0 | 2.4; 2.2 | 1.9: 1.7 | 3.0; 2.7 | 0.7; 0.7 | 0.9; 0.8 |
| $T_{1/2ß}$ [h] (lot A; lot B) | 17.1; 17.1 | 25.5; 25.1 | 19.0; 18.7 | 32.4; 31.6 | 18.7; 18.5 | 25.8; 25.4 |
| **PK parameters[1] (serum):** | 100 µg/kg | 1000 µg/kg | 100 µg/kg | 1000 µg/kg | 100 µg/kg | 1000 µg/kg |
| $AUC_{0-\infty}$ (µg/mL*h) | 49.6 | 947.6 | 67.4 | 960.8 | 59.3 | 795.6 |
| $T_{1/2\ ß}$ (h) | 16.8 | 23.7 | 18.6 | 30.9 | 18.7 | 25.1 |
| $C_{max}$ (µg/mL) | 2.9 | 36.7 | 3.3 | 31.3 | 2.6 | 24.7 |

[1] *To create comparability with PK studies in human whole blood concentration data generated in the three rabbit studies were converted into serum concentrations. Because the production lot comparison revealed comparable elimination profiles for the different production lots in all three experiments, serum PK parameter modelling for girentuximab was done based on mean combined serum concentration data of both lots in each study*.

The dose-dependent elimination half-life of girentuximab observed in the three rabbit-single-dose studies may be explained by the contribution of a saturable elimination pathway to overall elimination kinetics such as target binding and internalization. Dose-dependent elimination kinetics

of girentuximab was also described in humans (17) where a 2-mg dose eliminated faster than higher doses ranging from 5 to 50 mg. Dose-dependent elimination half-life was also seen in the Rhesus monkey 6-month toxicity study, where the low dose of 1.5 mg/kg eliminated with a significantly shorter terminal half-life compared with the two higher doses (5 and 15 mg/kg) (Internal Report 01-14005).

In the three in vivo biodistribution studies in rabbits strong binding of radioactive girentuximab to the duodenum was found. Positive duodenal expression of the CAIX-antigen corroborates the suitability of rabbit as model for the pharmacokinetics of radiolabelled girentuximab.

#### 1.2.2.5.2 Repeat-Dose Pharmacokinetics of Girentuximab in Rhesus Monkeys

The therapeutic use of girentuximab as an adjuvant treatment of ccRCC involves a single loading dose of 50 mg and subsequent maintenance doses of 20 mg administered once weekly over 6 months. Repeat-dose pharmaco-/toxicokinetic data were derived from pivotal toxicological primate studies. In particular, the girentuximab serum kinetics studies accompanying the 3-month and 6- month toxicity studies (Internal Reports 01-13001 and 01-13006) as well as a 4-week toxicity study in Macaca mulatta (Rhesus monkey) are described herein.

Pharmacokinetic parameters (Cmax, AUC0-∞ and t1/2β) of girentuximab were determined from the plasma concentration profiles obtained during Week 1 of a 12-week toxicity study in Rhesus monkey.

A two-compartment model best describes the pharmacokinetic plasma profile in monkeys. The same applies for human pharmacokinetics of girentuximab.

T1/2β values are within a comparable range in both species (mean t1/2β human: 176 hours (± 73 hours); mean t1/2β monkeys: 142 hours (± 41 hours).

These pharmacokinetic features of girentuximab in Rhesus monkey were confirmed in a 6-month chronic toxicity study with three girentuximab dose levels of 1.5, 5 and 15 mg/kg. Mean terminal half-lives determined in the 6-month chronic toxicity study in Rhesus monkeys were similar for males and females, but showed some dose-dependence, i.e. a prolongation with dose, as was observed in the rabbit model. Shorter elimination half-lives were observed in the low dose groups receiving girentuximab at 1.5 mg/kg compared with the two higher dose groups. Elimination half-lives, however, were similar in the medium and the high dose group. Shorter elimination half-lives of girentuximab at lower doses may be explained by the contribution to overall elimination of a saturable receptor-mediated pathway via binding to the CAIX-antigen followed by internalization, while at higher doses non-saturable pathways e.g. nonspecific clearance by the RES predominate. In line with the long serum half-life of girentuximab the antibody showed some accumulation in serum after weekly repeated administrations. Once steady state had been achieved after ≤ 6 weeks, however, no further accumulation of girentuximab occurred during the course of the study. This limited accumulation of girentuximab in serum was not associated with signs of toxicity throughout the study.

Consequently, the pharmacokinetic profile of girentuximab in Rhesus monkeys and humans appears to be similar.

The formation of Rhesus monkey-RhACA was measured following the sporadic observation of human anti chimeric antibodies (HACAs) in clinical trials. RhACA formation started after repeated

administration of the antibody, but was not associated with clinical symptoms or changes in laboratory parameters

#### 1.2.2.5.3 Product Metabolism

Studies on drug metabolism are inappropriate for protein drugs such as an antibody and no such studies have been conducted with girentuximab.

The metabolism of proteins, such as antibodies, is well known and can be confined to (i) ubiquitous enzymatic digestion to peptides and amino acids and (ii) modifications to this pathway in the case of ligand-receptor interactions or complex formation between antibody and surface antigen, if therapeutic protein binding is followed by endocytosis and intracellular degradation of the complex. Ubiquitous proteases are not likely to undergo pharmacologically relevant induction or inhibition.

#### 1.2.2.5.4 Elimination

Following enzymatic digestion of antibodies to antibody fragments, peptides, amino acids, carbohydrates, CO2 and NO2, the degradation products enter the major intermediate metabolic cycles, esp. in the hepatobiliary system, and/or are excreted with the urine or the faeces or exhaled.

Most of the radioactivity of the radiometal $^{89}$Zr will be excreted in faeces as it is hepatically cleared together with girentuximab during the first few days (approx. 5 days) after the infusion, but very small amounts may also be eliminated via saliva, sweat and urine.

#### 1.2.2.5.5 Toxicology

No particular studies on safety pharmacology were conducted with the naked girentuximab, the $^{124}$I- or the $^{89}$Zr-girentuximab.

No single dose toxicity study has been conducted. Information on (i.e. the absence of) single dose toxicity may, however, be derived from multiple administration studies. Acute reactions to girentuximab were investigated in the repeat-dose toxicity studies as well as the relationship of dose to systemic or local toxicity. No additional information was expected to be gathered from single dose administration of the antibody in non-human species.

##### 1.2.2.5.5.1 Girentuximab

Repeated dose toxicity studies for 30 days, 12 weeks and 6 months with doses administered once weekly were conducted in Macaca mulatta (Rhesus monkey): 6-month toxicity study of girentuximab after repeated slow bolus injection to Rhesus monkeys. Report dated 22 Sep 2008) Monkeys express cross-reactive CAIX antigen in a manner similar to humans, provide an

immunological environment reactive with the constant portions of the antibody and are therefore a suitable model for toxicity studies. The doses per body weight in the repeat dose monkey studies were several-fold higher than the proposed human doses in immunotherapy trials and produced exposures several-fold higher than in the human therapeutic setting. Girentuximab was well tolerated in the monkey studies and no relevant toxicity was observed. The safety in monkeys of chronic exposure at multi-fold higher levels than intended for imaging suggests safety of the single dose administration of radiolabelled girentuximab for imaging.

#### 1.2.2.5.5.1 $^{89}$Zr-girentuximab

In nonclinical rodent studies, imaging using 89Zr-girentuximab has proven to be safe and feasible (11,14,15). No local or systemic side effects were observed.

### 1.2.3 Effects in Humans

Imaging with radiolabelled girentuximab can lead to an accurate identification of ccRCC, or provide information to rule out ccRCC, and thus offers opportunities for less aggressive patient management.

*Table 3: Clinical Studies in Patients with Unlabelled girentuximab – Dose Regimen, Mean Steady-state Trough Levels, Clinical Benefit, Incidence of HACA Formation and Safety Profile*

| **Trial code** | **WX-2000-01-NAK** (Monotherapy) | **WX-2000-04-GIL** (Combination therapy with IL-2) | **WX-2001-05-IFN** (Combination therapy with IFN-α) | **WX-2003-07-HR** (Monotherapy) |
|---|---|---|---|---|
| **Design** | An open label single arm phase 2 study of girentuximab administered weekly by IV infusion to advanced renal cell carcinoma patients | A phase 1/2 study with girentuximab and low dose IL-2 plus periodic IL-2 pulsing in advanced and metastatic renal cell carcinoma patients | A phase 1/2 study with girentuximab and IFNα-2a in metastatic renal cell carcinoma patients | A randomized, double blind, phase 3 study to evaluate adjuvant girentuximab treatment versus placebo in patients with clear cell RCC and high risk of recurrence |
| **Clinical Phase** | 2 | 1/2 | 1/2 | 3 |
| **Patient Exposure (total of 545 patients, incl. patients from LUD 98-011)** | 36<br><br>36 Caucasian<br>12 female, 24 male | 35<br>34 Caucasian<br><br>1 Asian<br>8 female, 27 male | 31<br><br>31 Caucasian<br>8 female, 23 male | 431<br>403 Caucasian<br><br>10 Asian<br>10 African<br>8 Hispanic<br>156 female, 275 male |
| **Girentuximab dose regimen** | 50 mg once weekly for 12 weeks | 20 mg once weekly for 11 weeks | 20 mg once weekly for 11 weeks | Single loading dose of 50 mg (Week 1) followed by weekly infusions of |

| | | | | |
|---|---|---|---|---|
| | | | | 20 mg of girentuximab or placebo over 24 weeks |
| **Girentuximab plasma trough level (mean value)** | 15.9 µg/mL | 4.7 µg/mL | 6.7 µg/mL | ≥8.7 µg/mL (from Week 8 on) |
| **Clinical Benefit Rate* (Evaluated patients/ITT)** | 28% (9/32) 25% (9/36) | 23% (7/30) 20% (7/35) | 41% (11/26) 35.48% (11/31) | Not applicable |
| **Incidence of HACA formation** (%)** | 2 out of 36 (5.6%) no clinical symptoms | 2 out of 35 (5.7%) no clinical symptoms | None out of 31 (0%) | 51 patients out of 431** (11.8%) 37 patients out of 431*** (8.6%) with neutralising HACAs |
| **Safety profile** | • 13 SAEs, not treatment-related • 147 Non-Serious | • 4 SAEs, not treatment-related • 420 Non-Serious | • 6 SAEs, not treatment-related • 183 Non-Serious | • 72 SAEs, only 1 treatment-related (placebo) |

** Clinical Benefit Rate defined as Response rates (CR and PR) plus Stable Disease ≥ 24 weeks **Any HACA regardless of neutralising or non-neutralising properties*

**** Number of patients in the active treatment arm*

#### 1.2.3.1 Pharmacokinetics and Product Metabolism in Humans

The pharmacokinetics (PK) of intravenous girentuximab is best described using a two-compartment pharmacokinetic model with first-order elimination. The serum exposure to girentuximab is dose linear and dose proportional within the dose range 5 to 100 mg. The terminal serum elimination half- life is independent of dose within this range. A 2-mg dose produces disproportional low exposure and is eliminated with a faster serum half-life compared with higher doses. Doses of 10 mg or higher produce Cmin (trough) serum levels above 1 µg/mL one week after the dose. Girentuximab concentrations of 1 µg/mL reproducibly elicited ADCC in vitro. Thus, 20 mg girentuximab administered once weekly is suitable to warrant sufficient and sustained antibody concentrations adequate at inducing ADCC.

Continuous treatment with 20 mg girentuximab once a week over several months produces a mean serum concentration of approximately 9 µg/mL at steady state. This steady state is achieved between Week 4 and Week 8 and exceeds multi-fold the minimal concentration of 1 µg/mL which is adequate to elicit ADCC in-vitro.

Elimination parameters are similar comparing single dose and multiple dose elimination kinetics. Mean parameters registered in tumor-bearing renal cancer patients were approximately 19 ± 7 h for t1/2α, 180 ± 87 h for t1/2ß, 22 ± 10 mL/h for the clearance, 3.1 ± 0.7 L for the central volume of distribution and 4.6 ± 1.3 L for the volume of distribution at steady state. Exposure parameters AUC and Cmax are largely linearly correlated with the dose. Mean AUCinf values of 123 ± 46 µg*h/mL, 1671 ± 572 µg*h/mL, 2643 ± 462 µg*h/mL and 4519 ± 254 µg*h/mL were noted after infusion of girentuximab at relative doses of 5, 10, 25 and 50 mg/m2. Mean Cmax levels of 3.1 ±

0.2 µg/mL, 8.0 ± 1.2 µg/mL, 17.6 ± 4.3 µg/mL and 30.9 ± 3.6 µg/mL were noted at the four dose levels assayed.

A population pharmacokinetic study considering elimination profiles sourced from seven clinical studies was conducted to identify covariates influencing the pharmacokinetics of girentuximab. Age, gender, aspartate aminotransferase (AST), creatinine clearance (CRCL) and total bilirubin (TBIL) are not statistically significant predictors of clearance and the central volume of distribution. Furthermore, age, body weight, BSA, BMI, ideal body weight, AST, CRCL, and TBIL are not statistically significant predictors of the peripheral volume of distribution. The presence of the primary renal tumor in a patient increases the volume of distribution approximately 4-fold compared with healthy volunteers or nephrectomised renal cancer patients. The serum clearance, however, was not significantly influenced by the presence of tumor.

Pharmacokinetic analysis was performed with unlabelled girentuximab in main study WX-2007- 10-HV and with 131I-girentuximab in main study LUD 98-011. Results will be supported by pharmacokinetic data from historical studies with 131I-girentuximab.

In Study WX-2007-10-HV a single IV dose of unlabelled girentuximab was administered to healthy volunteers.

Study LUD 98-011 administered six weekly IV infusions of girentuximab at doses of 5, 10, 25, or 50 mg/m2 to patients with advanced RCC. The first and fifth doses of girentuximab were trace labelled with 131I (8-10 mCi).

A comparison of the pharmacokinetics in these two studies is provided in Table 5.

#### 1.2.3.1.1 Phase 1 Study: Single Dose intravenous administration of 20 mg girentuximab in Healthy Volunteers (Study WX-2007-10-HV)

The objective of the study was the determination of individual pharmacokinetic parameters and parameter ranges of girentuximab plasma elimination profiles observed in 12 healthy volunteers of both sexes after a single intravenous dose of 20 mg girentuximab. For each individual profile non- compartmental, two compartmental or three compartmental pharmacokinetic modelling was applied. The two-compartment model was found to fit measured profiles best. Concentration-time profiles of all healthy volunteers are shown in Figure 8. Serum concentration-time profiles of the study population showed a uniform and consistent shape during the initial and the terminal phase of elimination.

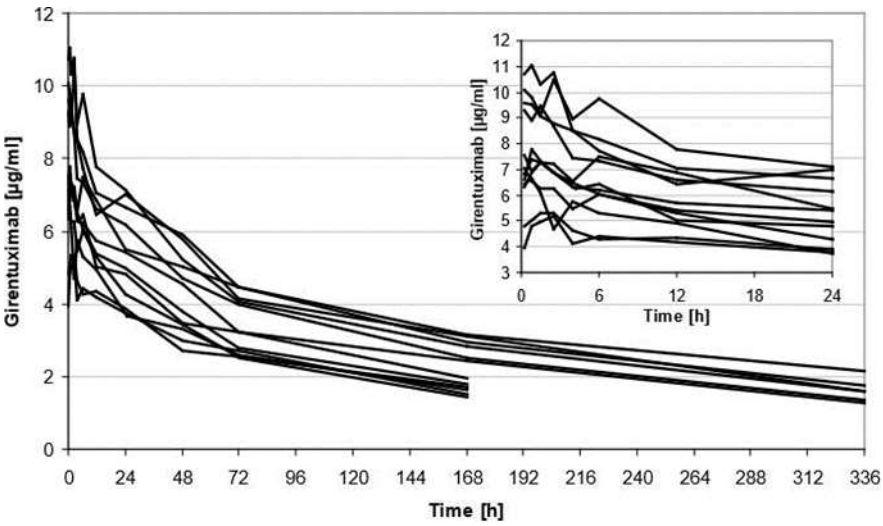



*Figure 7: Pharmacokinetics of unlabelled girentuximab in Healthy Volunteers*

At the 336-hour sampling time, quantifiable data were obtained from six volunteers. For the other six volunteers girentuximab concentrations were found to be below the limit of quantification. For these six volunteers the last sample considered for parameter calculation was the one taken at the sampling time point 168 hours. At the end of the pharmacokinetic sampling period (504 hours after start of infusion, data not shown) girentuximab concentrations were below the limit of quantification for all volunteers.

The two-compartment model was determined to be optimal for determination of the pharmacokinetic parameters of these healthy volunteers. A two-compartment model, as a bi-exponential model, generates two half-lives, the alpha (t1/2α) and beta (t1/2β) half-life. The alpha half-life is the distribution half-life, and the beta half-life is the overall elimination half-life in the terminal phase. t1/2α had a wide range (2.2 – 32 hours) with a mean of 15.1 hours (standard deviation [SD] of 11.5 hours). T1/2ß ranged from 91 to 269 hours with a mean of 176.4 hours (SD of 72.5 hours).

During steady-state there is equilibrium of the concentrations in all compartments with no net transport between them.

The serum profiles and PK parameters suggest little inter-individual variability, hence good predictability of exposure. Also, these data suggest no major influence of gender on girentuximab profiles. The serum Cmax values achieved have a narrow distribution around the population mean with a coefficient of variation (CV) of 26.3%. The mean terminal serum half-life (t1/2β) in this healthy volunteer population was approximately one week. This is similar to the mean terminal serum half- life reported for pre-surgery kidney cancer patients bearing their primary tumor in a study with $^{131}$I- girentuximab (t1/2α of 19.0±6.8 h and t1/2ß of 180.2±86.9 h) (Study LUD 98/011). The pharmacokinetics of girentuximab in healthy volunteers, is also similar to the pharmacokinetics reported in a population of nephrectomised metastatic ccRCC patients (11).

#### 1.2.3.1.2 Phase 1 Study: Multiple-dose, Dose Escalation Study of Girentuximab in Patients with Advanced Renal Cell Carcinoma (Protocol LUD 98-011)

Study LUD 98-011 was a phase 1, open-label, non-randomized, dose escalation study designed to evaluate the safety of escalating doses of girentuximab administered IV once weekly for 6 weeks to patients with advanced RCC. Secondary objectives included determination of blood pharmacokinetics, organ and tumor distribution of girentuximab, and evaluation of immune response and anti-tumor effects.

A total of 13 eligible male and female patients, 46-73 years of age, were enrolled.

Four dose groups received weekly IV infusions of girentuximab for 6 weeks at one of four doses (5, 10, 25, or 50 mg/m2). Three to four patients were treated per dose level. The first and fifth infusions at each dose level were trace-labelled with 8-10 mCi of $^{131}$I for the evaluation of biodistribution and other parameters. All patients showed $^{131}$I-girentuximab tumor localisation by gamma camera imaging in Weeks 1 and 5.

**Pharmacokinetics**

During Weeks 1 and 5, blood samples were collected for pharmacokinetic analysis immediately prior to the $^{131}$I trace-labelled study treatment infusion, 10 minutes, and 1, 2, and 4 hours after completion of the infusion. Samples for determination of radioactivity were also obtained on Day 1, on Day 2 or 3, on Day 4 or 5, and Day 6 or 7, in conjunction with gamma camera imaging.

Blood samples were collected in Weeks 1 through 6 immediately prior to infusion and 10 minutes following completion of infusion. In addition, a blood sample was collected in Week 8. Pharmacokinetic analyses were performed by measurement of serum $^{131}$I radioactivity and by ELISA assay of serum girentuximab. Thirteen patients were evaluable for pharmacokinetics.

Pharmacokinetic parameters from the first cycle of treatment (Week 1 and Week 5 infusions of 131I- labelled girentuximab as a pharmacokinetic probe) are presented in Table 4. Repeated intravenous doses of up to 50 mg/m2 of girentuximab were safe using once a week administration. There was no change in PK on repeated dosing and accumulation was low.

*Table 4: Pharmacokinetic Parameters of $^{131}$I-girentuximab Following Multiple Dose Administration in Patients with RCC (Study LUD 98-011)*

| Parameter, mean ± SD[1] | girentuximab Dose (mg/m²) | | | | |
|---|---|---|---|---|---|
| | 5 (N=3) | 10 (N=3) | 25 (N=4) | 50 (N=3) | All (N=13) |
| $t_{1/2\alpha}$ (h) | 21.3 ± 5.41 | 25.3 ± 3.39 | 17.9 ± 6.73 | 11.8 ± 4.93 | 19.0 ± 6.84 |
| $t_{1/2\beta}$ (h) | 122.9 ± 45.68 | 272.3 ± 114.55 | 184.9 ± 72.51 | 139.1 ± 41.94 | 180.2 ± 86.68 |
| AUC (h·µg/mL) | 309.9 ± 77.99 | 1671 ± 571.98 | 2643.2 ± 462.34 | 4519.3 ± 253.9 | NA |
| $C_{max}$ (µg/mL)[2] | 3.1 ± 0.21 | 8.0 ± 1.17 | 17.7 ± 4.29 | 30.9 ± 3.56 | NA |
| CL (mL/h) | 33.8 ± 15.98 | 16.7 ± 7.60 | 19.0 ± 4.72 | 21.6 ± 4.52 | 22.5 ± 10.28 |
| MRT (h) | 153.3 ± 51.6 | 360.3 ± 147.12 | 248.9 ± 96.73 | 193.6 ± 52.93 | 239.8 ± 113.66 |
| $V_1$ (mL) | 3149.9 ± 741.48 | 3206.1 ± 1103.90 | 2887.0 ± 895.91 | 3141.0 ± 441.93 | 3079.9 ± 739.24 |
| $V_{ss}$ (mL) | 4626.8 ± 240.93 | 5376.0 ± 2031.87 | 4542.4 ± 1708.3 | 4087.5 ± 906.65 | 4649.3 ± 1333.88 |

[1] Mean of Week 1 and Week 5 infusions
[2] $^{131}$I-labelled-girentuximab assay

Abbreviations: AUC: area under the concentration curve extrapolated to infinite time; Cmax: maximum serum concentration; CL: total serum clearance; MRT: mean serum residence time; NA: Not Applicable; SD: standard deviation; $T_{1/2\alpha}$ and $T_{1/2\beta}$: half-lives of the initial and termination phases of disposition, respectively. $V_1$: volume of central compartment; $V_{ss}$: volume of distribution at steady state.

No statistical differences were shown between dose levels for the mean (± SD) values of $T_{1/2\alpha}$, $T_{1/2\beta}$, CL (total serum clearance) or MRT (mean serum residence time) for $^{131}$I-labelled girentuximab (ANOVA, $p > 0.112$).

The $V_1$ (volume of central compartment) and $V_{ss}$ (volume of distribution at steady state) results also showed no differences between protein dose levels and were consistent with a two-compartment model for pharmacokinetic analysis.

The Cmax and AUC values showed a linear relationship to dose of girentuximab.

The $^{89}$Zr-girentuximab to be used as an imaging agent will be administered as a single dose; however, of note, infusions of girentuximab within a cycle were associated with increasing values for $C_{max}$ and $C_{min}$.

#### 1.2.3.1.3 Comparison of Pharmacokinetics in Study WX-2007-10-HV and Study LUD 98-011

The pharmacokinetic measurements determined from the two studies in healthy volunteers and in patients with RCC are compared in Table 5.

Despite the study population and dosing differences, the pharmacokinetics of unlabelled and $^{131}$I-radiolabelled girentuximab were similar between the two clinical studies. A two-compartment model appears to be appropriate for pharmacokinetic analysis of girentuximab, irrespective of radiolabel or disease state.

*Table 5: Pharmacokinetic Parameters of Naked girentuximab in Healthy Volunteers Compared with $^{131}$I-girentuximab in Patients with RCC (Studies WX-2007-10- HV and LUD 98-011)*

| **Parameter, mean ± SD[1]** | **Study WX-2007-10-HV 20 mg girentuximab in Healthy Volunteers** | **Study LUD 98-011**<br>**25 mg/m² $^{131}$I-girentuximab in Patients with RCC** |
|---|---|---|
| | **(N=12)** | **25 (N=4)** |
| $t_{1/2\alpha}$ (h) | 15.1 ± 11.5 | 17.9 ± 6.73 |
| $t_{1/2\beta}$ (h) | 176.4 ± 72.5 | 184.9 ± 72.51 |
| AUC (h·µg/mL) | 1202.3 ± 448.1 | 2643.2 ± 462.34 |
| $C_{max}$ (µg/mL) | 7.7 ± 2.0 | 17.7 ± 4.29 |
| CL (mL/h) | 17.9 ± 6.4 | 19.0 ± 4.72 |
| $V_{ss}$ (L) | 3.9 ± 1.1 | 4.5 ± 1.7 |

[1] For Study LUD 98-011, mean of Week 1 and Week 5 infusions;

*Abbreviations: AUC: area under the concentration curve extrapolated to infinite time; Cmax: maximum serum concentration; CL: total serum clearance; NA: Not Applicable; SD: standard deviation; t1/2α and t1/2β: half-lives of the initial and terminal phases of serum elimination, respectively. Vss: volume of distribution at steady state*

Compared with studies WX-2007-10-HV and LUD 98-011, shorter t1/2α and t1/2β values were observed in a previous clinical study performed in patients bearing the primary RCC (Steffens, 1997). The discrepancy in terminal serum half-life between the studies may relate to the sequestration of girentuximab from serum by binding to the primary tumor present in the patients investigated by Steffens et al. The concentration of residual serum girentuximab one week after

the dose (C168 h) may therefore be expected to be lower in kidney cancer-bearing patients than suggested by data generated in the healthy volunteer population (WX-2007-10-HV) or in a population of nephrectomized metastatic ccRCC patients (Study LUD 98-011).

Pharmacokinetics of $^{124}$I-girentuximab or $^{89}$Zr-girentuximab are not anticipated to be substantially different from those of girentuximab and $^{131}$I-girentuximab.

With girentuximab having a terminal half-life of approximately one week and $^{89}$Zr having a decay half-life of approximately 3.3 days, $^{89}$Zr-girentuximab provides good visualization on PET/CT images following clearance of background radiolabel from the sera.

#### 1.2.3.2 Safety and Efficacy

##### 1.2.3.2.1 Girentuximab

There is ample experience with radiolabelled tumor targeting in patients using the anti-CAIX antibody girentuximab. Although never observed in previous trials, allergic-type reactions are possible during and immediately following the administration of girentuximab.

As of November 2017, 545 patients have been treated with unconjugated girentuximab (Rencarex®) in five completed studies during the development program for Rencarex®. The trials were conducted with both the murine (mG250) and the chimeric version of the antibody (girentuximab), both of which are virtually indistinguishable in their ability to target RCC tumors and in their binding capacity to the CAIX antigen. One open label single dose phase 1 study (WX-2007-10-HV) in healthy volunteers (girentuximab monotherapy), two phase 1/2 studies in combination therapy with low dose Interleukin-2 (WX-2000-04-gil) and Interferon-α (WX-2001-05-IFN), both in patients with metastatic RCC, one phase 2 girentuximab monotherapy study (WX-2000-01-nak) in advanced RCC patients, and one adjuvant placebo-controlled double-blind phase 3 study in patients with clear cell RCC and high risk of recurrence (WX-2003-07-HR) were conducted. In addition, unlabelled girentuximab was administered in two further studies LUD 2002-003 and LUD 98-011, where patients received also radiolabelled girentuximab.

In a single-dose phase 1 study (Steffens et al., 1997), the injection of trace-labelled antibody was well tolerated and no side effects were seen.

In an open label Phase 1 study (WX-2007-10-HV) 12 healthy volunteers received a single IV dose of 20 mg of unlabelled girentuximab. Safety and tolerability of girentuximab in healthy volunteers as assessed by AEs, vital signs, 12-lead ECG, clinical laboratory evaluations, physical examination findings, and HACA. No safety concerns were raised.

In a multiple dose phase 1 study (LUD 98-011) repeated doses of up to 50 mg/m2 of girentuximab were administered for more than 6 weeks in patients with metastatic RCC without significant toxicity.

In a phase 2 study with a 12-weeks plus 8 weeks extension treatment regimen (WX-200001- NAK), the weekly IV administration of 50 mg girentuximab was safe and well tolerated by all patients without significant immunogenic effects or direct side effects. Intravenously administered girentuximab appears to be immune-silent and opens the possibility for repeated treatment.

The treatment with girentuximab in combination with IL-2 (WX-2000-04-GIL) or IFNα-2a (WX- 2001-05-IFN) appears to be safe in all investigated doses and schedules. The vast majority of adverse events were mild to moderate, transient, fully reversible and reflected mostly the constitutional side effects known to occur under treatment with IL-2 or IFNα-2a. Due to the combination of girentuximab with these cytokines, causal relationship can only be assessed to the combination regimen and not to girentuximab alone.

A total of 855 patients participated in study WX-2003-07-HR [ARISER trial]. Approximately half of these received girentuximab. HACA formation was followed and patients‟ HACAs were characterized for their neutralising properties and their impact on their girentuximab serum levels. 8.6% of the patients treated with girentuximab (37 patients out of 431) developed neutralising HACAs, whereas, only for 2.4% of the patients, the formation of neutralising HACAs had a relevant impact on their girentuximab level during the 24-week long treatment phase. The earliest time point when a girentuximab level was persistently no longer measurable was in Week 12 of the treatment period.

The AEs reported during study WX-2003-07-HR were predominantly as expected for this patient population. Overall, the frequency of patients reporting any AE, any SAE or any grade 3/4/5 AE, regardless of relationship, was comparable across both treatment arms. No treatment related deaths occurred during the study, and no patients were discontinued due to drug related SAEs. One girentuximab-treated patient who discontinued due to related cytokine release syndrome recovered completely. Overall, treatment with girentuximab was seen to be safe.

Table 6 lists the safety results for the four clinical studies in patients conducted with unlabelled girentuximab in the development program for Rencarex®.

*Table 6: Clinical Studies in Patients with Unlabelled girentuximab – Dose Regimen, Incidence of HACA Formation and Safety Profile*

| **Trial code** | **WX-2000-01-NAK** (Monotherapy) | **WX-2000-04-GIL** (Combination therapy with IL-2) | **WX-2001-05-IFN** (Combination therapy with IFN- α) | **WX-2003-07-HR** (Monotherapy) |
|---|---|---|---|---|
| **Design** | An open label single arm Phase 2 study of girentuximab administered weekly by IV infusion to advanced renal cell carcinoma patients | A Phase 1/2 study with girentuximab and low dose IL-2 plus periodic IL-2 pulsing in advanced and metastatic renal cell carcinoma patients | A Phase 1/2 study with girentuximab and IFNα-2a in metastatic renal cell carcinoma patients | A randomized, double blind, Phase 3 study to evaluate adjuvant girentuximab treatment versus placebo in patients with clear cell RCC and high risk of recurrence |
| **Clinical Phase** | 2 | 1/2 | 1/2 | 3 |
| **Patient Exposure (total of 545 patients, incl. patients from LUD 98-011)** | 36<br><br>36 Caucasian<br>12 female, 24 male | 35<br>34 Caucasian<br><br>1 Asian<br>8 female, 27 male | 31<br><br>31 Caucasian<br>8 female, 23 male | 431<br>403 Caucasian<br><br>10 Asian<br>10 African |

| | | | | |
|---|---|---|---|---|
| | | | | 8 Hispanic<br>156 female, 275 male |
| **Girentuximab dose regimen** | 50 mg once weekly for 12 weeks | 20 mg once weekly for 11 weeks | 20 mg once weekly for 11 weeks | Single loading dose of 50 mg (Week 1) followed by weekly infusions of 20 mg of girentuximab or placebo over 24 weeks |
| **Incidence of HACA formation* (%)** | 2 out of 36 (5.6%) no clinical symptoms | 2 out of 35 (5.7%) no clinical symptoms | None out of 31 (0%) | 51 patients out of 431* (11.8%)<br>37 patients out of 431**<br><br>(8.6%) with neutralising HACAs |
| **Safety profile** | • 13 SAEs, not treatment-related<br>• 147 Non-Serious AEs (12.2% related) | • 4 SAEs, not treatment-related • 420 Non-Serious AEs (81.9 % related)<br>• Most AEs typical<br><br>for IL-2 (e.g. fever, chills) | • 6 SAEs, not treatment-related • 183 Non-Serious AEs (39% definitely related) • Most AEs typical for IFN (e.g. fever,<br><br>chills, flulike symptoms) | • 72 SAEs, only 1 treatment-related (placebo)<br><br>• 66.5% of patients experienced AEs<br>• 21.6% were considered treatment-related (21.0%<br><br>placebo, 22.3% girentuximab) |

*Any HACA regardless of neutralising or non-neutralising properties ** Number of patients in the active treatment arm

Minimal HACA responses were observed and were not associated with clinical symptoms. No allergic reactions have been observed so far.

No causal relationship between deaths occurred during or after study termination and girentuximab was observed.

In conclusion, from the data collected in clinical studies, it appears that the chimeric antibody girentuximab is well tolerated, and safe. In addition, girentuximab has a low immunogenicity as the production of HACAs against the girentuximab antibody was only seen very rarely.

#### 1.2.3.2.2 Overview on Safety of $^{89}$Zr-TLX250 ($^{89}$Zr-TLX250)

The risks associated with the injection of Zirconium-89 are considered low. The mean effective dose after administration of a Zirconium-labelled monoclonal antibody is 0.6 mSv/MBq (18). Radiation exposure of 37 MBq $^{89}$Zr-labelled girentuximab will be approximately 22 mSv. Considering the patient category (majority older than 50 years and/or metastatic disease) the relative dose will be 22/5 = 4.4 mSv and this is an acceptable dose according to the ICRP 62 (ICRP, 1992).

In a single-centre, single-arm and open label proof-of-concept study (No. 08121986, ZIRDEE), conducted and recently completed by Radboud, 30 patients suspected of primary, recurrent or metastatic ccRCC and in whom conventional diagnostics were inconclusive, were included. A $^{89}$Zr-TLX250 PET/CT was acquired 4-5 days after single intravenous injection of 5 mg $^{89}$Zr- TLX250 (37 MBq) (19).

No dosing related AEs were reported for the 30 included patients. Three patients experienced mild diarrhoea of whom 2 patients had pre-existing bowel complaints such as stoma and colitis.

A bridging dosimetry study (ZIRDOSE), looking at both a 5 mg and a 10 mg mass dose of girentuximab, investigating the safety and diagnostic performance of $^{89}$Zr-TLX250 PET/CT in 10 patients with suspected ccRCC, based on incidental imaging evidence of a renal mass, is completed and in the analysing and reporting phase. Preliminary safety data did not prompt any safety concern over the treatment. In the course of the study, no treatment-related SAEs or SUSARs were reported from any of the 10 patients. The severe SAE of post operational bleeding was considered by the investigator as not related to the study treatment. A total of 7 AEs were experienced by 4 patients. An unlikely 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. Nausea is an expected side effect of girentuximab.

To date, the on-going ZIRCON phase 3 trial has dosed 29 patients with $^{89}$Zr-TLX250 where no treatment -related SAEs have been reported. The non-treatment related SAEs consisted of grade 3 anaemia, pleural effusion, increased C-reactive protein and a grade 5 arterial perforation through a surgical complication. Nine grade 1 AEs have been reported where only one incidence of diarrhea and abdominal pain were assessed as possibly related to the study drug. Two grade 2 events of abdominal pain and constipation were reported but were not considered to be related to $^{89}$Zr-TLX250.

#### 1.2.3.2.3 Girentuximab, Radiolabelled with Other Diagnostic Radionuclides

Altogether, during more than 2500 chimeric girentuximab administrations allergic-type reaction were not observed. In the radioimmunotherapy (RIT) trials, no side effects that could be attributed to the girentuximab itself were observed (7,12,13).

In radioimmunotherapy trials (RIT) with multiple administrations of radiolabelled girentuximab, Telix International Pty Ltd 40, HACA responses were observed in approximately 15% of patients (Stillebroer et al., 2013). Although HACA formation did not result in any clinical signs or symptoms or biochemical abnormalities, it prevented further treatment with radiolabelled girentuximab due to immune complex formation of the radiolabelled antibody in circulation and subsequent fast blood clearance of the immune complexes, causing reduced accumulation of radiolabelled girentuximab in the tumor (11).

In summary, there are a total of 265 unique patients from the three Wilex sponsored studies: WX/20- 001, LUD 2002-003, and LUD 98-011. It might be noteworthy that study LUD 98-011 administered girentuximab (four infusions) and $^{131}$I-girentuximab (two infusions).

The most comprehensive available safety data for radiolabelled girentuximab exist from patients which were undergoing diagnostic PET imaging with $^{124}$I-girentuximab, which was evaluated in a diagnostic Phase 3 study in 196 patients with suspected ccRCC (Protocol WX/20-001, an open- label, comparative study of positron emission tomography/computed tomography (PET/CT) versus diagnostic CT for the detection of ccRCC in pre-surgical patients with renal masses). Safety data from this study and two additional studies involving use of $^{124}$I- or $^{131}$I-girentuximab are presented.

All patients in the safety population received at least one dose of study treatment (i.e., $^{124}$I-girentuximab, girentuximab, or $^{131}$I-labelled girentuximab) (20).

In Study LUD 2002-003, one of the 25 patients tested developed HACA in response to the infusion. All physical examination results for this patient were normal.

Transient slight changes in blood pressure and pulse were recorded on day 1 for some patients during the infusion, but the vital signs of most patients were not affected. None of the systolic blood pressure readings met the criteria for decreasing the rate of or discontinuing the infusion.

In Study LUD 98-011, 13 patients were observed for at least 28 days after the first dose to assess toxicity. All patients were HACA negative after treatment except for one patient who developed HACA after the second weekly infusion.

In Study WX/20-001, 204 patients received a single 13.7 mg infusion of $^{124}$I-girentuximab, and 198 of these patients had at least one baseline and one follow-up (post-treatment) blood sample evaluable for HACA evaluation. Samples for HACA assessment were obtained at all of the study-defined time points for 169 of these patients. 142 out of 198 patients (71.7%) were negative for HACA at all time points, while 56 out of 198 patients (28.3%) were positive for HACA at one or more time points. Specifically, not all patients tested positive for HACA had a clinically relevant HACA conversion to positive for neutralising capability.

In reviewing positive HACA responses of the 56 positive HACA patients, 41 patients (20.7%; 41/198) had only one positive HACA sample and, of these forty-one patients, 10 were HACA positive within 48 hours before surgery. 30 patients had a positive HACA result at the last follow- up visit 8 to 9 weeks after surgery, and 1 subject 4 to 5 weeks after surgery. Only 15 patients (7.6%; 15/198) had more than 1 positive HACA sample.

In total, 44 of the 56 HACA converted patients had a positive sample at 8 to 9 weeks after surgery; while 11 patients (20%) who developed HACA antibodies reverted to HACA negative within approximately two months (study duration). For one patient the last follow up sample to be taken on Weeks 8-9 is missing. This observation of reconversion back to HACA negative is an expected immunologic response. The waning of HACA positivity could explain the absence of any clinically important findings in those patients who did convert to HACA positive versus those who remained HACA negative. Importantly, the patients remaining HACA positive [+] did not have any safety findings that differentiated them from HACA negative [-] patients implying HACA levels in these remaining HACA[+] patients were also decreasing.

The incidence of AEs considered to be related to $^{124}$I-girentuximab (12.5%) and of AEs that were severe or worse in intensity by CTCAE grading (21.4%) for these 56 HACA-positive patients was similar to that of all patients (13.3% and 16.4%, respectively). The incidence of SAEs was also similar (16.1% for patients with positive HACA results versus 14.6% for patients overall).

#### 1.2.3.2.4 Girentuximab and mG250, Radiolabelled with Therapeutic Radionuclides for RIT

Multiple studies have been conducted with $^{131}$I-labelled girentuximab in order to investigate the potential to use these antibodies for radioimmunotherapy (RIT). Due to the lack of efficacy, this development was discontinued.

#### 1.2.3.2.5 Overview on Safety

⇒ **Common Adverse Events**

Common AEs are defined as those occurring in more than 2% of patients in Study WX/20-001. The threshold of 2% was used due to the low incidence of AEs, the typical higher threshold e.g. 5%, would result in fewer listed "common" AEs. Low AE rates are expected in diagnostic radiopharmaceutical imaging agents and setting the threshold at the lower 2% allows the listing of the few AEs that were observed.

Safety information for Studies LUD 98-011 (administration of girentuximab with trace $^{131}$I-labelled girentuximab to patients with RCC) and WX2007-10-HV (administration of girentuximab to healthy volunteers) are presented as supportive data and can be found in the above sections.

To date, 230 patients have been treated with $^{124}$I-girentuximab in clinical trials. The following AEs, for which a causal relationship to the study medication could not be excluded, have been reported in at least 5% of patients. These adverse drug reactions (ADRs) are presented in descending order of frequency and are considered expected for $^{124}$I-girentuximab: headache, nausea, diarrhoea, dizziness, dysgeusia, pollakiuria, flushing and temporary increase in liver function tests (ALT, AST).

Single cases of mild administration site reactions (e.g. redness, pain, itching) and mild allergic reactions (e.g., rash, chills, feeling hot) have been reported and are also considered expected.

Table 7 presents the most commonly reported AEs in the two main studies for $^{124}$I-girentuximab (Studies WX/20-001 and LUD 2002-003). Adverse events are presented by decreasing SOC incidence in Study WX/20-001. AEs reported by at least one subject in Study LUD 2002-003 but reported by less than 2% of patients in Study WX/20-001 are presented for comparison.

*Table 7: Summary of Adverse Events Reported Following a Single Dose of $^{124}$I-girentuximab Reported in at Least 2% of Patients for Study WX/20-001 and at Least One Subject for Study LUD 2002-003 – Safety Population*

| | Study WX/20-001 | Study LUD 2002-003 |
|---|---|---|
| **MedDRA System Organ Class/ Preferred Term** | **N = 226 n (%)** | **N = 26 n (%)** |
| **Subject reporting at Least One Adverse Event** | **191 (84.5)** | **26 (100)** |
| | | |
| **Gastrointestinal disorders** | **96 (42.5)** | **2 (7.7)** |
| Abdominal pain | 26 (11.5) | 2 (7.7) |
| Abdominal distension | 7 (3.1) | 0 |
| Constipation | 26 (11.5) | 0 |
| Diarrhea | 19 (8.4) | 0 |
| Nausea | 44 (19.5) | 0 |
| Vomiting | 13 (5.8) | 0 |
| **Nervous system disorders** | **65 (28.8)** | **0** |
| Dizziness | 14 (6.2) | 0 |
| Dyseugesia | 12 (5.3) | 0 |
| Headache | 24 (10.6) | 0 |
| Hypoesthesia | 5 (2.2) | 0 |
| **General disorders and administration site conditions** | **63 (27.9)** | **0** |
| Fatigue | 26 (11.5) | 0 |
| Edema peripheral | 8 (3.5) | |
| Pain | 12 (5.3) | 0 |
| Pyrexia | 10(4.4) | 0 |
| **Injury, poisoning and procedural complications** | **61 (27.0)** | **0** |
| Incision site pain | 19 (8.4) | 0 |
| Procedural pain | 27 (11.9) | 0 |
| Urinary anastomotic leak | 5 (2.2) | 0 |
| **Respiratory, thoracic and mediastinal disorders** | **42 (18.6)** | **0** |
| Atelectasis | 11 (4.9) | 0 |
| Cough | 7 (3.1) | 0 |
| Dyspnea | 10 (4.4) | 0 |
| Pleural effusion | 11 (4.9) | 0 |
| Pneumothorax | 5 (2.2) | 0 |
| **Musculoskeletal and connective tissue disorders** | **39 (17.3)** | **0** |
| Back pain | 15 (6.6) | 0 |
| Flank pain | 6 (2.7) | 0 |
| Muscle spasms | 6 (2.7) | 0 |
| **Infections and infestations** | **35 (15.5)** | **0** |
| Urinary tract infection | 8 (3.5) | 0 |
| **Psychiatric disorders** | **33 (14.6)** | **0** |
| Anxiety | 14 (6.2) | 0 |
| Insomnia | 19 (8.4) | 0 |
| **Investigations** | **29 (12.8)** | **26 (100)** |
| Activated partial thromboplastin time prolonged | 0 | 2 (7.7) |
| Alanine aminotransferase increased | 3 (1.3) | 11 (42.3) |
| Aspartate aminotransferase increased | 2 (0.9) | 15 (57.7) |
| Blood alkaline phosphatase increased | 1 (0.4) | 2 (7.7) |
| Blood amylase increased | 1 (0.4) | 1 (3.8) |
| Blood bicarbonate decreased | 1 (0.4) | 2 (7.7) |
| Blood creatinine increased | 0 | 14 (53.8) |

*Table 7: Summary of Adverse Events Reported Following a Single Dose of $^{124}$I-girentuximab Reported in at Least 2% of Patients for Study WX/20-001 and at Least One Subject for Study LUD 2002-003 – Safety Population (...continued)*

| | Study WX/20-001 | Study LUD 2002-003 |
|---|---|---|
| **MedDRA System Organ Class/ Preferred Term** | **N = 226 n (%)** | **N = 26 n (%)** |
| **Subject reporting at Least One Adverse Event** | **191 (84.5)** | **26 (100)** |
| Hemoglobin decreased | 6 (2.7) | 26 (100) |
| International normalized ration increased | 0 | 5 (19.2) |
| Lipase increased | 1 (0.4) | 1 (3.8) |
| Platelet count decreased | 0 | 9 (34.6) |
| Weight decreased | 9 (4.0) | 0 |
| **Renal and urinary disorders** | **28 (12.4)** | **1 (3.8)** |
| Pollakisuria | 6 (2.7) | 0 |
| Hematuria | 5 (2.2) | 0 |
| Urinary retention | 0 | 1 (3.8) |
| **Skin and subcutaneous tissue disorders** | **26 (11.5)** | **1 (3.8)** |
| Exfoliative rash | 0 | 0 |
| Pruritus | 7 (3.1) | 0 |
| Rash | 10 (4.4) | 1 (3.8) |
| **Metabolism and nutrition disorders** | **23 (10.2)** | **26 (100)** |
| Anorexia | 4 (2.2) | 0 |
| Dehydration | 5 (2.2) | 0 |
| Hypercalcemia | 1 (0.4) | 1 (3.8) |
| Hyperkalemia | 0 | 3 (11.5) |
| Hyperglycemia | 3 (1.3) | 24 (92.3) |
| Hyponatremia | 0 | 5 (19.2) |
| Hypoalbuminemia | 1 (0.4) | 25 (96.2) |
| Hypocalcaemia | 8 (3.5) | 0 |
| Hypoglycemia | 1 (0.4) | 1 (3.8) |
| Hyperkalemia | 5 (2.2) | 5 (19.2) |
| Hypomagnesaemia | 0 | 1 (3.8) |
| Hyponatremia | 3 (1.3) | 5 (19.2) |
| Hypophosphatemia | 0 | 1 (3.8) |
| **Vascular disorders** | **22 (9.7)** | **0** |
| Hypertension | 6 (2.7) | 0 |
| Hypotension | 5 (2.2) | 0 |
| **Cardiac disorders** | **14 (6.2)** | **0** |
| **Blood and lymphatic system disorders** | **6 (2.7)** | **6 (23.1)** |
| Anemia | 5 (2.2) | 0 |
| Lymphopenia | 0 | 6 (23.1) |
| **Eye disorders** | **6 (2.7)** | **0** |
| **Immune system disorders** | **6 (2.7)** | **0** |
| **Reproductive system and breast disorders** | **5 (2.2)** | **0** |
| **Surgical and medical procedures** | **3 (1.3)** | **0** |
| **Endocrine disorders** | **2 (0.9)** | **0** |
| **Neoplasms benign, malignant and unspecified (incl. cysts and polyps)** | **2 (0.9)** | **0** |
| **Congenital, familial and genetic disorders** | **1 (0.4)** | **0** |
| **Hepatobiliary Disorders** | **0** | **9 (34.6)** |
| Hyperbilirubinemia | 0 | 9 (34.6) |

Notes: Adverse events were coded using MedDRA version 9.1 for Study WX/20-001, version 10.0 for Study LUD 2002-003.
AE = adverse event; incl. = including; MedDRA = Medical Dictionary for Regulatory Activities.

⇒ **Deaths**

No deaths were reported during Studies LUD 2002-003 and WX-2007-10-HV. A total of 5 patients had fatal SAEs in the respective other studies. All were considered unrelated.

Four (1.8 %) patients died during Study WX/20-001: disease progression, extravasation of urine, pulmonary embolism, and sepsis (one subject each). Although all four patients had AEs with an outcome of death, three of these patients had death as the reason for study discontinuation. The fourth subject (Subject WX/20-001/013006) discontinued from the study because of an SAE (disease progression), which began 5 days after infusion. The subject died 30 days later as a result of that SAE that was recorded as the reason for study discontinuation. None of the deaths were considered to be related to $^{124}$I-girentuximab.

One subject (Subject LUD 98-011/g250-20) in Study LUD 98-011 experienced a fatal SAE (acute renal failure, CTCAE Grade 4) that was considered unrelated to study treatment.

## 1.3 Rationale for conducting this study

$^{89}$Zr-Girentuximab is under clinical development as a PET/CT imaging radiopharmaceutical 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.

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

⇒ CAIX is expressed in several cancers. Especially, it is expressed in 70% to 90% of bladder cancers but not in normal urothelial tissue (8).
⇒ CAIX is expressed on the membrane of Non-Muscle-Invasive Bladder Cancer cells (8,21).

Klatte et al. showed that CAIX has a role as a diagnostic, prognostic, and therapeutic molecular marker. Because CAIX expression and is highly expressed in noninvasive, low-grade bladder cancer, the evaluation of CAIX may be a useful adjunct to diagnostic cytology, and intravesical CAIX targeted therapy may be effective in these high CAIX-expressing tumors. Likewise, because metastatic tumors express high CAIX, an evaluation of systemic CAIX-targeted therapy as a therapeutic approach is warranted (8).

The current project will consist of an intravesical instillation of anti-CAIX antibody (girentuximab) labeled with an activity of 37 MBq of positron-emitting zirconium-89.

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

Furthermore, it has been clearly shown that, after an intravesical instillation, a radiolabeled antibody remains confined in the bladder without extravasation in the general circulation (6,7). Thus radiation is limited to the bladder wall. In the clinical pilot study performed with an antibody

labeled with an activity of up to 740 MBq of an alpha-emitting radionuclide (bismuth-213) much more irradiating than zirconium-89 no toxicity at all has been observed. Thus no toxicity is expected in the current proposal consisting in an instillation of 37 MBq of zirconium-89 followed two hours later by a lavage of the bladder.

One of the objectives of this study is to assess the biodistribution of $^{89}$Zr-girentuximab in whole body if it exists.

After establishing the targeting properties of $^{89}$Zr-Girentuximab PET/CT imaging tracer in NMIBC, it should be interesting to develop a new targeted therapy using girentuximab- radiolabelled with a therapeutic radionuclide such as Astatine-211 ($^{211}$At-Girentuximab).

An alpha-immunotherapy project of non-muscle-invasive bladder cancer was initiated by Atonco with an alpha-emitting radionuclide labeled with astatine-211.

## 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

The extensive clinical experience with radiolabeled girentuximab has provided strong clinical evidence to support its use as a targeted PET imaging agent in clear-cell renal cell cancer.

CAIX, is a cell surface antigen expressed in 70% to 90% of bladder cancer NMIBC but not in normal urothelial tissue; therefore, it represents an optimal therapeutic target. There may be a role for CAIX as an intravesical target for instillation therapy(8).

Once $^{89}$Zr-Girentuximab has been shown to be safe, we will develop a treatment of bladder cancer NMIBC for therapeutic purposes with girentuximab radiolabeled with a therapeutic radionuclide such as: $^{211}$Astatine.

Especially as local treatments such as mitomycin C and BCG therapy are in short supply worldwide, it is important to offer alternative treatments to postpone cystectomy.

### 1.4.3 Overall risks

In previous clinical studies, intravenous administration of Zirconium-89 ($^{89}$Zr) labeled monoclonal antibodies was proven to be safe (18,22).

Although never observed in previous trials, allergic-type reactions are possible during and immediately following the intravenous administration of girentuximab. In a total of more than 2000

intravenous administrations of radiolabelled murine and chimeric girentuximab to humans, no allergic-type reaction was observed.

Moreover, it has been clearly shown that, after an intravesical instillation, a radiolabelled antibody remains confined in the bladder without extravasation in the general circulation (6,7) making an allergic reaction unlikely. No toxicities have been reported in these two studies.

In PERTINENCE study, even if **no toxicity related to intravesical $^{89}$Zr-Girentuximab administration is expected**, patients will be carefully monitored after intravesical $^{89}$Zr-Girentuximab administration.

Some adverse events related to intravesical administration are expected: mictional burning, deposition or scaling in the urine, pollakiuria, fatigue, fever and haematuria. Patient will be informed to contact the investigator if one of these adverse events occurs.

### 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 (15). 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.

The current PERTINENCE clinical trial will consist in intracavitary (intravesical) instillation of the same radiopharmaceutical "$^{89}$Zr-girentuximab" used in the Telix phase 3 ZIRCON clinical trial with systemic intravenous injection and using the same activity (37 MBq).

With regard to the potential toxicity following this administration route, we refer to two previous preclinical and clinical studies performed with a different antibody, namely anti-Epidermal Growth Factor Receptor (EGFR) labeled with bismuth-213, an alpha-emitting radionuclide.

In a preclinical study with an orthotopic human bladder carcinoma mouse model, an activity of up to 0,925 MBq was intravesically instilled (7). Excellent retention of the therapeutic radiopharmaceutical in the bladder with negligible systemic activity was observed.

It is important to note that no macroscopic urothelial lesions caused by a-particle irradiation was observed.
In a subsequent clinical pilot study, an activity of up to 821 MBq (22mCi) of the same radiopharmaceutical was intravesically instilled in 12 patients (6).

It was clearly shown that $^{213}$Bi activity remained in the bladder with no systemic uptake and reflux into the ureters as observed in the preclinical study (Figure 9).

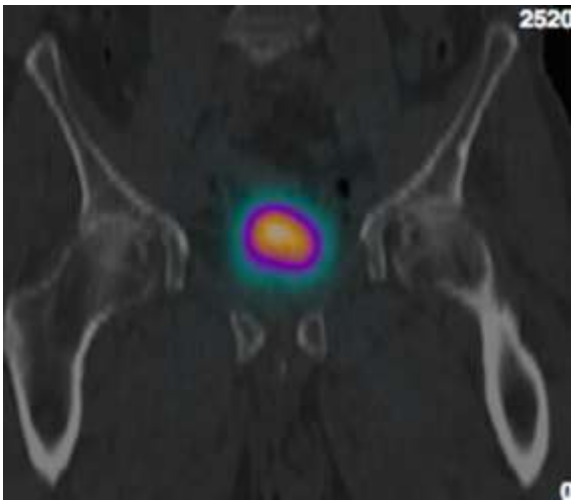


*Figure 9: Distribution of $^{213}$Bi-anti- EGFR MAb after intravesical instillation visualized via SPECT/ CT imaging: all activity remains in the urinary bladder.*

All 12 patients treated with the $^{213}$Bi-immunoconjugate showed excellent tolerance without any signs of adverse effects. $^{213}$Bi was not detected in blood samples taken from patients during 1 to 2 hours after treatment.

Overall, no toxicity at all was observed in both preclinical and clinical studies.
The antibody used in the PERTINENCE study is different but the same retention inside the bladder should be expected due to the size of the antibody molecule.
The radionuclide also is different but a higher toxicity could be expected with alpha-emitting bismuth-213 radionuclide than with positron-emitting zirconium-89 due to a much higher Linear Energy Transfer (LET).
Consequently, no toxicity at all is expected in the PERTINENCE study after intravesical instillation of 37 MBq of $^{89}$Zr-girentuximab.

According to the information described above, it appears that the benefit/risk ratio will be clearly in favour of the benefit to the patient.

The results of Pertinence study may contribute to the development of a new targeted therapy for Non-Muscle-Invasive Bladder Cancer (NMIBC) with girentuximab labelled with a therapeutic radionuclide such as astatine-211 ($^{211}$At-Girentuximab).

# 2. OBJECTIVES

## 2.1 Primary objective(s) and endpoint

### 2.1.1 Primary objective

The primary objective of this study is to assess the biodistribution of $^{89}$Zr-girentuximab.

### 2.1.2 Endpoints of primary objective

⇒ Bladder uptake will be evaluated quantitatively using SUVmax, SUVmean and (Bq/ml) at different time points (T0, T0+2h, T0+20h and T0+44h)

⇒ The activity retained by the bladder at T0 will be assessed by the difference between the instilled activity (Bq) and the activity after rinsing measured in the saline solution used for rinsing (Bq).

⇒ Biodistribution in whole body will be assessed visually and quantitatively by PET/CT imaging of whole body at T0+2H and quantitatively by assessing the $^{89}$Zr-Girentuximab activity in blood at T0+20h. Another PET/CT imaging of whole body will be performed at T0+44h in case of positive blood radioactivity at T0+20h.

## 2.2 Secondary objective(s) and endpoint

### 2.2.1 Secondary objectives

1. Assess the safety and tolerability of $^{89}$Zr-girentuximab administered by intravesical instillation.
2. Radiation protection management
3. Assess the degree of CAIX expression by immunohistochemistry of the tumor sample.

### 2.2.2 Endpoints of secondary objectives

1. The occurrence and the frequency of adverse events up to Day 30 after $^{89}$Zr-girentuximab administration.
2. Radiation protection management:

   ⇒ Radiation exposure of staff (extremities, lens and Whole body: µSv).

   ⇒ Radiopharmaceutical management from intravesical instillation to elimination (surfasic contamination: counts/cm$^2$ or Bq/cm$^2$).

3. The expression of CAIX will be assessed in TURBT or cystectomy tumor sample by immunohistochemistry (IHC).

# 3. 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.

## 3.1 Population description

Patients with Non-Muscle-Invasive Bladder Cancer (NMIBC) requiring a radical cystectomy will be included in this study just before their surgery. Cancer treatment will not be delayed by study participation.

## 3.2 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. Willingness and ability to comply with scheduled visits, treatment plans, laboratory tests, and other study procedures.
3. Female or male, Age ≥ 18 years at time of study entry.
4. PS: 0 or 1.
5. Clinical evidence of NMIBC based on cystoscopy and proven histologically of papillary tumors.
6. Histologically-confirmed pTa Grade 3 or pT1 Grade 3 bladder cancer patients relapsing without muscle invasion.
7. Negative sterile Urine cytobacteriological testing at baseline (T0).
8. Consent to use a contraception method for at least 42 days after administration of $^{89}$Zr-girentuximab.
9. Patient has valid health insurance.
10. Negative serum pregnancy test in female patients of childbearing potential at screening.

## 3.3 Non-inclusion criteria

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

1. Patient with urinary incontinence.
2. Known hypersensitivity to girentuximab.
3. Exposure to any experimental diagnostic or therapeutic drug within 30 days prior the date of planned administration of $^{89}$Zr-girentuximab

4. Exposure to **any** radiopharmaceutical within 30 days prior to the administration of $^{89}$Zr-girentuximab
5. Patients suffering from a bladder cancer at stage pT2, pT3 or pT4
6. Serious non-malignant disease that may interfere with the objectives of the study or with the safety or compliance of the patient; as judged by the investigator
7. Concomitant cancer in the past 5 years except cutaneous cancers (except melanoma) and in situ carcinoma
8. Prior chemotherapy, radiotherapy (other than short cycle of palliative radiotherapy), immunotherapy within 21 days of $^{89}$Zr-girentuximab administration
9. TransUrethral Resection of Bladder Tumor (TURBT), BCG or Mitomycin C endovesical instillations within 10 days before $^{89}$Zr-girentuximab
10. Patients with known HIV, HBV and HCV infections,
11. Pregnant or likely to be pregnant or nursing patient.
12. Mental impairment that may compromise the ability to give informed consent and comply with the requirements of the study
13. Persons deprived of their liberty, under a measure of safeguard of justice, under guardianship or placed under the authority of a guardian.

As patients are enrolled 10 days before the IMP administration, a confirmation of negative pregnancy test result from urine should be performed within 72 hours prior $^{89}$Zr-girentuximab administration.

Validated methods of contraception for both men and women are pill, condom, intra-uterine device.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.

## 3.4 Withdrawal of patients from study

### 3.4.1 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.

### 3.4.2 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.

## 3.5 Patients replacement

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

- Has not received $^{89}$Zr-TLX250 administration;
- Did not undergo $^{89}$Zr-TLX250 PET/CT imaging after intra-vesical 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 intra-vesical $^{89}$Zr-TLX250 will need to get all the examinations and blood draws as for the final study visit.

# 4. STUDY PROCEDURE $^{89}$Zr-TLX250 PET/CT SCAN

## 4.1 Study drug $^{89}$Zr-TLX250

### 4.1.1 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.
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.

### 4.1.2 Pharmaceutical Properties

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

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

### 4.1.3 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.

### 4.1.4 Packaging and Labelling

$^{89}$Zr-TLX250 solution for intravesical instillation 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.

### 4.1.5 Drug Logistics and Accountability

#### 4.1.5.1 Supply, Storage, Dispensation and Return

$^{89}$Zr-TLX250 solution for intravesical instillation 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) 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 manufacturer who shall arrange appropriate supply of IMP. Upon establishment of patient eligibility (see Section 3), the clinical site manager will order individualized doses of $^{89}$Zr-TLX250 solution for intravesical instillation, via the manufacturer 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 inform manufacturer. $^{89}$Zr-TLX250 for intravesical instillation 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 intravesical instillation 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 intravesical instillation 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.

#### 4.1.5.2 Drug Accountability

The radiopharmacist will confirm receipt of the study drug by e-mail 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.

## 4.2 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.

### 4.2.1 Dosage and Administration

This is sufficient to draw up the patient dose of 37 MBq of $^{89}$Zr- TLX250, adjusted to 10 mg of girentuximab at the time of injection.
The study medication will be prepared as individual syringe in ICO radiopharmacy by a dedicated pharmacist within one hour of administration.
The investigator should check that the urine cytobacteriological testing is negative before intravesical instillation to ensure the absence of germs.
The $^{89}$Zr-Girentuximab solution (about 10 ml) will be administered into the emptied bladder through a urethral catheter using natural urinary tract (see intravesical instillation procedure in appendix 7).
The syringe will be washed with 30 ml of saline solution which will be also injected into the bladder.
The urethral catheter should be removed.

Patients will be asked to turn over repeatedly to ensure equal distribution of $^{89}$Zr-Girentuximab in the bladder.
The $^{89}$Zr-Girentuximab should be maintained in the bladder for no more than 1.5 hours: duration of $^{89}$Zr-Girentuximab administration.
Then, the patient will be asked to drink water and urinate to rinse the bladder, the patient will drink water again and the first PET/CT imaging centered on the bladder will be performed about 2 hours after instillation.

The urethral catheter and the urine activity will be measured.
A whole body imaging should be performed 2 hours after $^{89}$Zr-Girentuximab administration (4 hours after instillation) (see intravesical instillation procedure in appendix 7).

The investigator or his/her designee will administer the study drug to the subjects, exercising accepted medical practices. Under no circumstances will the investigator allow study drugs to be used otherwise than as directed by this clinical study protocol. Study drug administration will be done under direct medical supervision.

### 4.2.2 $^{89}$Zr-TLX250 PET/CT Imaging

Imaging sessions are necessary to check the safety of intravesical application of $^{89}$Zr-TLX250 pharmaceuticals. Two aspects will be studied:

- **T0**: At the end of $^{89}$Zr-TLX250 administration: 2 hours after intravesical instillation, , a first imaging session centred on the **bladder** will be performed (with full bladder) and will allow to evaluate the possibility to identify the $^{89}$Zr girentuximab uptake surface and to measure tumor and normal bladder wall surface. Spatial distribution of PET/CT images will cover the lone bladder. PET acquisition duration will be adjusted to allow a sufficient count statistics for a good quality of activity measurements in main organs.
- **T0 + 2 hours**: a **whole body scan** will be performed (with empty bladder), from the skull base to the mid-thigh, to ensure that no residua $^{89}$Zr-TLX250 pharmaceutical occurred outside the bladder and spread in the rest of the body. During the whole body scan, the specific step including the bladder will be designed.
- **T0 + 20 hours (day 1)**: a new imaging session centred on the **bladder** will be performed (same protocol as first session) in order to quantify the remaining activity and calculate the decay of the later. The aim is to check that there is no residual radiopharmaceutical outside the bladder through the urethra.

  On day 1, a blood sample will be drawn in order to check the absence of activity from residual $^{89}$Zr-TLX250 in blood.
- **T0 + 44 hours (day 2)**:

  ⇒ If blood radioactivity testing on day 1 is **negative**, a **bladder scan** should be performed on day 2 (T0 + 44 hours).

  ⇒ If blood radioactivity testing on day 1 is **positive**, a **Whole body scan** should be performed on day 2 (T0 + 44 hours). This scan will permit to localise the uptake of the residual pharmaceutical.

In total, 4 imaging sessions will be performed, including a whole body scan and in case of residual activity in the day 1 blood sample, a second whole body scan will replace the bladder centred scan.

PET studies will be performed with a PET/CT hybrid system. A CT acquisition will be coupled to a PET acquisition for attenuation correction and anatomical tracking.

Low-dose CT acquisition parameters will be those usually used namely:

- Field: several fields of view allowing visualization of the bladder and whole body.
- Number: four acquisition times:
- Time 0: 2 hours after instillation:
  - Full bladder imaging: one step centred on the bladder after CT acquisition of the step.

⇒ Time 1: 4 hours after instillation:

- Whole body scan after scout view and low dose CT acquisition, to cover area from skull base to mid-thigh. The step on the bladder is included into this whole body scan.

⇒ Time 3: 20 hours after instillation (day 1):

- One step acquisition centred on bladder with low dose CT

⇒ Time 4: 44 hours after instillation (day2): depending on the day 1 blood sample results (in terms of activity in Bq/ml),

- In case of negative result (no activity i.e. background): One step acquisition centred on bladder with low dose CT.
- In case of positive result (activity > 2.5 times background): Whole body scan after scout view and low dose CT acquisition, to cover area from skull base to mid-thigh. The step on the bladder is included into this whole body scan.

Comment: These times should be adapted according to practical arrangement of intravesical instillation.

- Standard whole body scan = 100 kV, 5 mm, Acq 16x1,2 mm, pitch 1, CAREDOSE4D, CAREkV
- One step high accuracy CT: 120 kV, 4mm slices every 4 mm and 0.85 pitch CAREDOSE4D, CAREkVOne step low dose CT: 100 kV, 5mm, Acq 16×1.2mm, pitch, CAREDOSE4D, CAREkV

The CT acquisitions centred on the bladder will visualize a field of about 20 cm and consequently will deliver a low dose corresponding to a DLP of around 130mGy.cm and an effective dose of around 3 mGy.

The whole body CT acquisitions, from skull base to mid-thigh, will visualize a field depending of the height of the patient. For standard 170cm height, this will deliver a low dose corresponding to a DLP of around 350mGy.cm and an effective dose of around 5 mGy.

The total effective dose resulting from successive CT acquisitions for attenuation correction can be estimated from 14 to 16 mSv. This dose should be compared to a diagnostic CT acquisition dose to thorax and abdomen, which is about 15 to 16 mSv.

### 4.2.3 Imaging Analysis

#### 4.2.3.1 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 bladder lesion.

We would like to show that $^{89}$Zr activity remain in the bladder and that no systemic uptake and reflux into the ureters is observed as described in a previous clinical pilot study with intravesical instillation of $^{213}$Bi- anti- EGFR MAb (6).

#### 4.2.3.2 Semiquantitative $^{89}$Zr-TLX250 Tumor Targeting

SUVs will be calculated for the tumor bladder lesion, considering the measured tumor-bound activity, the injected activity dose, and the body weight and height of each patient.

#### 4.2.3.3 Quantitative $^{89}$Zirconium activity in blood

The $^{89}$Zirconium activity in the patient blood sample will be determined on day 1 by counting the $^{89}$Zr-TLX250 activity in blood in a calibrated gamma counter. Counting will be performed immediately after the end of blood collection in the local nuclear medicine department in order to check the absence of activity from residual $^{89}$Zr-TLX250 in blood.

#### 4.2.3.4 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 TURBT or cystectomy tumor sample by immunohistochemistry.

The degree of CAIX expression will be investigated and classified.

## 4.3 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".

## 4.4 Blinding

Not applicable, this is an open-label study.

## 4.5 Treatment Compliance

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

## 4.6 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 3 hours p.a., unless the investigator decides otherwise.

Excretion will occur during the rinsing stage after instillation. No excess excretion is expected after rinsing. If happens, only a low fractionation of leakage could occur days after. This is what will be studied with quantified imaging of the bladder. The leakage, if any, will be at very low level, lower than excretion of standard diagnosis examination after patient release.

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 for one week:

- Patients should be advised to observe rigorous hygiene in order to avoid risk of contamination of others using the same toilet facility.
- One shower per day.
- Change of underwear every day.
- A double toilet flush is recommended.
- Patients should wash their hands thoroughly every time after using the toilet.

After hospital discharge, **no distancing from and contact to other persons is required**.

The following precautions apply for health care workers:

Healthcare personnel are advised to 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).

A simple calculation with RAD PRO CALCULATOR software, shows that at 100cm from a 37MBq $^{89}$Zr point source, the dose rate is 4.5 µSv/h.Considering 10 cm of water as shielding due to the body, it reduces the dose rate to 3.8 µSv/h. In reality, a fraction of the activity will be remained into the bladder so we could expect one tenth or even one hundredth of the activity and so the dose rate at 100cm. As comparison, the releasing rule for a patient after iodine-131 treatment impose a maximum dose rate of 20 µSv/h at 100cm.

## 4.7 Total Radiation Exposure

$^{89}$Zr-Girentuximab will be administered by intravesical instillation, and should remain inside the bladder. The Low Dose CT scan will be performed using a hybrid camera. No dose to organs at risk is expected.

If in the worst case, there is a release of the product into the rest of the body, the dosimetry data shows a very low impact as it is not of the order of 37MBq (as for intravenous administration) but rather of 1 MBq (for intravesical instillation).

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 intravenous 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 (23). 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 (24) is used, the resulting effective dose will be 0.551 ± 0.030 mSv/MBq or 20.5 ± 1.1 mSv for 37 MBq.

## 4.8 Prior and Concomitant therapy

At baseline screening, all prior cancer-related treatments are to be recorded.
**All** bladder medications taken within 30 days before $^{89}$Zr- Girentuximab PET/CT exam should be recorded on the case report form.

### 4.8.1 Permitted concomitant therapy

TransUrethral Resection of Bladder Tumor (TURBT) in the 30 days after $^{89}$Zr- Girentuximab is permitted only if the patient's condition requires it.

### 4.8.2 Prohibited concomitant therapy

Chemotherapy, radiotherapy (other than short cycle of palliative radiotherapy), immunotherapy are prohibited within 21 days of $^{89}$Zr-girentuximab administration.
These treatments are prohibited within 30 days of $^{89}$Zr-girentuximab administration:

- Experimental diagnostic or therapeutic drugs.
- Any radiopharmaceutical exposure.

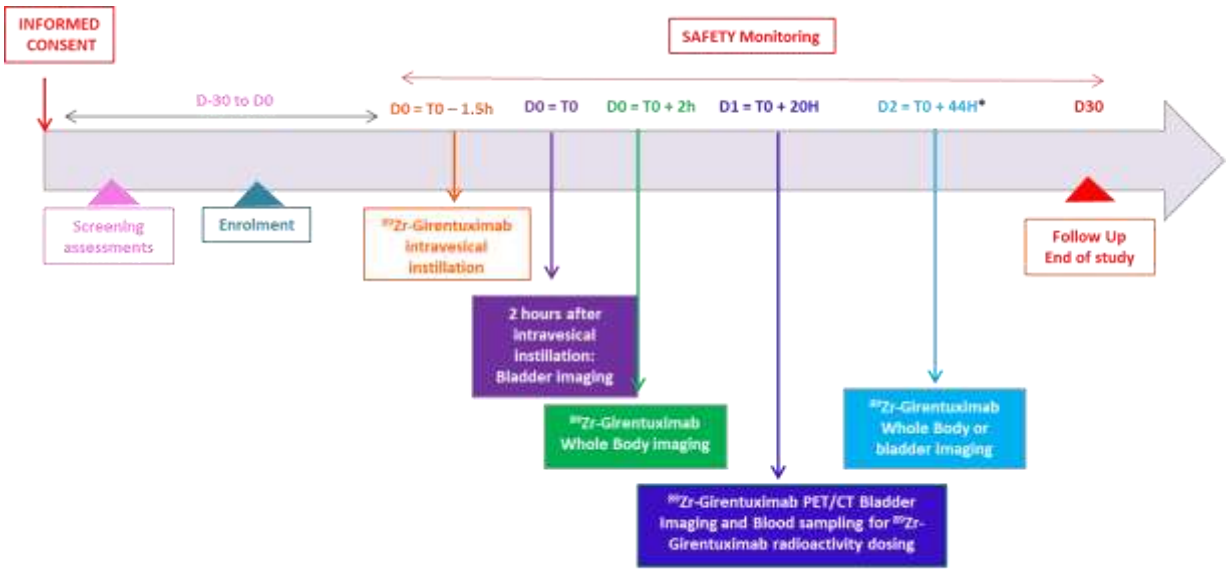
# 5. STUDY DESIGN

## 5.1 Overview of study design

The research presents the following characteristics:

- Feasibility pilot study
- Imaging trial
- Monocentric
- Non randomized
- Uncontrolled
- Open-label
- Prospective

## 5.2 Study design schedule




* If $^{89}$Zr-Girentuximab D1 Blood dosing is positive, a Whole body imaging should be done, if negative, a bladder imaging should be performed

Figure 1: Study design schedule

# 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 administration. 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 administration.

The following procedures will be performed during the Screening period:

- Informed Consent
- Subject registration and subject number assignment
- Review of eligibility criteria and confirm eligibility
- Demographics
- Record Medical History
- Record Disease History: Histopathological TURBT report
- Record NMIB - related treatments
- Perform full physical examination with ECOG Performance Status and Vital signs
- Review of prior/concomitant bladder medications
- Imaging exams: cystoscopy report + bladder diagram
- Standard laboratory samples for Haematology and serum Chemistry.
- Urine cytobacteriological testing: to be performed, before $^{89}$Zr-Girentuximab order: within 10 days before $^{89}$Zr-Girentuximab administration.
- Serum β-HCG pregnancy test in women of childbearing potential* and Urine Pregnancy test within 72 hours before $^{89}$Zr-TLX250 administration. Pregnancy test should not be performed for postmenopausal patients**.

* The women under 55 (<55) are considered with childbearing potential.
** A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

### 6.1.1 Medical history

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

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

Relevant bladder concomitant medications will be reported.

### 6.1.2 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, and temperature), Body weight and height will be evaluated.

### 6.1.3 Urine cytobacteriological testing

A urine cytobacteriological testing will be performed before $^{89}$Zr-Girentuximab order: within 10 days before $^{89}$Zr-Girentuximab administration. This testing should be negative before intravesical instillation to ensure the absence of germs before instillation.

### 6.1.4 Laboratory evaluations

The following laboratory tests will be performed:

⇒ Haematology: Haemoglobin, haematocrit, platelets, red blood cells, White blood cells and differential (neutrophils, basophils, eosinophils, lymphocytes, monocytes).

⇒ Chemistry: Creatinine, urea, SGOT (AST), SGPT (ALT).

⇒ Blood or urinary Pregnancy test

## 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: **CRGA0067**
- Study Sponsor number : ICO-2021-03
- 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**
**Tel: +33 (0)2 40 67 98 26**
**Project Manager: 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:

- Patient performance status.
- Pre-dose Vital signs.
- Blood or urine Pregnancy test (for female patients of childbearing potential) within 72 hours before $^{89}$Zr-TLX250 administration.
- Baseline findings.
- Bladder concomitant medications.
- Urine dipstick analysis before $^{89}$Zr- Girentuximab administration.

The $^{89}$Zr-girentuximab solution will be administered through an intravesical instillation using natural urinary tract (see section (5.2.1).

Vital signs will be assessed before; 2; 4; 20 and 44 hours after $^{89}$Zr-TLX250 administration.

Adverse event monitoring will be assessed within 2 hours post administration and reported in the medical file and the e-CRF.

### 6.3.2 $^{89}$Zr-TLX250 PET/CT Imaging and Dosimetry

Several PET/CT images acquisitions should be performed at the end of $^{89}$Zr-girentuximab administration on D0, D1 and D2 (see section 4.2.2).

Urine dipstick analysis at D1 and D2 of $^{89}$Zr- Girentuximab administration

A blood sample (one heparin tube of 4 ml) will be drawn on day 1 to evaluate quantitative biodistribution of $^{89}$Zr-TLX250 in blood (see section 4.2.3.3).

## 6.4 Follow-up visit: Day 30 following $^{89}$Zr-TLX250 administration

End of study visit will be performed 30 days after $^{89}$Zr-TLX250 administration.

All procedures bellow should be performed:

- Evaluation of Performance Status (WHO)
- Urine cytobacteriological testing

- Review of AE and SAE + concomitant medications

## 6.5 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:

- Occurrence of significant previously unknown adverse reactions or unexpectedly high intensity or incidence of known adverse reactions described such as: any adverse grade > 3 in 2 patients/3.
- recruitment of patients too low,
- poor quality of the data collected,

# 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.

In PERTINENCE study, **we don't expect any $^{89}$Zr-girentuximab adverse event** as it will be administered intravesically and rinsed out afterwards. It will not diffuse into the general circulation as with intravenous administration.

Clinical experience in 69 patients dosed with **a single intravenous 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 in case of single intravenous administration.

Expected adverse events related to intravesical administration are: mictional burning, deposition or scaling in the urine, pollakiuria, fatigue, fever and haematuria. Patient will be informed to contact the investigator if one of these adverse events occurs.

### 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 case 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 occurred and medications taken should be recorded in the e-CRF **from the date of administration of $^{89}$Zr- Girentuximab until 30 days after administration** regardless of whether they are related to $^{89}$Zr- Girentuximab PET/CT.

### 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 sections 7.1.2 and 7.1.5**:**

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

**- From $^{89}$Zr- girentuximab administration up to 30 days post-$^{89}$Zr- girentuximab 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- girentuximab 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.

In the case of the partner of a patient becomes pregnant, the pregnancy must also be reported to the sponsor in the same time frame. However, an authorization must be requested from the patient's partner in order to collect information about the pregnancy. This authorization must be kept in the patient's file.

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

## 8.1 Determination of sample size

As this study is an exploratory phase, 6 patients will be included in this study.

The limited sample size has been determined to take into account the fact that this study is a first-in-man intravesical procedure without intention to treat but necessary to confirm sufficient safety and utility for further patients.

This sample size was also used by Menke-van der Houven van Oordt et al (25) and should be able to reflect some inter-patients variability.

## 8.2 Selection of patients to be included in the statistical analysis

All included patients will be analysed.

## 8.3 Handling of missing, unused or invalid data

Special care will be taken to minimize any missing data for the 6 patients enrolled.

## 8.4 Description of analysis sets

Data will be summarized by frequency and percentage for categorical variables and by median, range, IQR, or mean and standard deviation for continuous variables.

# 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 endpoints:

⇒ Bladder uptake will be evaluated quantitatively using SUVmax, SUVmean and (Bq/ml) at different time points (T0, T0+2h, T0+20h and T0+44h)

⇒ The activity retained by the bladder at T0 will be assessed by the difference between the instilled activity (Bq) and the activity after rinsing measured in the saline solution used for rinsing (Bq).

⇒ Biodistribution in whole body will be assessed visually and quantitatively by PET/CT imaging of whole body at T0+2H and quantitatively by assessing the $^{89}$Zr-Girentuximab activity in blood at T0+20h. Another PET/CT imaging of whole body will be performed at T0+44h in case of positive blood radioactivity at T0+20h.

5) The presence of the major secondary objective endpoints:

1. The occurrence and the frequency of adverse events up to Day 30 after $^{89}$Zr-girentuximab administration.
2. Radiation protection management:

⇒ Radiation exposure of staff (extremities, lens and Whole body: µSv).

⇒ Radiopharmaceutical management from intravesical instillation to elimination (surfasic contamination: counts/cm$^2$ or Bq/cm$^2$).

3. The expression of CAIX will be assessed in TURBT or cystectomy tumor sample by immunohistochemistry (IHC).

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 IDF III** 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 (ATONCO)** 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 ATONCO** | |

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 ATONCO.

## 15. LIST OF REFERENCES

1. Kamat AM, Colombel M, Sundi D, Lamm D, Boehle A, Brausi M, et al. BCG-unresponsive non-muscle-invasive bladder cancer: recommendations from the IBCG. Nat Rev Urol. avr 2017;14(4):244-55.

2. Dinney CPN, Greenberg RE, Steinberg GD. Intravesical valrubicin in patients with bladder carcinoma in situ and contraindication to or failure after bacillus Calmette-Guérin. Urol Oncol. nov 2013;31(8):1635-42.

3. Brausi M, Oddens J, Sylvester R, Bono A, van de Beek C, van Andel G, et al. Side effects of Bacillus Calmette-Guérin (BCG) in the treatment of intermediate- and high-risk Ta, T1 papillary carcinoma of the bladder: results of the EORTC genito-urinary cancers group randomised phase 3 study comparing one-third dose with full dose and 1 year with 3 years of maintenance BCG. Eur Urol. janv 2014;65(1):69-76.

4. Veeratterapillay R, Heer R, Johnson MI, Persad R, Bach C. High-Risk Non-Muscle-Invasive Bladder Cancer-Therapy Options During Intravesical BCG Shortage. Curr Urol Rep. sept 2016;17(9):68.

5. Chatal J-F, Kraeber-Bodéré F, Chérel M, Haddad F. Alphatherapy, the new impetus to targeted radionuclide therapy? Eur J Nucl Med Mol Imaging. juill 2018;45(8):1362-3.

6. Autenrieth ME, Seidl C, Bruchertseifer F, Horn T, Kurtz F, Feuerecker B, et al. Treatment of carcinoma in situ of the urinary bladder with an alpha-emitter immunoconjugate targeting the epidermal growth factor receptor: a pilot study. Eur J Nucl Med Mol Imaging. juill 2018;45(8):1364-71.

7. Pfost B, Seidl C, Autenrieth M, Saur D, Bruchertseifer F, Morgenstern A, et al. Intravesical alpha-radioimmunotherapy with 213Bi-anti-EGFR-mAb defeats human bladder carcinoma in xenografted nude mice. J Nucl Med. oct 2009;50(10):1700-8.

8. Klatte T, Seligson DB, Rao JY, Yu H, de Martino M, Kawaoka K, et al. Carbonic anhydrase IX in bladder cancer: a diagnostic, prognostic, and therapeutic molecular marker. Cancer. 1 avr 2009;115(7):1448-58.

9. Turner KJ, Crew JP, Wykoff CC, Watson PH, Poulsom R, Pastorek J, et al. The hypoxia-inducible genes VEGF and CA9 are differentially regulated in superficial vs invasive bladder cancer. Br J Cancer. 22 avr 2002;86(8):1276-82.

10. van de Watering FCJ, Rijpkema M, Perk L, Brinkmann U, Oyen WJG, Boerman OC. Zirconium-89 labeled antibodies: a new tool for molecular imaging in cancer patients. Biomed Res Int. 2014;2014:203601.

11. Brouwers A, Verel I, Van Eerd J, Visser G, Steffens M, Oosterwijk E, et al. PET radioimmunoscintigraphy of renal cell cancer using 89Zr-labeled cG250 monoclonal antibody in nude rats. Cancer Biother Radiopharm. avr 2004;19(2):155-63.

12. van Dijk J, Uemura H, Beniers AJ, Peelen WP, Zegveld ST, Fleuren GJ, et al. Therapeutic effects of monoclonal antibody G250, interferons and tumor necrosis factor, in mice with renal-cell carcinoma xenografts. Int J Cancer. 15 janv 1994;56(2):262-8.

13. Surfus JE, Hank JA, Oosterwijk E, Welt S, Lindstrom MJ, Albertini MR, et al. Anti-renal-cell carcinoma chimeric antibody G250 facilitates antibody-dependent cellular cytotoxicity with in vitro and in vivo interleukin-2-activated effectors. J Immunother Emphasis Tumor Immunol. mai 1996;19(3):184-91.

14. Stillebroer AB, Franssen GM, Mulders PFA, Oyen WJG, van Dongen GAMS, Laverman P, et al. ImmunoPET imaging of renal cell carcinoma with (124)I- and (89)Zr-labeled anti-CAIX monoclonal antibody cG250 in mice. Cancer Biother Radiopharm. sept 2013;28(7):510-5.

15. Cheal SM, Punzalan B, Doran MG, Evans MJ, Osborne JR, Lewis JS, et al. Pairwise comparison of 89Zr- and 124I-labeled cG250 based on positron emission tomography imaging and nonlinear immunokinetic modeling: in vivo carbonic anhydrase IX receptor binding and internalization in mouse xenografts of clear-cell renal cell carcinoma. Eur J Nucl Med Mol Imaging. mai 2014;41(5):985-94.

16. Abou DS, Ku T, Smith-Jones PM. In vivo biodistribution and accumulation of 89Zr in mice. Nucl Med Biol. juill 2011;38(5):675-81.

17. Steffens MG, Boerman OC, Oosterwijk-Wakka JC, Oosterhof GO, Witjes JA, Koenders EB, et al. Targeting of renal cell carcinoma with iodine-131-labeled chimeric monoclonal antibody G250. J Clin Oncol. avr 1997;15(4):1529-37.

18. Börjesson PKE, Jauw YWS, de Bree R, Roos JC, Castelijns JA, Leemans CR, et al. Radiation dosimetry of 89Zr-labeled chimeric monoclonal antibody U36 as used for immuno-PET in head and neck cancer patients. J Nucl Med. nov 2009;50(11):1828-36.

19. Hekman MCH, Rijpkema M, Aarntzen EH, Mulder SF, Langenhuijsen JF, Oosterwijk E, et al. Positron Emission Tomography/Computed Tomography with 89Zr-girentuximab Can Aid in Diagnostic Dilemmas of Clear Cell Renal Cell Carcinoma Suspicion. Eur Urol. sept 2018;74(3):257-60.

20. Smaldone MC, Chen DY, Yu JQ, Plimack ER. Potential role of (124)I-girentuximab in the presurgical diagnosis of clear-cell renal cell cancer. Biologics. 2012;6:395-407.

21. Tafreshi NK, Lloyd MC, Bui MM, Gillies RJ, Morse DL. Carbonic anhydrase IX as an imaging and therapeutic target for tumors and metastases. Subcell Biochem. 2014;75:221-54.

22. Oosting SF, Brouwers AH, van Es SC, Nagengast WB, Oude Munnink TH, Lub-de Hooge MN, et al. 89Zr-bevacizumab PET visualizes heterogeneous tracer accumulation in tumor lesions of renal cell carcinoma patients and differential effects of antiangiogenic treatment. J Nucl Med. janv 2015;56(1):63-9.

23. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine. J Nucl Med. juin 2005;46(6):1023-7.

24. Andersson M, Johansson L, Eckerman K, Mattsson S. IDAC-Dose 2.1, an internal dosimetry program for diagnostic nuclear medicine based on the ICRP adult reference voxel phantoms. EJNMMI Res. 3 nov 2017;7(1):88.

25. Menke-van der Houven van Oordt CW, McGeoch A, Bergstrom M, McSherry I, Smith DA, Cleveland M, et al. Immuno-PET Imaging to Assess Target Engagement: Experience from 89Zr-Anti-HER3 mAb (GSK2849330) in Patients with Solid Tumors. J Nucl Med. juill 2019;60(7):902-9.

## 16. 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

**Appendix 7**: Intravesical instillation procedure