# Study Protocol

# Cancer in Pregnancy

# (CIP-study)

**June 15, 2018**

**PROTOCOL TITLE 'Cancer in pregnancy'**

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|---|---|
| **Protocol ID** | S25470 |
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| **Sponsor** | Universitaire Ziekenhuizen Leuven (UZ Leuven) |
| **Coordinating investigator/project leader** | **Prof. dr. Frédéric Amant**<br>UZ Leuven, Department of Gynaecological Oncology<br>Herestraat 49<br>3000 Leuven, België<br>Phone: +32 16 34 42 52<br>Fax: +32 16 34 76 87<br>E-mail: Frederic.amant@uzleuven.be |
| **Multicenter research**<br>Participating sites per country: https://www.cancerinpregnancy.org/international-network | |

## PROTOCOL SIGNATURE SHEET SPONSOR

| Name | Signature | Date |
|---|---|---|
| **Coordinating Investigator**<br>**Project leader**<br>**Principal Investigator at UZ Leuven:**<br><br>Prof. dr. F. Amant |  | **15-06-2018** |

## PROTOCOL SIGNATURE SHEET PARTICIPATING SITE

### Part I (Pregnancy, delivery and maternal health) – I.I.A. Registration study mother and neonate

- ○ No
- ○ Yes:

| Authorization of Ethical Committee/IRB | Authorization of Competent Authority | Insurance | Informed consent form |
|---|---|---|---|
| ○ Not required<br>○ Required | ○ Not required<br>○ Required | ○ Not required<br>○ Certificate available | ○ Not required<br>○ Required |

### Part I (Pregnancy, delivery and maternal health) – I.I.B. Effects of prenatal exposure to cancer treatment on fetal growth

- ○ No
- ○ Yes:

| Authorization of Ethical Committee/IRB | Authorization of Competent Authority | Insurance | Informed consent form |
|---|---|---|---|
| ○ Not required<br>○ Required | ○ Not required<br>○ Required | ○ Not required<br>○ Certificate available | ○ Not required<br>○ Required |

### Part I (Pregnancy, delivery and maternal health) – I.II. Measurement of maternal and paternal anxiety and emotional needs

- ○ No
- ○ Yes:

| Authorization of Ethical Committee/IRB | Authorization of Competent Authority | Insurance | Informed consent form |
|---|---|---|---|
| ○ Not required<br>○ Required | ○ Not required<br>○ Required | ○ Not required<br>○ Certificate available | ○ Not required<br>○ Required |

### Part I (Pregnancy, delivery and maternal health) – I.III. Biobank 'cancer and pregnancy'

- ○ No
- ○ Yes:

| Authorization of Ethical Committee/IRB | Authorization of Competent Authority | Insurance | Informed consent form |
|---|---|---|---|
| ○ Not required<br>○ Required | ○ Not required<br>○ Required | ○ Not required<br>○ Certificate available | ○ Not required<br>○ Required |

### Part I (Pregnancy, delivery and maternal health) – I.IV. Pharmacokinetics

- No
- Yes:

| Authorization of Ethical Committee/IRB | Authorization of Competent Authority | Insurance | Informed consent form |
|---|---|---|---|
| ○ Not required<br>○ Required | ○ Not required<br>○ Required | ○ Not required<br>○ Certificate available | ○ Not required<br>○ Required |

**PART II (Child) – Long term follow up of children born to mothers with cancer during pregnancy**

- No
- Yes:

| Authorization of Ethical Committee/IRB | Authorization of Competent Authority | Insurance | Informed consent form |
|---|---|---|---|
| ○ Not required<br>○ Required | ○ Not required<br>○ Required | ○ Not required<br>○ Certificate available | ○ Not required<br>○ Required |

| *Name Participating Site & Investigator* | *Declaration* | *Signature and Date* |
|---|---|---|
| _____<br>_____ | I hereby agree (1) with this protocol version (2) to submit this protocol version to the Ethical Committee (EC)/IRB or Competent authority, if required (3) to provide access to Prof. Amant upon his request, to the supporting documentation relating to EC/CA approval(s), ICF and insurance, where required. | _____<br>_____ |

## TABLE OF CONTENTS

The following chapters are not applicable for this study and have been removed from the CCMO template:

- Treatment of subjects
- Investigational product
- Non investigational product
- Structured risk analysis

The following chapters have been incorporated in chapter 7 per study part:

- Sample size calculation
- Study parameters/endpoints
- Statistical analysis (replaced to introduce description in chapter 7)

## LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

| | |
|---|---|
| **AAS** | **Atomic Absorbance Spectrometry** |
| **ANT** | **Amsterdam Neuropsychological Tasks** |
| **AR** | **Adverse Reaction** |
| **AUC** | **Area Under the Curve** |
| **AVLT** | **Auditory Verbal Learning Tests** |
| **BRIEF** | **Behavior Rating Inventory of Executive Functioning** |
| **BRIEF-P** | **Behavior Rating Inventory of Executive Functioning – Preschool Version** |
| **BSID** | **Bayley Scales of Infant Development** |
| **CA** | **Competent Authority** |
| **CBCL** | **Child Behavior Checklist** |
| **CIBERSORT** | **Cell-Type Identification By Estimating Relative Subsets Of RNA Transcripts** |
| **CIP** | **Cancer in Pregnancy** |
| **CMS** | **Children's Memory Scale** |
| **CRF** | **Case Report Form** |
| **CV** | **Curriculum Vitae** |
| **DSMB** | **Data Safety Monitoring Board** |
| **EU** | **European Union** |
| **GCP** | **Good Clinical Practice** |
| **HPLC** | **High-performance liquid chromatography** |
| **IB** | **Investigator's Brochure** |
| **ICF** | **Informed Consent Form** |
| **IHC** | **Immunohistochemistry** |
| **IUGR** | **Intrauterine growth retardation** |
| **LC-MS** | **Liquid Chromatography-Mass Spectrometry** |
| **NONMEM** | **NONlinear Mixed-Effects Modeling** |
| **PABC** | **Pregnancy Associated Breast Cancer** |
| **PRAC** | **Pregnancy Related Anxiety Questionnaire** |
| **CERQ** | **Cognitive Emotion Regulation Questionnaire** |
| **(S)AE** | **(Serious) Adverse Event** |
| **Sponsor** | **The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.** |
| **WMO** | **Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen** |

# 1. SUMMARY

**Rationale:**

Cancer is the second leading cause of death during the reproductive years and complicates between 1:1000 and 2000 pregnancies pregnancies. In the past no treatment during pregnancy was given, physicians would prefer termination of pregnancy when diagnosed in the first trimester, or delay of treatment in the second or third trimester, with the aim to start oncologic treatment postpartum. However, recent studies have shown that oncologic treatment is sometimes possible during pregnancy, without damaging fetal outcome. Results so far are based on relatively small case series, and more research is needed.

**Objective**:

To record the incidence, diagnosis and treatment of cancer during pregnancy and maternal (obstetrical and oncological) and fetal outcome after maternal cancer during pregnancy – with long term follow-up of both mother and child.

**Study design:** International multicentre prospective observational trial

This study consists of 2 study parts and 6 subparts, and physicians/patients can decide participation per study part.

**Study population:** All patients with a cancer diagnosis/cancer treatment during pregnancy.

**Intervention (if applicable)**: n.a.

**Main study parameters/endpoints:**

Mother: registration of diagnosis, treatment and outcome (both oncologic and obstetric).

Child: long term follow-up, up to the age of 18 years.

**Nature and extent of the burden and risks associated with participation, benefit and group relatedness:**

The nature and extent of the burden and risks associated with participation, benefit and group relatedness differs per study part:

Study parts:

**Part I. Pregnancy, delivery and maternal health**

Part I.I.A. Registration study 'Cancer during pregnancy' mother and neonate

*None*

Part I.I.B. Effects of prenatal exposure to cancer treatment on fetal growth. The association between placental pathophysiologic mechanisms (histopathology and immunohistochemistry), circulating maternal factors and fetal growth.

*From participating women, a maternal blood sample will be collected during or shortly after birth. Also, an umbilical cord blood sample and placental and umbilical cord biopsies will be drawn.*

Part I.II. Measurement of maternal and paternal anxiety and emotional needs when confronted with a cancer diagnosis during pregnancy

*The participants will be asked to complete questionnaires.*

Part I.III. Biobank 'cancer and pregnancy'

*From participating women, a tumour biopsy specimen before administration of neoadjuvant therapy will be collected at the time of diagnosis. Also, maternal blood samples will be collected before and/or after treatment.*

Part I.IV. Study on the pharmacokinetics of chemotherapeutic agents in pregnant women

*Approximately 10-12 additional peripheral venous blood withdrawals.*

**Part II. Child**

Long term follow up of children and adolescents in utero exposed to chemotherapy and/or radiotherapy

*Regular check-ups of the child, at the age of 18months, 3y, 6y, 9y, 12y, 15y, and 18y, and after the age of 18 years: 5-yearly cardiologic assessment and questionnaires (23y, 28y, 33y, 38y, and 43y). Optional at the ages of 9y, 12y, 15y and 18y: MRI session.*

## 2. INTRODUCTION AND RATIONALE

Cancer is the second leading cause of death during the reproductive years and complicates between 1:1000 and 2000 pregnancies [1]. The actual incidence of cancer during pregnancy is difficult to calculate due to the lack of central registries. As women in developed societies delay childbearing to the third or fourth decade of life, and the incidence of several malignancies rises with increasing age, this rare coincidence is likely to become more common.

The treatment of cancer in pregnancy has become more acceptable in the last decade, and an increasing number of reviews on this topic is being published. Meanwhile, original studies remain scarce, mostly due to the low incidence per centre.

The most frequently encountered types of cancer in women of childbearing age are breast cancer, cervical cancer, leukaemia, lymphoma and malignant melanoma. The maternal prognosis is suggested to be similar to non-pregnant women provided the same treatment strategies are applied [2]. These include surgery, systemic therapy and radiotherapy or a combination of these therapies. However, little data exist on the safety and efficacy of chemotherapy and radiotherapy during pregnancy. Among different physicians, different views exist on how a pregnant patient with cancer should best be treated (termination of pregnancy/treatment during pregnancy/preterm induction of labour and treatment postpartum) [3]. Results of a recently performed survey among European physicians showed that a significant number of physicians is reluctant to start oncologic treatment during pregnancy and would prefer iatrogenic preterm delivery [3]. Almost half of specialists (44%) would recommend termination of pregnancy as their first choice when cancer is diagnosed during the first or early second trimester of pregnancy. Preterm delivery in order to start cancer treatment in the postpartum period is preferred by 58%. Consistent with these findings is the opinion that 37% of respondents would not consider chemo- and/or radiotherapy during pregnancy. Although certain circumstances warrant this cautious approach to avoid oncologic treatment during pregnancy (e.g. termination of pregnancy during the first trimester, when advanced stage disease is diagnosed and treatment needs to start as soon as possible), several studies have shown that different types of oncologic treatment can be utilized during pregnancy without compromising fetal health [4-8].

Due to the low incidence, multicentre collaboration is needed to study this problem.

## 3. STUDY DESIGN

The proposed research concerns a multicentre prospective observational cohort study. European collaboration is utilized in order to include sufficient number of patients.

Time schedule:

This is an ongoing study, for which primary approval was granted in 2005 by UZ Leuven. UZ Leuven is the initiator of the study and the ethical committee of UZ Leuven is the central ethical committee. The UZ Leuven protocol is used in all centres, including the international collaborators.

As part 2 of this study includes the follow-up of children that were prenatally exposed to cancer or cancer treatment during pregnancy, the expected study end will be at least 18 years from the inclusion date. An important part of the follow up of the children is performed in the Netherlands, where the ethics commission approved the study in 2014. Consequently, we estimate the study to run until at least 2032.

This study consists of 2 study parts and 6 study subparts. **In the following, we describe the introduction, objective, methods, and statistical analysis separately for the different study parts. It should be noted that patients do not need to and sometimes cannot (when the clinical situation is not applicable) participate in all parts of the study.** Depending on the wishes and possibilities of the patient and the clinical situation, selected study parts will be chosen (flow chart 1 and 2).

**Study parts:**

**Part I Pregnancy, delivery and maternal health**

*Part I.I.A.* Registration study 'Cancer during pregnancy' mother and neonate

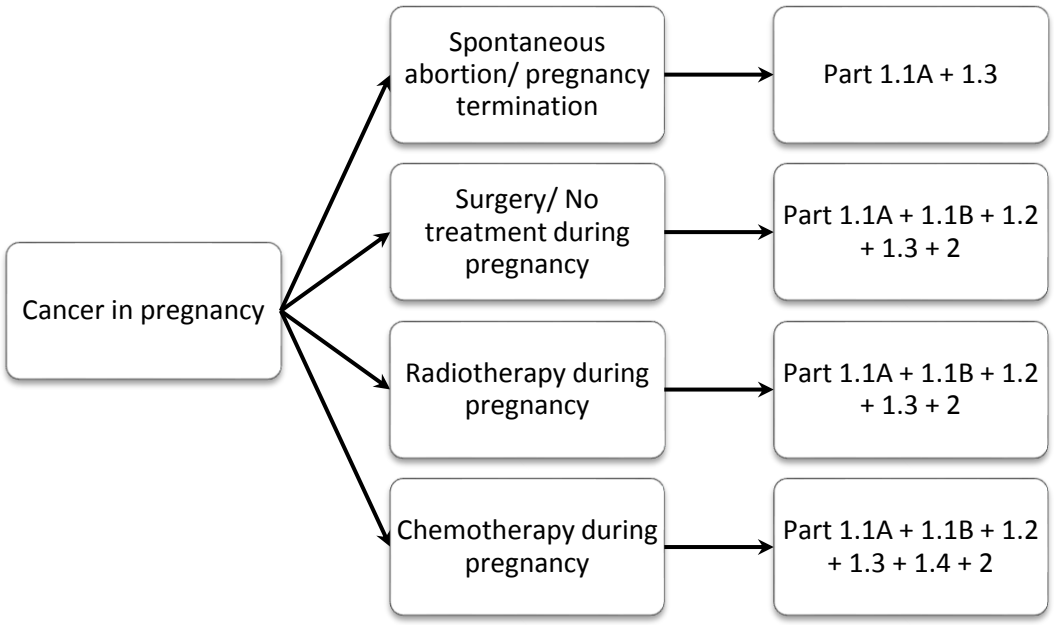
*Part I.I.B.* Effects of prenatal exposure to cancer treatment on fetal growth. The association between placental pathophysiologic mechanisms (histopathology and immunohistochemistry), circulating maternal factors and fetal growth.

*Part I.II.* Measurement of maternal and paternal anxiety and emotional needs when confronted with a cancer diagnosis during pregnancy

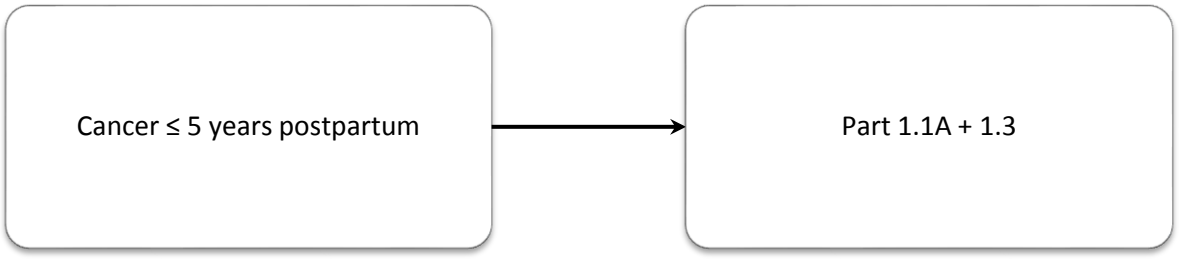
*Part I.III.* Biobank 'cancer and pregnancy'

*Part I.IV.* Study on the pharmacokinetics of chemotherapeutic agents in pregnant women

**Part II Child**Long term follow up (including Magnetic Resonance Imaging) of children and adolescents in utero exposed to chemotherapy and/or radiotherapy (including supportive medication and diagnostic procedures).




**Flow chart 1. Possibilities to participate in different study parts after** diagnosis of cancer during pregnancy.




**Flow chart 2.** Diagnosis ≤ 5 year postpartum.

# 4. STUDY POPULATION

## 4.1 Population (base)

All women diagnosed with cancer during pregnancy and in the first five years after delivery will be considered for inclusion. Cancer is diagnosed during pregnancy in approximately 1 in 1000 pregnancies [1]. With a birth rate around 127.000 neonates per year in Belgium, the incidence rate of cancer during pregnancy in Belgium is estimated at around 76 to 127 per year [9]. In Europe, this number translates into 3000 to 5000 new cases of cancer diagnosis during pregnancy yearly.

## 4.2 Inclusion criteria

In order to be eligible to participate in this study, a patient must meet all of the following criteria which will be listed per study part. Patients need to give their signed and written informed consent to participate in the trial after fully understanding the implication of the protocol.

## 4.3 Exclusion criteria

Mentally disabled or patients with significantly altered mental status that would prohibit understanding and giving informed consent will be excluded from participation in this study.

# 5. METHODS

## 5.1 Randomisation, blinding and treatment allocation

n.a.

## 5.2 Study procedures

For none of the study parts, it will be necessary to postpone diagnostic procedures or treatment. Participation in this study does not influence normal treatment.

## 5.3 Withdrawal of individual subjects

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

## 5.4 Replacement of individual subjects after withdrawal

n.a.

## 5.5 Follow-up of subjects withdrawn from treatment

n.a.

## 5.6 Premature termination of the study

No reasons for premature termination of the study are anticipated.

# 6. STATISTICAL ANALYSIS

Analysis will be performed with SAS (version 9.2), Statistical Package for Social Sciences for Windows (version 23) or R. Specific statistical methods will be listed per study part. Statistical analysis will be performed under supervision of an experienced statistician.

# 7. SPECIFICATIONS PER STUDY PART (1. PREGNANCY, DELIVERY AND MATERNAL HEALTH STUDIES AND 2. CHILD STUDIES) and SUBPART (6): introduction, objectives, inclusion criteria, sample size calculations, study procedures and statistical analysis

## 7.1 Part I.I.A. Registration study mother and neonate

### Introduction (Part I.I.A.)

The interest in cancer during pregnancy is increasing. Until a few years ago, mainly case studies were available and little prospective data were reported.

Although most studies report that the prognosis of women with cancer in pregnancy is similar to the non-pregnant patient, these data should be interpreted with caution. The reported series are not large enough to control for all prognostic factors and to draw firm conclusions. Regarding pregnancy-associated breast cancer (PABC), there is also data stating the contrary, that the overall survival of PABC patients is shorter than that of non-pregnant patients [10]. There is controversy as to whether pregnancy and/or lactation are independent prognostic factors. PABC has been described to be associated with a poorer prognosis [10], whereas other studies report similar survival, when matched for age, stage, and other prognostic factors [2, 11, 12].

*Results up to now: Publication regarding registration study (Part I)*

We reported in 2008 a series of 215 patients with cancer diagnosed during pregnancy [5]. This study showed an overall good outcome of the pregnancies complicated with cancer. In five (2.3%) of 215 patients, a miscarriage occurred at 10.7 ± 4.8 weeks of gestation, before cancer treatment was initiated. In 30 (14%) of 215 patients, the pregnancy was terminated at a gestational age of 10.9 ± 6.8 weeks. In 29 of 30 patients, maternal cancer was the reason for termination. In 58 (27%) of 215 patients, treatment was delayed until postpartum. In 122 (57%) of 215 patients, a single or a combination of treatment modalities was initiated during pregnancy. A total of 62 women received chemotherapy and 10 received radiotherapy. The most remarkable finding was the observation that 54.2% of children were born preterm, with a high rate of admission to the Neonatal Intensive Care Unit. In the vast majority (89.7%), the delivery was induced. The incidence of congenital malformations was not increased compared to the background risk in a general population.

*Current status (Part I.I.A.)*

At this stage (04-09-2017), 1682 women are included since the study started in Belgium (18 March 2005). These are patients with various types of cancer who have had different treatments during pregnancy, varying from termination of pregnancy, to delaying treatment until postpartum, to surgery, chemotherapy and/or radiotherapy during pregnancy.

Currently, 350 children who were exposed to chemotherapy and/or radiotherapy in utero, have been included for long term follow up (in Belgium, the Netherlands, Czech Republic and Italy).

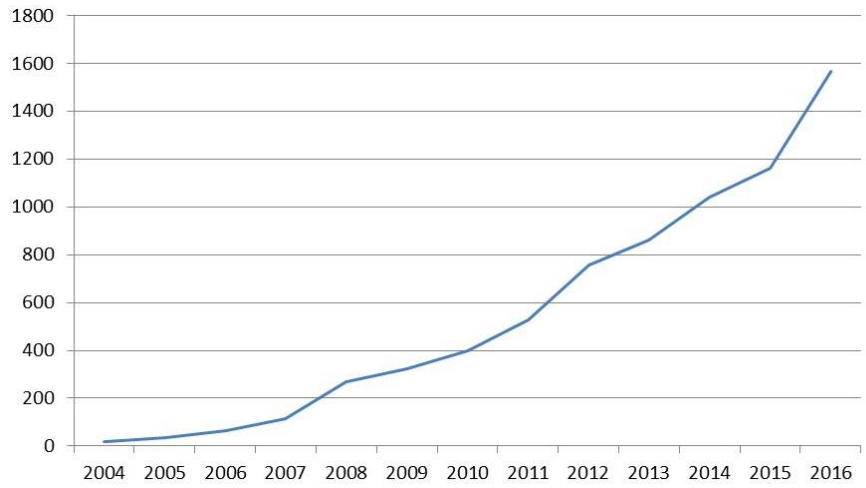



**Figure 1.** Inclusions of the multicenter CIP registration study up to 04-09-2017 (n=1682).

**Objectives (Part I.I.A.)**

*Primary Objective*

To evaluate the incidence of maternal (obstetrical and oncological) outcome of cancer occurring during pregnancy and compare oncological outcome with a matched control group of patients with non-pregnancy associated cancer.

To record the short and long term outcome of the child.

*Secondary Objective(s)*

To record the incidence and prognosis of age-matched women with cancer diagnosed in the first 5 years postpartum or outside the context of a pregnancy. Comparing oncological outcome with a matched control group of non-pregnancy associated cancer patients will allow us to

investigate whether different cancer types behave differently during, shortly after and outside pregnancy.

**Inclusion criteria (Part I.I.A.)**

Histological proven cancer in the premenopausal period under 45 years of age: this may be either during pregnancy, in the first 5 postpartum years or outside pregnancy. Retrospective data collection and anonymous registration will be performed to evaluate quality of care for patients that are no longer receiving treatment or have been deceased. For the control group, we aim to select one control patient for every pregnant and postpartum cancer patient matched on sex, age (premenopausal), tumour type, stage at diagnosis and year of diagnosis (+/- 5 years).

**Sample size calculation (Part I.I.A.)**

In Europe, the yearly total number of registered new cases of pregnancy-related cancer is 3000 to 5000. In the last year we registered 361 new cases in our study. We aim, during a period of 5 more years of registration (2017-2022), to register 1800 to 3600 new patients in Europe. We aim for one control patient per study patient.

**Study procedures (Part I.I.A.)**

In women with a cancer diagnosis during pregnancy or a cancer diagnosis within 5 years postpartum, the most important oncological and obstetrical data will be registered, as well as information on the maternal and paediatric outcome. Control patients are all other premenopausal women (≤45 years) diagnosed with cancer in the first 5 years after diagnosis of the matched case. All types of cancer and all types of treatment, including termination of pregnancy will be registered. Patients can be included both prospectively and retrospectively. For every pregnant and postpartum patient included, we will aim to register a matched non-pregnancy associated cancer patient with the same age, the same tumour characteristics and stage for whom we will register the same information except the obstetrical information. Since it is an anonymous registration of clinical data from the patients file, no informed consent is needed (not-WMO) but will be asked where possible.

*Recruitment of patients*

For retrospective recruitment of patients, in the past we have collaborated with the Foundation Cancer Registry and a medical insurance organization (National Alliance of Christian Sickness

Funds). Registration codes for chemotherapy and radiotherapy were linked to the code for delivery or in utero death (> 16weeks of gestation) with a maximum time interval of 9 months between both. The list of patients we obtained was fine-tuned in such a way that only the patients with a cancer diagnosis during pregnancy or the first postpartum year remained. Treating physicians of these patients were contacted by the Foundation Cancer Registry with a request to register their patients anonymously in this study. Control patients are found in the patients population of the oncological departments. In other countries several comparable registers or local cancer registries can be used.

Because information on pregnant state is not available in most cancer registries and because oncologic problems are not registered in perinatal data sets, prospective recruitment of patients is done as follows: networking, newsletters, presentation at conferences for obstetricians, oncologists, haematologists, and also a website for professionals (www.cancerinpregnancy.org) and for patients (www.kankerenzwangerschap.be).

We will collect the following data:

<u>At baseline:</u>

Oncological data including type of cancer, the date of diagnosis, tumour histology, the type and date of treatment.

Obstetrical data including gravidity and parity, gestational age at diagnosis, obstetrical complications, results of prenatal sonographic evaluations (biometry and morphology), gestational age at delivery, mode of delivery (induction, caesarean section, spontaneous labour) and breastfeeding start and end date.

Paediatric data that are collected include birth weight, gender, congenital malformations, admission to neonatal care unit and reason of admission.

<u>Annually until end of study:</u>

We will request information from the treating physician regarding patient's health status (relapse yes/no, site of relapse, treatment of relapse, date of relapse, date of death if applicable) and condition of the child.

This study does not involve any direct input or effort from the patient. Only the collection of existing information through the medical records is aimed for.

**Statistical analysis (Part I.I.A.)**

We plan to use descriptive statistics (mean +/- standard deviation), two-sided tests, and Wilcoxon rank sum test to compare continuous variables in two groups, for more than two groups, the Kruskal-Wallis exact test will be used. For comparing a proportion with an expected value, a binominal exact test will be used. Results are considered significant at P<.05.

## 7.2 Part I.I.B. Effects of prenatal exposure to cancer treatment on fetal growth. The association between placental pathophysiologic mechanisms (histopathology and immunohistochemistry), circulating maternal factors and fetal growth.

### Introduction (Part I.I.B.)

One of the findings of previous studies was that fetal growth restriction (FGR), defined as an estimated fetal weight lower than the $10^{th}$ percentile for gestational age, is frequent in pregnancies complicated by cancer and/or cancer treatment [5, 6, 13]. Our recent data on the pediatric outcome of 1170 patients with cancer during pregnancy show an increased risk for small for gestational age (SGA) when chemotherapy was administered during pregnancy. Consequently, an intensive obstetrical follow-up of fetal growth and placental function in a specialised centre is advisable in this high risk population [14]. FGR places an infant at significant risk of perinatal morbidity and mortality, and is known to have various potential causes [15]. The most common cause of FGR (80-90% of all cases) remains the compromised supply of nutrients and oxygen through the placenta to the fetus [16]. Placental dysfunction, deregulated metabolic adaptations, chronic inflammation, increased apoptosis and enhanced stress factors, are mechanisms that can contribute to decreased fetal growth [17, 18]. The exact underlying mechanism in patients with cancer diagnosis and treatment in pregnancy is so far unexplored. This hampers the identification of patients at risk, as well as the development of preventive measures.

### Objectives (Part I.I.B.)

1. Examine and compare whether FGR after chemotherapy exposure is associated with changes in placental histology between cancer patients with chemotherapy during pregnancy, without chemotherapy during pregnancy and pregnant women without cancer; all divided in with or without FGR.
2. Determine and compare the presence of biomarkers related to placental dysfunction and/or FGR between cancer patients with chemotherapy during pregnancy, without chemotherapy during pregnancy and pregnant women without cancer, using Polymerase Chain Reaction, Elisa and/or IHC analysis of placental tissue, cord blood and/or maternal blood.

**Inclusion criteria (Part I.I.B.)**

Women who are registered for part I.I.A. (pregnant women with histologically proven cancer) who reach at least a gestational age of 24 weeks are eligible for part I.I.B.

**Exclusion criteria (Part I.I.B.)**

Women with spontaneous abortion or pregnancy termination before 24 weeks of gestation (according to flow chart 1) cannot be included in part I.I.B.

**Sample size calculation (Part I.I.B.)**

We aim to include a total of 80 women with cancer during pregnancy (registered in part I.I.A; groups 1-4) and a total of 40 controls (group 5 and 6) within three years, matched for age, type of cancer type, maternal intoxications and comorbidities related to FGR (preeclampsia, SLE, hypertension, Crohn's disease, renal or cardial pathology).

**Methods (Part I.I.B.)**

*Main study parameter/endpoint*

Association of placental findings (placental weight, ischemic lesions, fibrin and thrombus deposition, macroscopic abnormalities (ie. placental tumours, abnormal cord insertions), villous immaturity/dysmaturity, viilitis and/or intrauterine infection and circulation disorders) with FGR in pregnant women with cancer.

*Secondary study parameters*

Association of levels of hormones, factors of angiogenesis, inflammation, apoptosis and proliferation in maternal blood and placenta biopsies with FGR in pregnant women with cancer.

**Study procedure (Part I.I.B.)**

There will be 4 groups of study subjects with cancer and/or chemotherapy and two control groups recruited from the CIP registry. Groups 1 and 2 concern children from women who have been treated with chemotherapy during pregnancy, respectively with FGR and without FGR. Groups 3 and 4 concern children from women with cancer without chemotherapy during pregnancy, respectively with FGR and without FGR. All newly registered subjects will be invited to participate; allocation to the appropriate group will take place after delivery. Groups 5 and 6 concern respectively children with FGR and without FGR from women

without a malignant disease during pregnancy. These women are invited to participate from the general obstetrical department of participating centers.

Maternal blood sampling, during or shortly after delivery will be taken simultaneously with standard of care blood samples to determine levels of circulating hormones, factors of angiogenesis and inflammation.

Umbilical cord blood sampling will be taken after birth, after clamping, to determine factors of growth and oxidative damage.

Placenta, umbilical cord and membranes will be collected and fixated with 4% paraformaldehyde to be examined by the local pathologist on different histology items. Afterwards, the standardly prepared formalin-fixed paraffin embedded blocks of placenta, umbilical cord and membranes will be collected and used for IHC studies on markers of angiogenesis, inflammation, apoptosis and proliferation.

**Statistical analysis (Part I.I.B.)**

Clinical data are collected anonymously in an online database (www.cancerinpregnancy.org).

*Primary study parameter*

To test for significant differences in placental pathology parameters (macro- and microscopic placental findings) between the 3 study groups of this study part (cancer with chemotherapy, cancer without chemotherapy and pregnant without cancer) the $\chi^2$ test will be used to compare the presence of ischemic lesions, fibrin deposition, macroscopic abnormalities as earlier mentioned, infection and circulation disorders between the study groups, the Kruskal-Wallis exact test will be used to compare continuous variables (placental weight, percentage of infarction or villous dysmaturity) between our 3 groups.

*Secondary study parameters*

For IHC, comparison of the number of cells scoring positive for investigated markers (factors of angiogenesis, inflammation, oxidative damage and apoptosis) between the 3 study groups will be performed using the Kruskal-Wallis exact test. For multiplex analyses, the inform software package will be used. To examine the effect of cancer and cancer treatment to the differential expression of the markers, multivariable analysis will be performed. Differences in patients' characteristics (general medical history and pregnancy-related complications) between the groups will be assessed using $\chi^2$ test.

## 7.3 Part I.II. Measurement of maternal and paternal anxiety and emotional needs when confronted with a cancer diagnosis during pregnancy

### Introduction (Part I.II.)

Currently, knowledge on the psychological impact and maternal needs when cancer is diagnosed during pregnancy is limited [19-21]. While there is an emotional burden for the pregnant woman when she is confronted with the diagnosis of cancer, nurses, psychologists and medical doctors have no guidelines to optimally support these mothers (and their partners). Moreover, high maternal stress and anxiety levels, maternal disease and its treatment may influence pregnancy and the (unborn) child [22-25]. Therefore, the results obtained in this CIP study will provide useful information on the psychological burden of these patients and therefore may provide useful tools to improve psychological support and treatment and to limit the adverse effects on the fetus.

Recently, we published a retrospective study on the distress, concerns and coping strategies of pregnant women diagnosed with cancer and their partners [19]. The results indicated that the patients and their partners had similar levels of distress. Patients and partners who mainly used internalizing cognitive coping strategies (e.g., high scores on rumination and catastrophizing) had the highest levels of distress and may therefore benefit from additional psychological support.

### Objectives (Part I.II.)

The study has three aims:

1) To compare the results of the retrospective study to those of newly diagnosed patients who are currently pregnant and their partners (prospective study).
2) To look at cross-cultural similarities and differences in distress and coping with cancer during pregnancy.
3) To validate our newly constructed questionnaire about concerns and distress related to a cancer diagnosis and treatment during pregnancy.

### Inclusion criteria (Part I.II.)

All patients and their partners newly diagnosed with cancer during pregnancy can be included. Patients will be included through the INCIP network collaboration between centres in Belgium, the Netherlands, USA and Spain. More centres are expected to join the study.

**Sample size calculation (Part I.II.)**

We aim to validate our newly constructed questionnaire with a total of 40 items. A total of 10 participants per item of the questionnaire is requested to validate the questionnaire and thus, 400 participants are aimed for. As both the patient and the partner fill out the questionnaire, we aim to collect questionnaires of 200 couples.

**Study procedures (Part I.II.)**

We developed a questionnaire on maternal concerns when cancer is diagnosed during pregnancy. This questionnaire is based on an existing questionnaire on maternal fear during pregnancy: the Pregnancy Related Anxiety Questionnaire (PRAQ). The PRAQ measures specific fears for: oneself and the partner, the integrity of the baby, the delivery, changes taking place and the future relationship with the baby and the partner. The PRAQ is designed and validated by Professor Van den Bergh (Tilburg University), who has an internationally recognized expertise in this area [24]. New items specifically addressing cancer during pregnancy topics were added, based on our clinical experience with patients. This questionnaire is designed for the patient, and an adapted version of the same questions is made for her partner. Furthermore, the validated Cognitive Emotion Regulation Questionnaire (CERQ) is added to investigate coping with cancer during pregnancy. This provides the opportunity to link the distress level of the patients and their partners with their cognitive coping strategies and to identify patients and partners at risk for high levels of distress based on their coping.

We will ask patients and their partners to complete the questionnaire in newly diagnosed cases. In the early phase of diagnosis, the complexity of the situation renders this questionnaire inappropriate at that stage. Therefore, the investigators plan to propose participation to the study once decisions on treatment are taken and once treatment is ongoing. At that stage, reflection on the process as a whole will be possible and acceptable.

*Heterogeneity of the study population*

The study is designed for any cancer case that is diagnosed during pregnancy. This will result in a wide variety of cancer types and treatment modalities used during pregnancy. This heterogeneity should not be a problem for the purpose of the study since the common denominator is the cancer diagnosis during pregnancy. In that respect, the group is homogeneous, a crucial issue with respect to interpretation and analysis of data.

**Statistical analysis (Part I.II.)**

1) The prospective data will be compared to the recently published retrospective data by means of ANOVA.
2) Cross-cultural similarities and differences between countries in distress and coping with cancer during pregnancy will be explored by means of ANOVA.
3) The newly constructed questionnaire will be validated by means of principal component analysis in order to use it as a tool for distress screening in patients diagnosed with cancer during pregnancy and their partners.

## 7.4 Part I.III. Biobank 'cancer and pregnancy'

**Introduction (Part I.III.)**

Prognosis of pregnancy-associated cancer has been the topic of several case-control and population based studies with inconsistent results. However, there is increasing evidence that breast and ovarian cancer diagnosed in the first 5 postpartum years have an increased risk of cause-specific death and thus worse prognosis, when compared to cancer diagnosed during pregnancy and outside the context of a pregnancy [26, 27] Up to now, few of these studies have taken all biological tumour characteristics into consideration. The (immuno)biology of most pregnancy-associated cancers remains poorly understood, and hence, it is unknown why some of these cancers, such as breast and ovarian cancer, have a poorer prognosis, or how they should be effectively targeted for improved survival. Examination of clinicopathological, molecular and immunological differences of the tumour is a method to examine tumour aggressiveness and invasiveness. In breast cancer, microarray-based gene expression studies have demonstrated that breast cancer is highly heterogeneous, both on the clinical and molecular level, comprising subtypes with distinct gene expression patterns that are associated with outcome [28]. Perou et al [29] identified four main subtypes: luminal-A, luminal-B (mainly oestrogen receptor positive), basal–like (mainly triple negative) and HER2-positive tumours. The correlation between molecular subtypes and clinical data has shown a significant difference in overall survival between different subtypes. Independent of subtype, postpartum breast cancer specifically has been associated with a poorer prognosis and an increased risk for metastasis and death compared to cancers diagnosed during or independent of a pregnancy [27]. However, the exact impact of a (recent) pregnancy on tumour immunology and biology in women is still unclear. The development of malignant tumours is controlled by a complex

biologic system that depends on genetic abnormalities as well as the interplay between tumour cells, stromal cells, and host inflammatory cells. For pregnancy-associated breast cancers for example, some studies pointed to a potential correlation between a *BRCA*-mutation and an increased amount of tumour infiltrating lymphocytes [30-33]. Although adequate immunological responses of the body can improve cancer prognosis, the impact of known alterations of the immune system during pregnancy on these mechanisms is unclear [34]. Thus it is arguable that the pregnancy milieu may exert an effect of the development and progression of cancer via hormonal, immune or other mechanisms. Extensive characterisation of the (immuno)biology of pregnancy-associated cancers may reveal novel (immuno)therapeutic targets for cancers diagnosed during pregnancy and up to 5 years postpartum.

**Objectives (Part I.III.)**

The 3 specific objectives of this study part are to:

1) Determine and compare the clinicopathological profile, and the metastatic and survival rates between cancer patients diagnosed during pregnancy, during the postpartum period, or independent of a pregnancy.
2) Identify and compare differentially expressed genes and pathways in breast tumour tissue of pregnant, postpartum and non-pregnant cancer patients, using whole transcriptome sequencing and *in silico* analyses.
3) Characterise and compare the biological and immunological microenvironment of breast tumour tissue of pregnant, postpartum and non-pregnant cancer patients using the whole transcriptome sequencing data from objective 2 combined with Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT) *in silico* analyses as well as via (multiplex) IHC analyses. In addition, we will investigate a potential correlation between BRCA-status, pregnancy-associated cancer and immune cell infiltration.

The 3 cohort groups that will be compared in each study part are: group 1 = cancer diagnosed during pregnancy; group 2 = cancer diagnosed ≤ 5 years postpartum; group 3 = non-pregnant patients with a cancer diagnosis ≥ 5 years before or after a pregnancy. These 3 cohort groups are matched for intrinsic molecular subtype and grade. We aim for 30-50% of these samples originating from Belgium.

**Inclusion criteria (Part I.III.)**

- Histological proven cancer during pregnancy or in the first 5 postpartum years.

- Tissue available (fresh frozen or formalin fixed tissue). Retrospectively collected tumour biopsies can be used, taken at the time of diagnosis (core biopsy) or surgery, and before neoadjuvant therapy was given. - For the prospective part of this study, a blood samples of the mother will be collected before and/or after cancer treatment.

**Study procedures and sample size (Part I.III.)**

*Clinicopathological characterisation*

The main objective of the first part is to compare the clinicopathological parameters, metastatic occurrence and overall survival of pregnant, postpartum and non-pregnant cancer patients. For each patient in the 3 groups the following data will be collected: tumour characteristics (data of diagnosis, date of local and/or distant recurrence, histological grade, clinical stage, tumour size, nodal involvement, histological and molecular subtype); therapy characteristics (type of surgery and admission of adjuvant and/or neoadjuvant chemo-,radio-, hormone- and targeted therapy); and patient characteristics (birth data of mother, date of delivery, gravidity, parity, miscarriages, clinical outcome and history of breastfeeding).

We aim for $\geq$ 200 patients per group.

*Molecular and immunological characterisation*

Nucleic acids extracted from FFPE tissues are fragmented and chemically modified, presenting an additional challenge to use in classical molecular analyses. Evaluation of DNA and RNA quality is a critical step in successful DNA/RNA sequencing. As a pilot project, the DNA and RNA isolation procedure and the subsequent DNA/RNA quality will be validated using fresh frozen and/or FFPE tissue from patients with variable ages and originating from different countries. For each participating country, we aim for 10 cancer samples.

Gene-expression analyses will be performed on formalin fixed and/or fresh frozen tumour samples of at least 50 patients per cohort (150 samples in total), matched for intrinsic molecular subtype, stage and grade. RNA will be extracted and transcriptome sequencing (Illumina HiSeq 2000, 1x50 bp single-end sequencing) will be performed, which enables two complimentary levels of analysis - gene expression and alternative splicing. With this method we aim to identify specific alterations in exon usage that may play a central role in disease mechanism and aetiology. We will use bioinformatics and statistical analysis to process the raw data into understandable biological data. First, using the transcriptome data, we will search for genes and

pathways that are specifically affected in postpartum cancers. Second, using the same transcriptome data as well as (multiplex) IHC, we will characterise components of the extracellular matrix that are known to influence tumour motility and invasion on one hand and investigate the composition and localisation of infiltrating immune cells and the presence of specific immune biomarkers in the tumour specimens on the other hand. Based on the molecular and immunological information retrieved from the analyses on the tumour tissue, we will prospectively investigate differences in inflammation and other significant immunological markers in blood samples from postpartum, pregnant and non-pregnant patients. In addition, for breast cancer, a potential correlation between *BRCA*-status, pregnancy-associated cancer and alterations in immune cell infiltration will be investigated in the three study groups. For a large number of patients the *BRCA*-mutation status has already been determined and can be retrieved from the patient databases. For the patients for whom *BRCA*-status is not available, we will extract DNA from fresh frozen and/or FFPE tissue and use the Multiplex Ligation-dependent Probe Amplification (MLPA) assay to determine variations in the DNA copy number of the *BRCA*-genes.

**Statistical analysis (Part I.III.)**

Regarding the clinicopathological characterisation, we aim to include at least 200 patients in each of the cohort groups.

To test for significant differences in clinicopathological prognostic characteristics between pregnant, postpartum and non-pregnant cancer patients, $\chi^2$ and One-Way ANOVA tests will be used. Differences in metastatic risk and overall survival will be determined by means of Kaplan-Meier curves and Cox Regression analyses. All statistical analyses will be performed using R software, 95% confidence intervals will be determined and a two-tailed p-value of 0.05 will be considered significant. Where necessary corrections for multiple testing will be applied.

For the molecular and immunological part of this project, we aim to include at least 50 patients per cohort group, matched for molecular subtype, stage and grade. Differential gene expression between the different patient groups will be evaluated using the Bioconductor package EdgeR. To identify general expression changes associated with progression of postpartum cancer, the gene probe expression values of cancer diagnosed during pregnancy, in the postpartum period or outside pregnancy, will be individually normalized by subtracting their $\log_2$ averages. Gene probe selection based on both an increase or decrease in expression by a factor of 2 ($\log_2$ 1/-1) and statistical significance ($p<0.05$) in at least one tumour stage based on Student's t-test as a

more stringent method of selection. To compare transcriptome profiles of pregnant, postpartum and non-pregnant cancer patients, we will perform a gene set enrichment analysis (GSEA) using the Gene Ontology and KEGG data bases [35]. We will apply the CIBERSORT computational method to qualify the relative levels of distinct cell types using the gene expression data of the patient cohorts [36]. Distributions of the estimated immune cell-type fractions will be compared across the cohorts using the Mann-Whitney U test. Per tumour type, the frequencies of tumour infiltrating lymphocytes (TILs) in H&E stained sections of the postpartum cancer patients and control patients will be compared by means of $\chi^2$ tests. For IHC, comparison of the number of cells scoring positive for investigated markers between the 3 cohorts will be performed using Fisher's Exact test and One-Way ANOVA. For multiplex analyses, the inFORM software package will be used. In addition, we will use the fully automated BioNumerics MLPA workflow for reliable *BRCA*-mutation analyses.

## 7.5 Part I.IV. Study on the pharmacokinetics of chemotherapeutic agents in pregnant women

**Introduction (Part I.IV.)**

Most anticancer drugs have a wide inter-individual pharmacokinetic variability and a narrow therapeutic window. Pharmacokinetic data from other patient-populations are difficult to extrapolate to pregnant women due to the important gestational changes. Those physiological changes may possibly alter the efficacy and toxicity of anticancer drugs administered during pregnancy.

*Results up to now- Publication regarding pharmacokinetics study (Part I.IV.)*

We reported interim results comparing pharmacokinetics between pregnant and non-pregnant patients [37]. The numbers of pregnant and non-pregnant patients were respectively 5 and 2, 7 and 5, 4 and 4 and 2 and 2 for paclitaxel, doxorubicin, epirubicin and carboplatinum, respectively. This study revealed an increased distribution volume and clearance and a decreased plasma drug exposure (area under the curve and peak plasma concentration) during pregnancy compared to non-pregnant state [37]. In a second report we used compartmental nonlinear mixed effect models to describe the pharmacokinetics of chemotherapeutics in pregnancy. Therefore a pooled analysis of pharmacokinetic data was carried out for pregnant (Pr) and nonpregnant (NPr) patients for doxorubicin (n = 16 Pr/59 NPr), epirubicin (n = 14

Pr/57 NPr), docetaxel (n = 3 Pr/32 NPr) and paclitaxel (n = 5 Pr/105 NPr)[38]. This analysis confirmed the reduced exposure in pregnancy, which was most apparent for docetaxel and paclitaxel. Some suggestions for dose adaptations were made, but should only be implemented after further confirmatory studies of the pharmacokinetics during pregnancy. Yet, in clinical practice preference is given to dose dense regimens.

Theoretically a reduced plasma drug concentration can lead to a suboptimal treatment. Albeit, current follow-up data do not show a significant difference in survival between women receiving their treatment in pregnant versus non-pregnant state[2, 13].

Further research is needed in order to enlarge the groups for statistical analysis and to study toxicity and oncological outcome.

**Objectives (Part I.IV.)**

*Primary Objective*

To investigate the difference in pharmacokinetics of cytotoxic drugs and the therapy related toxicity, compared between pregnant and non-pregnant patients.

*Secondary Objective(s)*

To correlate these results with outcome (disease free and overall survival, as registered in part I.I.A.).

**Inclusion criteria (Part I.IV.)**

Pregnant patients receiving chemotherapy during pregnancy.

**Sample size calculation (Part I.IV.)**

Following analysis of our published results, the sample size is calculated at 20 pregnant patients per chemotherapy type (epirubicin, doxorubicin, paclitaxel, docetaxel, platinum) in order to achieve statistical significance (p = 0.05; power 80% ). Data from historical nonpregnant controls will be used for comparison.

**Study procedures (Part I.IV.)**

The plasma concentration over time after drug administration will be determined based on a series of blood samples (min 5, max 11 – each time 4 ml) collected in the first 48 h after administration of chemotherapy. Sampling starts at the end of the infusion, though exact sampling times will depend on the infusion regimen of the chemotherapy (APPENDIX A). The

blood samples for the pharmacokinetics study are not part of standard care. Patients do not need to be hospitalized during the study.

Chemotherapy concentration will be determined in plasma samples by Liquid chromatography–mass spectrometry (LC-MS) .

Based on the plasma concentrations, following pharmacokinetic parameters will be calculated: terminal elimination half-life, apparent volume of distribution, maximal plasma concentration, whole body clearance and area under the curve.

**Statistical analysis (Part I.IV.)**

The report of the Netherlands Cancer Institute (APPENDIX B) confirmed that a number of twenty patients per group is needed (80% power, alpha=0.05) to have a statistically significant difference for the most important parameter Area Under Curve-D (Area Under the Curve corrected for Dosage) when using observed means and standard deviations of an earlier pilot study. Because the number of patients is small, population pharmacokinetics will be performed as well. To describe the evolution of plasma concentrations we use the state of the art analytic methods, "NONlinear Mixed-Effects Modeling" (NONMEM) method. With this NONMEM-analysis a population pharmacokinetic model can be developed from which rational dosing schedules for pregnant women can be deduced for the investigated cytotoxic drugs.

The NONMEM-analyses will be coordinated and executed by Prof. Dr. J.H. Beijnen and Dr. A.D. Huitema (department Pharmacy and Pharmacology, The Netherlands Cancer Institute (NKI)/Slotervaart Ziekenhuis, Amsterdam). This group has a vast experience with NONMEM-analysis and population PK/PD-studies.

## 7.6 Part II. Long term follow up of children and adolescents born to mothers diagnosed with cancer during pregnancy (including Magnetic Resonance Imaging of the brain)

**Introduction (Part II.)**

Preclinical studies in mouse, rat, and baboon models have shown that the placenta has a barrier function which protects the fetus by reducing the concentration of chemotherapy. Although passage rates vary considerably per drug, animal models show 1-10.8% transplacental transfer of anthracyclines [39, 40], and even less transfer of taxanes (0.8-2.4%) [41]. Carboplatin transplacental transfer is highest (43-56%) [37, 39-41].

Although several reports address the fetal outcome after prenatal exposure to chemotherapy or radiotherapy, there are no prospective studies on this subject, except the current study. Retrospective data suggest an overall reassuring short-term outcome after prenatal exposure to surgery, chemotherapy or radiotherapy. However stillbirth, premature birth and low birth weight occur more frequently [5, 6, 42-44]. Also, neonatal myelosuppression has been reported [5, 43]. Although dose-related cardiotoxicity after anthracycline-exposure in patients is a well-known problem, the effects of doxorubicin on the developing fetal heart need further investigation. Moreover, the development of the neural system continues throughout pregnancy and even after birth. Therefore, even when cancer treatment is administered after 14 weeks of gestation the development of the brain can be influenced by cytotoxic treatment. Concerning the long-term outcome, systematic studies are lacking. The available studies report a normal physical, neurological, psychological, haematological and immunologic outcome, without an increased occurrence of secondary malignancies, however, children were not examined in a standardized manner [4, 45-48].

*Results up to now - Publication regarding long term follow-up of the child (Part II.)*

We performed an interim analysis of 70 children who have undergone testing at the standard ages [8]. 236 cycles of chemotherapy were administered in 68 pregnancies (two twin pregnancies), median follow up period was 22.3 months (range 16.8-211.6 months). Our measurements of the children's behaviour, general health, hearing, and growth corresponded with those of the general population. Cardiac dimensions and functions were within normal ranges. Although neurocognitive outcomes were within normal ranges, cognitive development scores were lower for children who were born preterm than for those born full term. We identified a severe neurodevelopmental delay in both members of one twin pregnancy [8].

In 2015, we published results of a cohort study in which 129 children aged 12 to 42 months born to mothers diagnosed with cancer during pregnancy were compared to 129 matched controls born after a normal pregnancy[49]. General health, cognitive and cardiac development were comparable between study and control group. However, prematurity was related to a worse cognitive outcome in both study and control group. Moreover, children in the study group (especially if prenatally exposed to chemotherapy) were more likely to be born small for gestational age as compared to our control group, although not statistically significant[49]. This study was restricted to paediatric outcomes in toddlerhood. Long term follow-up is needed to

document health, neurocognitive or cardiac problems that may appear or become more apparent after several years.

**Objectives (Part II.)**

*Primary Objective*

To investigate general health, neurocognitive, cardiologic and sexual development, incidence and prevalence of secondary malignancies and hearing deficits in children from mothers diagnosed with cancer during pregnancy.

**Inclusion criteria (Part II.)**

-All children of mothers diagnosed with cancer during pregnancy, with or without cancer treatment (surgery, chemotherapy and/or radiotherapy) during pregnancy.

-Children born after an uncomplicated pregnancy and delivery matched for gender, age, gestational age and language. By matching for gestational age, we will be able to disentangle the effects of prematurity and cytotoxic treatment.

**Sample size calculation (Part II.)**

The sample size is n = 155 per age group (based on 85% power analysis for intelligence testing, this sample size will be used for all evaluations). During long term follow-up, one child can be included into consecutive age groups.

| | BE | NL | CZ | IT | Total |
|---|---|---|---|---|---|
| < 18 months | 15 | 8 | 3 | 3 | 29 |
| 18 months | 86 | 22 | 8 | 20 | 136 |
| 36 months | 37 | 8 | 2 | 11 | 58 |
| 6 years | 59 | 6 | 6 | 0 | 71 |
| 9 years | 28 | 5 | 2 | 0 | 35 |
| 12 years | 11 | 0 | 1 | 0 | 12 |
| 15 years | 7 | 0 | 0 | 0 | 7 |
| 18 years | 1 | 0 | 1 | 0 | 2 |

BE=Belgium, NL=the Netherlands, CZ=the Czech Republic, IT=Italy

**Table 1.** The current number of study children (prenatally exposed to chemotherapy and/or radiotherapy) included in the follow-up study in Belgium, the Netherlands, the Czech Republic and Italy.

Moreover, based on current recruitment data, we aim to enlarge the study group with 35 new children per year who will be prospectively included in the follow-up. Divided per country per year, this means 10 new study subjects in Belgium, 15 new study subjects in the Netherlands, 5 new study subjects in Czech Republic and 5 new study subjects in Italy. Canada is also involved in the collaboration, but they only include control subjects for the cardiac data.

**Study procedures (Part II.)**

*After birth*

At birth, an umbilical cord blood sample will be taken to evaluate the renal and hepatic function, coagulation, and haematological values, to evaluate the impact of chemotherapy exposure on the child. Within one week after birth, a paediatrician will perform a thorough physical (including birth weight, detection of congenital anomalies) and standard neurological examination and an ultrasound of the heart (performed by the paediatrician in the hospital where the patient delivers). Screening for hearing loss is commonly done in the first month after birth by means of an ALGO (automatic brainstem evoked response audiometry) or OAE (otoacoustic emissions) test. Results of this test will be requested. If the test is not standard of care, we will ask to do the test at the maternity unit. These examinations will provide information about short-term neonatal effects.

*Long term follow-up*

At the age of 18 months, 3 years, 6 years, 9 years, 12 years, 15 years and 18 years, the children will be invited to the hospital for a physical, neurologic and cardiac evaluation.

The children will be examined by a paediatrician, a cardiologist or trained technician supervised by the cardiologist, a trained Magnetic Resonance (MR) technician,and a psychologist. We will aim to plan the tests on the same day when possible. This will take about 2 hours (at the age of 18 months and 3 years) to 1,5 days for the older children. Parents are allowed to be present during the examinations.

Normal physical development and biometry will be assessed during the physical examination. Neuropsychological tests will be performed to evaluate their cognitive development. According

to the child's age, the following neuropsychological tests will be performed: BSID-III, WPPSI-III, WISC-III or WAIS-IV, ANT, AVLT, TEA-Ch, CMS CBCL and BRIEF / BRIEF-P (Table 2). Parents receive a report with the results of cognitive testing. An echocardiography will be performed to evaluate the morphology and function of the heart. No blood samples will be taken and no other invasive procedures will be done.

At the ages of 9, 12, 15 and 18 years, the children will be invited to participate in a MR neuroimaging examination. This examination consists of a high resolution T1-weighted 3D anatomical image and short FLAIR MR imaging to search for primary brain pathology, in combination with advanced diffusion weighted imaging (DWI) and resting-state functional MRI (rs-fMRI). Voxel-, surface- and/or deformation-based morphometry applied to the T1-weighted image allows to study volumetric and anatomical changes in grey matter (GM). Advanced DWI, a technique enabling the visualization and characterization of the white matter (WM) architecture via the self-diffusion of water molecules. allows to study potential chemotherapy-induced differences in WM microstructure[50]. Rs-fMRI uses the vascular response during rest to indirectly visualize brain activity, and allows us to determine the functional connectivity between the different brain regions and to assess possible differences in functional brain connectivity between subject groups[51]. The duration of the proposed scan protocol is 30-40 minutes, during which the child is supposed to lay supine in the scanner. No contrast agents will be used.

At the age of 9 years, we add a direct assessment of the attentional functions by means of an event-related potentials (ERP) study. Recording takes place using a multichannel (n=31) ERP, while children perform two tasks: Go/NoGo (measuring response inhibition) and posner (measuring cued attention).

Parents are asked at every test moment to fill out a self-constructed questionnaire about the child's general health and development (growth, age at puberty, and any developmental and medical problems).

Adults will be invited for a 5-yearly questionnaire and evaluation of cardiac function, performed at UZ Leuven (blood pressure, ECG and echocardiogram).

| | **General clinical exam** | **Questi on-naire** | **ECG and echoca rdiogr aphy** | **Neur o-logica l exam** | **Screeni ng for hearing loss** | **Neurocogniti ve and neuropsychol ogi-cal testing** | | **MRI** | **ERP** |
|---|---|---|---|---|---|---|---|---|---|
| birth | x | | x (anthra cycline - exposu re) | x | x | | | | |
| 18 months | x | x | | x | | x | BSID-III | | |
| 3 years | x | x | x | x | | x | BSID-III CBCL, BRIEF-P | | |
| 5-6 years | x | x | x | x | | x | WPPSI-III, ANT / CMS subtasks, CBCL, BRIEF | | |
| 3-yearly (9, 12 and 15 years) | x | x | x (at 12 yrs) | x | | x | WISC III, ANT / CMS subtasks, TEA-Ch, AVLT CBCL, BRIEF ERP | x | x (at 9 yrs) |
| 18 years | x | x | x | x | | x | WAIS-IV AVLT ANT | x | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | CBCL, BRIEF ERP | | |
| 5-yearly | | x | x | | | | | | |

**Table 2.** Tests per age group

*General and neurological clinical exam*

At birth: local hospital

From 18 months onwards: at national study centre

*Questionnaire*

Questions on general health, school performance, non-academic interests and social situation

*ECG*

Controls: Dickinson normal values

*Cardiac ultrasound*

Structural and functional evaluation

Controls: age- and gender-matched controls, selected at UZ Leuven and Sick Kids Toronto

*Developmental testing*

Bayley Scales of Infant and Toddler Development, third edition (BSID-III)

*Intelligence tests*

Wechsler Preschool and Primary Scale of Intelligence, third edition (WPPSI-III), Wechsler Intelligence Scale for Children, third edition (WISC-III),

Wechsler Adult Intelligence Scale, fourth edition (WAIS-IV)

*Memory*

Verbal:
- Subtask of Children's Memory Scale (CMS):
  - Numbers
- Auditory Verbal Learning Tests (AVLT)

Non-Verbal:
- Subtasks of Children's Memory Scale (CMS):
  - Picture Locations
  - Dot Locations
  - Faces

*Attention*

Subtasks of Amsterdam Neuropsychological Tasks (ANT):
- Baseline Speed
- Focused Attention Objects 2 keys

Focused Attention 4 Letters

Memory Search Objects 2 keys

Memory Search Letters

GoNoGo-task

Shifting Attentional Set-Visual

Test of Everyday Attention for Children (TEA-Ch)

*Behaviour*

Child Behavior Checklist (CBCL)

*Executive functions*

Behavior Rating Inventory of Executive Function – Preschool Version (BRIEF-P)

Behavior Rating Inventory of Executive Function (BRIEF)

Special attention is paid to hearing, because ototoxicity and hearing loss may be caused by exposure to cytotoxic drugs (mainly cisplatin).

Same testing is performed for the control group. Control children can be included at one time point or longitudinally, depending on the parent's and children's preferences. The aim is to disentangle the effects of prematurity and cytotoxic treatment on general health, neurological development and cardiac function.

Currently, the examinations after birth are part of standard care (except for the screening for hearing loss in some regions) and not extra for the study (umbilical cord blood test and physical examination). No standard long term follow-up exists of children who were exposed to chemotherapy and/or radiotherapy prenatally. Thus, the examinations at the age of 18 months, 3 years, 6 years, 9 years, 12 years, 15 years and 18 years are extra for the study.

If applicable, specific arrangements for participants are mentioned in APPENDIX C.

**Data analysis (Part II.)**

*Neuropsychological test battery*.

For the reports raw scores will be converted to standardized scores using published normative data for the specific age-group (provided by the respective tests). For the project, mean raw scores will be compared to those of the control group for the different age groups using ANOVA (F-statistic, $\alpha$=0.05, effect size: $R^2$). For in depth analysis of differences between our groups, contrast analysis will be performed using Tukey-Kramer (TK) for pair wise contrasts.

We will assess the relation between intelligence quotient (IQ) scores and gestational age at birth by linear (ordinary least squares) regression. The effect size will be estimated with the $\omega 2$

measure of explained variance. Age and sex will be added as covariates, together with a random effect for country.

*ERP.*

For a detailed description, we refer to the set-up and analysis described by researchers of our project: Myatchin and Lagae[52]. Adaptations will be made: we don't have patients with epilepsy and our children perform different subset of tests. Moreover, the analysis will be adapted to the current state of technology. The study and control group will be compared by ANOVA (F, $\alpha$=0.05, $R^2$), contrast analysis (TK), cluster and/or nonparametric statistics. This way, we will answer the question if there is a difference in the time trajectory between our groups.

*Bayley scores.*

Bayley scores are only calculated twice, but they have the same mean and standard deviation as the intelligence scores, so standardized Bayley and intelligence scores are comparable.

*MR imaging.*

State of the art image analysis approaches are used to investigate differences in GM/WM volume, WM microstructure, functional and/or structural connectivity. DWI images will be processed using analyses implemented in exploreDTI [53], MRtrix[54-56] and/or in-house developed software, to investigate WM microstructure and structural connectivity. Seed-based analysis, independent component analysis (ICA) and/or graph theory will be applied to analyze the rs-fMRI data and to assess functional brain connectivity [51, 57, 58]. Voxel-based [59], deformation-based [60] and/or surface-based [61] methods will be applied to investigate volumetric differences in the acquired T1 images.

Voxel-based statistical analysis using the general linear model and (non-)parametric statistics will be used to find significant differences ($p<0.05$) in imaging parameters between the subject groups. The association between the obtained MRI parameters and the performance on neurocognitive tests will be investigated using (voxel-based) correlation analysis. All analyses will be adapted to the current state of technology.

The anonymized neurocognitive and MR data can be reused for similar research.

## 8. SAFETY REPORTING

### 8.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited ethical committee if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

### 8.2 AEs, SAEs and SUSARs

*Definitions in Law of May 7, 2004 concerning experiments on the human person*

**Adverse reaction (AR):** all untoward and unintended responses to an investigational medicinal product or to an experiment and, when an investigational product is concerned, related to any dose administered;

**Adverse event (AE):** any untoward medical occurrence in a patient or subject of the treated group during an experiment, and which does not necessarily have a causal relationship with this treatment

**Unexpected adverse reaction (UAR):** an adverse reaction, the nature or severity of which is not consistent with the information on the experiment, and, when a clinical trial is concerned, with the applicable product information (e.g. investigator's brochure for an unauthorised investigational product or the patient leaflet joined to the summary of product characteristics for an authorised product);

**Serious adverse event (SAE) or serious adverse reaction (SAR):** any untoward medical occurrence or effect that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect, and this, when it is a clinical trial, at any dose;

**Suspected unexpected serious adverse reaction (SUSAR):** is an AR that is serious and unexpected (meaning that nature or severity of the AR is not consistent with the Investigational Medicinal Product reference safety information, which is the Investigator's Brochure) and is

judged by either the investigator or the sponsor as having a reasonable suspected causal relationship with the investigational medicinal product.

### 8.3 Adverse events

All adverse events caused by study interventions reported spontaneously by the subject or observed by the investigator or his staff will be recorded. We do not expect any study related adverse events for this study.

### 8.4 Serious adverse events (SAEs)

The investigator shall report all study intervention-related serious adverse events immediately, after first knowledge, to the sponsor except for those that the protocol or investigator's brochure identifies as not requiring immediate reporting. The immediate report shall be followed by detailed, written reports. The immediate and follow-up reports shall identify subjects by code numbers. For reported study intervention related deaths of a subject, the investigator shall supply the sponsor and the accredited ethics committee with any additional information requested.
The sponsor shall keep detailed records of all study intervention-related adverse events which are reported to him by the investigator or investigators. These records shall be submitted to the minister if the experiment is being conducted in Belgium, if he so requests.

Regarding those study intervention-related serious adverse events the Principal Investigator will take all reasonable measures, in consultation with Sponsor, to protect subjects at risk following the occurrence of such events.
Participation in this study holds an extremely low risk of AEs and study intervention-related SAEs since no invasive interventions are performed, except for venous punctures (for which also no SAE’s are expected). Since this study holds an extremely low risk of a study intervention-related SAE and no invasive interventions are performed, this present study is relieved of reporting non study related SAEs.

### 8.5 Data Safety Monitoring Board (DSMB)

Data safety monitoring board is not needed. Data monitoring will be performed by the appointed monitor.

# 9. ETHICAL CONSIDERATIONS

## 9.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI Ethical Principles for Medical Research Involving Human Subjects most recent version, the principles of ICH GCP (good clinical practice) and in accordance with each country specific legislation relating to clinical research and other guidelines, regulations and Acts.

In order to ensure the same quality and safety standards in patient care for clinical research as commonly applied by Sponsor in its regular activities in accordance with the Joint Commission International standards adopted by Sponsor, Sponsor shall comply with the following obligations: (a) Sponsor will use trained and qualified employees or contractors to manage and coordinate the Study; (b) Sponsor will ensure that multi-centre study reporting is reliable and valid, statistically accurate, ethical, and unbiased. The same requirements are applicable if multi-centre study data and multi-centre Study results are provided to Sponsor; (c) Sponsor will not grant incentives to Subjects or staff that would compromise the integrity of the research; (d) Sponsor is responsible for monitoring and evaluating the quality, safety, and ethics of the Study and will respect policies and processes when performing such monitoring and evaluation activities; (e) Sponsor will protect the privacy and confidentiality of the Subject data in accordance with all applicable laws and regulations.

## 9.2 Recruitment and consent

If required, the Study can and will be conducted only on the basis of prior informed consent by the Subjects, or their legal representatives, to participate in the Study. The Participating Site shall obtain a signed informed consent form (ICF) for all patients prior to their enrolment and participation in the Study in compliance with all applicable laws, regulations and the approval of the (local) Ethics Committee, if required. The Participating Site shall retain such ICFs in accordance with the requirements of all applicable regulatory agencies and laws.

In concrete, eligible patients will be informed about the study by the supervising physician (e.g. gynaecologist, surgeon or medical oncologist) primarily treating the malignancy. The patient will also obtain written information about the study. The patient can think about participation and discuss the study with her family. They will be given at least a week to consider their decision and are free to reconsider their decision at any moment during the trial. In case of participation the informed consent should be signed prior to registration of patient data. This is applicable for all study parts separately, patients can choose in which study part they wish to participate and are not obliged to consent to all parts. Separate written information and informed consent form exists for all study parts.

### 9.3 Objection by minors or incapacitated subjects (if applicable)

No incapacitated subjects will be included in the study.
Minors will be recruited for participation in study part III 'Long term follow up of children and adolescents of mothers with cancer during pregnancy'. Both parents and/or legal guardian need to give consent before participation. The child cannot be forced to undergo this study.

### 9.4 Benefits and risks assessment, group relatedness

As this study will not change treatment nor randomize patients, participation in this study will not affect patients' outcomes. As this is an observational study, no additional risks from the study are expected, except for the risk of hematoma after venous puncture for the pharmacokinetics study (study part I.IV).
For study part 2, minors are recruited. The risks associated with participation can be considered negligible because no invasive tests are performed; and the burden can be considered minimal because follow-up examinations are only performed 7 times in 18 years (18 months, 3y, 6y, 9y, 12y, 15y, 18y). The study is non-therapeutic, and can be regarded a group-related because it could not be conducted without the participation of subjects belonging to the group in question (children of mothers diagnosed with cancer during pregnancy).

### 9.5 Compensation for injury

Sponsor shall be liable, even without fault, for any damages incurred by a Study patient and linked directly or indirectly to the participation to the Study.
Sponsor shall enter into an insurance agreement in order to cover the liability for any damages incurred by a Study patient from a Belgian Participating Site.

If an insurance coverage is required by local laws of non-Belgian Participating Sites, these Participating Sites shall have and maintain in full force and effect during the term of this Agreement (and following termination of the trial to cover any claims arising from the trial) adequate insurance coverage for possible damages linked directly or indirectly to the patients' participation to the Study at Participating Sites..

### 9.6 Incentives

Eligible participants do not receive any special incentives that may encourage participation in this study.

## 10. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

### 10.1 Handling and storage of data and documents

The investigator records clinical data, using a paper or electronic case report form (CRF), all data is collected in an online database that is available through www.cancerinpregnancy.org. Participating physicians have a personal account to log in to the registration page and access the electronic CRF. They will have permanent access to their own series of patients. The participating physician ensures the confidentiality, accuracy, completeness, legibility and timeliness of the data recorded. Data handling and statistical analysis will be done anonymously by the investigator, with the subject identification code list only available to the local investigator (and research nurse if applicable) working in the local centre. The code will be based on the birth date and centre abbreviation (and not patient initials). The Sponsor will be the owner of the data.
Data will be kept for 20 years.

The Participating Investigator and the Participating Site shall treat all information and data relating to the Study disclosed to Participating Site and/or Investigator in this Study as confidential and shall not disclose such information to any third parties or use such information for any purpose other than the performance of the Study. The collection, processing and disclosure of personal data, such as patient health and medical information is subject to compliance with applicable personal data protection and the processing of personal data legislation (including but not limited to the EU Directive 95/46/EC and Belgian law of December 8, 1992 on the Protection of the Privacy in relation to the Processing of Personal Data).

### 10.2 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited ethical committee has been given. All amendments will be notified to the ethical committee that gave a favourable opinion.

A 'substantial amendment' is defined as an amendment to the terms of the ethical committee application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

If required by local law, all substantial amendments will be notified to the ethical committee and to the competent authority. If required by local law, non-substantial amendments will not be notified to the accredited ethical committee and the competent authority, but will be recorded and filed by the sponsor.

### 10.3 Public disclosure and publication policy

This study is registered with ClinicalTrials.gov, number NCT 00330447.

All publications from this study will be done according to the INCIP Publication Policy, publicly available on https://cancerinpregnancy.org/publication-policy

## 11. COMPENSATION

Depending on which part the Study Participating Site is participating, Participating Site shall receive a reasonable compensation for the work performed according to the attached budget & payment terms and conditions (APPENDIX D).

## 12. REFERENCES


1. Pavlidis, N.A., *Coexistence of pregnancy and malignancy.* Oncologist, 2002. **7**(4): p. 279-87.
2. Stensheim, H., et al., *Cause-specific survival for women diagnosed with cancer during pregnancy or lactation: a registry-based cohort study.* J Clin Oncol, 2009. **27**(1): p. 45-51.
3. Han, S.N., et al., *Cancer in pregnancy: a survey of current clinical practice.* Eur J Obstet Gynecol Reprod Biol, 2013. **167**(1): p. 18-23.
4. Avilés, A. and N. Neri, *Hematological malignancies and pregnancy: a final report of 84 children who received chemotherapy in utero.* Clin Lymphoma, 2001. **2**(3): p. 173-7.
5. Van Calsteren, K., et al., *Cancer during pregnancy: an analysis of 215 patients emphasizing the obstetrical and the neonatal outcomes.* J Clin Oncol, 2010. **28**(4): p. 683-9.
6. Cardonick, E., A. Usmani, and S. Ghaffar, *Perinatal outcomes of a pregnancy complicated by cancer, including neonatal follow-up after in utero exposure to chemotherapy: results of an international registry.* Am J Clin Oncol, 2010. **33**(3): p. 221-8.
7. Lataifeh, I.M., et al., *Management of cancer during pregnancy: obstetric and neonatal outcomes.* Int J Gynecol Cancer, 2011. **21**(6): p. 1159-64.
8. Amant, F., et al., *Long-term cognitive and cardiac outcomes after prenatal exposure to chemotherapy in children aged 18 months or older: an observational study.* The Lancet Oncology, 2012. **13**(3): p. 256-264.
9. Patel, B., S.K. Das, and M. Patri, *Neonatal Benzo[a]pyrene Exposure Induces Oxidative Stress and DNA Damage Causing Neurobehavioural Changes during the Early Adolescence Period in Rats.* Dev Neurosci, 2016. **38**(2): p. 150-62.
10. Rodriguez, A.O., et al., *Evidence of poorer survival in pregnancy-associated breast cancer.* Obstet Gynecol, 2008. **112**(1): p. 71-8.
11. Ibrahim, E.M., et al., *Pregnancy-associated breast cancer: a case-control study in a young population with a high-fertility rate.* Med Oncol, 2000. **17**(4): p. 293-300.
12. Beadle, B.M., et al., *The impact of pregnancy on breast cancer outcomes in women<or=35 years.* Cancer, 2009. **115**(6): p. 1174-84.
13. Loibl, S., et al., *Treatment of breast cancer during pregnancy: an observational study.* Lancet Oncol, 2012. **13**(9): p. 887-96.
14. de Haan J., Verheecke M. et al. Cancer during pregnancy: 20 years of oncological management and obstetrical outcome of 1170 patients - a cohort study. Accepted for publication in Lancet Oncology, 2017.
15. Cunningham, F.G., et al., *Fetal Growth Disorders,* in *Williams Obstetrics Study Guide*, F.G. Cunningham, Editor. 2010, Mc Graw-Hill Companies. p. 842-858.
16. Sankaran, S. and P.M. Kyle, *Aetiology and pathogenesis of IUGR.* Best Pract Res Clin Obstet Gynaecol, 2009. **23**(6): p. 765-77.
17. Wang, X., N. Athayde, and B. Trudinger, *A proinflammatory cytokine response is present in the fetal placental vasculature in placental insufficiency.* Am J Obstet Gynecol, 2003. **189**(5): p. 1445-51.
18. Wang, X., N. Athayde, and B. Trudinger, *Fetal plasma stimulates endothelial cell production of cytokines and the family of suppressor of cytokine signaling in umbilical placental vascular disease.* Am J Obstet Gynecol, 2003. **188**(2): p. 510-6.
19. Vandenbroucke, T., et al., *Psychological distress and cognitive coping in pregnant women diagnosed with cancer and their partners.* Psychooncology, 2016.
20. Ives, A., T. Musiello, and C. Saunders, *The experience of pregnancy and early motherhood in women diagnosed with gestational breast cancer.* Psychooncology, 2012. **21**(7): p. 754-61.
21. Henry, M., et al., *The psychological impact of a cancer diagnosed during pregnancy: determinants of long-term distress.* Psychooncology, 2012. **21**(4): p. 444-50.
22. DiPietro, J.A., et al., *Maternal psychological distress during pregnancy in relation to child development at age two.* Child Dev, 2006. **77**(3): p. 573-87.
23. Hollins, K., *Consequences of antenatal mental health problems for child health and development.* Curr Opin Obstet Gynecol, 2007. **19**(6): p. 568-72.
24. Van den Bergh, B.R., et al., *Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood.* Neuropsychopharmacology, 2008. **33**(3): p. 536-45.
25. Loomans, E.M., et al., *High levels of antenatal maternal anxiety are associated with altered cognitive control in five-year-old children.* Dev Psychobiol, 2012. **54**(4): p. 441-50.
26. Stensheim H, M.B., van Dijk T, Fossa SD, *Cause-specific survival for women diagnosed with cancer during pregnancy or lactation: a registry-based cohort study.* J Clin Oncol, 2009. **27**(1): p. 45-51.

27. Callihan, E.B., et al, *Postpartum diagnosis demonstrates a high risk for metastasis and merits an expanded definition of pregnancy-associated breast cancer.* Breast Cancer Res Treat, 2013. **138**(2): p. 549-59.
28. Ma, X.e.a., *Gene expression profiles of human breast cancer progression.* Proc Natl Acad Sci U S A, 2003. **100**(10): p. 5974-9.
29. Perou, C.M., et al, *Molecular portraits of human breast tumours.* Nature, 2000. **406**(6797): p. 747-52.
30. Peshkin BN, A.M.a.I.C., *BRCA1/2 mutations and triple negative breast cancers.* Breast Dis, 2010. **32**(1-2): p. 25-33.
31. Antoniou AC, e.a., *Parity and breast cancer risk among BRCA1 and BRCA2 mutation carrriers.* Breast Cancer Res, 2006. **8**(6): p. R72.
32. Cullinane CA, e.a., *Effect of pregnancy as a risk factor for breast cancer in BRCA1/BRCA2 mutation carriers.* 117, 2005. **6**(988-91).
33. Pruneri G, e.a., *Clinical validity of tumor-infiltrating lymphocytes analysis in patients with triple-negative breast cancer.* Ann Oncol, 2016. **27**(2): p. 249-56.
34. Azim, H.A., Jr., et al., *Tumour infiltrating lymphocytes (TILs) in breast cancer during pregnancy.* Breast, 2015. **24**(3): p. 290-3.
35. Ashburner, M., et al, *Gene ontology: tool for the unification of biology. The Gene Ontology Consortium.* Nat Genet, 2000. **25**(1): p. 25-9.
36. Newman, A.M., et al, *Robust enumeration of cell subsets from tissue expression profiles.* Nat Methods, 2015. **12**(5): p. 453-7.
37. Van Calsteren, K., et al., *Pharmacokinetics of chemotherapeutic agents in pregnancy: a preclinical and clinical study.* Acta Obstet Gynecol Scand, 2010. **89**(10): p. 1338-45.
38. van Hasselt JG, v.C.K., Heyns L, Han S, Mhallem Gziri M, Schellens JH, Beijnen JH, Huitema AD, Amant F, *Optimizing anticancer drug treatment in pregnant cancer patients: pharmacokinetic analysis of gestation-induced changes for doxorubicin, epirubicin, docetaxel and paclitaxel.* Ann Oncol, 2014. **25**(10): p. 2059-65.
39. Van Calsteren, K., et al., *Transplacental transfer of anthracyclines, vinblastine, and 4-hydroxy-cyclophosphamide in a baboon model.* Gynecol Oncol, 2010. **119**(3): p. 594-600.
40. Van Calsteren, K., et al., *Substantial variation in transplacental transfer of chemotherapeutic agents in a mouse model.* Reprod Sci, 2011. **18**(1): p. 57-63.
41. Calsteren, K.V., et al., *Transplacental transfer of paclitaxel, docetaxel, carboplatin, and trastuzumab in a baboon model.* Int J Gynecol Cancer, 2010. **20**(9): p. 1456-64.
42. Zemlickis, D., et al., *Maternal and fetal outcome after breast cancer in pregnancy.* Am J Obstet Gynecol, 1992. **166**(3): p. 781-7.
43. Cardonick, E. and A. Iacobucci, *Use of chemotherapy during human pregnancy.* Lancet Oncol, 2004. **5**(5): p. 283-91.
44. Cardonick, E., et al., *Breast cancer during pregnancy: maternal and fetal outcomes.* Cancer J, 2010. **16**(1): p. 76-82.
45. Aviles, A., et al., *Growth and development of children of mothers treated with chemotherapy during pregnancy: current status of 43 children.* Am J Hematol, 1991. **36**(4): p. 243-8.
46. Hahn, K.M., et al., *Treatment of pregnant breast cancer patients and outcomes of children exposed to chemotherapy in utero.* Cancer, 2006. **107**(6): p. 1219-26.
47. Chelghoum, Y., et al., *Acute leukemia during pregnancy: a report on 37 patients and a review of the literature.* Cancer, 2005. **104**(1): p. 110-7.
48. Luis, S.A., et al., *Pregnancy and radiotherapy: management options for minimising risk, case series and comprehensive literature review.* J Med Imaging Radiat Oncol, 2009. **53**(6): p. 559-68.
49. Amant, F., et al., *Pediatric Outcome after Maternal Cancer Diagnosed during Pregnancy.* N Engl J Med, 2015. **373**(19): p. 1824-34.
50. Le Bihan, D., et al., *Diffusion tensor imaging: concepts and applications.* J Magn Reson Imaging, 2001. **13**(4): p. 534-46.
51. van den Heuvel, M.P. and H.E. Hulshoff Pol, *Exploring the brain network: a review on resting-state fMRI functional connectivity.* Eur Neuropsychopharmacol, 2010. **20**(8): p. 519-34.
52. Myatchin, I. and L. Lagae, *Developmental changes in visuo-spatial working memory in normally developing children: event-related potentials study.* Brain Dev, 2013. **35**(9): p. 853-64.
53. Leemans, A., et al. *Explore DTI: a graphical toolbox for processing, analyzing, and visualizing diffusion MR data.* in *the 17th Annual Meeting of International Society Magnetic Resonance Medicine*. 2009. Hawaii, USA.
54. Jeurissen, B., et al., *Multi-tissue constrained spherical deconvolution for improved analysis of multi-shell diffusion MRI data.* Neuroimage, 2014. **103**: p. 411-26.

55. Raffelt, D.A., et al., *Investigating white matter fibre density and morphology using fixel-based analysis.* Neuroimage, 2017. **144**(Pt A): p. 58-73.
56. Christiaens, D., et al., *Global tractography of multi-shell diffusion-weighted imaging data using a multi-tissue model.* Neuroimage, 2015. **123**: p. 89-101.
57. Margulies, D.S., et al., *Resting developments: a review of fMRI post-processing methodologies for spontaneous brain activity.* MAGMA, 2010. **23**(5-6): p. 289-307.
58. Barkhof, F., S. Haller, and S.A. Rombouts, *Resting-state functional MR imaging: a new window to the brain.* Radiology, 2014. **272**(1): p. 29-49.
59. Mechelli, A., et al., *Voxel-based morphometry of the human brain: methods and applications.* Current Medical Imaging Reviews, 2005. **1**: p. 00-00.
60. Ashburner, J., et al., *Identifying global anatomical differences: deformation-based morphometry.* Hum Brain Mapp, 1998. **6**(5-6): p. 348-57.
61. Dahnke, R., R.A. Yotter, and C. Gaser, *Cortical thickness and central surface estimation.* Neuroimage, 2013. **65**: p. 336-48.

## 13. APPENDIX A

**Part I.IV. Sample scheme and reduced sample scheme according to administered chemotherapy**

# Pharmacokinetics of chemotherapeutics during pregnancy

**Name patient:** …… ……
**Date:** ……
**Cycle:** ……..... (e.g. FEC I)

Gestational age at the moment of administration (weeks) ……
Weight (kilogram) ……
Length ( cm) ……

Dose product 1:…… mg /m² = ..... mg
Dose product 2: …… mg /m² = ..... mg
Dose product 3: …… mg /m² = ..... mg

Time start product 1: ……
Time stop product 1: ……

Time start product 2: ……
Time stop product 2: ……

Time start product 3: ……
Time stop product 3: ……

**Processing of the blood samplings**

**TBC**: total blood count = determine Hb, Hct, thrombocyte count, leukocyte count, neutrophil count (in the lab of your hospital)

**Storage of plasma**: a volume of 4-6 ml blood is collected in an **EDTA-tube**, taken from a peripheral catheter (lock system flushed with saline) (not the catheter by which the chemo is administered or in case of peripheral infusion, not sampled from the same arm as the infusion is given).

- Label the blood tube with: patient's initials, date and hour of sampling
- If it is not possible to spin the samples immediately, they should be stored at 4-8°C (! maximum 2 hours).
- Spin the blood within 2 hours after sampling at 3000 rpm and 4°C for 10 minutes.
- Transfer the plasma to cryotubes (divide the total plasma amount in samples of of 1 – 1.5 ml each, label the cryotubes with patient's initials, date and hour of sampling). Store the cryotubes in the freezer, preferably at -80°C (or -20°C ).

- In case of analysis of **carboplatin**, the following technique should be performed:
    - Spin the blood within 2 hours after sampling at 2000 rmp at 18°C (room temperature)
    - Transfer 0.5ml of plasma to a cryotube.
    - Create an ultrafiltrate: Add 1-1.5ml of plasma to a 'Milipore filter'. Spin at 2000 rpm at 18°C (room temperature). The ultrafiltrate has to be stored in an Eppendorf benchtop and freezed at -80°C (or -20°C on transport), together with the cryotubes.
- In case of analysis of **paclitaxel and carboplatin** simultaneously, the following technique should be performed:
    - Spin the blood within 2 hours after sampling at 2000 rmp at 18°C (room temperature)
    - Transfer two samples of 0.5ml of plasma to a cryotube.
    - Create an ultrafiltrate: Add 1-1.5ml of plasma to a 'Milipore filter'. Spin at 2000 rpm at 18°C (room temperature). The ultrafiltrate has to be stored in an Eppendorf benchtop and freezed at -80°C (or -20°C on transport), together with the cryotubes.

**Schedule blood samples:**

The sampling time table will depend on the infusion regimen and the drugs we aim to use for pharmacokinetic analysis. Therefore, if you apply a new or different drug regimen then given in the examples below, please contact us before drug administration then we prepare an adapted sampling regimen.

**1. Paclitaxel 175mg/m², carboplatin AUC6**

Infusion regimen:

.Rinse 30 min with 1000 ml Glc5%

.Paclitaxel 175 mg/m² in 500 ml NaCl 0.9% over 3 hr

.Rinse 10 min with NaCl 0.9%

.Carboplatin AUC6 in 500 ml Glc 5% over 1 hr

.Rinse 10 min with NaCl 0.9%

(use in total 1000 ml NaCl 0.9% for rinsing)

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 0 = start pacliT | TBC + storage of plasma | | |
| | + 1 hr | storage of plasma | | |
| | + 2 hr | storage of plasma | | |
| | + 3 hr = stop pacliT | storage of plasma | | |
| | + 3.40 hr | storage of plasma | | |
| | + 4.10 hr = stop Carbo | storage of plasma | | |
| | + 5 hr | storage of plasma | | |
| | + 6 hr | storage of plasma | | |
| | + 8 hr | storage of plasma | | |
| | + 10 hr | storage of plasma | | |
| | + 12 hr | storage of plasma | | |
| Day 1 | + 24 hr | storage of plasma | | |
| Day 2 | + 48 hr | storage of plasma | | |
| Day 4 | + 4 d | TBC + storage of plasma | | |
| Day 7 | + 7 d | TBC + storage of plasma | | |
| Day 10 | + 10 d | TBC + storage of plasma | | |
| Day 12 | + 12 d | TBC + storage of plasma | | |
| Day 14 | + 14 d | TBC + storage of plasma | | |
| Day 16 | + 16 d | TBC + storage of plasma | | |
| Day 21 | + 21 d | TBC + storage of plasma | | |

**Reduced sampling scheme**

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 6 min (+0=start pacli) | TBC + storage of plasma | | |
| | + 3 hr 15 min | storage of plasma | | |
| | + 4 hr 30 min | storage of plasma | | |
| | *additional sample between + 5 and 12 hr | storage of plasma | | |

| | | | | |
|---|---|---|---|---|
| Day 1 | + 24 hr | storage of plasma | | |

**2. Paclitaxel ? mg/m², carboplatin AUC2**

Infusion regimen:

.Rinse 30 min with 500 ml Glc5%

.Paclitaxel ? mg/m² in 250 ml Glc 5% over 1 hr

.Rinse 10 min with Glc 5%

.Carboplatin AUC2 in 500 ml Glc 5% over 1 hr

.Rinse 10 min with Glc 5%

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 0 = start pacliT | TBC + storage of plasma | | |
| | + 1 hr = stop pacliT | storage of plasma | | |
| | + 2.10 hr = stop Carbo | storage of plasma | | |
| | + 4 hr | storage of plasma | | |
| | + 8 hr | storage of plasma | | |
| | + 12 hr | storage of plasma | | |
| Day 1 | + 24 hr | storage of plasma | | |
| Day 2 | + 48 hr | storage of plasma | | |
| Day 4 | + 4 d | TBC + storage of plasma | | |
| Day 7 | + 7 d | TBC + storage of plasma | | |
| Day 10 | + 10 d | TBC + storage of plasma | | |
| Day 12 | + 12 d | TBC + storage of plasma | | |
| Day 14 | + 14 d | TBC + storage of plasma | | |
| Day 16 | + 16 d | TBC + storage of plasma | | |
| Day 21 | + 21 d | TBC + storage of plasma | | |

**Reduced sampling scheme**

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 6 min (+0=start pacli) | TBC + storage of plasma | | |
| | + 1 hr | storage of plasma | | |
| | + 2.10 hr = stop Carbo | | | |
| | + 4 hr 30 min | storage of plasma | | |
| | *additional sample between + 5 and 12 hr | storage of plasma | | |
| Day 1 | + 24 hr | storage of plasma | | |

**3. Taxotere 100mg/m²**

Infusion regimen

. Rinse with Glc5%

. Taxotere 100 mg/m² in 250 ml Glc5% over 1 hour

. Rinse 10 min with Glc5%

(use in total 500 ml Glc 5% for rinsing)

| Day | Timing from start of infusion | Sample to collect | Time planned for sampling | Time actual sampling |
|---|---|---|---|---|
| Day 0 | + 0 = start | TBC + storage of plasma | | |
| | +1 hr = end | storage of plasma | | |
| | + 1.20 hr | storage of plasma | | |
| | + 1.40 hr | storage of plasma | | |
| | + 2 hr | storage of plasma | | |
| | + 3 hr | storage of plasma | | |
| | + 5 hr | storage of plasma | | |
| | + 9 hr | storage of plasma | | |
| | + 13 hr | storage of plasma | | |
| Day 1 | + 25 hr | storage of plasma | | |
| Day 2 | + 49 hr | storage of plasma | | |
| Day 10 | + 10 days | TBC | | |
| Day 12 | + 12 days | TBC | | |
| Day 14 | + 14 days | TBC | | |
| Day 16 | + 16 days | TBC | | |
| Day 21 | + 21 days | TBC | | |

**Reduced sampling scheme**

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 6 min (+0=start doce) | TBC + storage of plasma | | |
| | + 1 hr 15 min | storage of plasma | | |
| | + 1 hr 45 min | storage of plasma | | |
| | + 3 hr | storage of plasma | | |
| Day 1 | + 23 hr | storage of plasma | | |

### 4. Cisplatin 60 mg/m²

Dose 60 mg/m² in 500ml NaCl 0,9% over 3 hours

| Day | Timing from start of infusion | Sample to collect | Time planned for sampling | Time actual sampling |
|---|---|---|---|---|
| Day 0 | + 0 = start | TBC + storage of plasma | | |
| | +1h | storage of plasma | | |
| | + 2 h | storage of plasma | | |
| | + 3 h = stop | storage of plasma | | |
| | + 3.30 h | storage of plasma | | |
| | + 4 h | storage of plasma | | |
| | + 5 h | storage of plasma | | |
| | + 7 h | storage of plasma | | |
| | + 10 h | storage of plasma | | |
| | + 14 h | storage of plasma | | |
| Day 1 | + 26 h | storage of plasma | | |
| Day 2 | + 50 h | storage of plasma | | |
| Day 4 | + 4 days | storage of plasma | | |
| Day 7 | + 7 days | storage of plasma | | |
| Day 10 | + 10 days | TBC + storage of plasma | | |
| Day 12 | + 12 days | TBC + storage of plasma | | |
| Day 14 | + 14 days | TBC + storage of plasma | | |
| Day 16 | + 16 days | TBC + storage of plasma | | |
| Day 21 | + 21 days<br>= day 0 next cycle | TBC + storage of plasma | | |

**Reduced sampling scheme**

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 6 min (+0=start cispl) | TBC + storage of plasma | | |
| | + 1 uur 30 min | storage of plasma | | |
| | + 3 uur 25 min | storage of plasma | | |
| | + 5 uur 50 min | storage of plasma | | |
| Day 1 | + 24 uur | storage of plasma | | |

**5. Paclitaxel 330mg + Cisplatin 140 mg**

Premedication: Ranitidine 300mg po, promethasin 50mg im, palonosetron HCl 1A iv

Infusion regimen:

.Rinse 30 min with 500 ml NaCl 0.9%

.Paclitaxel 330mg in 500 ml NaCl 0.9% over 3 hr

.Rinse 1 hr with 500 ml NaCl 0.9%

.MgSO4 10%, 10ml + 500 ml NaCl 0.9% over 1 hr

.Cisplatin 100mg in 1000 ml Ringer over 2 hr

.Cisplatin 40mg in 500ml NaCl 0.9% over 1 hr

.Rinse 1 hr with 500 ml NaCl 0.9%

.Rinse 1 hr with 250 ml Mannitol 20%

.Rinse 2 hr with 500 ml Glc 5% + 20ml 7.5% KCl + 10ml MgSO4 10%

| Day | Timing from start of infusion | Sample to collect | Time planned for sampling | Time actual sampling |
|---|---|---|---|---|
| Day 0 | + 0 = start pacli | TBC + storage of plasma | | |
| | + 1 hr | storage of plasma | | |
| | + 2 hr | storage of plasma | | |
| | + 3 hr = stop pacli | storage of plasma | | |
| | + 3.30 hr | storage of plasma | | |
| | + 4 hr | storage of plasma | | |
| | + 5 hr = start cisplt | storage of plasma | | |
| | + 6.30 hr | storage of plasma | | |
| | + 8 hr = stop cisplt | storage of plasma | | |
| | + 9 hr | storage of plasma | | |
| | + 11 hr | storage of plasma | | |
| | + 15 hr | storage of plasma | | |
| | + 20 hr | storage of plasma | | |
| Day 1 | + 27 hr | storage of plasma | | |
| Day 2 | + 51 hr | storage of plasma | | |
| Day 4 | + 4 days | storage of plasma | | |
| Day 7 | + 7 days | storage of plasma | | |
| Day 10 | + 10 days | TBC + storage of plasma | | |
| Day 12 | + 12 days | TBC + storage of plasma | | |
| Day 14 | + 14 days | TBC + storage of plasma | | |
| Day 16 | + 16 days | TBC + storage of plasma | | |
| Day 21 | + 21 days | TBC + storage of plasma | | |

**Reduced sampling scheme**

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 6 min (+0=start pacli) | TBC + storage of plasma | | |
| | + 3 hr 15 min | storage of plasma | | |
| | + 3 hr 50 min | storage of plasma | | |
| | *additional sample between + 5 and 12 h | storage of plasma | | |
| Day 1 | + 24 hr | storage of plasma | | |

**6. Carbo mono**

Infusion regimen:

.Carboplatin AUC6 in 500 ml Glc 5% over 1 hr

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 0 = start Carbo | TBC + storage of plasma | | |
| | + 1 hr = stop Carbo | storage of plasma | | |
| | + 2 hr | storage of plasma | | |
| | + 3 hr | storage of plasma | | |
| | + 5 hr | storage of plasma | | |
| | + 8 hr | storage of plasma | | |
| | + 12 hr | storage of plasma | | |
| Day 1 | + 24 hr | storage of plasma | | |
| Day 2 | + 48 hr | storage of plasma | | |
| Day 4 | + 4 d | TBC + storage of plasma | | |
| Day 7 | + 7 d | TBC + storage of plasma | | |
| Day 10 | + 10 d | TBC + storage of plasma | | |
| Day 12 | + 12 d | TBC + storage of plasma | | |
| Day 14 | + 14 d | TBC + storage of plasma | | |
| Day 16 | + 16 d | TBC + storage of plasma | | |
| Day 21 | + 21 d | TBC + storage of plasma | | |

**Reduced sampling scheme**

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 6 min (+0 = start carbo) | TBC + storage of plasma | | |
| | + 1 hr | storage of plasma | | |
| | + 3 hr 30min | storage of plasma | | |
| | *additional sample between + 5 and 12 hr | storage of plasma | | |
| Day 1 | + 24 hr | storage of plasma | | |

7. **Paclitaxel 80 mg/m²**

Infusion regimen

. Rinse with Glc5%

. Paclitaxel 80 mg/m² in 250 ml Glc5% over 1 hour

. Rinse 10 min with Glc5%

(use in total 500 ml Glc 5% for rinsing)

| Day | Timing from start of infusion | Sample to collect | Time planned for sampling | Time actual sampling |
|---|---|---|---|---|
| Day 0 | + 0 = start | TBC + storage of plasma | | |
| | +1 hr = end | storage of plasma | | |
| | + 1.20 hr | storage of plasma | | |
| | + 1.40 hr | storage of plasma | | |
| | + 2 hr | storage of plasma | | |
| | + 3 hr | storage of plasma | | |
| | + 5 hr | storage of plasma | | |
| | + 9 hr | storage of plasma | | |
| | + 13 hr | storage of plasma | | |
| Day 1 | + 25 hr | storage of plasma | | |
| Day 2 | + 49 hr | storage of plasma | | |
| Day 10 | + 10 days | TBC | | |
| Day 12 | + 12 days | TBC | | |
| Day 14 | + 14 days | TBC | | |
| Day 16 | + 16 days | TBC | | |
| Day 21 | + 21 days | TBC | | |

**Reduced sampling scheme**

| Day | Time | Sample to collect | Planned time of sampling | Real time of sampling |
|---|---|---|---|---|
| Day 0 | + 6 min (+0=start pacli) | TBC + storage of plasma | | |
| | + 1 hr 15 min | storage of plasma | | |
| | + 1 hr 45 min | storage of plasma | | |
| | + 3 hr | storage of plasma | | |
| Day 1 | + 23 hr | storage of plasma | | |

Please send the filled form by email to liesbeth.leemans@uzleuven.be

When all samples of one patient are collected, please contact Liesbeth Leemans to arrange the transport of the samples.

**14. APPENDIX B**

**Part I.IV. Sampling time optimization of multiple anticancer drug combination treatments during pregnancy**

# Sampling time optimization of multiple anticancer drug combination treatments during pregnancy

*Coen van Hasselt December 3, 2012*

## 1 Objectives

The objective of this analysis was to develop a framework allowing simultaneous optimization of sampling times for multiple anti-cancer drugs (as present in one combination treatment), also taking into account the potential effect of pregnancy on PK parameters.

## 2 Methods

**Abbreviations** cyclo=Cyclophophamide; doxo=Doxorubicin; vinc=Vincristin; epi=Epirubicin; pac=Paclitaxel; eto=Etoposide; ifos=Ifosfamide; doc=Docetaxel; fu5=5-Fluorouracil; bleo=Bleomycin; vinb=Vinblastin; dac=Dacarbazine; cis=Cisplatin; mpred=Methylprednisone

**Software** Matlab R2011b with PopED 2.13.

**Implementation** A general PopED model specification file was written which allowed simulta- neous sampling time optimization for a maximum of 3 drugs given in one combination treatment. Different starting times of drug infusions can be specified. Subsequently an R script was used to generate different PopED specification files for scenarios with a varying number of possible samples, different gestational ages, and different drug combinations.

**Optimization specifications** A D-optimal design criterion was used, leading to maximization of expected parameter precision. All structural model parameters and between subject variabil- ity were included in the Fisher Information Matrix, whereas residual variability was excluded. Stochastic gradient search (50 iterations) in combination with a line search optimization (50 segments).

**Design specifications**

- The number of subjects for each optimization was set at 20 patients.
- All (optimized) sampling times are relative to administration of the first drug.

- Between 10-23h postdose, no sampling times were allowed (i.e. allowing sleeping)

**Model specification** Non-pregnant literature values for all drugs were retrieved and used as prior parameter estimates (Table 1).

**Treatment regimens** Treatment regimens and associated dose and infusion duration are given in (Table 2). The following drugs were excluded from the optimization (i.e. removed from the schedule): fu5, bleo, vinb, cyclo, vinc, ifos, eto, dac, cis, mpred.
With one exception (PacCarbo), at this moment, it is only planned to quantify only one drug simultaneously, even if multiple drugs have been administered. Nonetheless, this framework provides full support for combined optimization, taking into account multiple drugs simultaneously, if required.

Table 1: Parameter estimates of non-pregnant patients used for the prior model. NC=Number of compartments. NC=999 indicated no parameter estimates were available.

| | V1 | CL | V2 | Q2 | V3 | Q3 | FR | NC | treat |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 34.60 | 4.23 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | cyclo |
| 2 | 12.30 | 47.60 | 421.00 | 60.30 | 0.00 | 0.00 | 0.05 | 2.00 | doxo |
| 3 | 27.16 | 21.79 | 252.00 | 88.40 | 0.00 | 0.00 | 0.10 | 2.00 | vinc |
| 4 | 10.30 | 29.00 | 34.90 | 29.80 | 754.00 | 61.50 | 0.10 | 3.00 | epi |
| 5 | 3.64 | 6.71 | 881.00 | 44.70 | 0.00 | 0.00 | 0.10 | 2.00 | pac |
| 6 | 6.40 | 3.34 | 0.00 | 0.00 | 0.00 | 0.00 | 0.35 | 1.00 | eto |
| 7 | 26.00 | 5.60 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | ifos |
| 8 | 7.91 | 28.42 | 61.00 | 9.17 | 0.00 | 0.00 | 0.00 | 2.00 | doc |
| 9 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 999.00 | fu5 |
| 10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 999.00 | bleo |
| 11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 999.00 | vinb |
| 12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 999.00 | dac |
| 13 | 21.10 | 0.68 | 42.60 | 22.20 | 0.00 | 0.00 | 1.00 | 2.00 | cis |
| 14 | 96.50 | 1.83 | 18.20 | 436.80 | 0.00 | 0.00 | 1.00 | 2.00 | carbo |
| 15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 999.00 | mpred |

Table 2: Evaluated treatment regimens. Dur=Infusion duration (hours). tstart=Time of starting infusion (hours). Doses are given in mg. Relative doses are mg/m2. A typical BSA of 1.73 was used for computing the absolute dose.

| | treat | drug | dur | abs dose | tstart | rel dose |
|---|---|---|---|---|---|---|
| 1 | FEC1 | epi | 0.50 | 173.00 | 0.00 | 100.00 |
| 2 | FEC2 | fu5 | 0.17 | 865.00 | 0.67 | 500.00 |
| 3 | FEC3 | cyclo | 0.50 | 865.00 | 1.00 | 500.00 |
| 4 | FAC1 | fu5 | 0.17 | 865.00 | 0.00 | 500.00 |
| 5 | FAC2 | doxo | 0.50 | 86.50 | 0.33 | 50.00 |
| 6 | FAC3 | cyclo | 0.50 | 865.00 | 1.00 | 500.00 |
| 7 | PacCarbo1 | pac | 3.00 | 302.75 | 0.00 | 175.00 |
| 8 | PacCarbo2 | carbo | 1.00 | 1000.00 | 3.16 | 578.03 |
| 9 | ABVD1 | doxo | 0.50 | 43.25 | 0.00 | 25.00 |
| 10 | ABVD2 | bleo | 0.25 | 17.30 | 0.67 | 10.00 |
| 11 | ABVD3 | vinb | 0.25 | 10.38 | 1.08 | 6.00 |
| 12 | Taxotere | doc | 1.00 | 173.00 | 0.00 | 100.00 |
| 13 | AC1 | doxo | 0.33 | 103.80 | 0.00 | 60.00 |
| 14 | AC2 | cyclo | 0.50 | 1038.00 | 0.50 | 600.00 |
| 15 | Cis | cis | 3.00 | 103.80 | 0.00 | 60.00 |
| 16 | PacCis1 | pac | 3.00 | 330.00 | 0.00 | 330.00 |
| 17 | PacCis2 | cis | 2.00 | 140.00 | 5.00 | 140.00 |
| 18 | CHOP1 | vinc | 0.17 | 2.42 | 0.00 | 1.40 |
| 19 | CHOP2 | doxo | 0.50 | 86.50 | 0.00 | 50.00 |
| 20 | CHOP3 | cyclo | 0.50 | 1297.50 | 0.00 | 750.00 |

# 3 Results

**Sampling times** A minimum number of 4 - 5 samples is necessery to obtain adequate parameter precision accross all evaluated treatments (Figure 1). Final recommended sampling times are provided in (Table 3).

**Effect of gestational changes in PK on sampling times** The effect of gestation on change in optimal sampling times was marginal and can be ignored (Figure 2).
treat drug nsamples gestage times 1 FEC epi 3 1 0.18, 0.88, 23.02 2 FEC epi 3 20 0.83, 3.86, 24 3 FEC epi 3 30 0.83, 4.26, 24 4 FEC epi 3 40 0.83, 4.28, 24 5 FEC epi 4 1 0.1, 0.84, 3.25, 23.92 6 FEC epi 4 20 0.1, 0.83, 3.48, 24

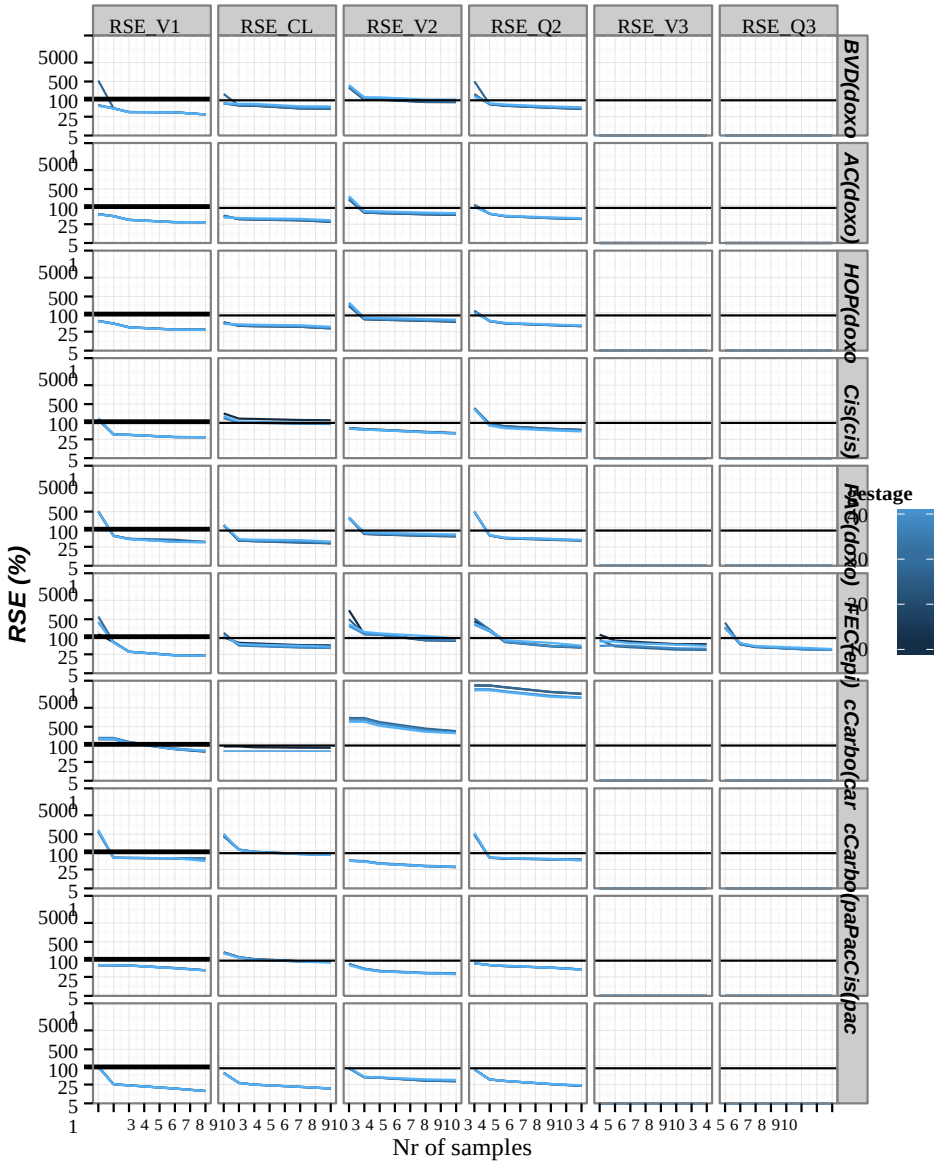



Figure 1: Expected parameter precision of prior population PK parameters. Stratiefied by treatment regimen (drug included in optimization). Grouped by gestational age (weeks).

Table 3: Sampling times for gestage=20 and nsample=4,5

| | treat | drug | nsamples | gestage | times |
|---|---|---|---|---|---|
| 6 | FEC | epi | 4.00 | 20.00 | 0.1, 0.83, 3.48, 24 |
| 10 | FEC | epi | 5.00 | 20.00 | 0.1, 0.65, 1.08, 3.52, 24 |
| 26 | FAC | doxo | 4.00 | 20.00 | 0.33, 1.11, 1.85, 23.02 |
| 30 | FAC | doxo | 5.00 | 20.00 | 0.33, 1.11, 1.88, 23.02 |
| 51 | PacCarbo | pac | 4.00 | 20.00 | 0.1, 3.25, 4.45, 24 |
| 52 | PacCarbo | carbo | 4.00 | 20.00 | 0.1, 3.25, 4.45, 24 |
| 59 | PacCarbo | pac | 5.00 | 20.00 | 0.1, 3.25, 4.44, 24 |
| 60 | PacCarbo | carbo | 5.00 | 20.00 | 0.1, 3.25, 4.44, 24 |
| 86 | ABVD | doxo | 4.00 | 20.00 | 0.1, 0.79, 1.47, 23 |
| 90 | ABVD | doxo | 5.00 | 20.00 | 0.1, 0.79, 1.48, 23 |
| 106 | Taxotere | doc | 4.00 | 20.00 | 0.1, 1.62, 3.01, 23 |
| 110 | Taxotere | doc | 5.00 | 20.00 | 0.1, 1.23, 1.74, 3.01, 23 |
| 126 | AC | doxo | 4.00 | 20.00 | 0.1, 0.68, 1.46, 23.9 |
| 130 | AC | doxo | 5.00 | 20.00 | 0.1, 0.68, 1.38, 23.84 |
| 146 | Cis | cis | 4.00 | 20.00 | 0.1, 1.75, 5.58, 24 |
| 150 | Cis | cis | 5.00 | 20.00 | 0.1, 1.52, 3.41, 5.88, 24 |
| 166 | PacCis | pac | 4.00 | 20.00 | 0.13, 3.25, 3.85, 24 |
| 170 | PacCis | pac | 5.00 | 20.00 | 0.1, 3.25, 3.86, 24 |
| 186 | CHOP | doxo | 4.00 | 20.00 | 0.1, 0.81, 1.5, 23 |
| 190 | CHOP | doxo | 5.00 | 20.00 | 0.1, 0.81, 1.49, 23 |

Figure 2: Optimal sampling times. Stratified by treatment regimen (drug included in optimization), and maximum number of samples. Between 10-23 hours postdose, no samples could be taken (indicated by dashed lines).

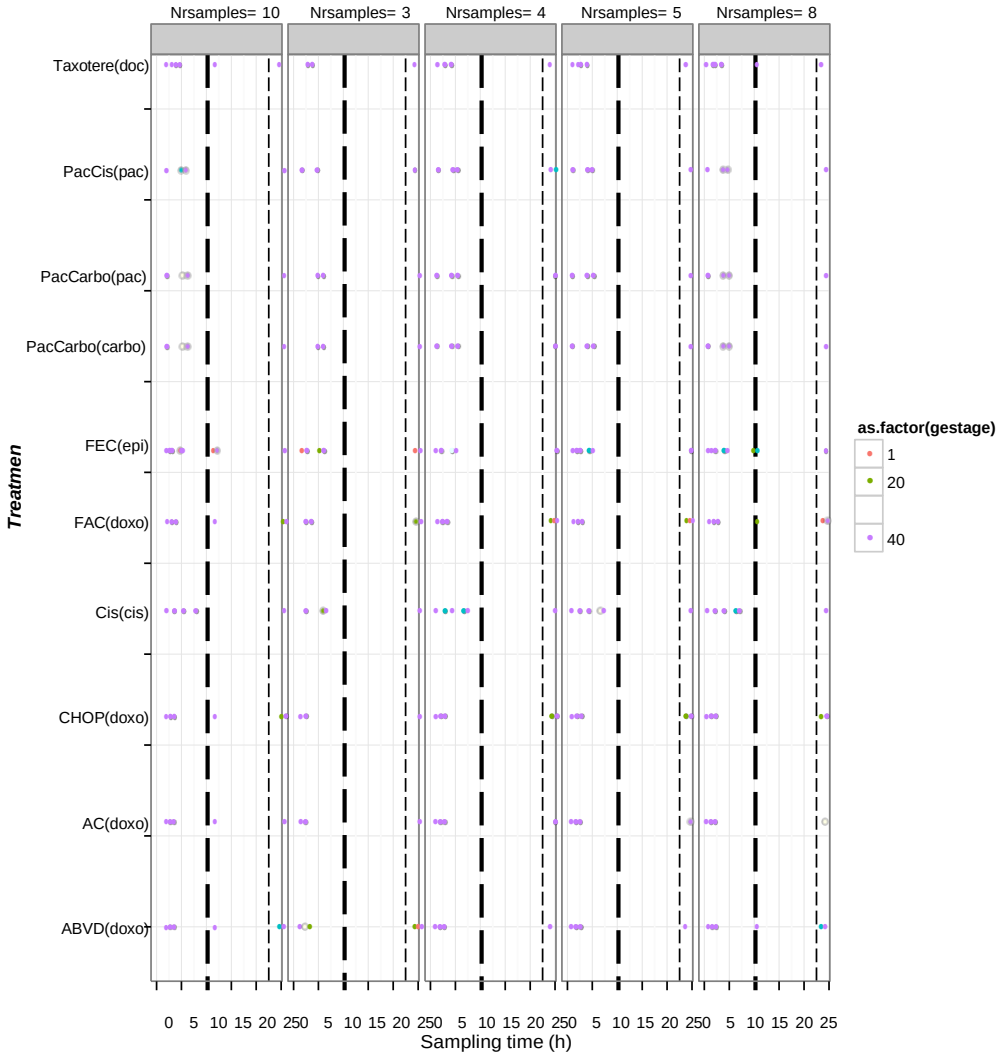


Table 4: Expected parameter precision for gestage=20 and nsample=4,5

| | treat | drug | nsamples | gestage | - RSE V1 | - RSE CL | - RSE V2 | - RSE Q2 | - RSE V3 | - RSE Q3 |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | FEC | epi | 4.00 | 20.00 | 14.04 | 10.61 | 27.82 | 40.59 | 9.83 | 11.57 |
| 10 | FEC | epi | 5.00 | 20.00 | 6.31 | 10.12 | 26.09 | 14.00 | 9.07 | 9.33 |
| 26 | FAC | doxo | 4.00 | 20.00 | 12.78 | 8.45 | 15.10 | 13.17 | 0.00 | 0.00 |
| 30 | FAC | doxo | 5.00 | 20.00 | 9.82 | 8.05 | 14.34 | 10.39 | 0.00 | 0.00 |
| 51 | PacCarbo | pac | 4.00 | 20.00 | 13.31 | 26.38 | 9.96 | 13.36 | 0.00 | 0.00 |
| 52 | PacCarbo | carbo | 4.00 | 20.00 | 38.22 | 13.12 | 208.92 | 3319.59 | 0.00 | 0.00 |
| 59 | PacCarbo | pac | 5.00 | 20.00 | 13.22 | 22.01 | 8.26 | 12.55 | 0.00 | 0.00 |
| 60 | PacCarbo | carbo | 5.00 | 20.00 | 27.16 | 12.54 | 147.23 | 2851.01 | 0.00 | 0.00 |
| 86 | ABVD | doxo | 4.00 | 20.00 | 10.05 | 12.88 | 21.91 | 14.40 | 0.00 | 0.00 |
| 90 | ABVD | doxo | 5.00 | 20.00 | 7.33 | 12.69 | 21.55 | 12.72 | 0.00 | 0.00 |
| 106 | Taxotere | doc | 4.00 | 20.00 | 5.06 | 5.72 | 9.29 | 7.64 | 0.00 | 0.00 |
| 110 | Taxotere | doc | 5.00 | 20.00 | 4.65 | 4.93 | 8.92 | 6.69 | 0.00 | 0.00 |
| 126 | AC | doxo | 4.00 | 20.00 | 9.87 | 7.68 | 13.50 | 12.02 | 0.00 | 0.00 |
| 130 | AC | doxo | 5.00 | 20.00 | 7.19 | 7.38 | 12.82 | 9.79 | 0.00 | 0.00 |
| 146 | Cis | cis | 4.00 | 20.00 | 7.48 | 20.41 | 11.60 | 18.15 | 0.00 | 0.00 |
| 150 | Cis | cis | 5.00 | 20.00 | 7.23 | 20.20 | 10.98 | 15.06 | 0.00 | 0.00 |
| 166 | PacCis | pac | 4.00 | 20.00 | 13.42 | 26.13 | 9.89 | 13.57 | 0.00 | 0.00 |
| 170 | PacCis | pac | 5.00 | 20.00 | 13.21 | 21.94 | 8.19 | 12.55 | 0.00 | 0.00 |
| 186 | CHOP | doxo | 4.00 | 20.00 | 9.98 | 8.30 | 14.52 | 12.23 | 0.00 | 0.00 |
| 190 | CHOP | doxo | 5.00 | 20.00 | 7.28 | 8.02 | 13.94 | 10.13 | 0.00 | 0.00 |

## 15. APPENDIX C

**Part II. Expenses and compensation for participants**

*Specific arrangements for participants in Belgium*

Participants will receive a compensation of €10 for each hour of examinations. Parents will be asked to write down their bank account number or that of their child on the day of consultation. The compensation will be paid by bank transfer after the consultation.

There are no compensations by medical companies, neither for the research doctor nor for the hospital department.

## 16. APPENDIX D

**Budget and Payment terms and conditions**

Sponsor will provide Participating Site compensation for its participation in this Study according to the following budget:

PART I.I.A. (Pregnancy, delivery and maternal health) - Registration study mother and neonate: €50 (per new completed case, including yearly update)

PART I.I.B. (Pregnancy, delivery and maternal health) – Effects of prenatal exposure to cancer treatment on fetal growth: None (tissue transport cost and biobanking will be paid by PI)

PART I.II. (Pregnancy, delivery and maternal health) – Measurement of maternal and paternal anxiety and emotional needs: Psychological questionnaire: €20 (per new complete case)

PART I.III. (Pregnancy, delivery and maternal health) – Biobank 'Cancer in pregnancy': depending on the particular study project, the tissue transport cost will be paid by PI

PART I.IV. (Pregnancy, delivery and maternal health) - Pharmacokinetics: : €150 (per new complete case: datasheet, lab results,…)

PART II. (Child) – Long term follow-up of children born to mothers with cancer during pregnancy: €50 (per new complete examination: neuropsychological testing, cardiac testing, paediatric testing,…)

The above mentioned amounts are exclusive of VAT and inclusive of Participating Site's overhead.

Payments will be made by Sponsor in euro via bank transfer once a year upon receipt of correct, accurate and complete dataset (including follow-up data) from Participating Site and upon receipt of a valid VAT invoice.

VAT will be regulated in accordance with the provisions foreseen in the European Directives of 2008/8/EC and 2006/112/EC. The regulations valid at the time of invoicing will be applicable.
In case services provided under this Agreement should be subject to VAT, the Participating Site shall be entitled to charge VAT at the legal rate in addition to the fees stated in this Agreement, provided the VAT is stated separately on the invoice made out to Sponsor.
Under the current provisions stated in the Directives mentioned above; reverse charges will be applicable; this last sentence does not apply in case invoices come from Belgian Participating Sites.

Sponsor VAT number: BE0419 052 173

Invoices will be sent to:
UZ Leuven
Clinical Trial Center (finance department)
Herestraat 49
3000 Leuven
Belgium

Invoices will contain the following payment details:

- Name Participating Site
- VAT ID number of Participating Site
- Address of Participating Site
- Payment currency: EUR
- Bank account holder name
- Bank account number
- IBAN
- Bank identifier code (SWIFT)
- Bank Name
- Bank Address
- Reference: S25470

**Participating Site acknowledges the fact that Sponsor received a limited ERC grant to finance this Study and Participating Site hereby agrees that if the limited grant is fully spent, Sponsor shall be under no obligation to reimburse Participating Site according to the above agreed payment schedule. As a consequence, Participating Site agrees to finance its own Study related costs.**

**If protocol amendments take place having significant financial implications, Participating Site shall be allowed to re-evaluate its commitment to participate in the Study.**

**In case Sponsor would receive, in the future, a new limited grant for this Study, reimbursement of the Participating Site shall be arranged in accordance with the principles of this Appendix.**

## 17. APPENDIX E

**DATA PROCESSING ANNEX ("DPA") TO THE PROTOCOL**

Definitions:

"**Protocol**" means the document entitled "Cancer in Pregnancy" containing the details of the academic study as developed by the Sponsor as approved by the relevant ethics committee.

"**Sponsor**" means Universitaire Ziekenhuizen Leuven.

Participating site acts as a data processor as defined under article 4, 8) of the Regulation (EU) 2016/679 ("**Data Processor**") for the Sponsor who acts as data controller as defined under article 4, 7) of the Regulation (EU) 2016/679 ("**Data Controller**").

"**Applicable Law**" means any applicable data protection or privacy laws, including:

(i) the Regulation (EU) 2016/679 also referred as the General Data Protection Regulation ("GDPR");

(ii) other applicable laws that are similar or equivalent to or that are intended to or implement the laws that are identified in (a) of this definition;

"**Personal Data**" means any information relating to an identified or identifiable natural person ('**Data Subject**'), including without limitation pseudonymized information, as defined in Applicable Law and described in the Protocol.

Rights and obligations:

1) The Data Processor is instructed to process the Personal Data for the term of the Protocol and only for the purposes of providing the data processing tasks set out in the Protocol.
2) The Data Processor must ensure that persons authorized to process the Personal Data have committed themselves to confidentiality or are under an appropriate statutory obligation of confidentiality.
3) The Data Processor shall implement appropriate technical and organizational measures to prevent that the Personal Data processed is:
   (i) accidentally or unlawfully destroyed, lost or altered,
   (ii) disclosed or made available without authorization, or
   (iii) otherwise processed in violation of Applicable Law.
4) The appropriate technical and organizational security measures must be determined with due regard for:
   (i) the current state of the art,
   (ii) the cost of their implementation, and
   (iii) the nature, scope, context and purposes of processing as well as the risk of varying likelihood and severity for the rights and freedoms of natural persons.
5) The Data Processor shall upon request provide the Data Controller with sufficient information to enable the Data Controller to ensure that the Data Processor's obligations under this DPA are complied with, including ensuring that the appropriate technical and organizational security measures have been implemented.
6) The Data Controller is entitled to appoint at its own cost an independent expert, reasonably acceptable to Data Processor, who shall have access to the Data Processor's

data processing facilities and receive the necessary information for the sole purpose of auditing whether the Data Processor has implemented and maintained said technical and organizational security measures. The expert shall upon the Data Processor's request sign a non-disclosure agreement provided by the Data Processor, and treat all information obtained or received from the Data Processor confidentially, and may only pass on, after conferral with Data Processor, the findings as described under 8) (ii) below to the Data Controller.

7) The Data Processor must give authorities who by Union or Member State law have a right to enter the Data Controller's or the Data Controller's processors' facilities, or representatives of the authorities, access to the Data Processor's physical facilities against proper proof of identity and mandate, during normal business hours and upon reasonable prior written notice.
8) The Data Processor must without undue delay in writing notify the Data Controller about:
   (i) any request for disclosure of Personal Data processed under the Protocol by authorities, unless expressly prohibited under Union or Member State law,
   (ii) any finding of (a) breach of security that results in accidental or unlawful destruction, loss, alteration, unauthorized disclosure of, or access to, Personal Data transmitted, stored or otherwise processed by the Data Processor under the Protocol, or (b) other failure to comply with the Data Processor's obligations, or
   (iii) any request for access to the Personal Data (with the exception of medical records for which the Data Processor is considered data controller) received directly from the data subjects or from third parties.
9) Such a notification from the Data Processor to the Data Controller with regard to a breach of security as meant in 8) (ii)(a) above will contain at least the following information:
   (i) The nature of the Personal Data breach, stating the categories and (by approximation) the number of Data Subjects concerned, and stating the categories and (by approximation) the number of the personal data registers affected (datasets);
   (ii) The likely consequences of the Personal Data breach;
   (iii) A proposal for measures to be taken to address the Personal Data breach, including (where appropriate) measures to mitigate any possible adverse effects of such breach.
10) The Data Processor shall document (and shall keep such documentation available for the Data Controller) any Personal Data breaches, including the facts related to the Personal Data breach, its effects and the corrective measures taken. After consulting with the Data Controller, the Data Processor shall take any measures needed to limit the (possible) adverse effects of Personal Data breaches (unless such consultation cannot be awaited due to the nature of the Personal Data breach).
11) The Data Processor must promptly reasonably assist the Data Controller (with the handling of (a) responses to any breach of security as described in 8) (ii) above and (b) any requests from Data Subjects under Chapter III of the GDPR (upon its entry into force), including requests for access, rectification, blocking or deletion. The Data Processor must also reasonably assist the Data Controller by implementing appropriate

technical and organizational measures for the fulfilment of the Data Controller's obligation to respond to such requests. The Data Processor must reasonably assist the Data Controller with meeting the other obligations that may be incumbent on the Data Controller according to Union or Member State law where the assistance of the Data Processor is implied, and where the assistance of the Data Processor is necessary for the Data Controller to comply with its obligations. This includes, but is not limited to, at the request to provide the Data Controller with all necessary information about an incident under 8) (ii), and all necessary information for an impact assessment in accordance with Article 35 and Article 36 of the GDPR.

Subprocessor:

12) The Data Processor may only engage a subprocessor, with prior specific or general written consent from the Data Controller. The Data Processor undertakes to inform the Data Controller of any intended changes concerning the addition or replacement of a subprocessor by providing a reasonable prior written notice to the Data Controller. The Data Controller may reasonably and in a duly substantiated manner object to the use of a subprocessor. The Data Processor must inform the Data Controller in writing of the discontinued use of a subprocessor.
13) Prior to the engagement of a subprocessor, the Data Processor shall conclude a written agreement with the subprocessor, in which at least the same data protection obligations as set out in this DPA shall be imposed on the subprocessor, including obligations to implement appropriate technical and organizational measures and to ensure that the transfer of Personal Data is done in such a manner that the processing will meet the requirements of the Applicable Law.
14) The Data Controller has the right to receive a copy of the relevant provisions of Data Processor's agreement with the subprocessor related to data protection obligations. The Data Processor shall remain fully liable to the Data Controller for the performance of the subprocessor obligations under this DPA. The fact that the Data Controller has given consent to the Data Processor's use of a subprocessor is without prejudice for the Data Processor's duty to comply with this DPA.