Study Protocol	
Protocol Number	AAA-Annexin-05 / NCT02677714
Investigational Product	Kit for the preparation of ^{99m}Tc-rhAnnexin V-128
Active substance	rhAnnexin V-128
Radiolabelled Imaging Product	^{99m}Tc-rhAnnexin V-128
Trial Phase	Proof of Concept and Phase II
Trial Title	^{99m}Tc-rhAnnexin V-128 Imaging of Apoptosis and Cardiotoxicity in Relationship to Ventricular Function in Patients with Early Stage Breast Cancer Receiving Doxorubicin-Based Chemotherapy
Short Trial Title	^{99m}Tc-rhAnnexin V-128 Imaging and Cardiotoxicity in Patients with Early Breast Cancer
Version and Date	v.7.0 dated 11 March 2018
Investigators	Principal Investigator: [redacted], MD, FRCPC, FACC Co-Investigators: [redacted], MD, FRCPC, FACC [redacted], PhD, MD [redacted], PhD [redacted], MD [redacted], MD [redacted], MD [redacted], PhD
Trial Sponsor	Advanced Accelerator Applications
The concepts and information contained herein or generated during the study are considered proprietary and shall not be disclosed in whole or in part without the expressed written consent of Advanced Accelerator Applications	
This study is to be completed according to the guidelines of Good Clinical Practice (GCP) and conducted in full compliance with the World Medical Association Declaration of Helsinki and its most recent amendments	

SPONSOR SIGNATORY APPROVAL PAGE

PROTOCOL TITLE: ^{99m}Tc-rhAnnexin V-128 Imaging of Apoptosis and Cardiotoxicity in Relationship to Ventricular Function in Patients with Early Stage Breast Cancer Receiving Doxorubicin-Based Chemotherapy

PROTOCOL NUMBER: AAA-Annexin-05

Signatures on this page denote approval of the study protocol outline by the respective Sponsor Department

Role	Name and contact	Date & Signature
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INVESTIGATOR ENDORSEMENT PAGE

I agree to conduct the study as outlined in the protocol entitled "^{99m}Tc-rhAnnexin V-128 Imaging of Apoptosis and Cardiotoxicity in Relationship to Ventricular Function in Patients with Early Stage Breast Cancer Receiving Doxorubicin-Based Chemotherapy" in accordance with the guidelines and all applicable government regulations. These guidelines and regulations include, but are not limited to:

1. Permission to allow the Sponsor and the regulatory agencies to inspect study facilities and pertinent records at reasonable times and in a reasonable manner that ensures participant confidentiality. If this study is to be inspected by a regulatory agency, the Sponsor should be notified as soon as possible;

2. Submission of the proposed clinical investigation, including the protocol and the consent form to a duly constituted Research Ethic Board (REB) as well as Health Canada (regulatory authority) for approval and acquisition of written approval prior to study conduct;

3. Use of written informed consent that is obtained prior to study conduct and that contains all the elements of consent as specified in the federal regulations and has been previously approved by the Sponsor, the REB and Health Canada;

4. Submission of any proposed change in or deviation from the protocol to the REB to be approved by the Sponsor. Any proposed changes or deviations from the protocol may require that the informed consent also reflect such changes or deviations and that the revised informed consent be approved by the REB and Health Canada;

5. Documentation and explanation of individual protocol deviations on the appropriate case report form page or in letters to the Sponsor;

6. Sending written reports of serious adverse events to the Sponsor/CRO within 24 hours by email ([redacted]) after the Investigator's awareness of the information;

7. Reporting of Serious Adverse Events (SAE) according to ICH/GCP and Regulatory Standards. SAEs will be reported from the signing of the informed consent and followed until resolution or determined to be not clinically significant.

8. Submission of timely progress reports to the REB and Sponsor at appropriate intervals on a schedule determined by the REB.

Regulations require an Investigator to prepare and maintain adequate and accurate case histories designed to record all observations and other data (such as investigational product accountability) pertinent to the investigation on each individual enrolled in the study. These records must be

maintained by the Investigator for a minimum period of 25 years or a period of time determined by the Sponsor following the date a marketing application is approved for the indication for which it is being investigated, or, if no application is to be filed or if the application is not approved for such indication, a minimum of 25 years or a period of time determined by the Sponsor after the investigation is discontinued and the appropriate regulatory authorities are notified.

In addition, I agree to provide all the information requested in the case report form in a manner to assure legibility and accuracy. To this end, I shall carefully follow the instructions for completing case report forms.

I also agree that all information provided to me by the Sponsor, including protocols, case report forms, and verbal and written information, will be kept strictly confidential and confined to the clinical personnel involved in conducting the study. It is recognized that this information may be related in confidence to the REB/regulatory authorities. I also understand that reports of information about the study or its progress will not be provided to anyone who is not involved in the study other than to the Principal Investigator, or in confidence to the REB or to the legally constituted regulatory authorities.

Principal Investigator Signature	Date of Signature

Table of Contents

1. Study Synopsis

Investigational Medicinal Product	Kit for the Preparation of ^{99m}Tc Recombinant Human Annexin V-128 for Injection
Title of the study	^{99m}Tc-rhAnnexin V-128 Imaging of Apoptosis and Cardiotoxicity in Relationship to Ventricular Function in Patients with Early Stage Breast Cancer Receiving Doxorubicin-Based Chemotherapy
Principal Investigator and Study Site	Dr. [redacted], MD, FRCPC, FACC University of Ottawa Heart Institute 40 Ruskin Street, Ottawa, Ontario K1Y 4W7, Canada
Sponsor	Advanced Accelerator Applications
Study Indication	Patients with early stage breast cancer receiving doxorubicin-based chemotherapy as part of their treatment
Objectives	Primary Objective: • To investigate ^{99m}Tc-rhAnnexin V-128 imaging of apoptosis in the evaluation of doxorubicin-induced cardiotoxicity in patients with early stage breast cancer. Secondary Objectives: • To determine the timing and extent of ^{99m}Tc-rhAnnexin V-128 imaging of apoptosis (^{99m}Tc-rhAnnexin V-128 myocardial uptake). • To determine the relationship between ^{99m}Tc-rhAnnexin V-128 myocardial uptake and the changes in left ventricular (LV) function measured by cardiac magnetic resonance imaging (CMRI) and the changes in cardiotoxicity biomarkers.
Rationale	Annexin V is an endogenous human protein that binds to phosphatidylserine (PS), a constitutive anionic phospholipid of the plasma membrane of all mammalian cells, that is only expressed only on the surface of physiologically stressed, depolarized, or apoptotic cells. Technetium-99m (^{99m}Tc) is a medical isotope widely used in diagnostic imaging. ^{99m}Tc-labeled annexin V imaging has the ability to image cellular stress and apoptosis such as myocyte damage in patients developing cardiotoxicity induced by chemotherapy.
Planned number of participants	Thirty evaluable patients with early stage (Stage I, II or III) breast cancer. Ten patients will be recruited in the first part of the trial, as a Proof of Concept (PoC) phase. The PoC phase will assess the potential of ^{99m}Tc-rhAnnexin V-128 in terms of imaging quality, uptake and medical relevance to enable the decision to continue or terminate the clinical investigation with the remaining 20 patients.
Study design and Methodology	This is a single-centre, PoC, Phase II study. Patients who have signed the informed consent and are eligible to participate in the study will undergo the following assessments:

	• Screening will be conducted within 2 weeks prior to chemotherapy treatment initiation. Eligible and consenting patients will undergo a medical review (including measurement of the height, weight, and BMI), vital signs (systolic and diastolic blood pressure, heart rate), blood analysis and urinalysis. The following blood analysis will be conducted: cardiotoxicity biomarkers troponin and NT-proBNP (N terminal pro B-type natriuretic peptide), assessment of anti-annexin V-128 antibodies (baseline value) and hematology / biochemistry as detailed in Section 10.3. Participants will undergo the first CMRI and planar and SPECT/CT ^{99m}Tc-rhAnnexin V-128 scans. • The planned chemotherapy treatment consists of doxorubicin 60 mg/m^2 in combination with cyclophosphamide 600 mg/m^2 IV (AC) every 2 or 3 weeks for 4 cycles to be followed by paclitaxel or docetaxel as per clinical practice, at the Ottawa Hospital Cancer Center, with potential dose adjustments. • After the 2nd cycle of doxorubicin and before the 3rd cycle, the participants will undergo a second series of study imaging procedures (CMRI,planar and SPECT/CT with ^{99m}Tc-rhAnnexin V-128). Blood sampling will be performed for the assessment of cardiotoxicity biomarkers (troponin, NT-proBNP). • After the 4th cycle of doxorubicin and within 2 weeks after the 4th cycle, participants will undergo the third series of study imaging procedures (CMRI, planar and SPECT/CT with ^{99m}Tc-rhAnnexin V-128). Blood sampling will be performed for the assessment of cardiotoxicity biomarkers (troponin, NT-proBNP). • At 12 weeks after the 4th cycle of doxorubicin, participants will undergo the fourth series of study imaging procedures (CMRI, planar and SPECT/CT with ^{99m}Tc-rhAnnexin V-128). Blood sampling will be performed for the assessment of cardiotoxicity biomarkers (troponin, NT-proBNP) and hematology / biochemistry as detailed in Section 10.3. Urinalysis will also be performed. Immunology blood sampling will be performed to rule out the development of anti-annexin V-128 antibodies. • The imaging procedures will be conducted at the University of Ottawa Heart Institute (UOHI) or at the Royal Ottawa Hospital (ROH). Safety assessments will be done at each procedure. The CMRI and the SPECT/CT imaging will be done on the same day or within one week of each other. • Relevant results (if available) from clinical laboratory assessments performed during the treatment period will be used for the study analysis. • After completion of the last follow-up visit at 12 weeks following chemotherapy in the first 10 participants, the Data Monitoring Committee (DMC) will conduct a visual assessment of the scans and provide recommendations for the continuation or termination of the Phase II study.

Treatment	After reconstitution and radiolabeling, ^{99m}Tc-rhAnnexin V-128 is administered as a single intravenous bolus of 350 MBq +/- 10% at baseline, after the 2nd cycle, after the 4th cycle and 12 weeks after AC chemotherapy.
Inclusion/ exclusion criteria	Inclusion criteria 1. Females □ 18 years of age with histologically confirmed early stage (Stage I, II or III) HER-2 negative breast cancer and planned for (neo)adjuvant doxorubicin-based chemotherapy (AC every 2 or 3 weeks x 4 cycles) 2. Eastern Cooperative Oncology Group Status (ECOG) □2 3. Able and willing to comply with the study procedures Exclusion criteria 1. Pregnancy or lactation 2. Moderate or severe valvular stenosis or regurgitation 3. History of atrial fibrillation or flutter 4. History of any disease or relevant physical or psychiatric condition which may interfere with the study objectives at the investigator judgment 5. Know hypersensitivity to the investigational product (IP) or any of its components 6. Prosthetic valve or pacemaker 7. Claustrophobia or inability to lie still in a supine position 8. Contraindication(s) to the CMRI procedure 9. Participation in another clinical trial within 4 weeks before study inclusion, except for patients who have participated or who are currently participating in a study without any study drug administration 10. Unwillingness to provide consent
Study duration and assessments	Maximum 26 weeks (+/- 4 weeks) including the screening and follow-up period for an individual participant.
Safety	Venous blood samples will be drawn for each participant at screening and 12 weeks after the end of chemotherapy for laboratory analysis (as detailed in section 10.3). Creatinine levels will be drawn for CMRI contrast standard practice as required if not available clinically. To rule out the development of anti-annexin V-128 antibodies, venous blood samples will be collected for each participant at screening and 12 weeks after the end of chemotherapy. Assays for anti-rhAnnexin V-128 IgG and IgM antibodies will be performed in serum samples by ELISA. For the assessment of cardiotoxicity biomarkers (troponin, NT-proBNP), venous blood samples will also be collected at the time of the first, second, third and fourth ^{99m}Tc-rhAnnexin V-128 scan.

	Results from clinical laboratory assessments performed during oncology treatment will be reviewed during the study participation to monitor any abnormalities. A medical review including the measurement of the height, weight, BMI and vital signs (systolic and diastolic blood pressure and heart rate) will be performed at screening and at the time of the first, second, third and fourth ^{99m}Tc-rhAnnexin V-128 scans. Changes in medical condition will also be recorded. Adverse events will be collected in the Electronic Case Report Form (e-CRF).
Statistics	Based on previous observations, the uptake of ^{99m}Tc-rh-Annexin-V 128 in normal females should be of about 1.2 +/- 0.2 % ID/gm and LVEF be 58 +/-7 %. For the comparison of ^{99m}Tc-rh-Annexin-V 128 uptake using a paired analysis, a sample size of 26 patients will allow detection of an absolute change of about 0.15% ID/gm with a two-sided 5% significance level and a power of 95%. For the comparison of left ventricular ejection fraction (LVEF) measured with CMR using a paired analysis, a sample size of 28 patients will allow to detect a difference of about 5 %LVEF units with a two-sided 5% significance level and a power of 95%. The final study population for recruitment size will be 30 patients, allowing for 5 to 10% patient attrition or incomplete imaging data. Primary endpoint of PoC: Image quality, imaging agent uptake and clinical relevance of apoptosis imaging in the evaluation of doxorubicin-induced cardiotoxicity in patients with early stage breast cancer using ^{99m}Tc-rhAnnexin V-128 will be determined by the DMC (visual image review and consensus). Primary endpoint of Phase II: The ability of ^{99m}Tc-rhAnnexin V-128 to detect doxorubicin-induced cardiotoxicity will be assessed by repeated analysis of uptake and LVEF compared to baseline. Secondary endpoints: Timing and extent of ^{99m}Tc-rhAnnexin V-128 myocardial uptake will be determined by an appropriate paired analysis of absolute value or Standardized Uptake Value (SUV) and Cardiac Uptake Ratio (CUR) at each follow-up visit compared to baseline. The LV function will be assessed by comparing CMRI LVEF at each follow-up visit compared to baseline using an appropriate paired analysis.

	^{99m}Tc-rhAnnexin V-128 uptake changes will be correlated with the changes in LVEF and cardiotoxicity biomarkers. Safety: Adverse events will be listed on an individual basis, including relationship, and severity, and will be summarized by MedDRA System Organ Class (SOC) and Preferred Term (PT). Participants with more than one adverse event within a particular SOC and PT will be counted only once for that SOC and PT. The incidence of adverse events will also be summarized by severity and relationship to the investigational imaging product. All other safety variables will be tabulated at each measuring time. Hematology, clinical chemistry, urinalysis, cardiotoxicity biomarkers and immunogenicity data will be analyzed with respect to the normal ranges of values provided by the laboratory. Similarly, descriptive statistics will also be provided for vital signs, physical examination, etc.

Table 1 – Visit Schedule

Study Procedures	**First UOHI Evaluation** (Screening within 2 weeks prior to the start of AC treatment)	**Second UOHI Evaluation** (After the 2nd cycle of doxorubicin and before the 3rd cycle)	**Third UOHI Evaluation** (After the 4thcycle of doxorubicin and within 2 weeks)	**Fourth UOHI Evaluation** (12 weeks after the 4th cycle of doxorubicin)
Written informed consent	x			
Inclusion/exclusion criteria	x			
Medical history/review	x	x	x	x
Concomitant medications	x	x	x	x
Height, weight, BMI[1]	x	x	x	x
Vital signs (BP, HR)	x	x	x	x
Lab analysis[2]	x			x
Immunogenicity by ELISA[3]	x			x
Cardiotoxicity biomarkers (troponin and BNP)	x	x	x	x
Pregnancy test	x	x	x	x
Cardiac Magnetic Resonance Imaging	x	x	x	x
rh-Annexin V-128 administration and planar and SPECT/CT imaging	x	x	x	x
Adverse Events	x	x	x	x

[1] The height will only be measured at screening.

[2] See Section 10.3. Additionally, results from laboratory analysis conducted during the oncology treatment will be used for research analysis.

[3] Anti-rhAnnexin V-128 IgG and IgM antibodies will be quantified in serum samples by ELISA. For this purpose, 10 mL blood samples will be collected at screening and 12 weeks after the last dose of 4 cycles of doxorubicin (before ^{99m}Tc-rhAnnexin V-128 administration). Serum will be prepared, divided into six aliquots and frozen (-80°C). Three out of six serum samples per time-point will be shipped to the central laboratory [redacted]

2. List of abbreviations

AC	Doxorubicin/cyclophosphamide chemotherapy
AE	Adverse Event
CMR	Cardiac Magnetic Resonance Imaging
eCRF	Electronic Case Report Form
CRO	Clinical Research Organization
CRP	C-Reactive Protein
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CUR	Cardiac Uptake Ratio
ECOG	Eastern Cooperative Oncology Group Status
EF	Ejection Fraction
ELISA	Enzyme-Linked Immuno-absorbent Assay
ERNA	Equilibrium RadioNuclide Angiography
FDA	Food and Drug Administration
GCP	Good Clinical Practice
Hb	Hemoglobin
Hct	Hematocrit
ICF	Informed Consent Form
ICRP	International Commission on Radiological Protection
IMP	Investigational Medicinal Product
ITLC	Instant Thin Layer Chromatography
IP	Investigational Product
LVEF	Left Ventricular Ejection Fraction
NT-proBNP	N Terminal pro B-type Natriuretic Peptide
MBq	Mega Becquerel
MIRD	Medical Internal Radiation Dose
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary For Regulatory Activities
PET	Positron Emission Tomography
PBS	Phosphate Buffered Saline
PoC	Proof of Concept
PS	Phosphatidylserine
PV	Pharmacovigilance
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell
RCP	Radiochemical Purity
REB	Research Ethic Board
ROI	Region of Interest
ROS	Reactive Oxygen Species
[redacted]	[redacted]
SAE	Serious Adverse Event

SD	Standard Deviation
SOP	Standard Operating Procedure
SPECT	Single-Photon Emission Computed Tomography
SUV	Standardized Uptake Value
TBR	Target to background ratio
TLC	Thin Layer Chromatography
WBC	White Blood Cell
WHO	World Health Organization
VOI	Volumes Of Interest

3. Background and Rationale

The 5-year survival for early stage breast cancer has increased from 79% in 1990 to 88% in 2012 (Canadian Cancer Statistics 2015. pg 61. www.cancer.ca). However, this improved cancer survival due to anthracycline-based chemotherapy is unfortunately associated with an increase in cardiovascular events [1]. In patients with breast cancer and surviving five years or longer after initial treatment, cardiovascular events represent the second major cause of mortality [2]. The incidence of heart failure is directly related to the cumulative dose of doxorubicin and estimated at 5%, 16% and 26% for doses of 400, 500 and 550 mg/m^2 [3]. Accordingly, clinical protocols limit the cumulative dose to 450 mg/m^2. Anthracycline therapy may cause cardiac damage in lower doses as cardiac biopsy data has shown significant changes in cell morphology with cumulative doses as low as 200 mg/m^2 [4]. Elevated troponin levels are a marker of myocyte necrosis and are elevated in some patients after one cycle of anthracycline therapy [5]. Thus, patients vary in terms of sensitivity to anthracycline therapy for myocyte damage and there is no safe threshold for all patients.

For many years, the accepted mechanism for anthracycline-induced cardiac toxicity was generation of excess reactive oxygen species (ROS) due to electron exchange between oxygen molecules and anthracycline [6] or between anthracycline complexes with iron [7]. However, antioxidants [8] and iron chelation [9] were ineffective in preventing cardiotoxicity in prospective randomized clinical trials. More recently, topoisomerase (Top) 2 was recognized as playing a major role in anthracycline-induced cardiotoxicity [10]. Top 2 is present in cardiac myocytes [11] and inhibition by anthracycline results in myocyte death. Doxorubicin treatment is associated with an increase in p53 phosphorylation and apoptosis [12]. Deletion of p53 or inhibition of the p53 pathway results in reduced apoptosis with better LV function after doxorubicin therapy [13, 14]. In Top2 -/- mice, doxorubicin therapy resulted in minimal changes and this supported the hypothesis that Top2 plays a major role in doxorubicin cardiotoxicity.

Primary prevention of anthracycline-induced cardiotoxicity has been pursued in several ways. Anthracycline can be administered by continuous infusion with less cardiotoxicity and similar tumor response rates [15]. Similarly, liposomal encapsulation has less cardiotoxicity but similar anti-tumor efficacy [16]. Alternatively, dexrazoxane can be administered as an effective cardioprotective agent [17] with less cardiotoxicity [18] and possibly less anti-tumor efficacy. [19]. Presently, dexrazoxane is approved by the FDA for women with metastatic breast cancer requiring additional doxorubicin and having received at least 300 mg/m^2. Conventional agents including betablockers, ACE inhibitors and ARBs have been used for primary prevention, with borderline or negative results [20-23].

Cardiotoxicity due to anthracycline therapy is clinically detected as changes in left ventricular ejection fraction (LVEF) measured by equilibrium radionuclide angiography (ERNA) [24], echocardiography [25, 26] or magnetic resonance imaging [27]. Cardiotoxicity is characterized by a decrease of LVEF from baseline of $\geq$5 % in patients with symptoms of heart failure or a decrease of LVEF $\geq$10% in asymptomatic patients to an EF <54% [28]. Of note, LVEF can vary with changes in afterload related to medications or other factors including sepsis, fluid overload and other heart disease. LVEF measured by ERNA has an associated exposure to radiation of 5

to 10 mSv per examination. Echocardiography has the advantage of no radiation and can provide information about valvular and pericardial disease. 2D echocardiography has less optimal reproducibility than ERNA with lesser ability to detect small changes in ejection fraction [29]. Recent small studies using 3D echocardiography have shown better reproducibility with less inter-observer variability for LVEF measurement compared to 2D echocardiography [25, 30].

Cardiac MR can be used to measure LVEF with a high degree of accuracy and reproducibility without radiation and also provide information about the pericardium [31]. However, availability of cardiac MR is relatively limited and not possible for patients with current cardiac devices including pacemakers and internal defibrillators or intracranial metal. Newer techniques have been developed to detect early cardiotoxicity and include global longitudinal strain by echocardiography [32, 33] and by cardiac MR [34]. Measurement of strain using echocardiography or cardiac MR appears to offer greater sensitivity for detection of small changes but is not well established.

Secondary prevention studies using betablocker or ACE inhibitor therapy suggests benefit with early initiation of therapy [35, 36].

Apoptosis is a form of programmed cell death with a series of cellular events including the exposure of phospholipid phosphatidylserine on the surface as an early event. Annexin-V is a small protein that binds to phosphatidylserine with high affinity. Annexin-V can be labeled with 99m-Tc for apoptosis imaging in heart and lung transplant rejection in animals and humans. [37-39]. Annexin-V imaging has been used for imaging of apoptosis in rats treated with acute and chronic doxorubicin therapy with a dose-dependent increase in tracer uptake and correlations with both histopathologic and immunohistochemical data as well as echocardiographic evidence of left ventricular dysfunction [40, 41]. In vivo planar and SPECT / CT imaging in rats demonstrated myocardial uptake of Tc-99m labeled annexin V at low doses of doxorubicin prior to the decrease in LV ejection fraction measured with echocardiography [41]. 99m-Tc hydrazine-nicotinamide (HYNIC) annexin-V has been used in patients for detection of acute myocardial infarction and endocarditis in cardiac patients and spontaneous and treatment induced apoptosis in cancer patients [42]. This approach with 99m-Tc-HYNIC-annexin-V had limitations with high uptake in the kidney resulting in large patient radiation dosing and significant abdominal scatter and has not advanced past phase II studies. A new annexin derivative, rhAnnexin V-128, has been developed permitting direct chelation of 99m-Tc to the protein, with a better biodistribution profile and less renal retention [43, 44]. We recently completed a phase I evaluation of ^{99m}Tc rh-Annexin-V 128 in 12 healthy adult volunteers and demonstrated that the radiotracer was a safe radiopharmaceutical with low participant radiodosimetry [45].

The development of cardiotoxicity is a major concern in patients with early stage breast cancer and treated with anthracycline therapy. Serial LVEF evaluation can detect cardiotoxicity usually after cardiac damage is present and most likely irreversible. Patients vary in cardiac sensitivity to anthracyclines. A better monitoring approach may be direct imaging of myocyte damage with annexin-V imaging to identify patients developing cardiotoxicity. These patients may benefit from changes in chemotherapy with dose alterations or changes to alternative drugs and treatment

with cardioprotective medications such as dexrazoxane or conventional medications such as betablockers or ACE inhibitors. We anticipate that greater amounts of apoptosis will be associated with greater changes in LV functional parameters measured with CMR. However, patients may vary in their cardiac sensitivity to a given dose of anthracycline and have varying degrees of apoptosis for a given dose.

This project builds on previous knowledge. Imaging of apoptosis with 99m-Tc labeled annexin-V has demonstrated increasing uptake of the radiotracer in association with worsening left ventricular dysfunction in rat models. Furthermore, we have demonstrated the safety of a new annexin derivative, rhAnnexin V-128, labeled with technetium-99m, in healthy volunteers and low radiodosimetry. Serial imaging of apoptosis with this new labeled annexin ^{99m}Tc-rhAnnexin V-128 may be useful for early detection of anthracycline cardiotoxicity.

4. Study Objectives

The primary objective of this investigation is:

- To investigate ^{99m}Tc-rhAnnexin V-128 imaging of apoptosis in the evaluation of doxorubicin-induced cardiotoxicity in patients with early stage breast cancer.

The secondary objectives are:

- To determine the timing and extent of ^{99m}Tc-rhAnnexin V-128 imaging of apoptosis (^{99m}Tc-rhAnnexin V-128 myocardial uptake).
- To determine the relationship between ^{99m}Tc-rhAnnexin V-128 myocardial uptake and the changes in left ventricular (LV) function measured by cardiac magnetic resonance imaging (CMRI) and the changes in the cardiotoxicity biomarkers.

5. Hypotheses

5.1 Primary Hypothesis

Patients with early stage breast cancer undergoing doxorubicin-containing (neo) adjuvant chemotherapy will have increased uptake of ^{99m}Tc- rhAnnexin V-128 after the 2nd and the 4th cycles of AC treatment, and 12 weeks after the last cycle of doxorubicin, compared to baseline uptake.

5.2 Secondary Hypotheses

1. Patients with early stage breast cancer undergoing doxorubicin-containing (neo) adjuvant chemotherapy will have worsening of LV function measured with CMRI after the 2nd and the 4th cycle of treatment and 12 weeks from the last dose of doxorubicin chemotherapy compared to baseline.
2. The uptake of ^{99m}Tc-rhAnnexinV-128 (percentage of injected dose per gram, % ID/gm) will correlate with changes in CMRI LV function parameters and changes in the cardiotoxicity biomarkers.

6. Study Design

This is a single centre, Proof of Concept (PoC), Phase II study. Patients with histologically confirmed early stage (Stage I, II and III) HER-2 negative breast cancer and scheduled to receive (neo) adjuvant doxorubicin-based chemotherapy (A- 60 mg/m2; C- 600 mg/m2; every 2 or 3 weeks x 4 cycles) to be followed by paclitaxel or docetaxel as per clinical practice [46, 47] will be recruited from the Ottawa Hospital Cancer Centre. Participants will be scheduled for cardiac MRI and ^{99m}Tc-rhAnnexin V-128 imaging prior to initiating doxorubicin-based chemotherapy. Participants will also undergo ^{99m}Tc-rhAnnexin V-128 imaging and CMRI after the 2nd and the 4th cycles of AC treatment and 12 weeks after the last cycle.

7. End of Study

The end of study is defined as the completion of all study procedures in the last enrolled participant (i.e. last visit, last participant).

8. Study Population

Approval for the study will be obtained from the Ottawa Hospital Science Network Research Ethics Board (OHSN-REB) and Health Canada. All participants will provide written informed consent prior to the initiation of any study procedures. It is foreseen to recruit thirty patients with early stage breast cancer.

8.1 Inclusion criteria

1. Females □18 years of age with histologically confirmed early stage (Stage I, II or III) HER-2 negative breast cancer and planned to receive (neo)adjuvant doxorubicin-based (A- 60 mg/m2; C- 600 mg/m2; every 2 or 3 weeks x 4 cycles) chemotherapy.
2. Eastern Cooperative Oncology Group Status (ECOG) □2 (see Appendix II)
3. Able and willing to comply with the study procedures

8.2 Exclusion criteria

1. Pregnancy or lactation
2. Moderate or severe valvular stenosis or regurgitation
3. History of atrial fibrillation or flutter
4. History of any disease or relevant physical or psychiatric condition which may interfere with the study objectives at the investigator judgment
5. Know hypersensitivity to the investigational product or any of its components
6. Prosthetic valve or pacemaker
7. Claustrophobia or inability to lie still in a supine position
8. Contraindication(s) to CMRI procedure
9. Participation in another clinical trial within 4 weeks before study inclusion, except for patients who have participated or who are currently participating in a study without any study drug administration
10. Unwillingness to provide consent

8.3 Source of Study Population

Patients from the Ottawa Hospital Cancer Centre will be enrolled. The Ottawa Hospital Cancer Centre sees about 1,000 patients per year with breast cancer (Stage I to III) and it is estimated that 4 to 5 per month would meet the study inclusion criteria. We plan to recruit 30 patients over 18 to 24 months, or approximately 1 to 2 patients per month.

8.4 Participant Study Identification

A unique participant identification number (Participant ID) will be assigned at the start of the screening period to each participant who signs the informed consent form. This number will identify the participant throughout the study. Participant IDs will include the 2-digit protocol number (05), the 2-letter code (CA) and a 3-digit participant number (ex: 05-CA001 for first participant in).

8.5 Premature Discontinuation

The withdrawal of a study participant is mandatory in the following cases:

- Pregnancy
- Protocol violation determined as critical
- Lost to follow-up
- Serious intercurrent illness or other safety reasons for which the Investigator considers it is in the best interest of the participant to withdraw from the study
- Screening failure
- Failure to complete prescribed cycle of anthracycline chemotherapy

A “screening failure” is a participant who has signed the informed consent, but who does not meet all selection criteria following the screening evaluations. For participants not considered eligible after the screening period, the reason for not being eligible must be documented. No additional assessments are needed. Participant information collected during screening will be entered in the eCRF and will be used in the study analysis.

A participant may withdraw from the study at any time. The primary reason for a participant’s withdrawal from the study should be determined if possible. The date and reason for discontinuation will be documented in the e-CRF.

8.6 Prohibitions and Restrictions

Patients who are pregnant will not be permitted to participate in this study. Pregnancy tests will be performed for all study participants (of child bearing potential) at baseline and prior to each IP administration.
All imaging will be performed in an ambulatory care setting. There are no specific precautions required for participants upon completion of Annexin and CMR imaging.

Any concomitant medication deemed necessary for the welfare of the participant during the study may be given at the discretion of the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded in full in the case report form, including any changes that have occurred during the study.

9. Investigational Product, Dose and Mode of Administration

The full name of the Investigational Drug is “Kit for the Preparation of Tc-99m Recombinant Human Annexin V-128 for Injection”.

9.1 Description of Investigational Product

The kit will be prepared, packaged and released according to the manufacturer’s Standard Operating Procedures (SOPs), and in compliance with the principles of the Good Manufacturing Practice (GMP) guidelines, ICH Good Clinical Practice (GCP) guidelines, and applicable local laws/regulations. The IP will be supplied as a sterile, single vial lyophilised kit for reconstitution at the clinical site with ^{99m}Tc solution eluted from a generator.

A single dose vial contains 0.4 mg of rhAnnexin V-128. The kit contains also stannous chloride (reducing agent), sodium □-D-Glucoheptonate dihydrate (transchelating agent), gentisic acid sodium salt hydrate (radiation stability enhancer), hydroxypropyl-□-cyclodextrin (solubilizing agent), sodium metabisulfite (antioxidant) and trehalose dihydrate (lyoprotectant and cake forming agent). Lactic acid is also present as buffering agent. The kit composition details are included in the Appendix I.

9.2 Handling, Preparation and Storage

The IP must be stored, handled and administered only by qualified/authorized personnel and must be prepared in accordance with pharmaceutical quality requirements, and radiation safety regulations. ^{99m}Tc-rhAnnexin V-128 must be administered at the investigational site. Based on the stability tests performed (Appendix I), the Annexin kit can be stored upon receipt at 5°C ± 3°C until the expiry date stated on the labels.

A single dose vial containing 0.4 mg of lyophilized rhAnnexin V-128, will be reconstituted with 2 mL ± 0.2 mL containing 740 MBq ± 74 MBq (20 mCi ± 2 mCi) of ^{99m}Tc eluted from the generator. Development studies have shown that this amount of radioactivity provides a sufficient amount of radioactivity for QC testing and the foreseen ^{99m}Tc rhAnnexin V-128 dose of 350 MBq up to 4 h after labeling. The administration volume (corresponding to 350 MBq) is calculated according to the estimated time of injection, on the basis of the physical decay of the radionuclide (half-life = 6.02 h). Stability studies performed on the Drug Product have demonstrated radiochemical purity at 6 h from labeling greater than 90%, which is in line with current specifications of the Kit for the preparation of ^{99m}Tc-rhAnnexin V-128 for injection. For the purpose of this study, it is recommended to administer the reconstituted solution within 4 hours after completion of the radiolabeling. It is also required to determine the amount of radioactivity injected to the patient by measuring the radioactivity before and after injection with an appropriate radioactivity calibration system.

The labeling reaction requires 90 minutes and the reconstituted radiolabeled product is stable for 6 hours.

Technetium-99m eluate should be obtained from an approved commercial Mo-99/Tc-99m generator that has been eluted within the past 24 hrs. The radiopharmacy will be directed to use the eluate within one hour of milking the generator.

9.3 Investigational Product Accountability

Drug inventory and accountability records for the "Kits for preparation of Technetium ^{99m}Tc-rhAnnexin V-128 for injection" will be kept by the Investigator/radiopharmacist, and must be documented throughout the study.

On an ongoing basis, the Investigator/radiopharmacist will conduct an investigational product (IP) supply inventory and to record the results of this inventory on the IP Accountability Record to reconcile delivery records with those of used and unused product. Any discrepancies must be accounted for. Appropriate forms of deliveries and returns must be signed by the person responsible for drug accountability.

Used/unused "Kits for preparation of Technetium ^{99m}Tc-rhAnnexin V-128 for injection" will be locally discarded.

Refer to Appendix I for detailed instruction on the Radiolabeled Imaging Product ^{99m}Tc-rhAnnexin V-128, Cautionary notes, Analytical and Biological Controls, Stability and Shelf Life.

9.4 Investigational Product Dose, Mode of Administration

The "Kit for preparation of ^{99m}Tc-rhAnnexin V-128 for injection" consists of 1 dose.
Participants will receive 1 dose of 350 MBq ± 10 % of^{99m}Tc-rhAnnexin V-128 administered as a single intravenous bolus over 10-20 seconds at each of the four scans.

10. Study Procedures

Voluntary written informed consent will be obtained prior to the initiation of any study-related procedures. Study conduct procedures will include screening and the imaging visits.

10.1 Baseline Assessments

- Each participant's date of birth (mm/yyyy), gender, ethnicity, weight, height, medical history, and relevant baseline characteristics will be recorded.
- Inclusion and Exclusion criteria will be checked.
- Results from any standard of care testing related to the participant's condition will be collected and will include baseline echocardiography when available.
- Women of childbearing potential must have negative pregnancy test at screening.
- Height, weight, BMI and vital signs will be recorded.

10.2 Safety Assessments

- Medical review including the measurement of the weight and BMI will be performed before each ^{99m}Tc-rhAnnexin V-128 administration.

- Measurement of vital signs (systolic and diastolic blood pressure and heart rate) will be performed 15 minutes before IP administration and at the end of the second imaging procedure, with each ^{99m}Tc-rhAnnexin V-128 administration.
- Women of childbearing potential must have negative pregnancy test at screening, before each IP administration and before each CMRI (in case the CMRI is not performed on the same day of IP administration).
- After signature of the ICF, any medical events defined as Adverse Events will be recorded and followed until the last study related procedure or until resolution.
- All medications taken from 2 weeks prior to the first administration date through the end of study will be recorded as concomitant medications.

10.3 Standard Laboratory Assessments

Blood samples for hematology, biochemistry and urinalysis will be obtained at baseline for screening, and 12 weeks after the end of chemotherapy and following any adverse effects attributed to the study procedures. Results from laboratory analysis conducted during the oncology treatment will be used for research analysis.

In the case of clinically significant abnormalities in baseline laboratory values, the participant will be declared as a screening failure.

Hematology	**Blood Chemistry**	**Urinalysis**
• WBC with differential • RBC • Platelets • Hb • MCV • Hematocrit[2]	• Total bilirubin • AP • AST/ASAT • ALT/ALAT • Gamma-GT • Creatinine[3] • ß-HCG at screening	• Dipstick test[1] • Pregnancy test *(before each injection of study product and before each CMRI, if applicable)*

[1] If any of the assessments of the dipstick test is positive, a microscopic analysis of the urine must be performed.
[2] An additional 5 ml blood sample will be collected at each CMRI visit for measurement of hematocrit to be used in conjunction with the T1 measurements to ensure accurate estimation of extracellular volume.
[3] Creatinine will be repeated for CMRI when required if not clinically available.

Laboratory Assessments, including cardiotoxicity biomarkers assessments, will be performed [redacted] at The Ottawa Hospital according to Ministry of Health and Long Term Care (MHLTC) standards.

10.4 Cardiotoxicity Biomarkers Assessments

Blood samples (3 ml) for cardiotoxicity biomarkers assessments (troponin, NT-proBNP) will also be collected at screening and before each injection of ^{99m}Tc-rhAnnexin V-128.

10.5 Immunogenicity Assessments

Anti-rhAnnexin V-128 IgG and IgM antibodies will be quantified in serum samples by ELISA. For this purpose 10 mL blood samples will be collected at screening and 12 weeks after the last dose of doxorubicin, before injection of ^{99m}Tc-rhAnnexin V-128. Serum will be prepared, divided into six aliquots and frozen (-80°C). Three out of six serum samples per time-point will be shipped to the central laboratory [redacted]. Remaining samples will be shipped only if required for analysis and destroyed at study end if not required.

10.6 ^{99m}Tc-rhAnnexin V-128 Planar and SPECT Imaging and Image Analysis

Administration of the ^{99m}Tc-rhAnnexin V-128 (350 MBq ± 10 %) will be as a single bolus via an intravenous catheter in an antecubital vein followed by a saline flush. All images will be acquired with a dual head SPECT/CT gamma camera with low-energy high-resolution collimators. The energy acceptance window for the ^{99m}Tc photopeak will be 140 keV (+/- 10%). A low dose CT scan (helical, 120 kVp, 1 mA with 1.9 pitch) will be acquired of the thorax for attenuation correction.

Planar imaging will be performed in the anterior view with acquisition using a 256 x 256 matrix, an energy window set for Tc^{99m} at 140 keV +/- 10% and a low energy, high resolution (LEHR) collimator. The image will be acquired for 1200s at one and two hours post injection of radiotracer.

Reconstruction will be done using iterative reconstruction incorporating CT-based attenuation correction and dual-energy-window scatter correction. Planar and SPECT images of the thorax will be acquired at 1 and 2 hours post-injection. Acquired images will be stored for off-line analysis using a Hermes Gold workstation (Hermes Medical Solutions). Myocardial uptake will be measured from regions of interest (ROIs) placed over the myocardium on the SPECT images co-registered with the corresponding CT images for anatomic delineation. Myocardial uptake will be expressed in absolute units (% injected dose/g or as a standardized uptake value (SUV). As a secondary analysis, a simpler approach without attenuation and scatter corrections will also be used by determination of a cardiac uptake ratio (CUR) as previously described [41] with a second group of ROIs placed over the axillary soft tissues to obtain a value representative of background.

$$CUR = \frac{\text{Myocardium (counts/pixel) - Soft tissue (counts/pixel)}}{\text{Soft tissue (counts/pixel)}}$$

Inter and intra-observer variability will be determined by repeated analysis of the images of all participants.

10.7 Cardiac Magnetic Resonance Imaging

Cardiac MRI will be performed with either a 1.5-T scanner (Siemens, Erlangen, Germany) or a 3.0-T Scanner (Siemens, Erlangen, Germany). Transverse images will be acquired with an inversion recovery prepared dark blood HASTE sequence (repetition time [TR] 600 ms, echo time [TE] 26 ms, 6 mm slice thickness, 1.8 mm interslice gap, matrix 256 x104). Cine bright-blood

images in the 4- and 2-chamber and long-axis planes will be performed with a breath-hold balanced steady-state free precession sequence (true fast imaging with steady-state precession, TR 42 ms, TE 1.2 ms, flip angle 70°, 6 mm slice thickness, matrix 192 x 174). Cine breath-hold balanced steady-state free precession short-axis images will be acquired for the entire LV from the base to the apex (stack of 10 sequential short-axis slices; TR 64 ms, TE 1 ms, flip angle 80°, 8 mm slice thickness, 1.6 mm interslice gap, matrix 192x 132) to obtain measurements of left ventricular ejection fraction.

To evaluate for myocardial edema, dark blood T2-weighted turbo spin echo short-axis images will be obtained (TR 1,800 to 2,100 ms, TE 74 ms, 8 mm slice thickness, 4 mm interslice gap, matrix 256 x 175). At each imaging session, a single 5 ml blood sample will be withdrawn for measurement of the hematocrit, to be used in conjunction with the T1 measurements to ensure accurate estimation of extracellular volume [49]. T1 maps in short-axis oblique and four chamber views using a modified Look-Locker inversion recovery, “MOLLI” will acquired before the administration of contrast to obtain native T1 values [49]. Late gadolinium enhancement (LGE) images will be obtained after 10 min of 0.2 mmol/kg injection of gadolinium (Gadovist®, Bayer Inc.) with a T1- weighted inversion recovery-prepared multislice true fast imaging with steady-state precession sequence with magnitude and phase-sensitive reconstruction. Images will be acquired sequentially in the short axis, followed by horizontal and vertical long-axis images (TR 700 ms, TE 1.0 ms, flip angle 40°, 8 mm slice thickness, 1.6 mm interslice gap, matrix 192 x 144). To quantify the myocardial mass of the LGE, the endocardial and epicardial borders of the short-axis view of the LV will be manually traced. The computer-assisted detection algorithm will be used to define LGE as any region with a signal intensity +/- 2 SD above a reference remote myocardial region. The LGE mass will be expressed as a percentage of the LV mass. 15 minutes post-injection, we will acquire post contrast T1 maps in short-axis oblique and four chamber views. All analysis including quantitative will be performed with dedicated computer software (CVI 42, Circle Cardiovascular Imaging, Calgary, Alberta, Canada) [48, 49].

10.8 Radiation Risk Assessment

Participants will receive radiation from 1) ^{99m}Tc-rhAnnexin V-128 (350 MBq) for a dose of 3.0 mSv (8.1 ± 0.8 µSv/MBq and 2) the CT scan for attenuation correction for a dose of 0.4 mSv. Total dose for the ^{99m}Tc-rh-Annexin-V 128 and CT will be 3.4 mSv for one imaging study and 13.6 mSv for the 4 administrations.

Since the annual background radiation dose at sea level in North America is 2.7 mSv, the total effect of dose for this study per patient radiation is equivalent to living 5 years in North America. This poses minimal risk to the participant and there are no expected consequences with this exposure.

11. Data Monitoring Committee

The Data Monitoring Committee (DMC) consists of investigators and Sponsor representatives, as well as external persons such as independent experts, if deemed necessary by the Sponsor. The main function of the committee will be to review the images of the first 10 participants in terms of image quality, visual efficacy (uptake) and clinical relevance of the study product, and determine

if there is a significant evidence of inadequate technical performance in this study group. The DMC will also determine if there is an excess of adverse events.
The DMC will promptly give recommendations to continue or terminate the study. The DMC report will be edited by the Sponsor and sent to the REB and regulatory authorities as required.

12. Statistical methods

12.1 Sample size and analysis

Sample size was estimated for the comparison of ^{99m}Tc-rh-Annexin-V 128 uptake in doxorubicin treated patients imaged after 2 cycles of doxorubicin versus normals baseline uptake using a 5% significance level and power of 95%. The measured heart uptake in 6 normal females (age 29.5 + 4.6 years, mean + SD) was 1.2 +/- 0.2% ID/gm in the Phase 1 study. Sample size estimations were done assuming results in the baseline studies of the patients will be similar to the normal females in the Phase 1 study.

For the comparison of ^{99m}Tc-rh-Annexin-V 128 uptake using a paired analysis and assuming a similar SD of 0.2%, a sample size of 26 patients will allow to detect a change of 0.15% ID/gm with a two-sided 5% significance level and a power of 95% (difference between two dependent means, G*Power 3.1.3).

For the comparison of left ventricular ejection fraction (LVEF) measured with CMR, a sample size of 28 patients will detect an absolute difference of 5% LVEF units with a two-sided 5% significance level and a power of 95% using a paired analysis and assuming a SD of 7 (difference between two dependent means, G*Power 3.1.3). Assumptions were based on representative CMR data for LVEF of 58 +/- 7% from 53 participants with breast cancer, lymphoma, leukemia and myelodysplastic syndrome [34].

The final study population for recruitment size will be 30 patients, allowing for 5 to 10% patient attrition or incomplete imaging data.

12.2 General statistical considerations

Continuous variables will be presented as number of non-missing values, mean, standard deviation, median, minimum, maximum and quartiles. For categorical variables, descriptive statistics will include counts and percentages per category. Confidence intervals will be computed when appropriate. Continuous variables will be compared using the most appropriate test such as one-way repeated measure ANOVA or Friedman tests with appropriate post-hoc testing if relevant. Proportions will be compared using the most appropriate test such as Chi squared test, Fisher exact test or McNemar test. Pearson's or Spearman linear regression analysis will be used to calculate the relationship between two data sets as appropriate. A p value less or equal to 0.05 will be considered significant.

No adjustment for multiplicity will be applied and missing data will not be replaced.

12.3 Demographics and Other Participant Characteristics

Demographic and other baseline data will be summarized descriptively. All background and demographic data will be listed in detail.

12.4 Efficacy

Primary endpoint of PoC:
Image quality, imaging agent uptake and clinical relevance of apoptosis imaging in the evaluation of doxorubicin-induced cardiotoxicity in patients with early stage of breast cancer using ^{99m}Tc-rhAnnexin V-128 will be determined by the DMC (visual image review and consensus).

Primary endpoint of Phase II:
The ability of ^{99m}Tc-rhAnnexin V-128 to detect doxorubicin-induced cardiotoxicity will be assessed by repeated analysis of uptake and LVEF compared to baseline using the most appropriate test such as one way repeated measures ANOVA or Friedman test, with appropriate post hoc testing if relevant.

Secondary endpoints:
Timing and extent of ^{99m}Tc-rhAnnexin V-128 myocardial uptake will be determined by an appropriate paired analysis of absolute value or Standardized Uptake Value (SUV) and Cardiac Uptake Ratio (CUR) after the 2nd and the 4thcycle of AC treatment and after 12 weeks of the last cycle of doxorubicin, compared to baseline. Differences of ^{99m}Tc-rhAnnexin V-128 myocardial uptake will be compared using the most appropriate test such as one way repeated measures ANOVA with post hoc testing

The worsening of LV function will be assessed by comparing CMRI LVEF after the 2nd and the 4thcycle of AC treatment and after 12 weeks of the last dose of doxorubicin compared to baseline. Differences of LV function will be compared using the most appropriate test such as one way repeated measures ANOVA with post hoc testing ^{99m}Tc-rhAnnexin V-128 uptake changes will be correlated with the changes in LVEF and in cardiotoxicity biomarkers using Pearson or Spearman correlation test as appropriate.

12.5 Safety

The statistical analysis of safety data will be mainly descriptive in nature.

12.5.1 Adverse Events / Serious Adverse Events

All original AE/SAE terms will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). System organ classes and preferred terms will be used in the analyses. Type and incidence of AEs/SAEs, as well as severity and relatedness to the IP will be tabulated.

12.6 Laboratory Tests

Descriptive statistics including shift tables will be generated for all laboratory tests performed i.e. the actual values and the changes from pre-injection by cross tabulations (with classes for below, within, and above normal range).

Laboratory data will be analyzed with respect to the normal ranges of values provided by the local laboratory and with respect to levels of change and significance in these values. Abnormal laboratory test results will be tabulated.

13. Adverse events and other safety aspects

13.1 Definition of Adverse Events

An AE is defined as any untoward medical occurrence in a participant and which does not necessarily have a causal relationship with the IP. An AE can therefore be any unfavorable and unintended sign (including an abnormal clinically significant laboratory finding), symptom, or disease, temporally associated with the use of an IP, whether or not causally related to the IP.

AEs will be reported, if applicable, from the signing of the informed consent until the last study-related procedure. AE severity will be assessed according to the grading system of the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE) as included in Appendix II.

13.2 Definition of Serious Adverse Events

A SAE is any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening;
  Note: “life-threatening” refers to an event in which the participant is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe;
- Results in persistent or significant disability/incapacity;
- Results in congenital anomaly or birth defect;
- Requires in-participant hospitalization or leads to prolongation of hospitalization, with the exception of elective pre-planned hospitalizations.

Serious Adverse Events (SAE) will be defined and reported according to ICH/GCP and Regulatory Standards. SAEs will be reported from the signing of the informed consent and followed until resolution or determined to be not clinically significant. The specific SAE Reporting Form should be used (Appendix IV).

13.3 Procedure in Case of Pregnancy

Prior to clinical study enrolment, women of childbearing potential must be advised of the importance of avoiding pregnancy during clinical study participation and the potential risks for an unintentional pregnancy. During the clinical study, all women of childbearing potential should use of a reliable means of contraception, and should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g. missed or late menstrual period).

The Investigator must report to the Sponsor any pregnancy associated with IP exposure including conceptions occurring until 30 days after the IP administration using the specific Pregnancy Form (Appendix V). Appropriate pregnancy follow-up procedures will be considered if indicated. The

Investigator should respond in accordance with the reporting procedure for SAEs including information regarding the outcome of pregnancy.

13.4 New Safety Information Affecting the Conduct of the Study

Any information or changes significantly affecting the conduct of the trial and/or increasing the risk to participants will be provided to all investigators, REB(s), and Regulatory Authorities. Depending on the nature of the information or the changes, the protocol and/or the participant information may necessitate an amendment.

13.5 Data Monitoring and Safety

The data collected for this study is observational in design and will not be used for clinical care or clinical decision making. No formal DSMB will be formed. However, the safety data will be part of the review and assessment by the DMC.

14. Termination of the study

Early termination of the study can occur in the following cases:

- When the visual review and analysis of the images of the first 10 participants by the DMC does not demonstrate the diagnostic potential of the study product in terms of quality or efficacy, the Sponsor may discontinue the clinical study by sending a written notice to the investigator and competent authorities.
- When the Sponsor is aware of new information on matters concerning the safety of the study product, as well as other important information that may affect proper conduct of the clinical study, the Sponsor may discontinue the clinical study and send a written notice of the discontinuation to the investigator and competent authorities.
- If the investigator intends to discontinue participation in the study, the investigator must immediately inform the Sponsor in written about the discontinuation and the reason for it.
- The study is stopped by a regulatory authority.
- The Sponsor reserves the right to discontinue the study at any time for any reason by sending a written notice of the discontinuation to the investigator and competent authorities.

15. Operational, Ethical and Administrative Considerations

15.1 Clinical Study Monitoring and Audit

Periodic study monitoring will be conducted by AAA to ensure that participants' human rights, safety, and well-being are protected, that the study is properly conducted in adherence to the current protocol and the principles of ICH GCP as well as Declaration of Helsinki, and that the data reported by the Investigator or designee are accurate, complete, and verifiable with the source documents. The assigned Clinical Study Monitor(s) will monitor the study in accordance with the monitoring guidelines. A copy of the Monitoring Log will be retrieved for the trial master file at the study close-out visits. The designated monitor will have the training and qualifications necessary to provide an appropriate and thorough verification of the study files. During the study, the Investigator will permit the Study Monitor to verify the progress of the study at the centre as

frequently as necessary. The Investigator will make the electronic data screens available, provide missing or corrected data, and sign the e-CRF. Key data transcribed into the e-CRF will be reviewed against the source documents. Personal information will be treated as strictly confidential and will not be made publicly available. Any inconsistency between the source data and the data recorded in the e-CRF will be corrected.

The Sponsor will ensure that appropriate Quality Control (QC) steps are included into the different clinical processes to guarantee adequate protection of the study participants and quality of the data.

The Investigator is responsible for the accuracy of the data entered on the e-CRFs. The relevant pages of each e-CRF should be fully completed within 5 working days of the participant's visit to which the data relate, to allow the Clinical Research Associates (CRAs) to review these pages promptly. Sufficient resources must be available to prompt e-CRF completion and collection. Request for clarifications to data (data queries) should be addressed within 5 working days of receipt.

The Investigator shall allow designated CRO/Sponsor representatives, representatives of the REB and regulatory bodies direct access to the source documents (paper or electronic) to verify the data reported in the e-CRFs and data queries (source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the participant and substantiate the integrity of the data collected during the trial). Source documents should be available to support all the data recorded in the e-CRF, unless otherwise specified in the e-CRF and data queries.

The Regulatory Authorities and/or the Ottawa Hospital Science Network Research Ethics Board (OHSN-REB) may review this study. This implies that auditors/inspectors have the right to inspect the study centre at any time during and/or after completion of the study and have access to source documents, including the participant's file.

15.2 Study Documents

Source data must be maintained at the study centre to document the existence of the study participants and substantiate the integrity of the collected study data. The author of an entry in the source documents must be identifiable.

The source documents should at least include the following information for each participant:

- Participant identification;
- Documentation of eligibility criteria;
- Consent process, signed and dated ICF;
- Dates of all visits, study procedures documentation;
- Images/scans;
- Investigational product administration time and date;
- Receipt, dispensation and destruction of used/unused investigational product;
- Record of all AEs, SAEs and other safety parameters;

- Date of study completion or reason for early discontinuation (if applicable).

15.3 Ethics and Protection of Participant Confidentiality

15.3.1 Ethical Conduct of Clinical Study

The Investigator(s) and all parties involved in this study will conduct the study in adherence to the ethical principles based on the Declaration of Helsinki, GCP, ICH guidelines, and the applicable laws and regulations.

ICH-GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting study activities that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of the participants are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the study data are credible.

The Investigator and all study staff will conduct the study in compliance with the REB and regulatory approved version of this protocol. The protocol, ICF, any information provided to the participant as well as any recruitment advertisements (if applicable), and any amendments to these items will have REB approval prior to their use in the study. Voluntary informed consent will be given by every participant in order to participate in the study-related procedures. The rights, safety, and well-being of the participants are the most important considerations and prevail over the interests of science and society. All personnel involved in the conduct of this study must be qualified by education, training, and experience to perform their assigned responsibilities.

15.3.2 Authorities

The protocol, name, and study centre of the Investigators, as well as other required documents will be submitted to Health Canada (Regulatory Authority), the Ottawa Hospital Science Network Research Ethics Board and the Ottawa Heart Institute Research Corporation according to requirements for review and approval before the beginning of the study. The Authorities will be informed about the end of the study and the results of the study in a way that guarantee confidentiality and Sponsor’s rights in terms of intellectual and/or industrial property.

15.4 Arrangement for Use of Information and Publication of Clinical Study Data

All information regarding the IP under study in the outlined protocol and the manufacturer’s operations, such as, but not limited to, patent applications, formulas, manufacturing processes, scientific data, or formulation information, supplied by the manufacturer and not previously published, are considered confidential and shall remain the sole property of the IP manufacturer. The Investigator agrees to use this information only to perform this study and will not use it for other purposes including publications and presentations without the IP manufacturer’s explicit written consent.

It is understood by the Investigator that the information developed during the conduct of this study including but not limited to study data and results is considered confidential and the sole property of the Sponsor, and will be used by the IP manufacturer for the development of the specified

investigational medication and may be disclosed as deemed necessary by the IP manufacturer to other Investigators, other pharmaceutical companies, and to governmental agencies.

The Investigator agrees that before he/she publishes any results or data of this study, including preliminary results, he/she shall send the draft manuscripts and copies of the information to be presented to the IP manufacturer at least 30 working days before submission to a publisher or presentation. The IP manufacturer reserves the right to review these materials before submission for publication or presentation. This is not intended to restrict or hinder publication or presentation but instead to allow the IP manufacturer to protect proprietary information and to provide comments based on information that may not yet be available to the Investigator(s).

15.5 Records Related to the Clinical Study and Confidentiality

The Investigator must retain e-CRFs and source documents of all enrolled participants (i.e. all participants who gave consent to be screened for the study), IP disposition, and other documents required by regulation, in his/her possession or in an accessible area for at least 25 years after the completion of this study.

Individual nominative participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Such information may only be given to a third party after approval of the participant, such as to the participant's general practitioner or to other appropriate medical personnel responsible for the participant's well-being.

All individuals and organizations involved in conducting the study and/or processing the study data will protect the participants' privacy with appropriate measures in accordance with the applicable local and regional laws.

15.6 Protocol Amendment and/or Revision and Protocol Deviations

Any deviation from the protocol that has not been approved by the Sponsor or designee and by the REB/regulatory authority may result in the discontinuation from the trial of the site involved. However, in the event of any medical emergency, the Investigator is free to perform any medical procedure deemed appropriate. Such events and procedures must be reported promptly to the Sponsor.

Any changes to the study, which arise after approval of the protocol, will be documented as protocol amendments and/or revisions. Depending on the nature of the amendment and/or revision, REB/ Regulatory Authority approval or notification is required. The changes will become effective only after the approval of the REB/Regulatory Authority (if applicable).

15.7 Qualification of the Investigators and delegation of responsibility

The Investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the study. He/she should meet all qualifications specified by the applicable regulatory requirements and should provide evidence of such qualifications through an up-to-date curriculum vitae (Principal as well as all other Investigators) and/or other relevant documentation requested.

The Investigator should be thoroughly familiar with the appropriate use of the IP, as described in the protocol, the current Investigator's Brochure, the product information, and other information sources provided by the IP manufacturer.

The Investigator should be aware of, and should comply with, ICH-GCP and the applicable regulatory requirements.

The Investigator should maintain a list of appropriately qualified persons to whom the Investigator has delegated significant study related duties.

15.8 Conflict of Interest and Financial Disclosure

There are no conflicts of interest to declare related to this study. The Principal Investigator is receiving financial support and the IP from the manufacturer to cover the cost of conducting this study.

Disclosable financial interests will be recorded on the Investigator Financial Disclosure Form.

Any Investigator(s) added as investigational staff must complete the Investigator Financial Disclosure Form at the beginning of his/her participation to the study. For any Investigator(s) leaving the site prior to study completion, an Investigator Financial Disclosure Form should be obtained at the end of his/her participation.

15.9 Investigator and Manufacturer Indemnity

The investigator and the Sponsor will provide appropriate medical treatment and care in the event of a study related injury or illness.

The Sponsor has covered this study by means of insurance for the participant according to national requirements. The name and the address of the relevant insurance company, the certificate of insurance, the policy number, and the sum insured are provided in the Investigator's File.

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Appendix I – Radiolabeling procedure of rh-Annexin V-128 and quality controls

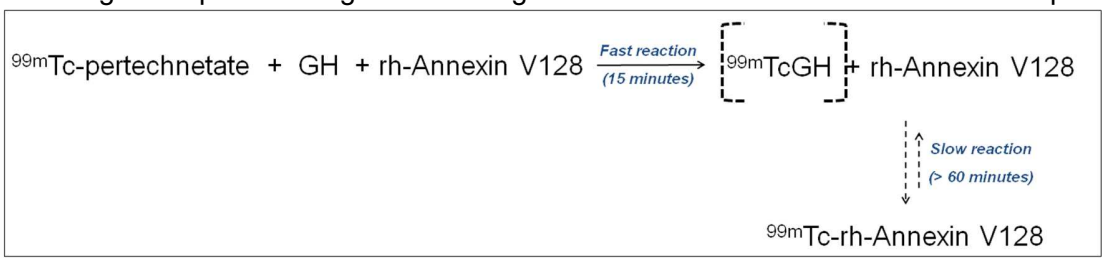
Radiolabeling procedure

The Technetium solution to be used for the labeling of rh-Annexin V-128 kit is the solution which can be obtained by commercially available Tc Generators compliant with the Ph.Eur. Monograph "Sodium Pertechnetate (^{99m}Tc) Injection (Fission)" and Tc 99m Injection USP.
During the product development, the following procedures and conditions have been tested and confirmed as adequate:

a) Radioactive concentration of the Pertechnetate solution added for labelling should be 370 MBq/mL (the reconstitution volume is 2 mL ± 0.2 mL).

Labelling at room temperature under slight rotation by introducing the vial in a shielded roller for 90 min: shorter incubation times may result in inadequate labelling.
Stability of the Radiolabelled compound up to 6h at room temperature (RCP 90 %).
Suitability of the Radio TLC method to determine the radiochemical yield and purity by using three different eluents: Acetone 100% ($^{99m}TcO_4^-$, R_f=1.0); Anticoagulant Citrate Dextrose solution (ACD) (^{99m}Tc-rhAnnexin V-128, R_f=0.0); PBS (^{99m}Tc-Glucoheptonate, R_f=1.0 and $^{99m}TcO_2$,R_f=0.0).

Labelling takes place through an exchange reaction with 99m-Technetium-Glucoheptonate.



Scheme of the exchange reaction between ^{99m}Tc-Glucoheptonate (^{99m}Tc-GH) and rhAnnexin V-128.

The instructions for the preparation of the ^{99m}Tc-rhAnnexin V-128 are in accordance with the following aseptic procedure:

1) Remove one rhAnnexin V128 Kit reaction vial from refrigerated storage and allow it to reach room temperature (from 5 to 10 minutes).
2) Waterproof gloves should be worn during the preparation procedure. Flip off cap from the rhAnnexin V-128 Kit vial and swab the top of the vial closure with an appropriate antiseptic to disinfect the surface, and then allow the stopper to dry.
3) Place the vial in a suitable radiation shield appropriately labelled with date, time of preparation, volume and activity.
4) Aseptically add 2 mL ± 0.2 mL of Sodium Pertechnetate Tc-99m solution containing 740 MBq ± 74 MBq (20 mCi ± 2 mCi) to the vial in the lead shield (**Do not shake**).
5) Remove the vial from the lead shield and place it in an appropriately shielded roller. Leave the vial under slow rotation for 90 min.
6) Remove the vial from the shielded roller, inspect visually for the absence of particulate matter and discoloration and place it again in a lead shield.

7) Aseptically withdraw material using a sterile shielded syringe. The so-obtained solution is stable for 6 hours after completion of radiolabelling reaction. For the purpose of this study it is recommended to use it within 4 hours after preparation.
8) Radiochemical purity should be checked prior to patient administration according to the Radio TLC Method.

Cautionary Notes:

- Tc-99m pertechnetate eluate should be obtained from a generator which has been eluted within the last 24 hours.
- Tc-99m pertechnetate eluate which is more than 6 hour old from the time of elution should NOT be used.

Stability and Shelf Life of the Kit for radiopharmaceutical preparation of ^{99m}Tc-rhAnnexin V-128

Stability evaluation was performed so far on the lab-scale, engineering and first GMP batches. Stability data indicate that the product is stable up to 12 months both at 5°C ± 3°C (long term condition) and up to 9 months at 25°C ± 2°C/60% RH ± 5% RH (accelerated conditions), with high radiochemical purity (RCP, well above 90%) and good biological activity (Biopotency, higher than 90%). Since the Drug Product confirmed to be stable after 12 months at the long term storage conditions (5°C ± 3°C), the current applied shelf-life is 12 months at 5°C ± 3°C.

The stability evaluation will be performed on future GMP batches in order to obtain complete stability data on at least three GMP batches of the Drug Product up to 12 months at the intended long term storage temperature (5°C ± 3°C). As mentioned above, the stability assessment is being also performed at the accelerated temperature condition (25°C ± 2°C/60% RH ± 5% RH), up to 9 months.

Stability of the Finished Product (^{99m}Tc-rhAnnexin V-128 radiolabelled Imaging Agent)

The stability of the Finished Product is 6 h after labeling at room temperature. At this time the Radiochemical Purity is still ≥ 90%. For the purpose of this study it is recommended to inject the product within 4 h after completion of the radiolabelling reaction.

Appendix II - Eastern Cooperative Oncology Group (ECOG) Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Revised: July 27, 2006

Appendix III - National Cancer Institute Common Terminology Criteria for Adverse Events

This is an extract of the whole document. For the complete CTCAE guide, version 4.0, please refer to the following website:
http://www.acrin.org/Portals/0/Administration/Regulatory/CTCAE_4.02_2009-09-15_QuickReference_5x7.pdf

Common Terminology Criteria for Adverse Events v4.0 (CTCAE)

Publish Date: May 28, 2009

Quick Reference

The NCI Common Terminology Criteria for Adverse Events is a descriptive terminology which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term.

Components and Organization

SOC

System Organ Class, the highest level of the MedDRA hierarchy, is identified by anatomical or physiological system, etiology, or purpose (e.g., SOC Investigations for laboratory test results). CTCAE terms are grouped by MedDRA Primary SOCs. Within each SOC, AEs are listed and accompanied by descriptions of severity (Grade).

CTCAE Terms

An Adverse Event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may *not* be considered related to the medical treatment or procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses. Each CTCAE v4.0 term is a MedDRA LLT (Lowest Level Term).

Definitions

A brief definition is provided to clarify the meaning of each AE term.

Grades

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

A Semi-colon indicates 'or' within the description of the grade.

A single dash (-) indicates a grade is not available.

Not all Grades are appropriate for all AEs. Therefore, some AEs are listed with fewer than five options for Grade selection.

Grade 5

Grade 5 (Death) is not appropriate for some AEs and therefore is not an option.

Activities of Daily Living (ADL)

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

† CTCAE v4.0 incorporates certain elements of the MedDRA terminology. For further details on MedDRA refer to the MedDRA MSSO Web site (http://www.meddramsso.com).

Serious Adverse Event (SAE) Reporting Form

ALL REPORTS MUST BE SIGNED AND DATED BY THE INVESTIGATOR.

Please fax or send by e-mail the form to AAA Global Pharmacovigilance within 24 hours from awareness of event.

E-mail: [redacted]
Fax N°: [redacted]

Study Name	EudraCT N°	Center N°	Investigator N°	Country

TYPE OF REPORT
☐ Initial ☐ Completion of data ☐ Follow-up

1. PATIENT DATA - DO NOT SEND PATIENT IDENTIFIABLE DATA				
Patient Initials (first letter of First Name and first letter of Last Name)**:** Last name ⌊_⌋ First name ⌊_⌋	**Patient N°:**	**Sex:**	**Year of Birth** (yyyy)**:** **Age at onset of event:**	**Weight** (kg)**:** **Height** (cm)**:**

2. EVENT DETAILS	
Date of onset (dd/mm/yyyy):	**Diagnosis:**
Description of SAE (please state date of first use)**:**	

Seriousness Criteria (check all that are relevant to the event):

☐ Participant died
☐ Hospitalisation or prolongation of existing hospitalisation
☐ Life-threatening
☐ Persistent or significant disability or incapacity
☐ Congenital anomaly/ birth defect
☐ Other significant medical events

Severity of event: ☐ Mild ☐ Moderate ☐ Severe

3. STUDY TREATMENT

Drug	Schedule	Route of administration	Start date (dd/mm/yyyy)	End date (dd/mm/yyyy)	Causally Related to Drug? *Tick either unrelated or possibly related*		Expected (Y/N)
					Unrelated	Possibly Related	
1.							
2.							
3.							
4.							

4. NIMPs (Non-investigational medicinal products)

Are there any additional medications **used as part of the protocol**? *Such medications are referred to as NIMPs*

Yes ☐ No ☐

If yes, please complete the table below

NIMP(s)	Dose/schedule	Route of administration	Start date (dd/mm/yyyy)	End date (dd/mm/yyyy)	Causally Related to NIMP? *Tick either unrelated or possibly related*		Expected (Y/N/NA)
					Unrelated	Possibly Related	
1.							
2.							
3.							

5. CONCOMITANT DRUGS RELEVANT TO THE SAE *(do not include therapy used to treat the SAE)*

☐ Tick box if no relevant concomitant medication

Drug name	Dose/schedule	Route of administration	Reason for use	Start date (dd/mm/yyyy)	End date (dd/mm/yyyy)	Continued? (Y/N)
1.						
2.						
3.						

4.						
5.						
6.						

6. MEDICAL HISTORY (list relevant medical history):

☐Tick box if no relevant medical history

Condition	**Start Date** (dd/mm/yyyy)	**End date** (dd/mm/yyyy)	**Ongoing (Y/N)**	**Medication required** Y/N
1.				
2.				
3.				
4.				

7. RELEVANT TEST/LABORATORY FINDINGS *(include only the results relevant to the SAE diagnosis or course of SAE)*

Test/lab finding	**Unit**	**Date** (dd/mm/yyyy)	**Value**	**Reference range**
1.				
2.				
3.				
4.				

Comment on test/laboratory findings (if none, mark as NA)

8. ACTION TAKEN (check all that are relevant to the SAE)		
☐ No action taken	☐ Drug permanently discontinued due to this SAE	☐ Concomitant medication taken
☐ Drug schedule adjusted/temporarily interrupted *If multiple drugs used, please record which drug(s) have been adjusted/interrupted:*	☐ Non-drug therapy given	☐ Hospitalisation/prolonged hospitalisation
9. OUTCOME OF SAE		
☐ Completely recovered Date of recovery (dd/mm/yyyy)	☐ Condition still present and unchanged	☐ Recovered with sequelae Date (dd/mm/yyyy) Describe sequale:
☐ Condition deteriorated	☐ Condition improving	☐ Death Date of death (dd/mm/yyyy)
Autopsy: Yes ☐ No ☐ N/A ☐ If Yes, include relevant information:		
10. ADDITIONAL INFORMATION		
11. INFORMATION SOURCE		
Name, address and telephone number of PI		
Date of report (dd/mm/yyyy)		
PI signature		

TO BE COMPLETED BY AAA GLOBAL PV (INTERNAL USE ONLY)

DATE OF RECEIPT: (dd/mm/yyyy)	
AAA CASE TRACKING NUMBER:	
INFORMATION COMPLETE: ☐ Yes ☐ No Name and signature:	FOLLOW-UP REQUESTED: ☐ YES ☐ NO Details:

PREGNANCY REPORTING FORM

ALL REPORTS MUST BE SIGNED AND DATED BY THE INVESTIGATOR.
Please fax or send by e-mail the form to AAA Global Pharmacovigilance within 24 hours from awareness of event.

E-mail: [redacted]
Fax N° : [redacted]

N.B : Fill the applicable fields and refer to Completion Guide of Pregnancy Reporting Form.

TYPE OF REPORT: ❒ Initial ❒ Completion of data ❒ Follow-up n° ……

Study Name	*EudraCT N°*	*Center N°*	*Investigator N°*	*Country*

1. PATIENT INFORMATION

Patient initials *(first letter of Last Name and first letter of First Name)*	**Patient N°:**	**Birthdate (YYYY):**	**Weight (kg):**
Last name ⌊_⌋ **First name** ⌊_⌋		⌊_⌋⌊_⌋⌊_⌋⌊_⌋ **Age**: ……	**Height (cm):**

2. PREGNANT WOMAN'S GENERAL MEDICAL HISTORY / BACKGROUND

BACKGROUND

Rhesus:	❒ Unknown ❒ Rh - ❒ Rh +
Smoking:	❒ Unknown ❒ No ❒ Yes …… *(pack-years)*
Alcohol:	❒ Unknown ❒ No ❒ Yes …… *(glasses/day)*
Drug abuse(s):	❒ Unknown ❒ No ❒ Yes …… *(detail)*

PRIOR IMMUNIZATIONS

Rubella:	❒ Unknown ❒ No ❒ Yes
Toxoplasma:	❒ Unknown ❒ No ❒ Yes

MEDICAL HISTORY

Hypertension:	❒ Unknown ❒ No ❒ Yes
Diabetes mellitus:	❒ Unknown ❒ No ❒ Yes
Epilepsy:	❒ Unknown ❒ No ❒ Yes
Psychiatric diseases:	❒ Unknown ❒ No ❒ Yes

VIRAL SEROLOGY

HIV:	❒ Unknown ❒ No ❒ Yes
Hepatitis:	❒ Unknown ❒ No ❒ Yes

3. PREGNANT WOMAN'S GYNECOLOGICAL & OBSTETRICAL HISTORY

GYNECOLOGICAL HISTORY

Used contraception: ❒ None ❒ Oral ❒ Local ❒ Intra-uterine device ❒ Other ……..…… *(detail)*

Regular menses: ❒ No ❒ Yes

Infertility treatment: ❒ No ❒ Yes ……..…… *(detail)*

OBSTETRICAL HISTORY

Gravidity: ❒ No ❒ Yes …….. *(detail)*

Parity: ❒ No ❒ Yes …….. *(detail)*

***In utero* demise:** ❒ No ❒ Yes …….. *(detail)*

Number of healthy live offspring: ……

Number of death offspring: ……

Number of malformated live offspring: ……

Abortions: ❒ No ❒ Yes

If yes, please precise:

❒ Spontaneous ……..…… (*detail*)

❒ Elective …….. (*detail*)

❒ Therapeutic ……..…… (*detail*)

4. MATERNAL & PATERNAL FAMILY HISTORY

Malformations: ❒ Unknown ❒ No ❒ Yes ……..…… *(detail)*

Prematurely died children: ❒ Unknown ❒ No ❒ Yes ……..…… *(detail)*

Psychomotor retardation: ❒ Unknown ❒ No ❒ Yes ……..…… *(detail)*

Consanguinity: ❒ Unknown ❒ No ❒ Yes ……..…… *(detail)*

Hereditary disease: ❒ Unknown ❒ No ❒ Yes ……..…… *(detail)*

Other: ……..…… *(detail)*

5. CURRENT PREGNANCY STATUS AT TIME OF DETECTION

Last menstrual period:

⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋

Estimated date of delivery:

⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋

Gestational age: ……

Ultrasound-estimated gestational age: ……

Polyzygotic pregnancy: ❒ No ❒ Yes

Ectopic pregnancy : ❒ No ❒ Yes

6. COURSE OF CURRENT PREGNANCY

DELETERIOUS EXPOSURES DURING PREGNANCY

Smoker: ❒ No ❒ Yes ……..…… *(pack-years)*

Alcohol: ❒ No ❒ Yes …….. *(glasses/day)*

Drug abuse(s) : ❒ No ❒ Yes ……..…… *(detail)*

Other : ……

PATHOLOGY (IES) DURING PREGNANCY

Hypertension : ❒ No ❒ Yes

Diabetes: ❒ No ❒ Yes

Infection : ❒ No ❒ Yes

Other : ……

MEDICATION RECEIVED AFTER PREGNANCY DETECTION

Name	Posology *(with units)*	Administration route	Indication(s)	Administration dates *(start/end)*

1.				
2.				
3.				
4.				
5.				

MEDICAL FOLLOW-UP

Hospitalization during pregnancy: ❒ No ❒ Yes …….. *(reason)*

Intrauterine growth retardation: ❒ No ❒ Yes …….. *(detail)*

Prenatal diagnosis: ❒ No ❒ Yes

Ultrasound: ❒ No ❒ Yes *If yes, please precise:*

Date: ⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Results: ……..

Date: ⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Results: ……..

Date: ⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Results: ……..

Invasive method: ❒ No ❒ Yes *If yes, please precise:* …….. *(method)*

Date: ⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Results: ……..

Toxicology screen: ❒ No ❒ Yes *If yes, please precise:* …….. *(drug / body fluid)*

Date: ⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Results: …….. (*level*)

7. OUTCOME OF CURRENT PREGNANCY

Live newborn: ❒ No ❒ Yes *If NO, please precise:*

❒ Spontaneous abortion Date: ⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Term: …… *(week)*

❒ Elective abortion Date: ⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Term: …… *(week)*

❒ Therapeutic abortion Date: ⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Term: …… *(week)*

❒ *In utero* demise Term: …… *(week)*

Malformations: ❒ No ❒ Yes …….. *(detail)*

Histopathology: ❒ No ❒ Yes …….. *(detail)*

8. DELIVERY

Delivery date:
⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋

Gestational age:
…… *(weeks)*

❒ Normal delivery
❒ Induced delivery
❒ Caesarean section

Fetal distress: ❒ No ❒ Yes *(If yes)* ❒ Chronic ❒ Acute

Normal placenta: ❒ No ❒ Yes ❒ Unknown

Amniotic fluid: ❒ Clear ❒ Turbid

Postpartum maternal condition: ❒ Normal ❒ Abnormal …….. *(detail)*

Intrapartum medication: ❒ Unknown ❒ No ❒ Yes …….. *(if yes, detail)*

9. NEWBORN

Sex : ❒ F ❒ M

Weight: ……..... *(unit)*

Length: ……..... *(unit)*

Cranial perimeter: ……..... *(unit)*

Preterm: ❒ No ❒ Yes

Dysmature: ❒ No ❒ Yes

APGAR score: ……..…. *(1 min)*

……..…. *(5 min)*

Intensive care: ❒ No ❒ Yes ❒ Unknown

Malformation : ❒ No ❒ Yes ……... *(detail)*

Neonatal pathology:

Breastfeeding: ❒ No ❒ Yes ……... *(detail)*

❒ No ❒ Yes

Transfer to NICU / pediatrics: ❒ No ❒ Yes …….. *(duration)*

Immediate outcome: ……..…….....

Department address: ……..…….. ……

Child's follow-up performed by: ……..…….. ……

10. INITIAL REPORTER

Occupation:

Full name:

Organization/Adress:

Telephone:

Fax:

Email:

Date & signature _____

<u>If the reporter is a patient or not a Healthcare Professional:</u>

Has the patient given the authorization to AAA to follow up the pregnancy with its treating Doctor? ❒ Yes ❒ No

Date & signature _____

<u>Details of the treating Doctor</u> *<u>(if different from the reporter)</u>*:

Occupation:

Full name:

Organization/Address:

Telephone/Fax:

Email:

ADMINISTRATIVE INFORMATION (for internal use only)

AAA Case #: ……

TYPE DE RAPPORT: ❒ Initial ❒ Follow up n°: ……

Reception date by AAA PV department

⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋

Reception date by a partner

⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋

<u>Initial reception by</u>:

NAME:

OCCUPATION:

LOCAL AFFILIATE/COUNTRY:

DATE: ⌊_⌋⌊_⌋/⌊_⌋⌊_⌋/⌊_⌋⌊_⌋⌊_⌋⌊_⌋

SIGNATURE _____

Data collected during a Pharmacovigilance investigation may be stored in a database according to Regulation (UE) no 520/2012, Regulation (CE) no 726/2004 and directive 2001/83/CE.