Study Protocol	
Protocol Number	AAA-Annexin-04 / NCT02667457
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 Planar and SPECT Imaging of Apoptosis in Asymptomatic Or Previously Symptomatic with TIA Patients with Carotid Atherosclerotic Plaque
Short Trial Title	^{99m}Tc-rhAnnexin V-128 imaging for Carotid Atherosclerosis
Version and Date	v.5.0 dated 2 February2018
Investigators	**Principal Investigator:** [redacted], MD, FRCPC, FACC **Co-Investigators:** [redacted], MD, FRCPC, FACC [redacted], MD, FRCPC, FACC [redacted], PhD, 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 Planar and SPECT Imaging of Apoptosis in Asymptomatic Or Previously Symptomatic with TIA with Carotid Atherosclerotic Plaque

PROTOCOL NUMBER: AAA-Annexin-04, Version 5.0, 2 February 2018

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

Role	Name and contact	Date & Signature
[redacted]	[redacted], PhD, DSc Advanced Accelerator Applications SA Tel: [redacted] Cell: [redacted] E-mail: [redacted]	
[redacted]	[redacted], MD, PhDAdvanced Accelerator Applications SA Tel : [redacted] E-mail: [redacted]	
[redacted]	[redacted], PharmD Advanced Accelerator Applications SA Tel: [redacted] Fax: [redacted] Cell: [redacted] E-mail: [redacted]	

INVESTIGATOR ENDORSEMENT PAGE

I agree to conduct the study as outlined in the protocol entitled "^{99m}Tc-rhAnnexin V-128 Planar and SPECT Imaging of Apoptosis in Asymptomatic Or Previously Symptomatic with TIA Patients with Carotid Atherosclerotic Plaque" 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. Reports of serious adverse events to the Sponsor/CRO within 24 hours by telephone and a written report of the serious adverse event within 72 hours after the Investigator's initial receipt of the information;

7. Reporting of Serious Adverse Events (SAE) according to ICH/GCP and regulator standards. SAE 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 Planar and SPECT Imaging of Apoptosis in Asymptomatic Or Previously Symptomatic with TIA Patients with Carotid Atherosclerotic Plaque
Principal Investigator and Study Site	Dr. [redacted], MD, FRCPC, FACC Ottawa Heart Institute, Ottawa Hospital, Division of Nuclear Medicine, 40 Ruskin Street, Ottawa, Ontario, Canada K1Y 4W7
Sponsor	Advanced Accelerator Applications
Study Indication	Patients with diagnosed asymptomatic carotid atherosclerotic plaque
Objectives	Primary Objectives • To determine the feasibility of imaging apoptotic activity in carotid atherosclerotic plaques of asymptomatic patients or patients that have been previously symptomatic with TIA only with significant carotid artery disease by using ^{99m}Tc-rhAnnexin V-128 and to report the prevalence of abnormal^{99m}Tc-rhAnnexin V-128 scans. Secondary Objectives: • To establish the uptake of ^{99m}Tc-rhAnnexin V-128 in a control group of participants with normal carotid ultrasounds. • To assess the ^{99m}Tc-rhAnnexin V-128 uptake versus plaque(s) echogenicity and echolucency. • To measure the aortic uptake of ^{99m}Tc-rhAnnexin V-128 and assess the correlation with its carotid uptake.
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 has demonstrated the ability to image cellular stress, membrane depolarization (i.e. acute and chronic pain in animal models and cell culture), apoptosis and necrosis in a variety of inflammatory diseases including atherosclerosis, myocarditis, acute heart transplant rejection, rheumatoid arthritis, prosthetic joint infection and Crohn's disease in both animal models and humans.
Planned number of participants	Thirty five evaluable asymptomatic or previously symptomatic with TIA only patients with diagnosed carotid atherosclerotic plaque. In addition, a control group of 10 participants with normal carotid ultrasound imaging will be enrolled.

	Fifteen patients and 5 normals will be recruited in the first part of the trial, as a Proof of Concept (PoC). The PoC study will assess the imaging potential of ^{99m}Tc-rhAnnexin V-128 in terms of imaging quality, uptake and medical relevance to enable the decision to continue the clinical investigation with the remaining 20 patients.
Study design and Methodology	This is a single-centre, single dose, PoC, Phase II study. Participants who have signed the informed consent and are eligible to participate in the study will undergo the following assessments: • A screening visit will be conducted within 8 weeks ^{99m}Tc-rhAnnexin V-128 imaging. Eligible and consenting participants will undergo a limited physical examination (height, weight, BMI), vital signs (systolic and diastolic blood pressure, heart rate), blood analysis and urinalysis. The following blood analysis will be conducted: CBC with automated differential, general chemistry panel (SMA-20) including, PT/PTT, INR (international normalized ratio for PT values) and assessment of anti-annexin V-128 antibodies. • Carotid ultrasound will be carried out within 8 weeks prior to the ^{99m}Tc-rhAnnexin V-128 planar and SPECT/CT imaging. Imaging visit: planar and dedicated SPECT/CT imaging of the carotids and aorta will be done 60 minutes after injection of tracer, and 120 minutes after injection. Vital signs assessment/participant monitoring will be done throughout the visit. • Within 24 hours after the administration of ^{99m}Tc-rhAnnexin V-128, participants will be called for the assessment of possible adverse events. • 30 +/- 3 days post ^{99m}Tc-rhAnnexin V-128 injection, all participants will return to clinic for a final blood sampling to rule out the development of anti-annexin V-128 antibodies. The following blood analysis will also be conducted: CBC with automated differential, general chemistry panel (SMA-20) including, PT/PTT, and INR. • Once the first 15 patients and 5 normals have been administered with ^{99m}Tc-rhAnnexin V-128 and have performed the imaging visits, the Data Monitoring Committee will review the images. The visual assessment of the scans in these first 20 participants will support the continuation or the 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 the imaging visit (Day 0).
Inclusion/ exclusion criteria	**Inclusion criteria** For all participants: 1. Males and females age 18 years or greater 2. Able and willing to comply with the study procedures 3. Negative pregnancy test for women of childbearing potential at screening and on the day of administration of ^{99m}Tc-rhAnnexin V-128.

	For participants with carotid artery disease: 4. Evidence of 50% or more carotid stenosis in one or more carotid arteries on carotid ultrasound within 2 years; For control participants: 4. No significant carotid artery disease on carotid ultrasound; **Exclusion criteria** 1. Previous carotid stenting or endarterectomy or stroke; 2. Diagnosis of vasculitis, dissection, or non-atherosclerotic carotid disease (Ehlers-Danlos, Marfans); 3. Pregnancy or lactation; 4. History of any disease or relevant physical or psychiatric condition or abnormal physical finding which may interfere with the study objectives at the investigator judgment; 5. Know hypersensitivity to the investigational product or any of its components; 6. Claustrophobia or inability to lie still in a supine position; 7. 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; 8. Unwillingness to provide consent.
Study duration and assessments	Maximum 3 months (+/- 3 days) including the screening period for an individual participant
Safety	Venous blood samples will be drawn for each participant at screening and at 30 +/- 3 days post IP administration for laboratory analysis (CBC with diff., SMA-20, PT/PTT, INR). To rule out development of anti-annexin V-128 antibodies, venous blood samples will also be drawn for each participant at screening and 30 +/- 3 days post ^{99m}Tc-rhAnnexin V-128 injection. Assays for anti-rhAnnexin V-128 IgG and IgM antibodies will be performed in serum samples by ELISA. For this purpose, 10 mL of blood will be collected for each assay. 24 hours post IP injection, each participant will be called to review any signs or symptoms of AEs. If any, adverse event(s) will be reported and recorded in the Electronic Case Report Form (e-CRF). All participants will undergo a physical examination (height, weight, BMI) and vital signs (systolic and diastolic blood pressure, heart rate) at screening visit and before and after injection of the radiotracer. Adverse events will be collected in the e-CRF.

Statistics	A sample size of 30 patients produces a 96 to 98% confidence interval equal to the sample proportion plus or minus 0.17 to 0.20 when the estimated proportion is 0.3 to 0.5. Sample size was also estimated for the comparison of ^{99m}Tc-rh-Annexin-V 128 uptake in asymptomatic patients with carotid disease versus controls using a 5% significance level and power of 95%. Based on the results from the Phase I clinical trial in 12 healthy volunteers and by allowing attrition, we plan to recruit 10 participants in the control group and 35 patients with carotid plaque. Among the 35 patients and 10 normals, 15 patients from the disease group and 5 normals from the control group will be enrolled in the PoC phase, which is believed to provide sufficient data to demonstrate the potential of ^{99m}Tc-rhAnnexin V-128 imaging in carotid atherosclerotic plaque. Primary endpoint of PoC: The feasibility of imaging apoptotic activity in carotid atherosclerotic plaques using ^{99m}Tc-rhAnnexin V-128 will be assessed by the data monitoring committee (visual image review and consensus). The frequency and severity of abnormal carotid scans will be determined in the patient and normal groups. Primary endpoint of Phase II: To determine the prevalence of abnormal ^{99m}Tc-rhAnnexin V-128 planar and SPECT/CT imaging. Secondary endpoints: Descriptive statistics of ^{99m}Tc-rhAnnexin V-128 uptake in control participants will be presented by areas of interest to assess the background uptake profile. ^{99m}Tc-rhAnnexin V-128 carotid uptake will be related to the ultrasound data of plaque echogenicity and echolucency. ^{99m}Tc-rhAnnexin V-128 uptake in carotids will be correlated to aortic uptake in all participants. Safety: Adverse events will be listed on an individual basis, including relationship, and severity, and will be summarized by 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. All other safety variables will be tabulated at each measuring time. Hematology, clinical chemistry, urinalysis and immunogenicity data will be analysed 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	**Screening** (Within 8 weeks prior to Day 0)	**Planar and SPECT/CT Visit** (Day 0)	**24 hours post IP injection**[1]	**30 ± 3 days post IP injection** (Day 30)
Written informed consent	x			
Inclusion/exclusion criteria	x	x		
Medical history	x			
Concomitant medications	x	x	x	x
Physical examination (height, weight, BMI)	x	x		
Vital signs (BP, HR)	x	x		
Lab analysis (hematology, PT/PTT/INR, biochemistry, urine)	x			x
Immunogenicity by ELISA[2]	x			x
Pregnancy test	x	x		
Carotid ultrasound[3]	x			
rh-Annexin V-128 administration		x		
Planar & SPECT/CT imaging		x		
Adverse events assessment		x	x	x

[1] Within 24 hours after injection of ^{99m}Tc-rh-Annexin V-128, each participant will be called to review any signs or symptoms of AEs.

[2] 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 Day 30 (±3 days) post administration of investigation product. Serum will be prepared, divided into six aliquots and frozen (-80°C). Three out of six serum samples per time-point will then be shipped to the [redacted] central laboratory.

[3] Within 8 weeks prior to ^{99m}Tc-rh-Annexin V-128 imaging.

2. List of abbreviations

AE	Adverse Event
BUN	Blood Urea Nitrogen
e-CRF	Electronic Case Report Form
CRO	Clinical Research Organization
CRP	C-Reactive Protein
CT	Computed Tomography
DMC	Data Monitoring Committee
ELISA	Enzyme-Linked Immuno-absorbent Assay
ESR	Erythrocyte Sedimentation Rate
FDA	Food and Drug Administration
FDG	18-fluorodeoxyglucose
GCP	Good Clinical Practice
Hb	Hemoglobin
HPLC	High-Performance Liquid Chromatography
IBD	Inflammatory Bowel Disease
ICF	Informed Consent Form
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ICRP	International Commission on Radiological Protection
IEC	Independent Ethics Committee
IL-6	Interleukine-6
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
IRB	Institutional Review Board
ITLC	Instant Thin Layer Chromatography
MBq	Mega Becquerel
MIRD	Medical Internal Radiation Dose
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary For Regulatory Activities
NOAEL	No Observed Adverse Effect Level

PET	Positron Emission Tomography
PBS	Phosphate Buffered Saline
PoC	Proof of Concept
PS	Phosphatidylserine
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PV	Pharmaco Vigilance
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell
RCP	Radiochemical Purity
REB	Research Ethic Board
ROI	Region of Interest
RR	Respiratory Rate
[redacted]	[redacted] (CRO)
SAE	Serious Adverse Event
SEC-HPLC	Size-Exclusion HPLC
SD	Standard Deviation
SOP	Standard Operating Procedure
SPECT	Single-Photon Emission Computed Tomography
TBR	Target to background ratio
TLC	Thin Layer Chromatography
US	Ultra Sounds
UV/Vis	Ultraviolet/Visible
WBC	White Blood Cell
WFI	Water For Injection
WHO	World Health Organization
VOI	Volumes Of Interest

3. Background and Rationale

Atherosclerosis is projected to become an internationally leading cause of mortality[1-3]and is the underlying pathophysiology responsible for angina, myocardial infarction, transient ischemic attacks and stroke. Atherosclerosis is an inflammatory disease process that begins with the formation of lipid-rich fatty streaks in vascular endothelium, and eventually resulting in complexly remodeled plaques[4] consisting of a central lipid rich and necrotic core bound by a thin fibrous cap[5, 6]. The fibrous cap is prone to eroding or rupturing leading to thrombus generation and resulting in severe ischemic injury of the tissue bed supplied by the thrombosed vessel[7, 8]. Rupture-prone plaque is referred to as vulnerable plaque[9].

Vascular inflammation can be imaged with 18-fluorodeoxyglucose positron emission tomography (FDG PET).FDG uptake imaged with PET is considered to be a marker of macrophage expression within plaque[10]. Macrophages are one of the main cellular components of inflamed atherosclerotic plaque[11]. However, a major limitation of FDG PET is the normal diffuse uptake in non-vascular structures (myocardium and neck musculature) that leads to poor specificity with false positive results. This extravascular uptake represents a challenge for imaging vasculature, particularly the coronaries and carotids[12]. Other cells adjacent to the target of interest and within the field of view may also take up FDG, resulting in difficulty with separating FDG uptake in inflamed lesions from surrounding tissues.

Immunohistochemical examination of ruptured vulnerable plaques has demonstrated that there is increased expression of apoptotic and disintegrated macrophages within the lipid rich necrotic core[13-15]. In addition to macrophages, smooth muscle cells undergoing apoptosis or programmed cell death have been localized within the fibrous components of plaque[16, 17]. Further, expansion of the necrotic core may be related to plaque destabilization[15, 18, 19]. Thus, imaging apoptosis may identify vulnerable plaque and be a more specific imaging approach for advanced and complicated plaque than FDG PET.

Apoptosis is an energy-dependent process of programmed cell death that ensues following the initiation of an internal and regulated suicide program[20]. A hallmark feature of apoptosis is the externalisation of phosphatidylserine (PS) as the cell transitions towards shrinkage, degradation of DNA and eventual fragmentation[20]. Annexin V is an endogenous human protein (molecular weight 36 kDalton) produced by epithelial cells. Annexin V binds with very high affinity to PS, and serves the purpose of labeling cells for phagocytosis[21]. Radiotracers that bind specifically to Annexin V have been developed for the non-invasive detection of apoptotic activity using imaging modalities such as SPECT. Early clinical cardiovascular studies with ^{99m}Tc labeled Annexin V and SPECT imaging demonstrated significant radiotracer uptake in 7 patients with acute myocardial infarction[22] and in 4 patients with carotid artery atherosclerosis[23]. Validation studies in experimental rabbit models of atherosclerosis correlated uptake of ^{99m}Tc labeled Annexin V with histological degree of apoptosis, atherosclerotic lesion severity and macrophage burden[24] and plaque vulnerability[25]. Thus, ^{99m}Tc labeled Annexin V has potential for imaging atherosclerotic lesions and identifying advanced lesions with greater apoptosis and vulnerability.
^{99m}Tc-HYNIC-Annexin V was the early ^{99m}Tc labeled Annexin V used successfully in several

clinical studies but had a major limitation with very high renal uptake resulting in a high patient radiation dose and limiting abdominal imaging[26]. A novel form of Annexin-V with an endogenous ^{99m}Tc chelating site (rhAnnexin V-128) has been developed[27]. ^{99m}Tc labeled rhAnnexin V-128has more rapid renal clearance than ^{99m}Tc-HYNIC-Annexin V and similar detection of apoptosis in animal models[27].

^{99m}Tc labeled rhAnnexin V-128 has been evaluated in a Phase I clinical trial at the University of Ottawa Heart Institute in 12 normal volunteers. No significant adverse effects were observed. Blood clearance was rapid. Greatest radiotracer uptake was in the kidneys, bladder (due to rapid kidney clearance), liver and lungs. Total effective dose (IRCP 60) for ^{99m}Tc labeled rhAnnexin V-128 was 7.7 + 0.8 uSv/MBq and was significantly less than 11.0 + 0.8 uSv/MBq reported for ^{99m}Tc-HYNIC-Annexin V-128[28].

Ultrasound imaging can provide further insight into the morphology of plaque. B-mode ultrasound derived measures of plaque echogenicity can predict the content of soft lipid tissue and the amount of calcification in carotid plaque[29, 30]. Echogenic plaque is typically composed of fibrous tissue and calcification, while echolucent plaque is lipid-rich[29, 30]. Plaque morphology by ultrasound has predictive value for identifying patients at increased risk of ischemic cerebrovascular events independent of degree of stenosis and vascular risk factors[31]. A recent review of ultrasound, computed tomography and magnetic resonance imaging of the carotid arteries concluded that ultrasound was preferable at the present time due to high spatial and temporal resolution, safety, low cost and wide availability[32].

The goal of the present investigation is to assess the feasibility of^{99m}Tc-rhAnnexin V-128 planar and SPECT imaging as a technique for detection of apoptotic carotid plaque in asymptomatic patients with significant carotid artery disease. This investigation will determine the prevalence of significant apoptotic activity in carotid plaque in patients with carotid disease. ^{99m}Tc-rhAnnexin V-128 uptake in the carotid arteries as a measure of apoptotic activity will be compared to plaque ultrasound-based echogenicity and echolucency, measures of plaque vulnerability. Aortic uptake of ^{99m}Tc-rhAnnexin V-128 will be imaged and related to carotid uptake of ^{99m}Tc-rhAnnexin V-128.

4. Study Objectives

The primary objective of this investigation is:

- To determine the feasibility of imaging apoptotic activity in carotid atherosclerotic plaques of asymptomatic or previous symptomatic with TIA only patients with significant carotid artery disease by using ^{99m}Tc-rhAnnexin V-128 imaging and to report the prevalence of abnormal ^{99m}Tc-rhAnnexin V-128 scans.

The secondary objectives are:

- To establish the uptake of ^{99m}Tc-rhAnnexin V-128 in a control group of participants with normal carotid ultrasounds.
- To assess the ^{99m}Tc-rhAnnexin V-128 uptake versus plaque echogenicity and echolucency.
- To measure aortic uptake of ^{99m}Tc-rhAnnexin V-128 and assess the correlation with its

carotid uptake.

5. Hypotheses

5.1 Primary Hypothesis

^{99m}Tc-rhAnnexin V-128 imaging can be used to detect apoptosis in carotid plaque in asymptomatic or previously symptomatic with TIA only patients with at least 50% carotid stenosis as defined by ultrasound. In this population, the anticipated prevalence of carotid apoptosis will be 0.3 to 0.5.

5.2 Secondary Hypotheses

- ^{99m}Tc-rhAnnexin V-128 uptake will be increased in plaque with greater echogenicity
- ^{99m}Tc-rhAnnexin V-128 uptake will be decreased in plaque with greater echolucency
- ^{99m}Tc-rhAnnexin V-128 imaging can be used to detect apoptosis in the aorta in asymptomatic patients with at least one 50% carotid stenosis as defined by ultrasounds.
- Aortic uptake of ^{99m}Tc-rhAnnexin V-128 will be correlated with carotid uptake of ^{99m}Tc-rhAnnexin V-128

6. Study Design

This is a single-centre, single dose, Proof of Concept (PoC), Phase II study. Participants will receive a single intravenous bolus of 350 MBq ± 10 % of ^{99m}Tc-rhAnnexin V-128 followed by planar and SPECT/CT imaging of the carotids and aortas. Carotid plaque echogenicity and echolucency will be assessed by carotid ultrasound carried out within 8 weeks of ^{99m}Tc-rhAnnexin V-128 imaging. Planar and SPECT/CT ^{99m}Tc-rhAnnexin V-128 carotid imaging will be obtained in 35 asymptomatic participants with at least one $\geq$ 50% carotid stenosis and in 10 participants with normal carotid ultrasounds.

7. End of Study

The end of the study is defined as the completion of all study procedures on 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. Thirty-five participants with carotid stenosis and ten age and sex matched control participants will be enrolled.

8.1 Inclusion criteria

For all participants:

1. Males and females age 18 years or greater;
2. Able and willing to comply with the study procedures;
3. Negative pregnancy test for women of childbearing potential at screening and on the day of administration of ^{99m}Tc-rhAnnexin V-128.

For participants with carotid artery disease:

4. Evidence of 50% or more carotid stenosis in one or more carotid arteries on carotid ultrasound within 10 years;

For control participants:

4. No significant carotid artery disease on carotid ultrasound;

8.2 Exclusion criteria

1. Previous carotid stenting or endarterectomy, or stroke;
2. Diagnosis of vasculitis, dissection, or non-atherosclerotic carotid disease (Ehlers-Danlos, Marfans);
3. Pregnancy or lactation;
4. History of any disease or relevant physical or psychiatric condition or abnormal physical finding which may interfere with the study objectives at the investigator judgment;
5. Know hypersensitivity to the investigational product or any of its components;
6. Claustrophobia or inability to lie still in a supine position;
7. 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;
8. Unwillingness to provide consent.

8.3 Source of Study Population

The carotid artery study population will be adult male and female patients who are referred to the outpatient cardiology clinics and/or the non-invasive Diagnostic Imaging Department at the University of Ottawa Heart Institute. The control population will be healthy volunteers who do not have carotid atherosclerosis as assessed by ultrasound and whose lab values are within the normal range.

8.4 Participants Study Identification

Each participant will be identified with a participant ID number. 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 (04), the 2-letter code (CA) and a 3-digit participant number (ex: 04-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 inter-current illness or other safety reasons for what the Investigator considers it is in the best interest of the participant to withdraw from the study
- Screening failure

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 at the screening visit will be entered in the eCRF and will be used in the study analysis.

The participants may withdraw from the study if they decide to do so, at any time and irrespective of the reason, or this may be the Investigator's decision. 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

The most important required restriction for study participants is pregnancy.
Imaging is performed ambulatory. After imaging, participant may go back home or go back to work without any particular precautions.
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 investigational product 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 α-D-Glucoheptonatedihydrate (transchelating agent), gentisic acid sodium salt hydrate (radiation stability enhancer), hydroxypropyl-β-cyclodextrin (solubilizing

agent), sodium metabisulfite (antioxidant) and trehalosedihydrate (lyoprotectant and cake forming agent). Lactic acid is also present as buffering agent. The kit composition details are included in the Appendix II.

9.2 Handling, Preparation and Storage

The investigational product 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 II 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 one dose of 350 MBq± 10 % of ^{99m}Tc-rhAnnexin V-128 administered as a single intravenous bolus.

10. Study Procedures

Voluntary written informed consent will be obtained prior to the initiation of any study-related procedures. Study conduct procedures will include a screening visit, the imaging studies, a phone visit within 24 hour post ^{99m}Tc-rhAnnexin V-128 administration visit and a visit at 30 days.

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.
- Results from any standard of care testing related to the participant's condition will be collected.
- Women of childbearing potential must have negative pregnancy test at screening and before the investigational product administration.
- Limited physical exam (height, weight, BMI) and vital signs (systolic and diastolic blood pressure, heart rate), will be recorded.

10.2 Laboratory Assessments

Blood samples for hematology, biochemistry and urinalysis will be obtained at baseline for screening, 30 +/- 3 days post IP administration and following any adverse events.

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

Hematology	Coagulation	Blood Chemistry	Urinalysis
• WBC with differential • RBC • Platelets • Hb • MCV • Hematocrit	• PT • PTT • INR	• BUN • Uric acid • Albumin • Direct bilirubin • LDH • Calcium • Chloride • Phosphorus • Total cholesterol • Total protein • Serum creatinine • Total bilirubin • AP • AST/ASAT • ALT/ALAT • Gamma-GT • Sodium • Potassium • Glucose • ß-HCG at screening	• Dipstick test[1] • Pregnancy test *(before injection of study product, if applicable)*

[1] In case one of the assessments of the dipstick test is positive, a microscopic analysis of the urine must be performed.

Laboratory Assessments will be performed by the [redacted] The Ottawa Hospital according to Ministry of Health and Long Term Care (MHLTC) standards.

10.3 ^{99m}Tc-rhAnnexin V-128 Planar and SPECT Imaging of the Carotid Arteries and Aorta and Image Analysis

Planar and SPECT imaging of the carotids and aorta will be performed 60 and 120 minutes after injection of tracer.

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. Negative pregnancy test for women of childbearing potential will be confirmed on the day of administration.

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 neck, thorax and abdomen for attenuation correction.

Reconstruction will be done using iterative reconstruction incorporating CT-based attenuation correction and dual-energy-window scatter correction. Planar and SPECT images of the carotid arteries and thoracic and abdominal aorta 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). Volumes-of–interest (VOIs) will be placed over the carotids and the thoracic and abdominal aortic sections to determine image counts from the attenuation and scatter corrected images. A ^{99m}Tc source of known activity will be imaged and used to determine the imaging system calibration factor for converting reconstructed image counts into an activity concentration (MBq/cc or % injected dose/gram).

A normal database of carotid and aortic uptake will be determined from 10 control participants and used to identify abnormal uptake in carotid artery disease participants.

Inter and intra-observer variability will be determined by repeated analysis of the images of 20 participants. Two observers will carry out the assessment of inter-observer variability. Images will be anonymized prior to analysis and observers will be blinded to all clinical data.

10.4 ^{99m}Tc-rhAnnexin V-128 Radiation Risk Assessment

Participants will receive radiation from 1) ^{99m}Tc-rhAnnexin V-128 (350 MBq) for a dose of 2.2 mSv (7.7 $\pm$ 0.8 uSv/MBq) and 2) the CT scan for attenuation correction for a dose of 1.8 mSv. Total dose for ^{99m}Tc-rhAnnexin V-128 and CT components will be 4.0 mSv.

10.5 Ultrasound Examination

Clinical evidence of 50% or more carotid stenosis in one or more carotid arteries on carotid ultrasound within 10 years prior to enrolment is required for inclusion in the trial.
Evidence of 50% or more carotid stenosis has to be confirmed in a more recent US imaging within 8 weeks prior to ^{99m}Tc-rhAnnexin V-128 administration. If not confirmed, the participant will be declared as a screening failure.

B-mode and color Doppler ultrasound studies will be acquired of both carotid arteries and performed with an ultrasound scanner equipped with a 5- to 7-MHz transducer. Plaque morphology, as echogenicity, defined as reflectance of the emitted ultrasound signal, will be assessed as previously described[29] and graded from 1 to 4 as echolucent, predominantly echolucent, predominantly echogenic, or echogenic. The vessel lumen will be used as the reference structure for defining echolucency, and the bright echo zone produced by the media-adventitia interface in the far wall will be used as the reference structure for defining echogenicity. Interobserver reproducibility of plaque morphology in stenotic arteries has been previously assessed (κ=0.56, 95% CI 0.38 to 0.74)[31].

The degree of stenosis will be calculated by the following equation: (1−PSVr/PSVs)×100%, where PSVr denotes peak systolic velocity at the point of reference (here, the distal carotid artery) and PSVs the peak systolic velocity in the stenosis[31].

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 DMC will review and evaluate the images of the first 15 patients and the first 5 healthy participants in terms of image quality, carotid target uptake and clinical relevance of the study product. The DMC will also review the safety data. The frequency of abnormal carotid scans will be determined in the participant and normal groups. The study will be terminated if there is a low frequency of positive scans in the carotid participant group or high frequency of positive scans in the normal group.

The DMC will promptly give recommendations to continue or terminate the study. The DMC report will be prepared by the Sponsor and sent to the REB and regulatory authorities as required. Preliminary results can be used by the Sponsor for publication purpose, before the end of the main trial.

12. Statistical methods

12.1 Sample size and analysis

The primary hypothesis is that ^{99m}Tc-rhAnnexin V-128 SPECT carotid imaging can detect apoptosis. We anticipate that the prevalence of positive ^{99m}Tc-rhAnnexin V-128 SPECT carotid scans will be about 0.3 to 0.5 of the patients. A sample size of 30 patients produces a 95% confidence interval with a precision of about 0.17 to 0.20 when the estimated proportion is 0.3 to 0.5. Sample size was also estimated for the comparison of ^{99m}Tc-rh-Annexin-V 128 uptake in asymptomatic patients with carotid disease versus normals using a 5% significance level and power of 95%. The measured neck uptake in 12 normals was 0.8 $\pm$ 0.27% ID/gm in the Phase I study. Assuming a similar SD of 0.27% ID/gm in the patients and a minimal clinically important difference (MCID) of 50% or a change of 0.4 in uptake, the required sample size is 9 normals and 25 patients. Allowing for attrition, we plan to recruit 10 normals and 35 patients.

12.2 General statistical considerations

Statistical methods will be detailed in the Sponsor statistical analysis plan.

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 paired analysis Mann-Whitney-Wilcoxon U test or paired analysis student-t test. 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:
The feasibility of imaging apoptotic activity in carotid atherosclerotic plaques using ^{99m}Tc-rhAnnexin V-128 will be determined by the data monitoring committee (visual image review and consensus).

Primary endpoint of Phase II:
Efficacy of ^{99m}Tc-rhAnnexin V-128 planar and SPECT/CT imaging will also be evaluated by determining the prevalence of abnormal ^{99m}Tc-rhAnnexin V-128 scans.

Secondary endpoints:
Descriptive statistics of ^{99m}Tc-rhAnnexin V-128 uptake in control subjects will be presented by areas of interest to assess the background uptake profile.

^{99m}Tc-rhAnnexin V-128 uptake will be related to ultrasound data of plaque echogenicity and echolucency, by direct comparison and by using correlation test and/or diagnostic test performance methods (McNemar test) if appropriate.

^{99m}Tc-rhAnnexin V-128 uptake in carotids will be correlated to its aortic uptake in all participants.

Efficacy analysis of the primary endpoint will be mainly descriptive in nature. Inferential statistics (such as comparisons, correlations…) will be mainly conducted as explorative analyses to serve as the basis for future confirmatory trials.

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. The type and incidence of AEs/SAEs, as well as severity and relatedness to the investigational product will be tabulated.

12.5.2 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 analysed 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 investigational product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease, temporally associated with the use of an investigational product, whether or not causally related to the investigational product.

AEs will be reported, if applicable, from the signing of the informed consent until the last study-related procedure.

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 or incapacity;
- Results in congenital anomaly or birth defect;
- Requires inpatient hospitalization or prolongation of hospitalization.

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.

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 investigational product exposure including conceptions occurring until 30 days after the IP administration. 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, the REB, 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 15 patients and 5 normals by the Data Monitoring Committee 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 with written notice 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 subject 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(s) at any time during and/or after completion of the study and have access to source documents, including the participant's file.

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).

15.2 Ethics and Protection of Participant Confidentiality

15.2.1 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.

15.2.2 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.

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

All information regarding the investigational product 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 investigational product 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 investigational product 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 investigational product manufacturer for the development of the specified investigational medication and may be disclosed as deemed necessary by the investigational product 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, he/she shall send the draft manuscripts and copies of the information to be presented to the investigational

product manufacturer at least 30 working days before submission to a publisher or presentation. The investigational product 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 investigational product manufacturer to protect proprietary information and to provide comments based on information that may not yet be available to the Investigator(s).

15.4 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), investigational product 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.

15.5 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 authorities 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).

15.6 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 investigational

product, as described in the protocol, the current Investigator's Brochure, the product information, and other information sources provided by the investigational product 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.

15.7 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 investigational product 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.

15.8 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

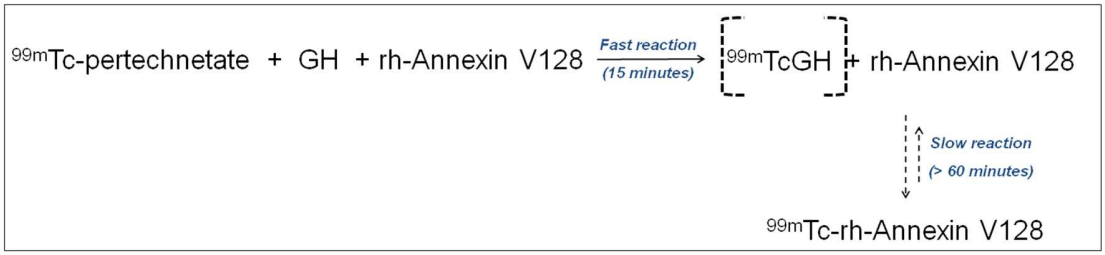
The technetium solution to be used for the labeling of rh-Annexin V-128 kit is the solution which can be obtained from commercially available Tc generators compliant with the Ph.Eur. Monograph “Sodium Pertechnetate (^{99m}Tc) Injection (Fission)” and Tc 99m Injection USP.

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).

b. 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.
c. Stability of the radiolabelled compound: up to 6hours at room temperature (RCP 90 %).
d. 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).

The 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 of storage at the accelerated temperature conditions (25°C ± 2°C/60% RH ± 5% RH), 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 hours after completion of the radiolabelling reaction.

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

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: Severe	☐ Mild	☐ Moderate	☐

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	☐ Concomitant medication taken

	SAE	
☐ 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: [illegible] [illegible]	
INFORMATION COMPLETE: ☐ Yes ☐ No Name and signature:	FOLLOW-UP REQUESTED: ☐ YES ☐ NO Details:

[illegible]

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° ……

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

Rhesus:	❒ Unknown ❒ Rh - ❒ Rh +	**Rubella:**	❒ Unknown ❒ No ❒ Yes
Smoking:	❒ Unknown ❒ No ❒ Yes …… *(pack-years)*	**Toxoplasma:**	❒ Unknown ❒ No ❒ Yes
Alcohol:	❒ Unknown ❒ No ❒ Yes …… *(glasses/day)*		
Drug abuse(s):	❒ Unknown ❒ No ❒ Yes …… *(detail)*		
Hypertension:	❒ Unknown ❒ No ❒ Yes	**HIV:**	❒ Unknown ❒ No ❒ Yes
Diabetes mellitus:	❒ Unknown ❒ No ❒ Yes	**Hepatitis:**	❒ Unknown ❒ No ❒ Yes
Epilepsy:	❒ Unknown ❒ No ❒ Yes		
Psychiatric diseases:	❒ Unknown ❒ No ❒ Yes		

3. PREGNANT WOMAN'S GYNECOLOGICAL & OBSTETRICAL HISTORY

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

Regular menses: ❒ No ❒ Yes

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

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

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

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

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

Other : ……

Hypertension : ❒ No ❒ Yes

Diabetes: ❒ No ❒ Yes

Infection : ❒ No ❒ Yes

Other : ……

Name	Posology *(with units)*	Administration route	Indication(s)	Administration dates *(start/end)*
1.				
2.				
3.				

4.				
5.				

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)*

Preterm: ❒ No ❒ Yes

Dysmature: ❒ No ❒ Yes

Intensive care: ❒ No ❒ Yes ❒ Unknown

❒ No ❒ Yes …….. *(detail)*

Length: …….. *(unit)* **Cranial perimeter:** ……..... *(unit)*	**APGAR score:** ……..…. *(1 min)* ……..…. *(5 min)*	**Malformation :** ❒ No ❒ Yes …….. *(detail)* **Neonatal pathology:** ❒ No ❒ Yes **Breastfeeding:**
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:

AAA Case #: …… **TYPE DE RAPPORT:** ❒ Initial ❒ Follow up n°: ……	**<u>Initial reception by</u>:** NAME: OCCUPATION: LOCAL AFFILIATE/COUNTRY: DATE: ⌊ ⌋⌊ ⌋/⌊ ⌋⌊ ⌋/⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋
Reception date by AAA PV department ⌊ ⌋⌊ ⌋/⌊ ⌋⌊ ⌋/⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋	
Reception date by a partner ⌊ ⌋⌊ ⌋/⌊ ⌋⌊ ⌋/⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋	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.