Services de Médecine Nucléaire
Et de Rhumatologie

Clinical trial study

Protocol Amendment n°4; 16 December 2014
Replaces Protocol Amendment 3; 08 April 2014

A Phase I-IIa study of safety, tolerance, pharmacokinetics, dosimetry and benefice of early nuclear medicine imaging of ^{99m}Tc-rhAnnexin V-128 in patients with rheumatoid arthritis or ankylosing spondylitis

Study number: 1002 / NCT number: NCT02328027

Study Phase: I-IIa

Principal investigator

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Patients coming from department of

Prof. [redacted], MD, [redacted], [redacted]

Signature: ……

Sponsor

Advanced Accelerator Applications Switzerland S.A., 4 rue du Tour de l'Ile, 1204 Geneva

Investigational Medicinal Product (IMP)

Kit for the Preparation of Tc-99m Recombinant Human Annexin V-128 for Injection

Manufacturer of Product

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STUDY ACKNOWLEDGMENT

Protocol N°1002/Protocol Amendment n°4, 16 December 2014

1. I have carefully read this protocol entitled "A Phase I-IIa study of safety, tolerance, pharmacokinetics, dosimetry and benefice of early nuclear medicine imaging of ^{99m}Tc-rhAnnexin V-128 in patients with rheumatoid arthritis or ankylosing spondylitis", and agree that it contains all the necessary information required to conduct the study. I agree to conduct this study as outlined in the protocol.
2. I understand that this study will not be initiated without approval of the appropriate Institutional Review Committee/Ethics Committee and that all administrative requirements of the governing body of the institution will be complied with fully.
3. I confirm that I will conduct the study in accordance with the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines and the provisions of the Helsinki Declaration; copies of these documents have been given to me by the Sponsor.
4. I will also ensure that sub-investigator(s) and other relevant members of my staff have access to copies of this protocol, the ICH GCP guidelines, and the Helsinki Declaration to enable them to work in accordance with the provisions of these documents.
5. Written informed consent will be obtained from all participating subjects in accordance with institutional and ICH Guidelines for Good Clinical Practice.
6. I will enroll patients who meet the protocol criteria for entry and who can be followed up in accordance with this protocol.
7. I understand that my signature on each completed Case Report Form indicates that I have carefully reviewed each page and accept full responsibility for the contents thereof.
8. I understand that the information presented in this study protocol is confidential and I hereby assure that no information based on the conduct of the study will be released without prior consent from the Sponsor unless this requirement is superseded by the Health Authority and/or the European Medicines Agency.

Investigator's Name and Signature	Date
AAA Switzerland, CEO, Name and Signature	Date
AAA, Head of BD and Pre-clinical Development, Name and Signature	Date
AAA, Head of Clinical Development, Name and Signature	Date

SYNOPSIS

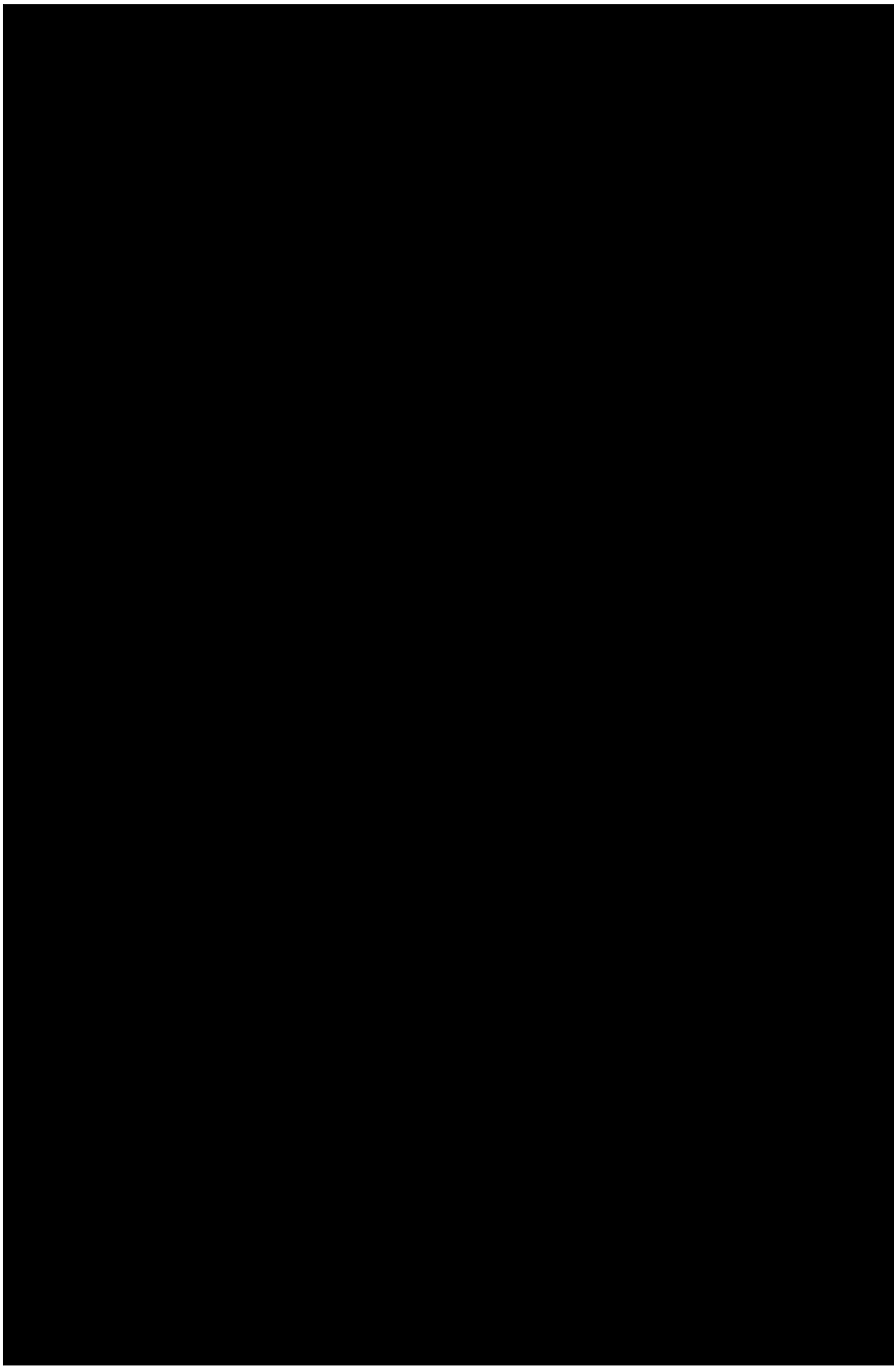
Investigational Medicinal Product	Kit for the Preparation of Tc-99m Recombinant Human Annexin V-128 for Injection
Title of the study	A Phase I-IIa Study of safety, tolerance, pharmacokinetics, dosimetry and benefice of early nuclear medicine imaging of ^{99m}Tc-rhAnnexin V-128 in patients with rheumatoid arthritis or ankylosing spondylitis
Study centre	Nuclear Medicine Department, CHUV, 1011 Lausanne, Switzerland
Sponsor	Advanced Accelerator Applications Switzerland S.A., 4 rue du Tour de l'Ile, 1204 Geneva
Subject's Indication	Rheumatoid Arthritis (RA) or Ankylosing Spondylitis (AS)
Objectives	Primary Objectives: • To determine the safety and tolerability of imaging with ^{99m}Tc-rhAnnexin V-128. Secondary Objectives: • To determine the biodistribution, pharmacokinetics and radiation dosimetry of ^{99m}Tc-rhAnnexin V-128 • To demonstrate the time-dependent distribution and localization of ^{99m}Tc-rhAnnexin V-128 both in RA and AS patients • To determine ability of early imaging with ^{99m}Tc-rhAnnexin V-128 to evaluate the presence of lesions before and after short term treatment of either RA or AS patients
Rationale	Annexin V is an endogenous human protein that binds to phosphatidylserine, a molecule expressed only on the surface of apoptotic cells. Technetium-99m (Tc-99m) is a medical isotope widely used in diagnostic imaging. ^{99m}Tc-labelled annexin V has demonstrated the ability to image cellular apoptosis and necrosis of, among others, rheumatoid arthritis or ankylosing spondylitis conditions.
Planned number of patients	20 evaluable patients (patients who have completed Day 56 assessments) will be included in the study with either rheumatoid arthritis or with ankylosing spondylitis. Ten enrolled patients, independently from the group (RA or AS), will take part in adosimetric and pharmacokinetics (PK) sub-study, after providing written approval. Out of the first 6 enrolled patients, at least 3 patients must take part in the sub-study.
Study design and Methodology	This is a monocentric, open label, Phase I-IIa study Subjects who have signed the informed consent and are eligible to participate in the study will be subjected to the following assessments: • At screening, patients will have a physical examination, vital signs, blood analysis (haematology, coagulation, chemistry and immunology), urine analysis, a bone scintigraphy if not already performed within 8 weeks before rhAnnexin V-128 administration, an ECG, a pregnancy test for women with childbearing potential, BASDAI, BASFI, BASMI for

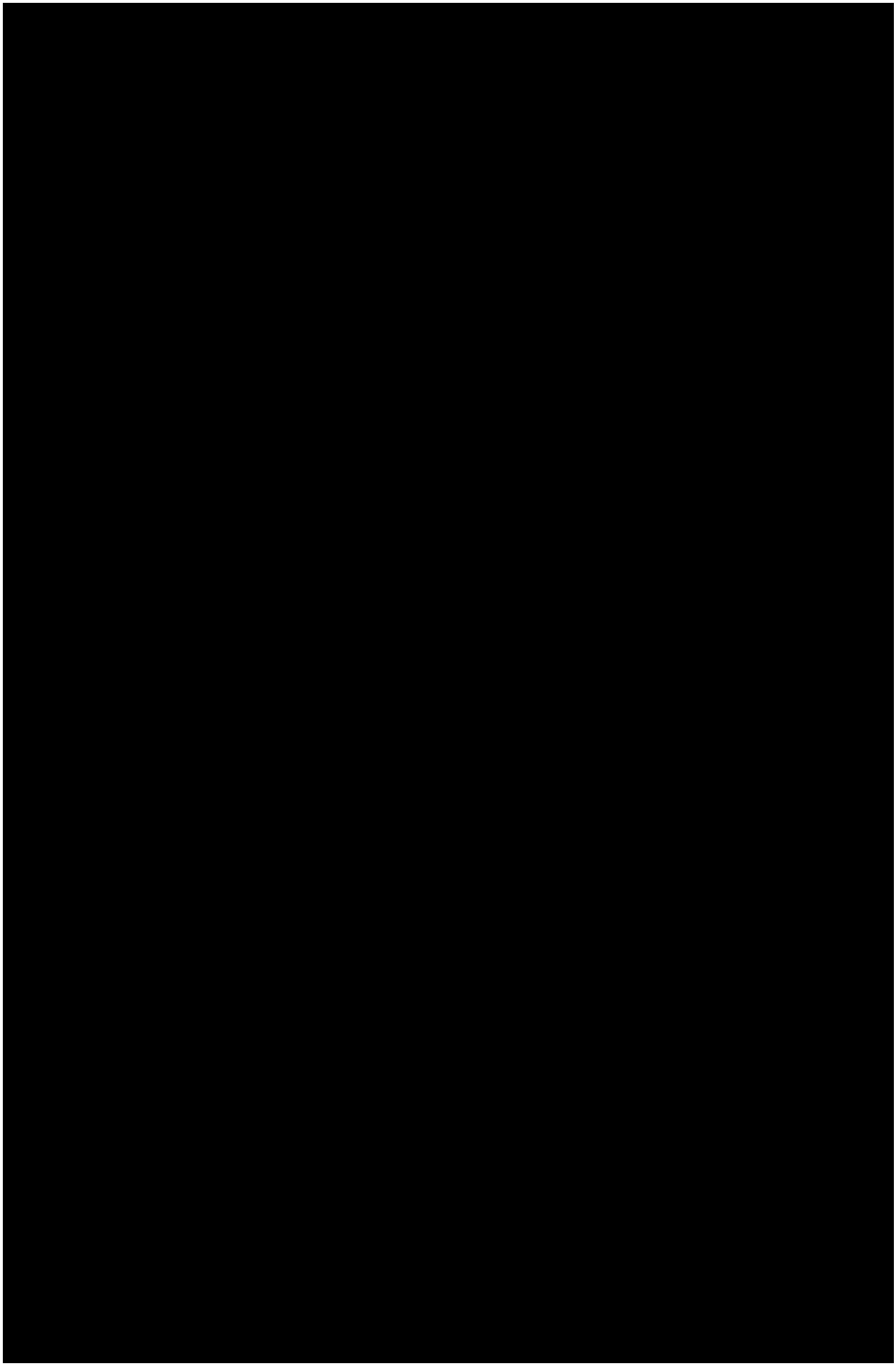
	patients with AS, DAS28 Disease Activity Score assessments for patients with RA. • On Day 1, patients will have whole body scintigraphies with ^{99m}Tc-rhAnnexin V-128, vital signs assessment and an ECG. A pregnancy test will also be performed prior to rhAnnexin V-128 administration. The first 3 patients (who will not take part in the sub-study) will be imaged at 60 min and at 2 hrs after administration. Next enrolled patients will be imaged at a time in line with the results of the image acquisition in the first 3 patients who will have participated in the sub-study. Additional images, blood and urine samples will be taken for the 10 patients participating in the dosimetric and PK study. • At Day 2, patients will be called over the phone to inquire if they experienced allergic reactions such as edema or rash. Patients who will participate in the PK and dosimetric sub-study will be interviewed in face to face on site meeting during the visit on Day 2. For these last patients, blood/urine analysis, whole body scintigraphy with ^{99m}Tc-rhAnnexin V-128 will be repeated 24 hours later for the dosimetric and pharmacokinetics analyses. Vital signs and ECG will also be assessed. • At Day 30+/- 3 days, all patients will have immunological evaluations as well as blood (haematology and biochemistry) and urine analysis, ECG and vital signs. • At 6 weeks (Day 42) ± 2 weeks, a whole body scintigraphy with ^{99m}Tc-rhAnnexin V-128 at a time in line with the results of the image acquisition in the first 3 patients who will have participated in the sub-study, ECG, physical examination, vital signs, blood/urine analyses, a pregnancy test for women with childbearing potential, BASDAI, BASFI, BASMI for patients with AS, DAS28 Disease Activity Score assessments for patients with RA will be performed in all patients. • At Day 43, patients will be called over the phone to inquire if they experienced allergic reactions such as edema or rash. • At 8 weeks (Day 56) ± 2 weeks, vital signs and immunological evaluation will be performed. • At Day 90+/- 2 weeks, all patients will have immunological evaluations, ECG, physical examination and vital signs, blood and urine analysis, BASDAI, BASFI, BASMI for patients with AS, DAS28 Disease Activity Score assessments for patients with RA.
Treatment	The single dose vial (rhAnnexin V-128: 0.40 mg) will be reconstituted with 740 MBq (20 mCi) of ^{99m}Tc. The labelling reaction requires 90 minutes and the reconstituted radiolabelled product is stable for 6 h. For the purpose of this study, it is recommended to administer the reconstituted solution within 4 hours after completion of the labelling reaction.The drug is administered at a dose of 250 MBq as a single intravenous bolus over 10-20 seconds at Day 1 and at 6 weeks (42 days) +/- 2 weeks after the first administration.
Inclusion/ **exclusion criteria**	**Inclusion criteria** 1. Patients diagnosed with RA based on ACR/EULAR 2010 criteria with a score ≥ 6, OR Patients diagnosed with AS based on the ASAS criteria. Patients with RA must have serology assessment performed and documented at the time of enrolment. 2. Patient with RA active disease (DAS > 2,6) and the introduction of a Bi-DMARD treatment should be indicated. RA patients must at least be treated with DMARD (methotrexate,

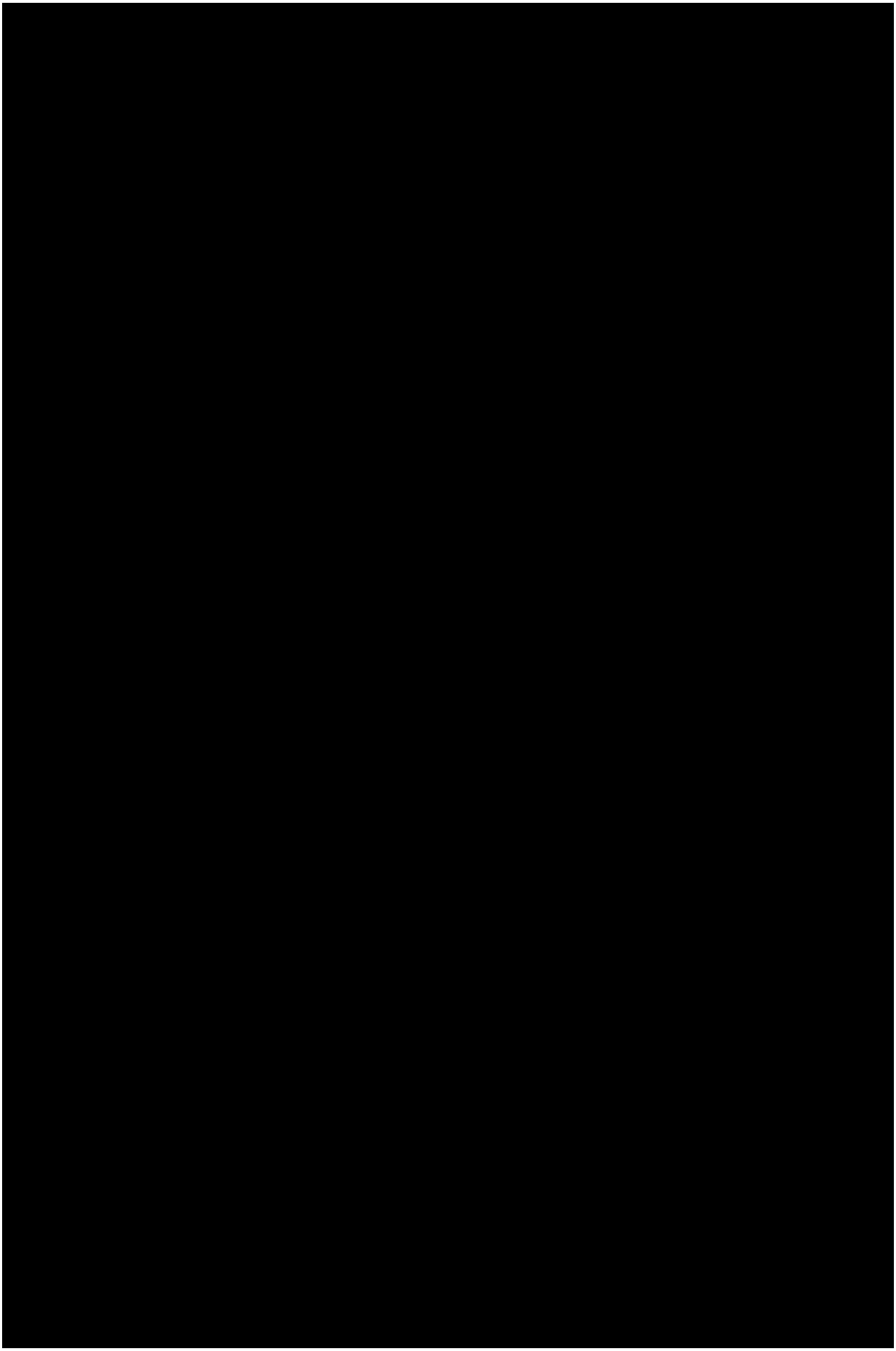
	leflunomide and sulfasalazine) or combination of these treatments for at least 3 months. Treatment will be pursued while on study. OR RA patients must have been previously treated with Bi-DMARD before initiation of the new Bi-DMARD treatment. The non-response of the previous Bi-DMARD treatment must be documented. OR Patients with ankylosing spondylitis with insufficiently controlled disease while under NSAID and indication for Bi-DMARD. These patients must be under NSAID for at least 3 months and under the same NSAID for at least 1 month prior to enrolment. 3. ≥ 18 years old 4. Karnofsky ≥ 80% 5. Negative pregnancy test for women with childbearing potential, 6. For women with childbearing potential, use of two reliable means of contraception (e.g., hormonal contraceptive, patch, vaginal ring, intrauterine device, associated with other barrier method of contraception such as the use of condoms) throughout their participation to the study 7. Absence of ECG anomaly 8. Written ICF signed **Exclusion criteria** 1. Pregnancy or lactation 2. Liver impairment (ALT, AST or Bilirubin > 2 ULN) at screening visit or baseline 3. Kidney impairment (serum creatinine > 1.5 mg/dL) 4. History of congestive heart failure (NYHA III & IV) 5. History of malignant disease within 5 years 6. 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 7. Know hypersensitivity to the investigational drug or any of its components 8. Participation to another clinical trial within 4 weeks before study inclusion except for patients who have participated or who are currently participating in an interventional study without any study drug administration.
Study duration and assessments: maximum 4 months (+/- 2 weeks) including the screening period	
Dosimetry	The quantitative in vivo biodistribution of the ^{99m}Tc-rhAnnexin V-128 will be determined through whole-body planar imagings. In addition, a SPECT/CT will be performed on the abdominal/pelvis regions at 3 hours post administration for the first 3 patients included in the sub-study in order to generate relevant dosimetry data after extrapolation for the reconstruction with OLINDA software. Depending upon the results in those 3 patients, the SPECT/CT on the abdominal/pelvis regions will be performed at 5 hours post administration Reconstruction will be done using iterative reconstruction incorporating CT-based attenuation correction and dual-energy-window scatter correction. Dynamic planar imaging will be acquired during the first 10 minutes followed by

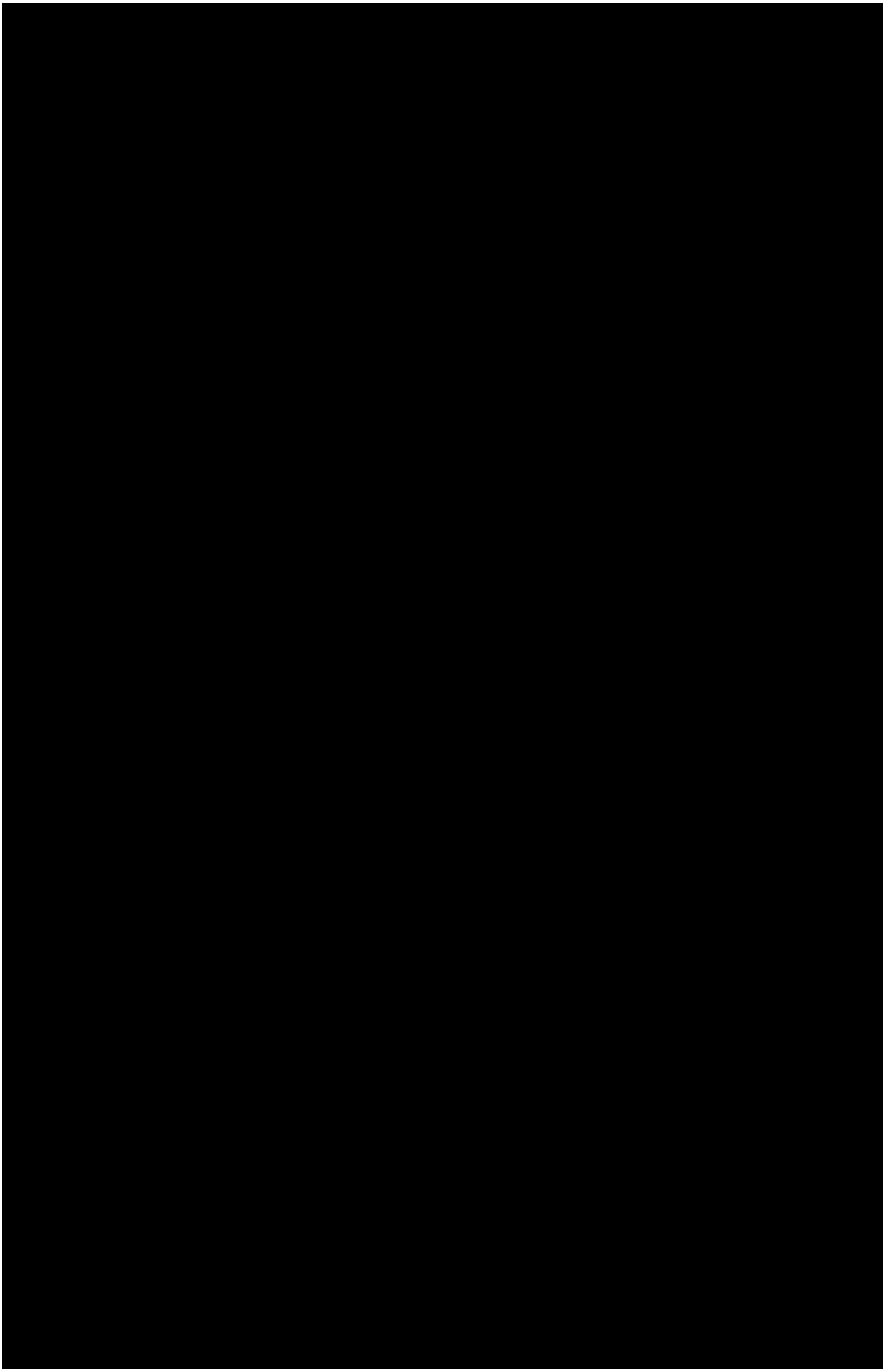
	whole body planar images at 30min, 60 min, 2hrs, 4 hrs and 24 hrs post-injection for the first 3 patients included in the sub-study. Depending upon the results in those 3 patients, the timeframe for dosimetry measurements and PK will be confirmed or modified with a time-point at 3 hrs (instead of 2 hrs) and 6 hrs (instead of 4 hrs) for the following 7 enrolled patients in the sub-study. . Radiation dose to the organ of interests (particularly the kidneys), effective dose equivalent and effective dose will be calculated following the MIRD committee guidelines using the OLINDA/EXM software. **Blood and urine collection and counting:** Venous blood samples will be drawn for each subject at screening, Day 30, Day 42 and Day 90 for lab analysis (Haematology, biochemistry). For patients included into the subgroup of PK and dosimetric study, blood sample will also be collected for lab analysis (Haematology, biochemistry) at Day 2. For the first 3 patients, a further 10 ml of venous blood samples will be drawn at nominal times of 0, 5, 10, 15, 30, 60 minutes as well as 2 hrs, 4hrs and 24 hrs post administration. Depending upon the results in those 3 patients, the timeframe for dosimetry measurements and PK will be confirmed or modified with a time-point at 3 hrs (instead of 2 hrs) and 6 hrs (instead of 4 hrs) for the following 7 enrolled patients in the sub-study. The following whole blood and serum aliquots will be prepared as described in Appendix 2, for radioactivity measurements, and PK. For immunogenicity assays, venous blood samples will be drawn for each subject at screening, at Day 30 ± 3 days, at Day 56 ± 14 days and Day 90 ± 14 days. Total quantity of collected blood samples for patients (participating or not in the PK and dosimetric sub-study) is described in Appendix 3. Urine sample will be collected within 24 hrs prior to ^{99m}Tc-rhAnnexin V-128 administration, preferable just prior to its administration (0 h sample) to achieve bladder emptying. Urine collections will be obtained and volume recorded, possibly in the time intervals 0-1 h, 1 h – 4 hrs, 4 hrs -16 hrs, 16 hrs – 24 hrs post administration. Dual 2 mL aliquots of urine from each period will be counted in a gamma counter at local laboratory. Urine samples (10 mL) will also be analyzed by SEC-HPLC technique at local laboratory. Blood clearance fractions and effective half-life estimates from multi-term non-compartmental exponential analysis will be calculated from serial measurements of radioactivity in the whole blood and serum.
Pharmacokinetics	Pharmacokinetic analysis will be also performed based on rhAnnexin V-128 serum concentration determined by ELISA method. As mentioned above, venous blood samples of 10 ml volume each will be taken from the first 3 patients at nominal times of 0, 5, 10, 15, 30, 60 minutes as well as 2 hrs, 4hrs and 24 hrs post administration. Depending upon the results in those 3 patients, the timeframe fordosimetry measurements and PK samples will be confirmed or modified with a time-point at 3 hrs (instead of 2 hrs) and 6 hrs (instead of 4 hrs) for the following 7 enrolled patients in the sub-study. The blood will be processed and aliquoted as described in Appendix 2. Two aliquots of at least 0.6 mL of serum per time-point will be frozen at -80°C.

	After complete decay of radioactivity, one out of two serum samples per time-point will be shipped to the central laboratory and analyzed by ELISA to determine the rhAnnexin V-128 concentration in serum (method detailed in the Laboratory Manual).
Immunogenicity assessments	Assays for anti-rhAnnexin V-128 IgG and IgM antibodies will be performed in serum samples by ELISA. For this purpose 10mL blood samples will be collected at baseline, at Day 30 ± 3 days and at Day 56 ± 14 days. An additional sample is required 3 months after the first administration of the ^{99m}Tc-rhAnnexin V-128 (Day 90 ± 14 days) in case of positive anti-Annexin antibodies outcome in previous examinations. Serum will be prepared, divided into six aliquots and frozen (-80°C) until complete decay. Three out of six serum samples per time-point will be then shipped to the centralized laboratory, where the analysis for anti-rhAnnexin V-128 antibodies will be performed (methods detailed in the Laboratory Manual). 24 hours after each treatment injection (at Day 2 and Day 43), each patient will be interviewed over the phone (in face to face meeting at Day 2 for patients who will participate into the PK and dosimetric sub study) in order to inquire if they experienced signs or symptoms of allergic reactions (edema, rash). If this is the case, Adverse Event(s) should be reported and recorded in the Case Report Form.
Safety and tolerability	Adverse events will be collected in the Case Report Form (CRF).









Visit schedule – Table 1

Study Procedures	Screening (±4 weeks)	Day 1	24 hours[1]	Day 30 ±3 days	Day 42 ±2weeks	Day 56 ±2weeks	Day 90 ±2weeks
Written informed consent	x						
Inclusion/exclusion criteria	x						
Medical history	x						
Concomitant medications including RA and AS treatment	x	x	x	x	x	x	x
Disease assessment (BASDAI, BASFI, BASMI for AS for AS patients, DAS28 scales including Ultrasound assessments for RA patients)	x				x		x
Physical examination	x				x		x
Vital signs (BP and HR)	x	x	x	x	x	x	x
Lab analysis (haematology, biochemistry; urine)	x		x	x	x		x
Venous/urine sampling (PK and dosimetry)[2]		X[1]	x				
Immunogenicity by ELISA[3]	x			x		x	x
Urine SEC-HPLC analysis[1,4]		x	x				
Pregnancy test	x	x			x		
Standard 12-lead ECG	x	x	x	x	x		x
rh-Annexin V-128 administration[6]		x			x		
Bone scintigraphy[7]	x						
Whole Body scintigraphic imaging[5]		x	x		x		
Adverse Events	x	x	x	x	x	x	x

[1] Only for patients participating to the dosimetric and PK substudies.

[2] Venous blood samples of 10 ml volume each will be taken from the first 3 patients at nominal times of 0, 5, 10, 15, 30, 60 minutes and 2 hrs, 4hrs and 24 hrs post administration as described in Appendix 2 for radioactivity measurements, and PK. Depending upon the results in those 3 patients, timeframe of radioactivity measurements and PK will be confirmed or modified with a time-point at 3 hrs (instead of 2 hrs) and 6 hrs (instead of 4 hrs) as required for the following 7 enrolled patients in the sub-study. The whole blood and serum samples (bullet points 1 and 2) will be counted in an auto-gamma counting system at the CHUV/NUC laboratory.

[3] Anti-rhAnnexin V-128 IgG and IgM antibodies will be performed in serum samples by ELISA. For this purpose 10mL blood samples will be collected at baseline, Day 30 (±3 days), Day 56 (±2weeks) and Day 90 (±2weeks) post administration of test agent (sample at 90 days is required only in case of positive anti-annexin antibodies outcome in previous examinations). Serum will be prepared, divided into six aliquots and frozen (-80°C) until complete decay. Three out of six serum samples per time-point will be then shipped to the central laboratory.

[4] One urine sample will be collected within 24 hrs prior to ^{99m}Tc-rhAnnexin V-128 administration, preferable just prior to the administration of study

drug (0 h sample) to achieve bladder emptying. Quantitative urine collections will be obtained (and volume recorded) possibly in the time intervals 0-1 h, 1 h – 4 hrs, 4 hrs – 16 hrs, and 16 hrs – 24 hrs post administration. Dual 2 mL aliquots of urine from each period will be counted in a gamma counter at the CHUV/NUC laboratory.

Urine samples (10 mL aliquot) will be analysed as a function of time by SEC-HPLC technique at the local laboratory in order to gain information on the chemical status of ^{99m}Tc-rhAnnexin V-128 and on the presence of ^{99m}Tc-rhAnnexin V-128-related species.

[5] The first 3 patients participating to the dosimetric and PK sub-study will be imaged post administration at 30min, 60 min, 2 hrs, 4 hrs and 24 hrs after the first administration. Depending upon the results in those 3 patients, timeframes of the radioactivity measurements and PK will be confirmed or modified with a time-point at 3 hrs (instead of 2 hrs) and 6 hrs (instead of 4 hrs) as required for the following 7 enrolled patients in the sub-study. The other first 3 patients (not taking part to the sub-study) will be imaged on Day 1 at 60 min and at 2 hrs after administration. Next patients will be imaged at a time defined on the basis of the data collected in the first 3 patients participating to the dosimetric and PK sub-study. On Day 42 ± 2 weeks, all patients will be imaged at a suitable time, defined on the basis of the data collected on the first 3 patients participating to the dosimetric and PK sub-study.

[6] 24 hours after each treatment injection, each patient will be contacted to inquire if they experienced signs or symptoms of allergic reactions (edema, rash).

[7] The bone scintigraphy must be performed at screening except for patients who already had a bone scintigraphy within 8 weeks prior to ^{99m}Tc-rhAnnexin V-128 administration. In that case, the bone scintigraphy report must be collected (after patient anonymity) and will be reviewed.

LIST OF ABBREVIATIONS

AE	Adverse Event
ACD	Anticoagulant Citrate Dextrose Solution
ACR	American College of Rheumatology
ALAT	Alanine Aminotransferase
ALT	Alanine Transaminase
Anti-CCP AB	Anti-cyclic citrullinated peptide antibodies
AP	Alkaline Phosphatase
AS	Ankylosing Spondylitis
ASAS	Assessment of Spondylo-Arthritis Society
ASAT	Aspartate Aminotransferase
AST	Aspartate Transaminase
BASDAI	Bath Ankylosing Spondylitis Disease Activity Index
BASFI	Bath Ankylosing Spondylitis Functional Index
BASMI	Bath Ankylosing Spondylitis Metrology Index
Bi-DMARD	Biological Disease Modifying Anti-Rheumatic Drug
BUN	Blood Urea Nitrogen
CHUV	Centre Hospitalier Universitaire Vaudoix
CRF	Case Report Form
CRO	Clinical Research Organisation
CRP	C-Reactive Protein
CT	Computed Tomography
CV	Cardiovascular
DAS28	Disease Activity Score
DMARD	Disease Modifying Anti-Rheumatic Drug
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic Acid
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ESR	Erythrocyte Sedimentation Rate
EULAR	European League Against Rheumatism

FDA	Food and Drug Administration
FDG	Fluorodesossi- Glucose
Gamma-GT	Gamma-Glutamyl Transpeptidase
GCP	Good Clinical Practice
GE	General Electric
GI	Gastrointestinal
GP	General Practitioner
Hb	Hemoglobin
HPLC	High-Performance Liquid Chromatography
HR	Heart Rate
HYNIC	Hydrazino-Nicotinamide
IBD	Inflammatory Bowel Disease
ICF	Informed Consent Form
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICH	International Conference on Harmonisation
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 Normalised Ratio
IRB	Institutional Review Board
ITLC	Instant Thin Layer Chromatography
LDH	Lactate Dehydrogenase
MIRD	Medical Internal Radiation Dose
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary For Regulatory Activities
MRI	Magnetic Resonance Imaging
NSAID	Non Steroidal Anti-Inflammatory Drug
NOAEL	No Observed Adverse Effect Level
OFSP	Office Fédéral de la Santé Publique
PBS	Phosphate Buffered Saline
PK	Pharmacokinetics
PS	Phosphatidylserine

PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PV	Pharmaco Vigilance
QA	Quality Assurance
QC	Quality Control
RA	Rheumatoid Arthritis
RBC	Red Blood Cell
RCP	Radiochemical Purity
ROI	Region of Interest
RR	Respiratory Rate
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SEC-HPLC	Size-Exclusion HPLC
SOP	Standard Operating Procedure
SPECT	Single-Photon Emission Computed Tomography
TBR	Target to background ratio
TLC	Thin Layer Chromatography
TNF	Tumour Necrosis Factor
US	United States
UV/Vis	Ultraviolet/Visible
WBC	White Blood Cell
WFI	Water For Injection
WHO	World Health Organization
VOI	Volumes Of Interest

Table of Contents

1 INTRODUCTION

Background and rationale

Apoptosis and necrosis are the two major cell death processes. Apoptosis is an energy-dependent process of cell death with activation through an internal and regulated suicide program. Necrosis differs from apoptosis and is accidental cell death induced by a variety of chemical or physical insults. Necrosis is characterized by progressive swelling of the cell, denaturation of proteins in the cytoplasm and disruption of the plasma membrane integrity usually associated with an inflammatory response [1]. In contrast, apoptosis proceeds by a cascade of events including externalisation of phosphatidylserine (PS), shrinkage of the cytoplasm, condensation of nuclear proteins, degradation of DNA and fragmentation of the cell [2]. Apoptosis is a form of programmed cell death and useful to the organism as a means of selectively removing unwanted cells. However, apoptosis occurs excessively in disease states including acute myocardial infarction, stroke and inflammatory conditions [3, 4].

Annexin V, an endogenous human protein with a molecular weight of 36 kDalton (320 aa), is produced by the epithelial cells of many tissues and blood cells (placenta, umbilical vessels, liver, spleen, kidney, heart, uterus, skeletal muscle, erythrocytes, leukocytes, endothelial cells, and platelets). Physiologically it plays a role in binding cells undergoing apoptosis/necrosis and acts as an “eat me” signal for macrophage phagocytosis. This function is mediated by the high affinity binding (*K*d = 7 nM) of Annexin V to phosphatidylserine (PS). PS is a phospholipid physiologically present in the inner leaflet of cell membrane and maintained in this position by a series of enzymes (floppase/flippase). Apoptosis plays a critical role in the pathogenesis of a number of disorders (including cerebral and myocardial ischemia, autoimmune and neurodegenerative diseases, viral illness, organ and bone marrow transplant rejection, and tumor response to chemotherapy and radiation), leading to the activation of proteolytic enzymes (caspases) and to the changes of plasma membrane structure. As a result, there is rapid redistribution of PS from the inner membrane leaflet to the outer membrane leaflet of apoptotic cells, exposing the anionic head group of PS to Annexin V binding. Such changes occur within 30-120 min of apoptotic signalling *in vitro.* The externalization of PS is a general feature of apoptosis occurring prior to membrane bleb formation, deoxyribonucleic acid (DNA) degradation and nuclear condensation. On the other hand, PS is also accessible for Annexin V binding in necrotic cells because of disruption of the plasma membrane.

Rheumatoid arthritis (RA) is associated with apoptosis caused by the influx of autoimmune cells which stimulates an inflammatory synovitis that is a hallmark of early RA [5-7]. Rheumatoid Arthritis is a chronic relapsing incurable disease of unknown aetiology that affects at least 2% of the adult population in the U.S. and Europe [8]. There are few specific preclinical and clinical biomarkers of RA. Anti-CCP (cyclic citrullinated peptide) antibodies are a good example of that kind of biomarker. They can be found in patients many months before patients clinically develop RA [9, 10].

In contrast to established disease, diagnosis of early RA is problematic. Therefore classification criteria are used to identify RA as early as possible; those criteria are a mix of clinical, biological and immunological data. All current imaging techniques including conventional (plain film) radiography, MRI, ^{99m}Tc-DPD bone scans, and F-18 FDG-PET cannot directly evaluate preclinical (pre-articular phase) RA limiting the information on this stage of disease [11-15]. In the modern rheumatology, rheumatologists are regularly using ultrasound examinations of joints to evaluate infraclinical synovitis or erosions, which could not be seen on conventional radiography; both of them – synovitis and erosions - are found in RA. Ultrasounds are used to improve and to complete the quality of the clinical assessment of RA.

Diagnosis of early stage RA prior to the erosive bone changes, which are a radiographic hallmark of the disease is difficult with a sensitivity of as low as 50%. Many clinicians now believe that early institution of anti-RA therapy may reduce the severity of the disease and, in some cases, may even prevent the recurrence of the symptoms.

The first step in the treatment of the disease is to introduce as soon as possible a disease-modifying anti-rheumatic drug (DMARD) to prevent the development of a destructive arthritis that could lead to the loss of function of a joint. Methotrexate, leflunomide and sulfasalazine belong to that category of medication; unfortunately they all need a couple of weeks until they can act as an anti-inflammatory drug, therefore glucocorticoids or non steroidal anti-inflammatory drugs (NSAID) are prescribed in the very first steps of the treatment of RA to control inflammation; both categories will be step down in a second time. If DMARDs fail to achieve what they are aiming at, biological treatments – anti-tumor-necrosis factor (TNF) therapies, anti-CD 20 positive lymphocytes treatments, anti-interleukine 6 or abatacept - can be added as second line agents[16].

Ankylosing spondylitis (AS) is a chronic, disabling rheumatic disease characterized by inflammatory back pain, restricted spinal mobility, and frequently peripheral arthritis, enthesitis, and extra-articular manifestations like acute anterior uveitis. Patients fulfil classification criteria for AS if characteristic radiological changes of the sacroiliac (SI) joints are present, together with defined clinical symptoms, physical findings and the presence of HLA-B27.

An earlier diagnosis of ankylosing spondylitis is required because there is still a 5-7 year delay between first symptoms and diagnosis, and new effective treatments are available for active disease.

Radiographic diagnosis is complicated by the slow evolution of radiological changes in many patients. Thus, sacroiliac radiographs may be normal in the early phase of disease and may stay normal in some patients for many years. Hence, two sets of criteria that allow earlier diagnosis in patients with predominant axial and peripheral manifestations were developed: the ASAS criteria[17].

In AS, there are different therapeutical options depending on the extension of the disease. In case of AS limited to axial skeleton, different NSAIDs are used as first line agents associated to physiotherapy. For AS with isolated appendicular skeleton manifestations, therapeutical strategies as in RA can be tried. Anti-TNF drugs are second line agents for diseases with axial and appendicular skeleton manifestations[18].

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

^{99m}Tc-labelled Annexin V radiopharmaceuticals have been developed for imaging apoptosis [19]. RhAnnexin V-128 is a protein produced by recombinant techniques in *Escherichia coli*. It has been prepared by Dr. John Tait as a mutant of Annexin V (wild type, human Annexin), in which six amino acids have been added to the amino terminus of rh-Annexin V to form an endogenous ^{99m}Tc-binding site. Published data in preclinical studies in mice show that ^{99m}Tc-rhAnnexin V-128 has only 6-8% renal localization at 1 hour post intravenous injection compared to 45-50% renal localization of ^{99m}Tc-HYNIC-Annexin V [20]. Moreover, ^{99m}Tc-rhAnnexin V-128 shows almost twice the target-to-background localization at apoptotic targets *in vivo* as demonstrated by ^{99m}Tc-HYNIC-Annexin V.
Annexin V binds with high affinity to phosphatidylserine exposed on the cell surface as an early step in the apoptotic cascade [19]. ^{99m}Tc-hydrazino-nicotinamide (HYNIC) Annexin V has been developed and used in Phase II clinical studies in patients with acute myocardial infarction [21-23], in patients with lymphoma [24], head and neck carcinoma [25, 26] and in patients with non-small-cell lung cancer receiving chemotherapy [27]. ^{99m}Tc-HYNIC-Annexin V has limitations including high uptake in the kidney resulting in a large patient radiation dose and significant abdominal scatter [28].
New improved Annexin V-based imaging agents have been developed [20, 29] and have improved biodistribution profiles with less renal uptake compared to ^{99m}Tc-HYNIC-Annexin V and similar

detection of apoptosis [24, 25]. This advantage of decreased renal retention of the radiolabelled mutant Annexin compound with decreased abdominal background and renal dosimetry needs to be demonstrated in humans.
Preclinical characterization of ^{99m}Tc-rhAnnexin V-128 safety profile indicated that the product can be safely administered, both from the point of view of the proposed radioactive dose (250 MBq) and also considering the total drug substance (protein) present in a 250 MBq dose (0.4 mg). In particular, the radiation safety assessment performed based on extrapolations from animal biodistribution and dosimetry data showed that, for the proposed injected dose of 250 MBq, the estimated kidney absorbed dose in human is around 40 mGy, which is below the level recommended by FDA for the single dose per organ (0.05 Sv, as generally recognized as safe in the FDA Guidance for Industry and Researchers, 2010) and considerably lower than the threshold for nephrotoxicity reported in literature, that is 14 Gy (corresponding to 14 Sv for ^{99m}Tc) when delivering radiation dose in 2 fractions, as reported by Emami et al., 1991, for tolerance dose 5/5 (the probability of 5% complications within 5 years of irradiation) reported in literature [30]. These estimated kidney absorbed doses are also lower than those achieved with some technetiated diagnostics currently used in the Nuclear Medicine practice (e.g. ^{99m}Tc MoAb antiCEA has kidney as critical organ with an absorbed dose in mGy/MBq of 0.1-0.4 (that is 100-400 mGy for an injected dose of 1000 MBq).
The estimation of effective dose for total human body, from an injected dose of 250 MBq, is 2.8 mSv, that is far below the FDA single-study limit of 30 mSv for research subjects, as per FDA Guidance for Industry and Researchers, 2010 [31].
Therefore, animal data from biodistribution/dosimetry studies and extrapolation of human kidney absorbed and total body effective dose, along with the current Nuclear Medicine practice concerning technetiated diagnostics, support the proposed single dose of 250 MBq of ^{99m}Tc Annexin V-128 for the Phase I-IIa study.
Regarding the toxicity profile of the non-radiolabelled protein, a package of pre-clinical studies was designed in order to investigate the potential toxicity of rhAnnexin V-128. Preliminary 7-days toxicity studies conducted in rats and dogs, showed that rhAnnexin V-128, administered intravenously once daily, was well tolerated up to the highest tested dose, that is 450 fold (in rats) and 200 fold (in dogs) the proposed human dose.
The main repeat-dose toxicity studies were conducted in rats and dogs as well, and consisted of 2-weeks daily intravenous administrations of rhAnnexin V-128, at doses that were approximately 25, 50 and 100 times the intended human dose. The toxicity profile was in general favourable, with some treatment-related findings in the spleen (germinal centres hyperplasia) and in the liver (periportal eosinophilic cell infiltration) in the rats at the intermediate and high dose, and some periportal subchronic inflammation in the dog liver at the high dose. These signs were in general reversible after 2 weeks recovery period. The No-Observed-Adverse-Effect-Level (NOAEL) was defined to be 0.2 mg/kg/day in rats (25 fold the intended human dose) and 0.4 mg/kg/day in dogs (50 fold the intended human dose).
These findings meet the safety margins recommended in the relevant guideline[32], which indicates that the mass dose intended to be used in the Phase I studies should be at least 100 times lower than the NOAEL found in the acute toxicity studies in the rat and at least 25 times lower than the NOAEL found in the repeated toxicity studies in rat and dog.

As described in sections 5.3.1 and 5.3.2 of the investigator Brochure, it is worth noticing that no adverse reaction, including any sign of allergic or anaphylactic reaction, was reported in animals during in vivo phase of any of the toxicity studies performed in rats and dogs. As a reference the dose range tested in animals was within 25 to 450 times the intended human dose (section 5.3 of the IB).

In addition to the in vivo toxicology studies mentioned above, the safety of rhAnnexin V-128 was evaluated in a whole blood cytokine release ex-vivo assay (section 5.3.3 of the investigator Brochure). The results showed that rhAnnexin V-128 did not induce any cytokine release.

An open label, single dose, Phase I clinical study of safety, tolerability, pharmacokinetics and nuclear

medicine imaging of ^{99m}Tc-rhAnnexin V-128 intravenous administration of 370 MBq single intravenous bolus in 12 healthy adult volunteers has been conducted. A series of images for up to 24 hours from the time of injection and blood samples were obtained from time of screening, dosing day and 24 hours after injection, with follow up visits at 72 hrs, 7 days and 30 days post injection. A physical examination was conducted 72 hrs, 7 days and 30 days post administration of ^{99m}Tc - rhAnnexin V-128. No abnormal findings have been noted after administration of the studied imaging product and during the observation period. No immunology response has been reported in any of the 12 treated subjects (15 and 30 days post administration). The Phase I Clinical Trial Report is in preparation.

2 STUDY OBJECTIVES AND HYPOTHESIS

This Phase I-IIa study will be undertaken to determine the safety, tolerability, biodistribution and ability of early imaging with ^{99m}Tc-rhAnnexin V-128 to predict response to treatment. Patients with diagnosed rheumatoid arthritis (10 patients) or ankylosing spondylitis (10 patients) will be imaged on Day 1 and after a suitable interval time (6 weeks +/- two weeks) post-first administration of the imaging product.

Ten patients, independently from the group (RA or AS), will participate to a dosimetry and PK sub-study after written approved consent. These patients will have additional images and blood sampling at Day 1 and 24 hrs after administration of ^{99m}Tc-rhAnnexin V128. In these patients urines will be also analysed as a function of time by SEC-HPLC.

Out of the first 6 patients, at least 3 patients must be enrolled in the dosimetric and PK sub-study.

The Study objectives are:

Primary Objectives:

- To determine the safety and tolerability of imaging with ^{99m}Tc-rhAnnexin V-128.

Secondary Objectives:

- To determine the biodistribution, pharmacokinetics and radiation dosimetry of ^{99m}Tc-rhAnnexin V-128
- To demonstrate the time-dependent distribution and localization of ^{99m}Tc-rhAnnexin V-128 both in RA and AS patients
- To determine ability of early imaging with ^{99m}Tc-rhAnnexin V-128 to evaluate the presence of lesions before and after short term treatment of either RA or AS patients

Study hypothesis:

^{99m}Tc-rhAnnexin V-128 could be used as an adjunct for the diagnosis of early stage rheumatoid arthritis and ankylosing spondylitis and for the assessment of response to treatment of these diseases.

Study justification:

For the patient, the availability of an *in vivo* biomarker of early RA and AS with high sensitivity and specificity in the early stages of the disease could support the timely initiation of disease-modifying treatment, and could allow improved control of risk and cost on a patient-by-patient basis of the anti-rheumatic agents now in clinical use.

3 STUDY DESIGN

Study Outline

This is a monocentric, open label, Phase I-IIa study.
Patients who have signed the informed consent and are eligible for study participation according to the inclusion and exclusion criteria will receive a single intravenous bolus of ^{99m}Tc-rhAnnexin V-128 on Day 1 and a single intravenous bolus of ^{99m}Tc-rhAnnexin V-128 6 weeks later (+/- 2 weeks).
All patients will start a new RA or AS treatment on Day 2.
In order to minimize the risks for the study population, patients will be enrolled with a minimum of 2-week interval.
Details on study assessments are provided in Protocol Section 6 and Table1.

Study duration and End of Study

Study duration is of 4 month +/- 2 weeks (including the screening period).
Eligible patients will receive two injections: one on Day 1 and a second administration 6 weeks +/- 2 weeks later. The end of the study is defined as the moment that the last enrolled patient has completed 18 days follow-up after completing the second ^{99m}Tc-rhAnnexin V-128 administration An additional blood sample for immunological assay is required 3 months after the first administration of the ^{99m}Tc-rhAnnexin V-128 (Day 90 ± 14 days) in case of positive anti-Annexin antibodies outcome in previous examination.

4 PATIENTS SELECTION

Approval for the study will be obtained from the Research Ethics Committee in Lausanne, Swissmedic and the OFSP. All patients will provide written informed consent prior to the initiation of any study procedures.

Inclusion Criteria

1. Patients diagnosed with RA based on ACR/EULAR 2010 criteria (score ≥ 6), OR
   Patients diagnosed with AS based on the ASAS criteria. Patients with RA must have serology assessment performed and documented.at the time of enrolment.
2. Patient with RA active disease (DAS > 2,6) and the introduction of a Bi-DMARD treatment should be indicated. RA patients must at least have been treated with DMARD (methotrexate, leflunomide and sulfasalazine) or combination of these treatments for at least 3 months. Treatment will be pursued while on study.
   OR
   RA patients must have been previously treated with Bi-DMARD before initiation of the new Bi-DMARD treatment. The non-response of the previous Bi-DMARD treatment must be documented. OR
   Patients with ankylosing spondylitis with insufficiently controlled disease while under NSAID and indication for Bi-DMARD. These patients must be under NSAID for at least 3 months and

under the same NSAID for at least 1 month prior to enrolment.
3. ≥ 18 years old
4. Karnofsky ≥ 80%
5. Negative Pregnancy test for women with childbearing potential
6. For women with childbearing potential, use of two reliable means of contraception (e.g., hormonal contraceptive, patch, vaginal ring, intrauterine device, associated with other barrier method of contraception such as the use of condoms) , throughout their participation in the study
7. Absence of ECG anomaly
8. Written ICF signed

Exclusion Criteria

1. Pregnancy or lactation
2. Liver impairment (ALT, AST or Bilirubin > 2 ULN) at screening visit or baseline
3. Kidney impairment (serum creatinine > 1.5 mg/dL)
4. History of congestive heart failure (NYHA III & IV)
5. History of malignant disease within 5 years
6. 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
7. Known hypersensitivity to the investigational drug or any of its components
8. Participation to another clinical trial within 4 weeks before study inclusion except for patients who have participated or who are currently participating in an interventional study without any study drug administration.

Number of patients

Twenty evaluable patients (patients who have completed Day 56 assessments) will be recruited diagnosed either with Rheumatoid Arthritis (RA) or Ankylosing spondylitis (AS). Patients will be recruited with a ratio of 1:1 RA:AS.

Details on treatment of the patients:

Patients with RA will be diagnosed based on ACR/EULAR10 and will thereafter be evaluated with DAS 28. Patients with AS will be diagnosed based on the ASAS criteria. Each patient will be anonymized and identified with a patient ID number.

A unique subject identification number (Patient ID) will be assigned at the start of the Screening Period to each subject who signs the informed consent form until the study termination of the patient. This number will identify the subject throughout the study.
Patient IDs will include the 2-digit country code number (SW) and a 3-digit subject number (ex: SW001 for first subject In).

In both groups of patients – i.e. RA and AS patients – the introduction of a Bi-DMARD treatment should be indicated at the moment of inclusion in the study. The Bi-DMARD therapy should start on Day 2 of the study. RA patients must have been treated at least with one DMARD (methotrexate, leflunomide and sulfasalazine) or a combination of them in the last 3 months – before inclusion - and this/these DMARD(s) will be continued in this study. Otherwise, RA patients must have been previously treated with Bi-DMARD before administration of the new Bi-DMARD treatment. The non-response of the previous Bi-DMARD treatment must be documented.
AS patients must at least have been treated with NSAID in the last 3 months before inclusion and they

should be under the same NSAID treatment during the last 4 weeks before inclusion.
For RA patients, anti-tumour necrosis factor (TNF) antibodies, anti-interleukine 6 (IL-6) antibodies and abatacept therapies are to be considered and are authorized in Switzerland as first line Bi-DMARD agents in RA. On the contrary, anti-CD 20 positive lymphocytes antibodies can only be used, if one anti-TNF therapy failed.
In the AS patients group, only anti-TNF antibodies can be considered, if NSAID are not sufficient.

Ten patients enrolled in the study will be asked to participate in a dosimetry and PK sub-study, irrespective of the group they are part of (RA or AS).

Screening Failures and Drop Outs patients

For the purpose of this study, an “enrolled patient” is a patient who has signed the informed consent and meets all inclusion and exclusion criteria to participate in the study.

A “screening failure” is a patient who has signed the informed consent, but who does not meet all selection criteria and has not received any study medication as part of this protocol.
For patients not considered eligible after the screening period, the reason for not being eligible must be documented. No additional assessments are needed.

A “drop-out” is a patient who has received the study medication and withdrawn consent during the participation in the trial. A “drop-out” may also be a patient who has been “lost to follow-up” and is presumed to have withdrawn consent prior to the end of the trial procedures. However, in the case where a patient who has signed the informed consent and has received study medication as part of this protocol, but who is (later) found not to meet all selection criteria, this will be considered a “protocol violation”.

Discontinuation Criteria for Individual Patients

The patient is free to withdraw from the study for any reason and at any time without giving reason for doing so and without penalty or prejudice. It is also possible that the Sponsor or Swissmedic request termination of the study if there are concerns about conduct or safety.
A patient may be withdrawn from the study if:

- A serious adverse event (SAE) occurs.
- The patient fails to comply with the evaluations or other requirements of the study.
- The patient starts treatment with one of the medications disallowed. The final decision to withdraw a patient who starts treatment with disallowed medication will be made by the Sponsor following consulting of the Investigator.

A patient must be withdrawn from the study if:

- The Investigator considers it, for safety reasons, to be in the best interest of the patient.
- The patient withdraws his/her consent.
- The patient is pregnant.

If withdrawal occurs for any reason, the Investigator must determine the primary reason for a patient’s withdrawal from the study. The date and reason for discontinuation must be documented in the CRF.
For patients who are lost to follow-up (i.e., those patients whose status is unclear because they fail to appear for study visits without stating an intention to withdraw), the Investigator should show ‘due diligence’ by documenting in the source documents steps taken to contact the patient, e.g., dates of telephone calls, registered letters, etc.

Prohibition and Restrictions

Previous Annexin V-based agents, such as ^{99m}Tc-BTAP-rhAnnexin V and ^{99m}Tc-HYNIC-rhAnnexin V, have been used in several clinical trials as a tool for both diagnosis and follow up of drug therapy in oncological, autoimmune, transplanted and cardiopatic patients. No significant effects on vital signs, coagulation parameters or measured haematology or chemistry values have been observed.
Existing Phase I study data in healthy volunteers, indicates that ^{99m}Tc-HYNIC-rhAnnexin V is a safe radiopharmaceutical [28].
The good safety profile of ^{99m}Tc-rhAnnexin V-128 has been confirmed by the extensive package of bioanalytical, immunological and other preclinical studies performed by the Sponsor which indicate adequate safety margin for use in man at the concentrations and doses foreseen in this study.

Furthermore, no adverse reactions were observed in a Phase I study in 12 healthy volunteers who have been administered 370 MBq of ^{99m}Tc-rhAnnexin V-128.

The most important required restriction for study patients is pregnancy.
Imaging is performed ambulatory. After imaging, patient may go back home or go back to work without particular precaution for him or his relatives.

Any concomitant medication deemed necessary for the welfare of the subject 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.

5 STUDY MEDICATION AND TREATMENT

Packaging and Labelling

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

The kit will be prepared, packaged and released according to Sponsor Standard Operating Procedures (SOPs), Good Manufacturing Practice (GMP) guidelines, ICH Good Clinical Practice (GCP) guidelines, and applicable local laws/regulations.

The investigational product will be supplied by Sponsor as a sterile, single vial lyophilised kit for reconstitution with ^{99m}Tc solution eluted from a generator.

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

Composition of the Kit for the preparation of ^{99m}Tc-rhAnnexin V-128 for injection

Component	Amount mg/ vial	Function	Reference to Standards
rhAnnexin V-128	0.400	Drug Substance	In-house
Sodium α-D-Glucoheptonate dihydrate	3	Transchelating agent	In-house
Gentisic acid sodium salt hydrate	0.020	Radiation Stability Enhancer	In-house
D(+)-Trehalose dihydrate	50	Lyoprotectant/Cake forming agent	Ph. Eur. 07/2010:2297
Sodium metabisulfite	0.020	Lyoprotectant	Ph. Eur. 01/2008:0849
Hydroxypropyl-β-Cyclodextrin	5	Solubilizing agent	Ph. Eur. 01/2009:1804

Stannous chloride dihydrate	0.010	Reducing agent	Ph. Eur. 01/2008:1266
Lactic acid	5.406	Buffering agent	Ph. Eur. 01/2008: 0458
Sodium hydroxide	1.320	pH regulation agent	Ph. Eur. 01/2008: 0677
Nitrogen	qs	Inert blanket	Ph. Eur. 01/2008:1247

Handling of Study Medication

^{99m}Tc-rhAnnexin V-128 must be administered at the investigational site.

The study medication 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.

Based on the stability tests performed (Appendix 1), the Annexin Kit can be stored upon receipt at 5°C ± 3°C at least up to 6 months..

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. Stability studies have demonstrated radiochemical purity at 6 h from labelling greater than 90%, which is in line with current specifications of the Kit for the preparation of ^{99m}Tc-rhAnnexin V-128 for injection. The administration volume (corresponding to 250 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). For the purpose of this study, it is recommended to administer the reconstituted solution within 4 hours after completion of the labelling reaction.

IMP Packaging and Storage

The site personnel will maintain shipping, dispensing, and collection logs. All investigational product will be stored and inventoried according to the protocol instructions and applicable state, regional and federal regulations and will be stored in a secure, locked location with limited authorized access at the investigational site.

Precautions and recommendation for use

Technetium-99m eluate should be obtained from a commercially available Mo-99/Tc-99m generator, approved by regulatory authorities, that has been eluted within the past 24 hrs. The radiopharmacist will be directed to use the eluate within one hour of milking the generator.

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/the radiopharmacist, and must be documented throughout the study.

On ongoing basis the Investigator/radiopharmacist agrees to conduct a study medication supply inventory and to record the results of this inventory on the IMP Reconciliation Form. It must be possible to reconcile delivery records with those of used and unused medication. Any discrepancies must be accounted for. Appropriate forms of deliveries and returns must be signed by the responsible person.

Used/unused “Kits for preparation of Technetium ^{99m}Tc-rhAnnexin V-128 for injection” will be locally discarded after monitoring by the Clinical Research Associate.

Refer to Appendix 1 for detailed instruction on the Radiolabelled Imaging Product ^{99m}Tc-rhAnnexin V-

128, Cautionary notes, Analytical and Biological Controls, Stability and Shelf Life.

Study Medication Dose, Mode of Administration, Batch Number

The "Kit for preparation of ^{99m}Tc-rhAnnexin V-128 for injection" consists of 1 patient dose.

The single dose vial (0.40 mg rh-Annexin V-128) will be reconstituted with 2 mL ± 0.2 mL containing 740 MBq ± 74 MBq (20 mCi ± 2 mCi) of ^{99m}Tc. The labelling reaction requires 90 minutes and the reconstituted radiolabelled product is stable for 6 h. For the purpose of this study, it is recommended to administer the reconstituted solution within 4 hours after completion of the labelling reaction,

Patients will receive 2 administrations of ^{99m}Tc-rhAnnexin V-128 , one on day 1 and one on day 42 (±14 days).

When investigational product is received at the site, the Investigator, Pharmacist or authorized designee shall check for accurate delivery and acknowledge receipt by signing and dating the documentation provided by the Sponsor and returning it to the Sponsor or designee. A copy of this documentation shall be retained for the Investigator file.

The dispensing of the investigational product shall be carefully recorded on an investigational product accountability form provided and an accurate accounting must be available for verification by the Clinical Research Associate at each monitoring visit.

Unused investigational product must not be discarded or used for any purpose other than the present study.

The Clinical Research Associate will periodically collect the Drug Accountability Forms and will check all investigational product (both unused and used) before making arrangements for authorizing their destruction at the investigational site.

6 ASSESSMENTS

Assessments will take place in the Nuclear Medicine Department and in the Rheumatology department CHUV, Lausanne, Switzerland.
Subjects will be evaluated for safety and tolerability in accordance with Table 1 and the following Visits Schedule:

- Voluntary written informed consent will be obtained from every subject prior to the initiation of any study-related procedures.
- Each subject's demography: gender, ethnicity, weight, height, medical history, and relevant baseline characteristics will be recorded at screening visit.
- Inclusion/exclusion criteria will be checked at Screening.
- Women of childbearing potential must have negative urine pregnancy test at screening and before each ^{99m}Tc-rhAnnexin V-128 administration.
- All patients will have a baseline DPD-99m-Tc bone scan prior to any treatment. Patients will also perform a 3 hours post injection whole body scan as described below (Imaging section). The ^{99m}Tc-DPD bone and whole body scan are not required in case the patients already performed them within 8 weeks prior to the ^{99m}Tc-rhAnnexin V-128 administration.
- Vital signs will be taken at all study visits.
- ECGs will be recorded at all study visits except for Day 56 visit to measure the different ECG intervals (RR, PR, QRS, and a more extended QT evaluation according to ICH E14, and heart rate, HR). A single ECG will be taken supine, after 5 minutes rest, and not immediately after a meal. The parameters will be measured as a mean value of minimally 3 beats. The Investigator will note in the CRF whether the ECG is normal or abnormal, as well as the clinical relevance of abnormal ECGs. Clinically relevant abnormalities will be recorded on the Adverse Event page of the CRF.
- Physical examination will be conducted at Screening, Day 42 and Day 90.

- BASDAI, BASFI, BASMI for AS, DAS28 Disease Activity Score assessments for RA will be performed at baseline, Day 42 and Day 90.
- Blood samples for haematology, coagulation, biochemistry and urinalysis will be obtained at screening, at Day 30, Day 42 and Day 90, as described in the table below. For PK subgroup of patient's blood samples will also be collected pre-injection, 24 hours post injection as described above.
- All medications taken from 2 weeks prior to the first administration date through the end of study are to be recorded as medical history and concomitant medications, included therapies for RA and AS
- Any adverse events post-injection will be followed until resolution. 24 hours after each treatment injection (at Day 2 and Day 43), each patient will be contacted by phone (except at Day 2 for patients participating in the PK and dosimetric sub-study who will be interviewed during in site visit) in order to inquire if they experienced signs or symptoms of allergic reactions (edema, rash).

In more details, all patients will undergo the following assessments:

General laboratory analysis will be performed and blood samples collected at screening, on Day 30, Day 42 and Day 90 Table 2 summarizes general laboratory analysis to be performed.

Haematology	Coagulation	Blood Chemistry	Urinalysis
• WBC with differential • Platelets • Hb • MCV • Haematocrit	• PT • PTT • INR	• BUN • Serum creatinine • Creatinine Clearance • Uric acid • Albumin • Total bilirubin • AP • AST/ASAT • ALT/ALAT • Gamma-GT • Sodium • Potassium • LDH • GlycoHb • fT4 • CRP • Calcium • Glucose	• RBC/hpf • WBC/hpf • Casts/lpf • Protein by dipstick test • Pregnancy test *(at screening, if applicable and before injection of study product)*

Clinical assessment

For RA diagnosed patients a clinical evaluation will be made at the screening visit, at Day 42 and Day 90. The DAS-28 (3items: tender joints, swollen joints and ESR) will be calculated. For AS beside the clinical evaluation including the search for enthesopathy – using the Maastricht Score -, assessments with BASDAI, BASFI and BASMI will be made.
The clinical assessment will be completed by the measurement of biological markers of inflammation (ESR, CRP and CBC).

Ultrasound assessment

For RA patients only, an ultrasound examination of the 28 joints included in the DAS 28 will be made at screening, Day 42 and Day 90. Synovitis, power-doppler activity and erosions will be checked.

Imaging

Bone scintigraphy: all patients will have a baseline DPD-99m-Tc bone scan prior to any treatment.. Standard quality control of the camera will be performed every day before patient imaging. Patient will be injected with 9MBq/Kg of DPD-99m-Tc. Peak will be centered on 140KeV with a 20% windowing; a LEHR collimator will be used. It will be a triple phase bone scan including angiogram, blood pool imaging (10 minutes after injection) and 3 hours delayed imaging. Angiogram will be centered on the most symptomatic joint and/or and associated inflamed tendinous structures; acquisition will be started immediately after DPD-99m-Tc intravenous injection. Then 16 frames of 15 seconds will be acquired (128x128 matrix; zoom 1). Blood pool images will be acquired 10 minutes after injection and will be centered on the most symptomatic joint. A complement of imaging could be performed at this time centered on another joint. It will be images of 2 minutes (256x256 matrix, zoom 1). Delayed images will be performed 3h post injection. They will be focused on the same joints visualized on blood pool images, with the same acquisition parameters. Centered images will be completed by a whole body scan (10cm/min, 1024x256 matrix; zoom 1). SPECT images could be acquired 3h after injection centred on the most symptomatic joints (60 projections of 10 seconds, 128x128 matrix, zoom 1). On planar images, region of interest will be drawn on symptomatic joints and non symptomatic joints and ratios of activity will be measured. All patients will be subjected to early and delayed planar anterior and posterior images centered on the hands and the feet. On SPECT images, VOI will be drawn on symptomatic joints.

^{99m}Tc-rhAnnexin V-128 administrations:patients will be injected intravenously with 250 MBq of ^{99m}Tc-rhAnnexin V-128 and thenthewhole body planar imagings (GE Infinia Hawkeye IV camera, (8 cm/min, 1024x256 matrix; zoom 1) will be performed and repeated acquisitions on symptomatic joints. Standard quality control of the camera will be performed every day before patient imaging. Peak will be centered on 140KeV with a 20% windowing; a LEHR collimator will be used. A dual-SPECT would be acquired centered on the thorax and abdomen at 1h after injection centered (2*60 projections of 10 seconds each, 128x128 matrix, zoom 1). The use of the whole-body and SPECT data for dosimetry will be discussed further in the Dosimetry section

Semi-quantitative assessment of inflammatory joints will be performed in terms of TBR as well as by calculating the relative uptake between pre and post treatment in ROI/VOIs. ROI and VOI defined and use in the different scintigraphies will strictly have the same pixel size and localization; they will be measured in counts.

In case a suspected anomaly is found at imaging, it would be reported to the rheumatologist and the patient's general practitioner.

Immunogenicity assessments

Assays for Anti-rhAnnexin V-128 IgG and IgM antibodies will be performed in serum samples by ELISA at screening visit (before test agent administration at the latest) at Day 30 (+/- 3 days) and at Day 56 (+/- 2 weeks), i.e. 2 weeks after second injection. An additional sample is required 3 months after the first administration of the 99mTc-rhAnnexin V-128 (Day 90 ± 14 days) in case of positive anti-annexin antibodies outcome in previous examinations.
For this purpose, 10 mL of blood will be collected per sample.
Serum will be prepared (following the same procedure as that used for PK samples – see Appendix 2), divided into six aliquots and frozen (-80°C) until complete decay. Three out of six serum samples per time-point will be then shipped to the local laboratory, where the analysis for anti-rhAnnexin V-128 antibodies will be performed (methods detailed in the Laboratory Manual).

Patients participating to the dosimetry and PK studies will undergo the following additional assessments:

Pharmacokinetics (blood and urine)

Venous blood samples of 10 ml each will be taken just before administration of ^{99m}Tc-rhAnnexin V-128 and then at the following time-points: 5, 10, 15, 30, 60 min, 2 hrs, 4 hrs and 24 hrs post the first rhAnnexin V-128 administration for the first 3 patients included in the sub-study. Depending upon the results in those 3 patients, the timeframe for dosimetry measurements and PK will be confirmed or modified with a time-point at 3 hrs (instead of 2 hrs) and 6 hrs (instead of 4 hrs) for the following 7 enrolled patients in the sub-study. The following whole blood or serum aliquots will be prepared as described in Appendix 2:

1) 2 x 1 mL of whole blood will be used for radioactivity measurements
2) 2 x 1 mL of serum aliquots will also be used for radioactivity measurements
3) 2 x 0.6 mL at least of serum aliquots to be frozen at -80°C, will be used for pharmacokinetics measurements. After complete decay of radioactivity, one out of 2 aliquots/time-point will be shipped to the centralized laboratory and analyzed by ELISA to determine the rhAnnexin V-128 concentration in serum (See the Laboratory Manual for further instruction).

The whole blood and serum samples (bullet points 1 and 2) will be counted in a gamma counting system (CHUV/NUC laboratory) and compared to the count rate from a representative percentage of the injected activity inside the same counter.
Blood clearance fractions and effective half-life estimates from multi-term non-compartmental exponential analysis will be calculated from serial measurements of radioactivity in whole blood and serum. Pharmacokinetic analysis will be also performed based on rhAnnexin V-128 serum concentration determined by ELISA method.

Urine samples will be collected as follows (see also Appendix 2). One sample will be collected within 24 hrs prior to ^{99m}Tc-rhAnnexin V-128 administration, preferably just prior to the administration of study drug (0 h sample) to achieve bladder emptying. Quantitative urine collections will be obtained (and volume recorded) possibly in the time intervals 0-1 h, 1 h – 4 hrs, 4 hrs – 16 hrs, and 16 hrs – 24 hrs post administration. Dual 2 mL aliquots of urine from each period will be counted in a gamma counter at the CHUV/NUC laboratory. The mean between the two ^{99m}Tc radioactivity measurements and the measured volume of urine will be used to calculate the amount of ^{99m}Tc excreted in that period and the cumulative percent injected dose (radioactivity).

SEC-HPLC urine assessment

Urine samples will be also analysed as a function of time by SEC-HPLC technique at local laboratory, in order to gain information on the chemical status of ^{99m}Tc-rhAnnexin V-128 and on the presence of ^{99m}Tc-rhAnnexin V-128-related species. For this purpose, 10 mL aliquot from each urine sample will be analyzed through SEC-HPLC (CHUV/NUC laboratory).

Dosimetry

Administration of the ^{99m}Tc-rhAnnexin V-128 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 GE Infinia Hawkeye 4 dual-head integrated SPECT/CT gamma camera with low-energy high-resolution collimators. The

energy acceptance window for the ^{99m}Tc photopeak will be 140 keV (+/- 10%).

The quantitative in vivo biodistribution of the ^{99m}Tc-rhAnnexin V-128 will be determined through whole-body planar imaging following the recommended procedures provided in MIRD Pamphlet No 16 [20]. Reconstruction will be done using iterative reconstruction incorporating CT-based attenuation correction and dual-energy-window scatter correction. Dynamic planar imaging will be acquired during the first 10 minutes centered on the thorax. Then whole body planar images post administration will be acquired at 30 min, 60 min, 2 hrs and 4 hrs at Day 1 and at 24 hrs post-injection for the first 3 patients of the sub-study. Depending upon the results in those 3 patients, timeframe for dosimetry measurements and PK will be confirmed or modified with a time-point at 3 hrs (instead of 2 hrs) and 6 hrs (instead of 4 hrs) for the following 7 enrolled patients in the sub-study. In addition, a SPECT/CT will be performed on the abdominal/pelvis regions at 3 hours post administration for the first 3 patients in order to generate relevant dosimetry data after extrapolation for the reconstruction with OLINDA software. Depending upon the results in those 3 patients, the SPECT/CT on the abdominal/pelvis regions will be performed at 5 hours post administration. Patient's bladder should be voided before this acquisition. A ^{99m}Tc source of known activity will be imaged before the first acquisition with the patient [33].The activity of this source will be measured in a dose calibrator prior to imaging and used to determine the imaging system calibration factor for converting reconstructed image counts into an activity concentration. In all patients, the source regions will be the brain, lungs, heart, liver, spleen, gastrointestinal (GI) tract, kidneys and urinary bladder contents and volumes of interest (VOIs) will be drawn around these. An additional VOI will be drawn around the whole body. The salivary glands, thyroid gland and gall-bladder will be defined as 190 source regions when visible and VOIs drawn about them. The difference between the number of counts in the whole-body VOI and the sum of those in the specified VOIs will define the number of counts in the "remainder" category. In addition, a VOI will be drawn over the quadriceps femoris (to provide a measure of soft tissue uptake and washout as describe below). Background will be estimated from a separate SPECT acquisition with no activity present in the field of view. We assume the VOI set about the GI tract will demonstrate a rapid and non-specific uptake by soft tissue which will clear (in parallel with observed washout from soft tissues) to be replaced by activity in the contents arising from hepatobiliary transport. In order to isolate the activity within the GI tract contents, the washout of activity from the GI tract tissues will be modelled by the measured washout of activity from the VOI set over the quadriceps femoris, rescaled to that of the gastrointestinal tract at the time of the first image acquisition, when there was no activity in the gastrointestinal tract contents, and then subtracted from the activity measured in the GI tract VOI. This procedure provides the activity due to the GI contents alone.
For each source region, the measured activity at each time point will be normalized to that administered, decay-corrected to the time of injection, and least-squares-fit by an analytical function. Physical decay of ^{99m}Tc will then be introduced to these analytical fits of the normalized activities and the function integrated to yield the cumulated activity normalized to the amount of administered activity (numerically equivalent to the residence time). The cumulated activity in the combined urinary bladder contents and voided urine will be calculated using a dynamic urinary bladder model [33] for a 3.5 hour urinary bladder voiding interval [34]. The cumulated activity in the gastrointestinal tract [35] with the assumption that all activity enters the duodenum via the hepatobiliary transport will be calculated.

Radiation dose to the organ of interests (particularly the kidneys), Effective Dose Equivalent (ICRP 30), Effective Dose (ICRP 60) will also be calculated.

The internal radiation dosimetry for the adult human will be calculated through the normalized cumulated activities for each patient provided as input to OLINDA. The derived ensemble of organ absorbed doses will then be used to calculate the Effective Dose per unit administered activity [36] with the following modifications: 1) gonadal absorbed dose will be taken to be the arithmetic mean of the testicular and ovarian absorbed doses [34]; 2) absorbed dose to the thymus will be used as a surrogate for the absorbed dose to the oesophagus [36]; and 3) absorbed dose to the wall of the upper large intestine will be removed from the remaining organs category and the mean absorbed dose to the colon

wall, given by the mass-weighted absorbed doses to the walls of the upper and lower large intestine, used in the Effective Dose [36].
Because of the non-specific uptake by the walls of the GI tract, further dosimetry analysis of these tissues will be performed. While the MIRD schema specifies the walls of the GI tract compartments as target regions, they are not specified source organs. As such, their photon contributions to the dosimetry will be assimilated within those categorized as “remaining tissues”. However, the “self dosing” to the GI tract walls due to non-penetrating particulate radiations from ^{99m}Tc uptake within the walls will be estimated from the measured GI tract wall cumulated activities using decay data summarized by Stabin [37] and downloaded from the RADAR website. These masses of the three GI tract compartments’ walls (small, upper large and lower large intestine) will be taken from [38]. The organ absorbed doses and Effective Doses for the individual patients will be averaged.

7 DATA MONITORING COMMITTEE (DMC)

Ten patients, independently from the group (RA or AS), will participate in the dosimetry and PK sub-study. Out of the first 6 patients, at least 3 patients will be enrolled in the PK and dosimetric sub-study. Preliminary data will be analyzed as soon as these 3 patients have completed the sub-study. Depending upon the results in those 3 patients, the time schedule for imaging acquisition and PK will be confirmed or revised.
Results of dosimetry, PK and immunologic assessments will be monitored by the DMC to assess safety and adequacy of rhAnnexin V-128 technical performance. The data monitoring committee consists of two Site clinicians (one of them being a Nuclear Medicine Physician). These clinicians are not trial investigators. The main function of the committee will be to determine if there is a premature high evidence of an excess of adverse events or the presence of adequate/inadequate technical performance in this patients group.
DMC will advise on the safety of study patients and the patients yet to be recruited to the study as well as maintaining validity and scientific merit of the study. The DMC will review examinations of safety data and promptly give recommendations to continue, continue with modification, or terminate the study.
The DMC report will be sent to the Sponsor and the ethics committee as well as competent authorities
Preliminary results can be used by Sponsor for publication purpose, before the end of the main trial.

8 STATISTICAL METHODS

The statistical analysis of the present study will be performed in accordance with the principles stated in the Consensus-Guideline E9 (Statistical Principles for Clinical Trials) of the International Conference on Harmonisation (ICH).

Sample size

The number of patients in this study is not based on statistical power considerations. The planned sample includes 20 patients with Day 56 assessments which are believed to provide sufficient data to assess the general safety and tolerability of ^{99m}Tc-rhAnnexin V-128. Patients will be recruited with a ratio of 1:1 RA:AS.
Any patient who has not completed Day 56 assessment will be replaced by a patient from the same group (RA or AS). It is expected that about 10% of patients will not complete their Day 56 assessments; therefore, approximately 23 patients will be recruited.
In addition, 10 patients enrolled in the study will be asked to participate in a dosimetry and PK study, irrespective of the group they are part of (RA or AS).
Out of the first 6 patients, at least 3 patients must take part in the dosimetric and PK sub-study.

Demographics and Other Subject Characteristics

Demographic and other baseline data will be summarized descriptively. Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, minimum, and maximum will be presented. All background and demographic/RA-AS diagnosis data will be listed in detail.

Concomitant Medications

Concomitant medications (including treatments for the underline disease) will be coded using World Health Organization (WHO) dictionary. Type and incidence of concomitant medications will be tabulated (generic terms).

Laboratory Tests

Laboratory tests (haematology, coagulation, blood chemistry and urinalysis) will be monitored before and during the study days mentioned in the study chart. A total volume of 100ml will be collected during the study. For each patient participating to the dosimetric and PK sub-study a total of 205 ml will be drawn throughout the study.

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 pre-defined levels of change in these values.
Abnormal laboratory test results will be tabulated.

Vital Signs and ECG

Vital signs will be taken at each study visits, while twelve-lead ECG will be performed.
Descriptive statistics (N, mean, median, minimum, maximum, 95% confidence interval for the mean, etc.) of the observed values as well as for the changes from baseline value will be created. Frequency tabulations with values within, below or above the normal ranges will be made.
ECG parameters will include heart rate (HR), RR interval, PR interval, QRS width and QT interval.
QT intervals will be corrected for heart rate. ECG results will also be evaluated by means of descriptive statistics (mean, median, 95% confidence interval for the mean, etc.) and frequency tabulations.
Graphical presentations might be created to facilitate the interpretation.

Physical Examination

Physical examination results will be tabulated; abnormalities will be listed.

Safety

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

Adverse Events

All original AEs 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, as well as severity and relatedness to the study medication will be

tabulated. Special attention will be given to those patients who prematurely discontinue the study or the study medication due to an AE, or who experience a severe AE or an SAE.

Dosimetry

Descriptive statistics for the dosimetry parameters will be reported.
Dosimetry data for liver, spleen, kidneys, testes, and whole body will be determined using image quantification according to the methodology presented in MIRD Pamphlet No. 16 [39].

Pharmacokinetics and immunology

PK/immunology analysis plan and report will be created.

9 ADVERSE EVENTS AND OTHER SAFETY ASPECTS

Definition of Adverse Events

An AE is defined as any untoward medical occurrence in a patient and which does not necessarily have a causal relationship with the study medication. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease, temporally associated with the use of a study medication, whether or not causally related to the study medication.
AEs will be reported from signing the informed consent onwards until the last study-related procedure. If the information of an untoward medical occurrence is collected before starting the intake of study medication, this information will be listed as a pre-treatment AE during statistical analysis.

Definition of Serious Adverse Events

An 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 patient 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-patient hospitalisation or leads to prolongation of hospitalisation.

  Hospitalisation or prolongation of hospitalisation will not be considered as SAE in the following cases:
    - Hospitalisation planned before the patient inclusion,
    - Hospitalisation less than 24 h
    - Hospitalisation needed for the routine follow-up of the patient disease.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These events, including those that may result in disability, should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for severe allergic reactions that do not result in hospitalization.
The Investigator must report all SAEs by sending a completed SAE Reporting Form (Appendix 5) to the Safety Officer designee within 24 h of becoming aware of the event.
All SAEs must be addressed to Advanced Accelerator Applications EuQPPV, [redacted] with the following contact details:

Tel: [redacted];
Fax: [redacted]
Mobile : [redacted];
E-mail: [redacted]

Criteria for Causal Relationship to the Clinical Study Medication

The investigator should express his / her opinion as to the causality of the SAE in section 12 of the SAE Reporting Form:

12. Possible Causes of the Serious Adverse Event

Check off all that apply		**Specify**
Pre-existing / underlying disease	❑	
Study treatment	❑	
Other treatment	❑	
Protocol-related procedure	❑	
Other (e.g., accident, new / intercurrent illness)	❑	

Investigator Reporting Requirements

Throughout the study, the study staff will question the patient in a non-directive way as to the occurrence of AEs. The patient will also be instructed, when signing the informed consent, as from that moment, to contact the Investigator to report any study medication or non-study medication-related adverse or unusual event that occurs during participation to the study.
The study staff will record all these events in the patient's medical records and e-CRF, whether observed by the Investigator, the investigational staff, or spontaneously reported by the patient.
The Investigator will provide a complete description of the event in standard medical terminology, the date of onset and termination, severity, relationship to the study medication, action taken regarding the study medication, any treatment given, the outcome, and whether or not the event is considered an SAE. If known, the Investigator should report the underlying illness or disorder rather than the individual signs and symptoms.

Reporting of Serious Adverse Events

In the case of an SAE, the Investigator must immediately (at the earliest possible time point within 24h of awareness) complete the SAE section of the CRF, reporting all information that is required by the Regulatory Authorities and contact the delegated Safety Officer designee, if needed. The name and contact details of the delegated Safety Officer designee will be available in the Investigator File (and will be updated when needed).
The minimum information required for immediate reporting is the event description, the patient ID, the study medication concerned, and the identifiable reporter (Investigator or designee). Even if not all the facts are known, an initial report should be made. The Investigator must provide follow-up information as soon as possible. If requested by the delegated Safety Officer designee, documents relevant to the diagnosis, treatment, and course of the event must be submitted (e.g. technical investigation reports, histology findings, hospital discharge documents). All documents must be anonymized with respect to

the patient's personal data identification.

When the Investigator determines that there is not more information likely to be available, a final report should be provided.
The Sponsor or delegated CRO will assume responsibility for appropriate reporting of AEs to Swissmedic and Independent Ethics Committee(s)/Institutional Review Board(s) (IEC/IRB) according to local laws and regulations.
SAEs will be collected following the patient written consent to participate in the study. The collection of SAE information will continue to be reported by the Investigator for each patient until 30 days after the last study medication administration.

Follow-up of Adverse Events

All AEs occurring during the study are to be followed up until resolved or judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized. An assessment should be made at the last study-related visit for each patient. Certain long-term AEs cannot be followed until resolution within the settings of this protocol. In these cases follow-up will be the responsibility of the treating physician.
Since it is unpredictable how long such a follow-up might take, data from this follow-up generated after the patient's last study-related visit will be recorded by the Investigator. Full details regarding this follow-up will be described in the Clinical Study Report, if necessary.
If during AE follow-up the case has progressed to the level of "SAE", or if a new SAE whose relationship to the study medication could not be ruled out is observed, the situation must be reported immediately by the Investigator becoming aware of the information (considering that the "date of SAE onset" is the date of the first manifestations of that AE).

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 risk factors for an unintentional pregnancy. For all women to whom the study has been proposed, it will be ensured they are using two reliable means of contraception (e.g., hormonal contraceptive, patch, vaginal ring, intrauterine device, associated with other barrier method of contraception such as the use of condoms). During the clinical study, all women of childbearing potential 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 any pregnancy associated with investigational product exposure including conceptions occurring until 30 days after the last study medication administration. The report should be carried out within 24 hours of pregnancy confirmation by sending a completed Pregnancy Reporting Form to the Safety Officer designee.
Appropriate pregnancy follow-up procedures should be considered if indicated. The Investigator must report follow-ups within 24 hours of the receipt of any new information on the course of the pregnancy, including perinatal and neonatal outcome, by sending a completed Pregnancy Reporting Form to the Safety Officer designee.
When the outcome of the pregnancy, delivery, and newborn fulfill the criteria of SAEs [e.g., spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly (including anomaly in a miscarried fetus)], the Investigator should respond in accordance with the reporting procedure for SAEs described in section 9.5. Additional information regarding the outcome of pregnancy (which is categorized as an SAE) is mentioned below.
Death of a newborn within 1 month after birth should be reported as an SAE regardless of its relationship with the study medication.
If an infant dies more than 1 month after birth, it should be reported if a relationship between the death and intrauterine exposure to the study drug is judged as reasonably related by the Investigator, provided that the date of conception was prior to injection of the product or within one month of

monitoring the patient.
If the date of conception is more than a month after the injection, all cases of child deaths are not included.
In case of a delivery of a living newborn, the newborn's condition is evaluated at birth. The miscarried fetus is evaluated by visual examination unless test results which indicate a congenital anomaly are obtained prior to miscarriage.

New Safety Information Affecting the Conduct of the Study

When new information, including "Dear Doctor Letters" but not limited to that, necessary for conducting the clinical study properly will lead to a protocol amendment, the Sponsor should inform all Investigators involved in the clinical study, the head of the study site, ethics committees, and Regulatory Authorities of such information, and when needed, should amend the patient information and let the patient sign the updated version of the Patient Information.

10 TERMINATION OF THE STUDY

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

- When the Sponsor is aware of information on matters concerning the quality, efficacy, and safety of the study drugs, 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 along with the reasons to the investigator and applicable authorities.
- If the investigator intends to discontinue participation in the study, the investigator must immediately inform the Sponsor of the discontinuation and the reason for it.
- The Sponsor reserves the right to discontinue the study at any time for failure to meet expected enrolment goals.

11 OPERATIONAL, ETHICAL, AND ADMINISTRATIVE CONSIDERATIONS

Study personnel

All study staff will be informed of the study protocol through meeting and internal study initiation. Their task will be supported by internal SOPs specific to the study.
The study nurse will be asked to collect blood and urine samples and to ship/send them to the appropriate laboratory.

Data collection

The study will be monitored by Advanced Accelerator Applications according to the current SOP for the monitoring of studies. Shortly before the study starts, the Study Monitor will meet with the Investigator and Investigational Staff involved reviewing the procedures regarding study conduct and recording of data in the CRF. 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 CRFs. Key data transcribed into the 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 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 patients and quality of the data.
An independent Quality Assurance (QA) department, Regulatory Authorities and/or IECs/IRBs 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 patient's file. By participating in this study, the Investigator(s) agree(s) to this requirement.
For any data transfer, measures will be undertaken to protect patient data handed over against disclosure to unauthorized third parties and patient confidentiality will be maintained at all times.

Data Review

All data relating to the study must be recorded in the CRFs provided by the Sponsor. These CRFs should always reflect the latest observations on the patient's participation in the study. Therefore, CRFs are to be completed as soon as possible after (or during) the patient's visit. To avoid inter-observer variability, every effort should be made to ensure that the study determinations are completed by the same individual who made the initial ones at baseline. The Investigator must verify that all data entries in the CRFs are accurate and correct.
The monitor will review the CRFs and evaluate them for completeness and consistency. The CRF will be compared with the source documents to ensure that there are no discrepancies between critical data. All entries, corrections and alterations are to be made by the responsible Investigator or his/her designee. The monitor cannot enter data in the CRFs.

Data Clarification

If corrections to a CRF are needed, the responsible monitor or data manager will raise a query. The appropriate investigational staff will answer queries.

Source Documents

Source data must be available at the study centre to document the existence of the study patients and substantiate the integrity of the study data collected. They must include the original documents relating to the study, as well as the medical treatment and medical history documentation of the patient.
The source medical records should at least include the following information for each patient:

- Patient identification (name, date of birth, gender);
- Documentation of eligibility criteria, i.e. medical and medication history, physical examination;
- Participation in study (including study number);
- Study discussed, signed and dated ICF;
- Dates of all visits;
- Images/scans and reports;
- Documentation that protocol-specific procedures were performed;
- Study medication administration time and date;
- Receipt, dispensation and destruction of used/unused study medication;
- Record of all AEs and other safety parameters;
- Record of all previous and concomitant therapies;
- Date of study completion or reason for early discontinuation (if applicable).

The following documents are considered as source documents as well: nurse records and worksheets.
The author of an entry in the source documents must be identifiable. Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded on the CRF are consistent with the original source data.

Clinical Study Monitoring

Advanced Accelerator Applications or designee is responsible for periodic monitoring of the study to

ensure that patients' human rights, safety, and well-being are protected, that the study is properly conducted in adherence to the current protocol and ICH GCP, 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 their Monitoring Log will be obtained at the study close-out visits.

Direct Access to Source Data/Documents

The Investigator and the study centre must accept monitoring and auditing by the Sponsor or deputy as well as inspections from the IEC(s)/IRB(s) and/or relevant Regulatory Authorities. They must provide all study-related records, as well as source documents to these instances when they are requested to. The confidentiality of the patient's identity shall be well protected and consistent with local and national regulations when the source documents are patient to direct access.

Data Management

Data management activities will be coordinated by Sponsor with the support of a CRO as necessary.
All study-specific processes and definitions will be described in the Data Management Plan. Coding of AEs and Medical History terms will be performed using MedDRA; previous and concomitant medication will be coded using WHO codes.

Ethical Conduct of Clinical Study

The Investigator(s) and all parties involved in this study should 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 patients. Compliance with this standard provides public assurance that the rights, safety, and well-being of the patients 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 IEC/IRB approved version of this protocol. The protocol, ICF, any information provided to the patient, recruitment advertisements, and any amendments to these items will have IEC/IRB approval prior to their use in the study. Voluntary informed consent will be given by every patient in order to be screened for the study and prior to the initiation of any study-related procedures. The consent process must meet all applicable local laws. The rights, safety, and well-being of the patients 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.

Authorities

The protocol, name, and study centre of the Investigators, the votes of the IEC(s)/IRB(s), as well as other locally required documents will be submitted to SwissMedic, according to local requirements for review and approval before the beginning of the study. SwissMedic will be informed about the end of the study.

Patient Confidentiality

Individual patient 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 patient, to the patient's general practitioner or to other appropriate medical personnel responsible for the patient's well-being.
The Sponsor, its board members, and its personnel shall not disclose any confidential information on patients obtained during the performance of their duties in the study without justifiable reasons.
All individuals and organizations involved in conducting the study and/or processing the study data must pay very careful attention to protect the patients' privacy with appropriate measures, for example, by prohibiting the use of any private information that may identify a patient (e.g. name or address). These details shall be processed in accordance with the applicable local and regional laws.

Patient Information/Written Informed Consent

According to ICH GCP (CPMP/ICH135/95) the patient must give consent to participate in the study, only after having been fully informed by the Investigator of the nature, significance, and implications of the study, as well as to the associated risks involved. Such meetings must be carried out on an individual basis, and adapted to the educational background and previous knowledge of the patient. Participation to this meeting will be documented in the patient's clinical chart. In the present protocol, patient will be informed by the rheumatologist (CHUV) about the scope and goals of the study based on the patient information sheet. Patient will have at least 24 hours to consider his participation to the protocol before signing the informed consent. Any study-related procedure will only take place after signed patient informed consent has been collected.
The patient will be instructed by the Investigator that the consent for study participation can be withdrawn at any time, without having to justify a reason, and that no disadvantageous consequences will follow regarding further medical treatment. The Investigator shall ask for the reason of premature termination without violating the patient's rights (ICH GCP Definition 4.3.4).
Furthermore, the patient must be informed about insurance coverage and the corresponding patient obligations (see Patient Information). The ICF must be personally dated and signed by both the Investigator/Delegate and the patient. The patient receives 1 copy of the original patient information and Informed Consent Form signed and dated by both Investigator/Delegate and the patient.
The original of the ICF will be retained by the Investigator in the Investigator's File, who will confirm the patient's consent in the CRF. The patient will only be included in the study after written consent is given.
Furthermore, the Investigator is recommended to inform the patient's general practitioner of his/her participation in the study, provided that the patient has a general practitioner and the patient agrees to disclose this information. For this purpose, a copy of the patient information sheet and the patient's signed informed consent will be sent to the GP if the patient agrees with this and has ticked the appropriate tickbox on the Patient Information Consent Form.

Arrangement for Use of Information and Publication of Clinical Study Data

The present study will be registered on http://clinicaltrials.gov/ and its results will be published.
All information regarding the investigational product under study in the outlined protocol and Sponsor's operations, such as patent applications, formulas, manufacturing processes, basic scientific data, or formulation information, supplied by the Sponsor and not previously published, are considered confidential by the Sponsor and shall remain the sole property of the Sponsor. 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 Sponsor's written consent.
It is understood by the Investigator that the information developed during the conduct of this study is considered confidential and will be used by the Sponsor for the development of the specified investigational medication. This information may be disclosed as deemed necessary by the Sponsor to other Investigators, other pharmaceutical companies, and to governmental agencies. To allow for the use of the information derived from this study and to ensure complete and thorough analysis, the Investigator is obligated to provide the Sponsor with complete test results and all data developed in this study, and to provide direct access to source data/documents for study-related monitoring, audits,

IEC/IRB review, and regulatory inspection.
Any publication or public presentation of the results of this study must be according to the Sponsor's standards. The first publication is coordinated by the Sponsor. The Investigator agrees that before he/she publishes any results of this study, he/she shall send the draft manuscripts and copies of the information to be presented to the Sponsor at least 30 working days before submission to a publisher or presentation. The Sponsor 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 Sponsor to protect proprietary information and to provide comments based on information that may not yet be available to the Investigator(s).

Documents and Records Related to the Clinical Study

The Investigator must retain CRFs and source documents of all enrolled patients (i.e. all patients who gave consent to be screened for the study), study medication disposition, and other documents required by regulation, in his/her possession or in an accessible area for at least 10 years after the completion of this study. The Sponsor will notify the Investigator when the records no longer need to be kept.
The Investigator should take measures to prevent accidental or premature destruction of these documents.
Under no circumstance shall the Investigator relocate or dispose any study documents before having obtained the Sponsor's written approval.
If it becomes necessary for the Sponsor or the appropriate Regulatory Authority to review any documentation relating to this study, the Investigator must permit access to such reports.
Any difficulty in archiving and storage of clinical study documents must be discussed with the study monitor prior to the initiation of the study.
The data and information collected during this study will be reported in Clinical Study Report(s) by the Sponsor.

Sampling collection

Biological samples collected at site for routine lab analysis (Heamatology and Biochemistry) will be discarded after study analysis, according to local rules. For samples that are to be sent to a centralized foreign laboratory, i.e. for ELISA analysis, the Clinical Trial Agreement will be adapted according to local rules. These collected samples will be stored for up to 10 years (counting from when the last subject performed the last study visit) and then discarded, unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent.
In case of patient consent withdrawal to the use of personnel data, biological samples will not be analyzed and will be destroyed.
Samples collected will only be used for study purposes as per protocol.

Protocol Amendment and/or Revision

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

Qualification of the Investigators

The Investigator(s) should be qualified by education, language, 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(s), as described in the protocol, the current Investigator's Brochure, the product information, and other information sources provided by the Sponsor.
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.

Financial Disclosure

The disclosed financial interest of the Investigator must be collected prior to enrolment of the first patient into the study, following study centre completion, and 1 year following study completion. The Investigator should promptly update this information if any relevant changes occur during this period.
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.

Insurance of Patients and Others

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

Investigator Indemnity

The Sponsor shall be liable towards the patients in accordance with the provisions of the Clinical Study Law. Notwithstanding the foregoing; the Sponsor does not, however, agree to indemnify, defend, or hold the Investigator harmless against liability, damage, loss, cost or expense (including reasonable attorney's fees and expenses), including liabilities arising out of or in connection with claims of any nature by third parties, including, without limitation, in respect of bodily injury or death, arising out of or in connection with the negligence, wrongful acts or omissions or willful misconduct of the Investigator, the Institution, or its affiliates.
Including but not limited to:

- The making of unauthorised representations and warranties concerning the study medication or the study;
- The failure to obtain appropriate informed consent;
- Non-compliance with applicable rules or regulations;
- Failure to conduct the study in accordance with this protocol.

A condition of this indemnity obligation is that, whenever the Investigator has information from which it may be reasonably concluded that an incident of bodily injury, sickness, disease, or death has occurred, the Investigator must immediately notice the Sponsor of all pertinent data surrounding any such incident, and, in the event a claim is made or a suit is brought, the Investigator will assist the Sponsor and cooperate in gathering information with respect to the time, place, and circumstances, and in obtaining the names and addresses of the injured parties and available witnesses.
The Investigator shall not, except at his/her own cost, voluntarily make any payment or incur any expense in connection with any such claim or suit without the prior written consent of the Sponsor.

12 QUALITY ASSURANCE

The Sponsor is implementing and maintaining QA and QC systems with written SOPs to ensure that studies are conducted and data are generated, documented (record), and reported in compliance with the protocol, GCP, and applicable regulatory requirement(s).

The Sponsor or designee may arrange to inspect or audit the study centre. The auditor is independent from the clinical monitoring and project management team at the Sponsor's site. The audit may include on-site review of regulatory documents, CRFs and source documents. The auditors will have direct access to these documents.

Medical imaging is the main research tool used in the present study. These tools are subject to strict quality control laws such as regular controls performed by the producer and regular internal controls.

Appendix 1

Radiolabelled Imaging Product ^{99m}Tc-rhAnnexin V-128

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:

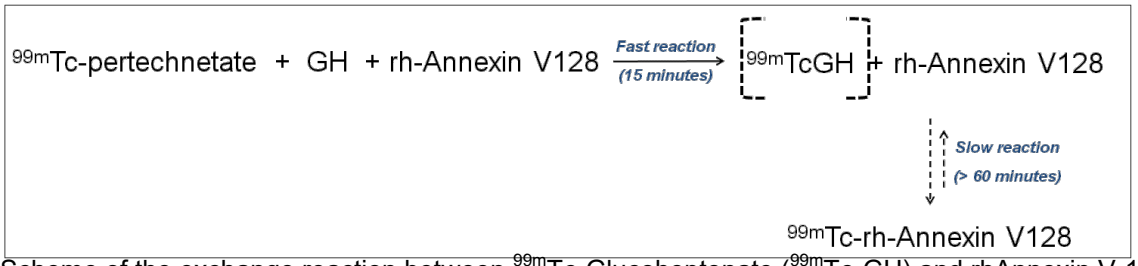
a) Radioactive concentration of the Pertechnetate solution added for labelling should be 370 MBq/mL (the reconstitution volume is 2 mL ± 0.2 mL).

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 6h 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).

Labelling takes place through an exchange reaction [40, 41] 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 an 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 h**
- **Tc-99m pertechnetate eluate which is more than 6 hour old from the time of elution should NOT be used [40-42]**.

Analytical and Biological Controls

Specifications for the Kit for the preparation of ^{99m}Tc-rhAnnexin V-128

Test	Method	Acceptance Criteria
Colour of the freeze dried cake	Visual Ph. Eur.	White
Appearance of the reconstituted solution	Visual Ph. Eur.	Clear, colourless
Time of reconstitution	Visual Ph. Eur.	< 30 sec
Leak test	Visual Ph. Eur.	Conforms
Particulate matter (sub-visible particles)	Light Obscuration Particle count test (Ph.Eur. 2.9.19 method 1)	≥ 10 µm: ≤ 6000/vial
		≥ 25 µm: ≤ 600/vial
pH	pH meter	5.0 – 5.8
Uniformity of content	Internal SOP (by weighing)	Theoretical content (65.17 mg/vial) ± 10%
Residual moisture	Karl Fisher (Ph. Eur. 2.5.12).	≤ 5.0%
Total Tin	ICP-OES	0.0047 – 0.0058 mg/vial
Sn^{++} / Sn total	UV/Vis Spectrophotometry	> 50%
Sodium α-D-Glucoheptonate dihydrate	HPLC/MS-MS-detector	3.0 mg/vial ± 10%
Gentisic acid sodium hydrate	RP-HPLC - UV	0.020 mg/vial ± 10%
Identity D(+)-Trehalose dihydrate	HPLC/ MS-MS-detector	Conforms to reference
Identity Hydroxypropyl-β-Cyclodextrin	HPLC - Evaporative Light Scattering Detector (ELSD)	Conforms to reference
Sodium Metabisulfite	Titrimetric determination with titration pipette (MColortest™)	10-30 ppm/vial
Identity Lactic acid	HPLC - UV	Conforms to reference
Identity rh-Annexin V-128 protein	Western Blot	Conforms to reference
rh-Annexin V-128 protein concentration	Bradford Assay	0.400 mg/vial ± 10%
Chemical Purity (% rh-Annexin V-128 monomer)	SEC-HPLC – UV	≥ 90.0%
	RP-HPLC – UV	
Chemical Purity (% rh-Annexin V-128 dimer)	SEC-HPLC – UV	≤ 10.0%
	RP-HPLC – UV	
Radiochemical Purity (% ^{99m}Tc-rh-Annexin V-128)#	ITLC	≥ 90.0%
Radiochemical Purity (% $^{99m}TcO_4$ + $^{99m}TcO_2$)#		≤ 8.0%
Radiochemical Purity (% ^{99m}Tc-glucoheptonate)#		≤ 10.0%
Radiochemical Purity (% ^{99m}Tc-rh-Annexin V-128)#	SEC-HPLC	≥ 90.0%

Biopotency (%)[#]	RBC binding assay	90 - 110 %
Bioaffinity	RBC Displacement Assay	ratio IC_{50} 0.5-2 to reference
Sterility	Ph. Eur. 2.6.1	Sterile
Bacterial Endotoxin Test (LAL)	Ph. Eur., 2.6.14, USP <85>	<175 EU/vial*

*The specification for Bacterial Endotoxins for Radiopharmaceuticals to be administered by i.v., according to Ph. Eur. and USP, is 175 IU/maximum administered volume (Vmax). In the case of this Drug Product 175 IU/Vmax corresponds to 175 IU/vial or 175 EU/vial.

[#] Test performed right after the completion of labelling reaction (T=0) and 6 hours after (T=6h).

Specifications of the Radiolabelled Imaging Product (^{99m}Tc-rhAnnexin V-128) (test to be performed at the clinical site)

Test	Method	Acceptance Criteria
Radiochemical Purity (% ^{99m}Tc-rhAnnexin V-128)	ITLC	≥ 90.0%
Radiochemical Purity (% $^{99m}TcO_4$ + $^{99m}TcO_2$)		≤ 8.0%

Analytical and Biological methods for Quality Control

Test	Method
Appearance	Appearance is tested following the Ph. Eur. 2.2.1. method. The method consists in a visual check of the vial size, colour, crimp type, freeze dried cake appearance and clarity and degree of opalescence of the reconstituted vials. The time of reconstitution is also checked.
Leak	The test is performed by means of vacuum- pressure treatment and methylene blue dye solution. No change in the aspect of the vial has to be observed.
Sub-visible particles test	The insoluble particulate matter test follows the Ph.Eur. 2.9.19 method 1 (Light Obscuration Particle Count Test). This method is performed using the instrument HYAC-Royco mod. 9703 Liquid Particle Counter and it is based on the principle of light blockage which allows an automatic determination of the size and number of particles. The particles with size ≥ 10 µm have to be ≤ 6000/vial and those with size ≥ 25 µm must be ≤ 600/vial.
pH	The pH control is performed by measuring the $[H^+]$ with a pH microelectrode (sensitivity = 0.1 unit), according to the Ph. Eur. 2.2.3 method. The pH test is performed on a lyophilized kit after reconstitution with Saline solution for injection. The pH value must be within the following range: 5.0 to 5.8.
Uniformity of content	The uniformity of content is checked on 3 vials. The aluminum crimp cap is removed and each vial is weighed. The content is then completely removed, the vial and the stopper are properly rinsed and dried. The weight of each vial + stopper is measured again and the content calculated as follows: [Weight of the intact (filled) vial+stopper] – [Weight of the empty vial+stopper]. The content must be within 90%-110% of the nominal weight.
Residual moisture	The percentage of residual moisture that is an expression of the water content of the lyophilized vials is performed according to Ph. Eur. 2.5.12. (by Karl Fisher method). The water content must be not more than 5%.
Total Tin	The Tin present in the sample is analyzed by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES), technique based on the atomic emission spectroscopic characteristics typical of every chemical element. The concentration of the samples (mg/L) is determined at the wavelength corresponding to the Tin emission line (189.927 nm) and calculated by interpolation on the standard curve ($r^2 \geq 0.995$). Total Tin content must be in the range: 0.0047 to 0.0058 mg/vial.

Sn^{++} / Sn total	This quantitative analysis is performed by UV/VIS spectrophotometry. The reading is performed at the wavelength of 700 nm. The method exploits the reducing action of Sn^{2+} on the phosphomolybdic acid with consequent formation of "Molybdene blue". The intensity of the blue colour developed is directly proportional to the Sn^{2+} concentration. The samples concentration (mg/mL) is calculated by interpolation on the standard curve ($r^2 \geq 0.990$). Stannous Tin / Total Tin ratio must be higher than 50%.
Sodium α-D-Glucoheptonatedihydrate	The quantification of the sodium α–D-Glucoheptonate dihydrate used as transchelating agent is performed by HPLC assay using a MS-MS detector. The amount of sodium α–D-Glucoheptonate dihydrate is calculated by interpolation on the standard curve ($r^2 \geq 0.995$) preliminary prepared using 5 different concentration of α–D-Glucoheptonic acid sodium salt. The amount must be 3 mg/vial ± 10%.
Gentisic acid sodium salt hydrate	The quantification of the Gentisic acid sodium hydrate used as radiation stability enhancer is performed by RP-HPLC assay using an Ultra Violet (λ= 220 nm and 254 nm) detector. The amount of Gentisic acid sodium hydrate, retention time 4.5 ± 0.5 min, is calculated by comparing three chromatograms of the sample with the average value obtained from the system suitability test (six injections of Gentisic acid sodium salt hydrate standard solution: 0.020 mg/mL). The amount must be 0.020 mg/vial ± 10%.
Identity D(+)-Trehalose dihydrate	The identification of the D(+)-Trehalose dihydrate used as lyoprotectant/cake forming agent is performed by HPLC assay using a MS-MS detector. Three HPLC runs of the D(+)-Trehalose dihydrate standard solution are performed. Three chromatographic runs on the sample to be analysed are also performed. The chromatograms of the sample are compared to the chromatograms of the reference standard. Identification is considered valid if the averages of the retention times of the sample runs and of the standard runs do not differ by more than 0.5 min.
Identity Hydroxypropyl-β-Cyclodextrin	The identification of the Hydroxypropyl-β-Cyclodextrin used as solubilizing agent is performed by HPLC using an Evaporative Light Scattering Detector (ELSD). Three HPLC runs of the Hydroxypropyl-β-Cyclodextrin (HPβCD) (5 mg/mL) standard solution are performed. Three chromatographic runs on the sample to be analysed are also performed. The chromatograms of the sample are compared to the chromatograms of the reference standard. Identification is considered valid if the signal corresponding to the HPβCD (RT about 25 min) is present and peaks interfering with HPβCD signal are absent or do not compromise the target molecule identification..
Sodium Metabisulfite	The quantification of Sodium Metabisulfite is performed using a titrimetric determination method with titration pipette. In sulfuric solution by titration with potassium iodate solution against starch as the indicator, iodide ions are oxidized to iodine, which in turn oxidizes sulphite ions to sulfate ions. At the titration end-point, excess iodine forms a blue complex with the indicator. The sulfite concentration is determined from the consumption of titration solution (iodometric determination). The amount of sodium

	metabisultife is calculated by interpolation on the standard curve (correlation coefficient or $r^2 \geq 0.990$) preliminary prepared using 3 different concentrations of sodium metabisulfite (including matrix). The amount must be within a range of 10-30 ppm/vial.
Identity Lactic acid	The identification of the Lactic acid used as buffering agent is performed by RP-HPLC using a UV (λ= 210 nm) detector. Three HPLC runs of the Lactic acid standard solutions are performed. Three chromatographic runs on the sample to be analysed are also performed. The chromatograms of the sample are compared to the chromatograms of the reference. Identification is considered valid if the averages of the retention times of the sample runs and of the standard runs do not differ by more than 1 min.
Identity rhAnnexin V-128	The identification of the rhAnnexin V-128 protein is performed by "Western Blot" using a specific anti-rhAnnexin V-128 antibody developed in rabbit. The sample and a molecular weight standard are run electrophoretically on a Tris-Glycine gel under denaturing conditions, where proteins migrate based on their molecular weight. The proteins are then transferred into a PVDF membrane and incubated with a primary antibody specific for Annexin V128 followed by a secondary antibody (anti-rabbit) labelled with peroxidase. The substrate 4-chloro-1-naphthol (4CN) is added making visible the band corresponding to Annexin V128. The identity is conform when only 2 bands at 36kDa and 72kDa are visible.
Bradford assay rhAnnexin V-128 protein concentration	The quantification of the protein is performed by UV/VIS spectrophotometry (λ=595 nm). The method exploits the ability of Coomassie Brilliant Blue G-250 to bind proportionally to protein: determinations are made by comparison to the colour response of protein assay standards. Results are interpolated against the standard curve. The correlation coefficient (or r^2) of the standard curve must be > 0.990 to accept the test. The amount of rhAnnexin V-128 must be 0.400 mg/vial ± 10%.
Chemical Purity by SEC-HPLC and RP_HPLC (% rhAnnexin V-128 monomer)	The determination of the % of the rhAnnexin V-128 monomer and dimer/high molecular weight species is performed using a Size Exclusion column by analytical HPLC and Reverse Phase (RP_HPLC). Detection of the peaks is made by UV detector (λ=220 nm and 280 nm both for SEC-HPLC and RP-HPLC). The Chemical Purity is calculated by integration of the rhAnnexin V-128 monomer peak (t_R= 13.0 ± 0.5 min for SEC-HPLC and t_R= 26.0 ± 0.5 min for RP-HPLC) and rhAnnexin V-128 natural dimer/high molecular weight species peak (t_R= 11.5 ± 0.5 min for SEC-HPLC and t_R= 27.5 ± 0.5 min for RP-HPLC). The percentage of rhAnnexin V-128 monomer must be ≥ 90.0% and consequently the percentage of dimer/high molecular weight species must be ≤ 10%.
Chemical Purity (% rhAnnexin V-128 dimer/high molecular weight species)	
Radiochemical Purity (ITLC)	The Radiochemical Purity (RCP) test is performed in compliance with the Ph. Eur. by ITLC. The three different eluents are used as follow: Acetone 100% (Tc-99m, Rf=1.0); ACD (^{99m}Tc-AxV128, Rf=0.0); PBS (^{99m}Tc-Glucoheptonate, Rf=1.0) and $^{99m}TcO_2$, Rf=0.0). The RCP is calculated by integration of ^{99m}Tc-rhAnnexin V-128 peak that must be ≥ 90.0%; sum of ^{99m}Tc-Glucoheptonate + ^{99m}Tc-free + $^{99m}TcO_2$ must be ≤ 10% (where sum of ^{99m}Tc-free + $^{99m}TcO_2$ must be ≤ 8.0% and ^{99m}Tc-glucoheptonate must be ≤ 10.0%).

Radiochemical Purity (SEC-HPLC)	The RCP test is performed by analytical SEC-HPLC using Ultraviolet and radiometric detectors in series. The RCP is calculated by integration of ^{99m}Tc-rhAnnexin V-128 peak (t_R= 13.5 ± 0.5 min) that must be ≥ 90.0%.
Biopotency (%)	The % of rhAnnexinV-128 capable to bind to PS (PhosphatidylSerine) is calculated by the Biopotency assay: a fixed amount of labelled rhAnnexin V-128 and a fixed amount of preserved RBC (expressing PS on their surface) are incubated either in the presence of calcium (positive binding) or of chelating agent EDTA (negative binding). The degree of binding, after RCP adjustment, is calculated: {[(Negative Binding counts - Positive Binding Count) / Negative Binding counts] x 100}/(% rhAnnexin V-128 RCP in ITLC) = Binding % corrected. The % of binding must be within the range 90 - 110%.
Bioaffinity	The affinity of rhAnnexin V-128 in binding the PS (PhosphatidylSerine) in the presence of Calcium is determined by the displacement of FITC (Fluorescein Isothiocyanate)-AnnexinV. The IC_{50} ratio to reference value must be between 0.5 and 2.
Sterility	The method is compendial and complies with the current Ph. Eur. Monograph: 2.6.1 “Sterility”.
Bacterial Endotoxin Test (LAL)	The method employs the “LAL GEL CLOT” test. The method is compendial and adherent to the current Ph. Eur. Monograph: 2.6.14 “Bacterial Endotoxins”.

Specifications justification

- **Sub-visible particles test:** The specification is ≤ 6000 counts/vial for particles ≥ 10µm and ≤ 600 counts/vial for particles ≥ 25µm. These levels ensure that there is not an excess of insoluble particulates in the reconstituted vial previous injection.
- **pH**: The specification for the pH measurement is 5.0 to 5.8. This range was determined to be the suitable one for the labeling with Tc-99m.
- **Residual Moisture:** The specification is fixed as NMT 5%. The stability of the pharmaceuticals in lyophilized form is demonstrated to be strictly correlated to the water content after freeze-dry cycle. Data in the literature confirm that for the proteins a value ≤ 5% is appropriate.
- **Total Tin:** The specification is 0.0047 – 0.0058 mg/vial. This corresponds to 0.009 – 0.011 mg/vial in term of $SnCl_2$ x $2H_2O$. The stability of the excipient Tin Chloride Dihydrate is reflected in the specification corresponding to the ± 10% standard range stipulated for the content of excipients in drug products.
- **Sn^{++}/Sn Total:** The specification is not less than 50%. During the development phase it was demonstrated that, when fresh, even an amount equal to 3.2 µg is sufficient to reach a good Radiochemical purity (> 85.0%).
- **Sodium α-D-Glucoheptonate dihydrate amount:** The specification is 3 mg/vial ± 10%. During the development phase increasing amounts of this transchelating agent were tested, going from 0.027 mg/vial (which was the amount present in the previous formulation) up to 6 mg/vials (about 200-fold more concentrated compared to the previous formulation). The concentration of sodium α-D-glucoheptonate dihydrate that showed the best performance in

terms of labelling efficiency (and therefore radiochemical purity) is the 3 mg/vials. It is important to note that this concentration is inferior to the one present in other approved drugs (for example Draximage kit). A variation of ± 10% guarantees a good labeling yield.

- **Gentisic acid sodium salt hydrate:** The specification is 0.020 mg/vial ± 10%. This amount is sufficient to stabilize the formulation for at least 6 months (as shown by preliminary stability studies). A variation of ± 10% guarantees a good stability.
- **Identity D(+)-Trehalose dihydrate:** The specification is “conforms to reference in HPLC”. The quantification of this excipient is not essential.
-
- **Identity Hydroxypropyl-β-Cyclodextrin:** The specification is “conforms to reference in HPLC”. The quantification of this excipient is not essential.
- **Sodium Metabisulfite:** The specification is 10 – 30 ppm/vial (20 ppm/vial ± 50%). This amount is sufficient to maintain the active pharmaceutical ingredient (rhAnnexin V-128) in the monomeric form, blocking formation of the Disulphuric Bridge between two rhAnnexin V-128 molecules (dimer). The 10 ppm/vial is the minimum amount to guarantee the desired reduced status of the protein; the 30 ppm/vial higher limit was chosen in order to guarantee the antioxidant effect over time.
- **Identity Lactic Acid:** The specification is “conforms to reference in RP-HPLC”. The quantification of this salt is not essential: the pH of the reconstituted lyophilized product in saline (5.0 to 5.8) indicates that the formulation is perfectly buffered.
- **Identity rhAnnexin V-128:** The specification is “conforms to reference” by Western Blot. The protein rhAnnexin V128 needs to be recognized by the specific antibodies and run at the right molecular weights in its two forms: 36kDa for the monomer and 72kDa for the dimer.
- **rhAnnexin V-128 content:** The specification is 0.400 mg/vial ± 10%. This range guarantees a good radiochemical purity, yield and specific activity.
- **Chemical Purity:** The specification is ≥ 90.0%. The main detectable impurity in the product is the rhAnnexin V-128 dimer formed through a sulphur bridge (S-S) between the cysteins of two rhAnnexin V-128 molecules. The cysteine residue cannot be blocked, since it is essential for the chelation of the ^{99m}Tc. However, the formation of sulphur bridge between the two cysteines is limited (≤ 10%) by using inert gas to remove oxygen, acidic pH of the solution (that decreases the reactivity of the cysteine), sodium metabisulfite and sodium gentisate as antioxidant agent.

  Non covalent high molecular weight forms of Annexin can also be detected, by SEC-HPLC, in the Drug Product, in a very limited percentage. The sum of Annexin dimer and high molecular weight species (if any) is always ≤ 10%.
- **Radiochemical Purity (ITLC):** The specification is ≥ 90.0%. The radiochemical impurities are ^{99m}Tc-Sodium Glucoheptonate, $^{99m}TcO_4^-$ (^{99m}Tc free pertechnetate) and $^{99m}TcO_2$. The main impurity is represented by ^{99m}Tc-Sodium Glucoheptonate that is known to be rapidly excreted following administration by the kidneys [45, 46]This impurity cannot be avoided because its formation is essential for a successful labelling. The sum of the other two by-products ($^{99m}TcO_4^-$ + $^{99m}TcO_2$) is always ≤ 8.0 and ^{99m}Tc-glucoheptonate is always ≤ 10.0.
- **Radiochemical Purity (SEC-HPLC):** The specification is ≥ 90.0%. *See Radiochemical Purity (HPLC) justification of specification*.
- **Biopotency:** The specification is 90 - 110%. A value of red blood cells binding ≥ 90% ensures that the protein has retained its biological activity, namely binding specifically to anionic

phosphatdylserine exposed on the cellular membrane of cells in the presence of the optimal calcium concentration.

- **Bioaffinity**: the specification is 0.5 to 2 expressed as IC_{50} ratio to reference value. It is performed on the API material as well as on the Drug Product to ensure that the protein has retained its biological activity throughout the process.
- **Sterility:** The specification is “Sterile”. The acceptance criteria set are in line with the provisions of Section "2.6.1 Sterility" of the current Ph. Eur.
- **Bacterial Endotoxin:** The specification is 175 IU/Vmax corresponding to 175 IU/vial. It is in compliance with Ph. Eur. (01/2008: 20614).

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

The target for the stability of the Drug Product (Kit for radiopharmaceutical preparation of ^{99m}Tc-rhAnnexin V-128) is 18 months at 5°C ± 3°C.

The stability evaluation has been performed on lab-scale and engineering batches of the Drug Product and is now ongoing on the batch manufactured in GMP conditions . (CT001 14002). Drug Product stability will be assessed up to 18 months (every 3 months for the first year and every 6 months for the second year, up to 18 months) at the intended storage temperature (5°C ± 3°C). Furthermore, stability assessment will be also performed at the accelerated temperature condition (25°C ± 2°C), up to 9 months.

The stability assessment of the first GMP batch CT001 14002 will cover the stability of future GMP batches that will be produced for clinical trial use.

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 2

Dosimetry and Pharmacokinetics: Manual for Procurement, Storage and Handling of Blood and Urine Samples

Blood samples (10 mL) will be collected just before administration of the therapeutic dose and then at the following time intervals: 5, 10, 15, 30, 60 minutes, 2 hrs, 4 hrs and 24 hours post the first rhAnnexin V-128 administration for the first 3 patients of the sub-study. Depending upon the results in those 3 patients, the timeframe for dosimetry measurements and PK will be confirmed or modified with a time-point at 3 hrs (instead of 2 hrs) and 6 hrs (instead of 4 hrs) for the following 7 enrolled patients in the sub-study The following whole blood or serum aliquots have to be prepared.

1) 2 x 1 mL of whole blood (for radioactivity measurements)
2) 2 x 1 mL of serum (for radioactivity measurements)
3) 2 x 0.6 mL at least of serum (for ELISA assay)

Therefore, out of the 10 mL, 2 x 1 mL of whole blood will be collected in tubes with anticoagulant and counted in a gamma-counter at the CHUV/NUC laboratory.
The remaining 8 mL will be collected in serum tubes (preferably serum tubes with gel separator) and centrifuged for 5 min at 1500 rpm at room temperature. The serum will be divided in 2 aliquots of 1 mL each and in 2 aliquots of at least 0.6 mL each. The 1 mL serum aliquots will be counted in the gamma-counter at the CHUV/NUC laboratory.
The 0.6 mL serum samples will be frozen at -80°C (**please note that these samples should be put at -80°C within 1 hour from blood withdrawal).** After complete decay of radioactivity, one out of two samples per time-point will be shipped to the centralized laboratory for ELISA assay.
Avoid freeze-thawing of the serum samples.

Urine samples will be collected as follows. One sample will be collected within 24 hrs prior to ^{99m}Tc-rhAnnexin V-128 administration, preferable just prior to the administration of study drug (0 h sample) to achieve bladder emptying. Quantitative urine collections will be obtained (and volume recorded) possibly in the time intervals 0-1 h, 1 h – 4 hrs, 4 hrs – 16 hrs, and 16 hrs – 24 hrs post the first administration. Dual 2 mL aliquots of urine from each period will be counted in a gamma counter at the CHUV/NUC laboratory. The mean between the two ^{99m}Tc radioactivity measurements and the measured volume of urine will be used to calculate the amount of ^{99m}Tc excreted in that period and the cumulative percent injected dose (radioactivity).
Urine samples (10 mL aliquot from each urine sample) will be also analysed as a function of time by an SEC-HPLC technique at the CHUV/NUC laboratory, in order to gain information on the chemical status of ^{99m}Tc-rhAnnexin V-128 and on the presence of ^{99m}Tc-rhAnnexin V-128-related species. For this purpose, 10 mL aliquot from each urine sample will be transferred to the CHUV-NUC laboratory where the SEC-HPLC analysis will be performed as soon as possible to avoid stability issues.

The collection of blood and urine will be recorded in the time-schedule template shown below.

Dosimetry and Pharmacokinetics: Time schedule for biological samples (after first ^{99m}Tc-rhAnnexin V-128 administration)

Patient ID: _____
weight: _____kg; height:_____cm;

Date of injection_____ time: _____
Administered activity (background corrected) _____GBq

BLOOD SAMPLES - 1 ml/sample			URINE COLLECTION - COMPLETE		
n.	Time	date	n.	Time interval	date
1	Before administration	Day 1	1	Before treatment	Day 1
2	5 min	Day 1	2	Up to 1 h	Day 1
3	10 min	Day 1	3	From 1h → 4 hrs	Day 1
4	15 min	Day 1	4	4 hrs→ 16 hrs	Day 1
5	30 min	Day 1	5	16 hrs → 24 hrs	Day 1-2
6	60 min	Day 1			
7	2 hrs	Day 1			
8	4 hrs	Day 1			
9	24 hrs	Day 2			

URINE COLLECTION: Please ask the patient to void the urinary bladder BEFORE the acquisition of each scintigraphic image, especially within the first 24 hrs post injection, when the activity elimination rate is high.

Dosimetry and Pharmacokinetics: Time schedule for biological samples in case of modified time-points after results of the first 3 patients. (after first ^{99m}Tc-rhAnnexin V-128 administration)

Patient ID: _____
weight: _____kg; height:_____cm;
Date of injection_____ time: _____
Administered activity (background corrected) _____GBq

BLOOD SAMPLES - 1 ml/sample			URINE COLLECTION - COMPLETE		
n.	Time	date	n.	Time interval	date
1	Before administration	Day 1	1	Before treatment	Day 1
2	5 min	Day 1	2	Up to 1 h	Day 1

3	10 min	Day 1	3	From 1h → 4 hrs	Day 1
4	15 min	Day 1	4	4 hrs→ 16 hrs	Day 1
5	30 min	Day 1	5	16 hrs → 24 hrs	Day 1-2
6	60 min	Day 1			
7	3 hrs	Day 1			
8	6 hrs	Day 1			
9	24 hrs	Day 2			

URINE COLLECTION: Please ask the patient to void the urinary bladder BEFORE the acquisition of each scintigraphic image, especially within the first 24 hrs post injection, when the activity elimination rate is high.

Appendix 3
Total quantity of collected blood

Patients who will **not** participate in the PK and dosimetric sub-study:

Analysis	Screening	Day 30	Day 42	Day 56	Day 90
Haematology and biochemistry	15 ml	15 ml	15 ml		15 ml
Immunogenicity analysis	10 ml	10 ml		10 ml	10 ml
TOTAL	100 ml				

Patients who will participate in the PK and dosimetric sub-study:

Analysis	Screening	Day 1	Day 2	Day 30	Day 42	Day 56	Day 90
Heamatology and biochemistry	15 ml		15 ml	15 ml	15 ml		15 ml
PK and dosimetry sub-study		80 ml (10 ml * 8 time points between 0 and 6h)	10 ml				
Immunogenicity analysis	10 ml			10 ml		10 ml	10 ml
TOTAL	205 ml						

Appendix 4

References

1. Majno, G. and I. Joris, *Apoptosis, oncosis, and necrosis. An overview of cell death.* Am J Pathol, 1995. **146**(1): 3-15.
2. Saraste, A. and K. Pulkki, *Morphologic and biochemical hallmarks of apoptosis.* Cardiovasc Res, 2000. **45**(3): 528-37.
3. Saikumar, P., et al., *Apoptosis: definition, mechanisms, and relevance to disease.* Am J Med, 1999. **107**(5): 489-506.
4. Kam, P.C. and N.I. Ferch, *Apoptosis: mechanisms and clinical implications.* Anaesthesia, 2000. **55**(11): 1081-93.
5. Eguchi, K., *Apoptosis in autoimmune diseases.* Intern Med, 2001. **40**(4): 275-84.
6. Post, A.M., et al., *Imaging cell death with radiolabeled annexin V in an experimental model of rheumatoid arthritis.* J Nucl Med, 2002. **43**(10): 1359-65.
7. Yamanishi Y., F.G.S., *Apoptosis in rheumatoid arthritis* in *Apoptosis and autoimmunity* J.W. Sons, Editor. 2003, Kalden J.R. and Herrmann 169-196.
8. Lawrence, R.C., et al., *Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States.* Arthritis Rheum, 1998. **41**(5): 778-99.
9. Britsemmer, K., et al., *Validation of the 2010 ACR/EULAR classification criteria for rheumatoid arthritis: slight improvement over the 1987 ACR criteria.* Ann Rheum Dis, 2011. **70**(8): 1468-70.
10. De Rycke, L., et al., *Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations.* Ann Rheum Dis, 2004. **63**(12):1587-93.
11. Guillemin, F., et al., *Reproducibility and sensitivity to change of 5 methods for scoring hand radiographic damage in patients with rheumatoid arthritis.* J Rheumatol, 2005. **32**(5): 778-86.
12. Ejbjerg, B.J., et al., *The smallest detectable difference and sensitivity to change of magnetic resonance imaging and radiographic scoring of structural joint damage in rheumatoid arthritis finger, wrist, and toe joints: a comparison of the OMERACT rheumatoid arthritis magnetic resonance imaging score applied to different joint combinations and the Sharp/van der Heijde radiographic score.* Arthritis Rheum, 2005. **52**(8): 2300-6.
13. Bruynesteyn, K., et al., *Radiography as primary outcome in rheumatoid arthritis: acceptable sample sizes for trials with 3 months' follow up.* Ann Rheum Dis, 2004. **63**(11):1413-8.
14. Beckers, C., et al., *Assessment of disease activity in rheumatoid arthritis with (18)F-FDG PET.* J Nucl Med, 2004. **45**(6): 956-64.
15. Beckers, C., et al., *(18)F-FDG PET imaging of rheumatoid knee synovitis correlates with dynamic magnetic resonance and sonographic assessments as well as with the serum level of metalloproteinase-3.* Eur J Nucl Med Mol Imaging, 2006. **33**(3): 275-80.
16. Goekoop-Ruiterman, Y.P., et al., *Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial.* Arthritis Rheum, 2005. **52**(11): 3381-90.
17. Sieper, J., et al., *The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis.* Ann Rheum Dis, 2009. **68 Suppl 2**: 1-44.
18. Zochling, J., et al., *Current evidence for the management of ankylosing spondylitis: a systematic literature review for the ASAS/EULAR management recommendations in ankylosing spondylitis.* Ann Rheum Dis, 2006. **65**(4): 423-32.
19. Lahorte, C.M., et al., *Apoptosis-detecting radioligands: current state of the art and future perspectives.* Eur J Nucl Med Mol Imaging, 2004. **31**(6): 887-919.
20. Tait, J.F., C. Smith, and F.G. Blankenberg, *Structural requirements for in vivo detection of cell death with 99mTc-annexin V.* J Nucl Med, 2005. **46**(5): 807-15.

21. Steinmetz N, T.R., Hendel RC, et al, *Simultaneous dual isotope 201TI/99mTc-Annexin (apomateTM) SPECT in detection of acute myocardial infarction: initial results of a phase II multicenter trial* J Nucl Med 2002. **43**(5): 4P.
22. Taillefer R, P.D., Duong DH, et al, *99mTc-Annexin V scintigraphy in detection of acute myocardial infarction (MI): repeat imaging after the onset of acute symptoms in order to evaluate the persistence of abnormal radiotracer uptake* J Nucl Med 2002. **43**(5):5P.
23. Thimister PWL, P.M., Janssen D, Heidendal GAK, *Disappearance of apoptosis in the sub acute phase of acute myocardial infarction (abstract).* Eur J Nucl Med Mol Imaging 2002. **29(suppl I)**: S49.
24. Haas RLM, V.-O.R., De Jong D, Zerp SF, Van den Heuvel IJ, Hoefnagel CA, Bartelink H, Verheij *Radiation induced apoptosis in follicular lymphoma subjects assessed by 99mTc-Annexin V scintigraphy* Eur J Nucl Med Mol Imaging 2003. **30**(suppl. 2):S197.
25. van de Wiele, C., et al., *Quantitative tumor apoptosis imaging using technetium-99m-HYNIC annexin V single photon emission computed tomography.* J Clin Oncol, 2003. **21**(18): 3483-7.
26. Vermeersch, H., et al., *99mTc-HYNIC Annexin-V imaging of primary head and neck carcinoma.* Nucl Med Commun, 2004. **25**(3): 259-63.
27. Van den Heuvel IJ, P.B., Valdés-Olmos R, Haas R, *Labelling and imaging aspects of 99mTc-rh-Annexin V in tumour-apoptosis detection.* Eur J Nucl Med Mol Imaging 2003. **30**(Suppl. 2): S167.
28. Kemerink, G.J., et al., *Safety, biodistribution, and dosimetry of 99mTc-HYNIC-annexin V, a novel human recombinant annexin V for human application.* J Nucl Med, 2003. **44**(6): 947-52.
29. Tait, J.F., et al., *Improved detection of cell death in vivo with annexin V radiolabeled by site-specific methods.* J Nucl Med, 2006. **47**(9): 1546-53.
30. Emami, B., et al., *Tolerance of normal tissue to therapeutic irradiation.* Int J Radiat Oncol Biol Phys, 1991. **21**(1): 109-22.
31. DeGrado, T.R., et al., *Synthesis and evaluation of (18)F-labeled choline analogs as oncologic PET tracers.* J Nucl Med, 2001. **42**(12): 1805-14.
32. Services, U.S.D.o.H.a.H., et al., *Guidance for Industry - Developing Medical Imaging Drug and Biological Products - Part 1: Conducting Safety Assessments.* 2004.
33. Cloutier, R.J., et al., *Dose to the fetus from radionuclides in the bladder.* Health Phys, 1973. **25**(2):147-61.
34. Protection, I.C.o.R., *Age-dependent Doses to Members of the Public from Intake of Radionuclides - Part 1*, ed. I. Publication. 56. 1990: Pergamon Press.
35. Protection, I.C.o.R., *Limits for Intakes of Radionuclides by Workers*. Ann. ICRP. Vol. 30 (Part 1). 1979.
36. Chen Z. P., W.S.P., Tang J., Li X. M., Liu C. Y., Xu X. J., Cao G. X., *Simplified method for determining radiochemical purity of 99mTc-TRODAT-1*. Journal of Radioanalytical and Nuclear Chemistry.. 277(3). 2008.
37. Stabin, M.G., R.B. Sparks, and E. Crowe, *OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine.* J Nucl Med, 2005. **46**(6): 1023-7.
38. Ahlgren, S., K. Andersson, and V. Tolmachev, *Kit formulation for 99mTc-labeling of recombinant anti-HER2 Affibody molecules with a C-terminally engineered cysteine.* Nucl Med Biol, 2010. **37**(5): 39-46.
39. Siegel, J.A., et al., *MIRD pamphlet no. 16: Techniques for quantitative radiopharmaceutical biodistribution data acquisition and analysis for use in human radiation dose estimates.* J Nucl Med, 1999. **40**(2): 37S-61S.
40. Millar, A.M., *Effect of source and age of sodium pertechnetate Tc 99m on radiochemical purity of technetium Tc 99m exametazime.* Am J Hosp Pharm, 1993. **50**(1): 103-6.
41. Hung, J.C., et al., *Generator eluate effects on the labeling efficiency of 99mTc-sestamibi.* Nucl Med Biol, 1995. **22**(7): 949-51.
42. Hung, J.C. and L.M. Thorson, *Effects of generator eluate age on the radiochemical purity of fractionated 99Tcm-MAG3.* Nucl Med Commun, 1995. **16**(3): 157-60.
43. Rottey S., Slegers G., Van Belle S., Goethals I., *Van de Wiele C.,Sequential 99mTc-Hydrazinonicotinamide-Annexin V Imaging for Predicting Response to Chemotherapy*, J. Nucl. Med. 2006; 47: 1813-1818.
44. Hung, J.C. and L.M. Thorson, *Effects of generator eluate age on the radiochemical purity of fractionated 99Tcm-MAG3.* Nucl Med Commun, 1995. **16**(3): p. 157-60.

45. de Kieviet, W., *Technetium radiopharmaceuticals: chemical characterization and tissue distribution of Tc-glucoheptonate using Tc-99m and carrier Tc-99.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 1981. **22**(8): p. 703-9.
46. Qiu, K., et al., *99mTc-labeled HAb18 McAb Fab fragment for radioimmunoimaging in nude mice bearing human hepatocellular carcinoma.* World journal of gastroenterology : WJG, 1998. **4**(2): p. 117-120.

Appendix 5

Sponsor: Advanced Accelerator Applications (AAA)

Clinical Trial Identification	Eudract N°	Center N°	Investigator N°	Country

Please fax or send by e mail the completed form to the AAA Pharmacovigilance Unit within 24 hours.
E-mail: [redacted] **; Fax N°:** [redacted] **; Urgency: EUQQPV:** [redacted] **or** [redacted]
Type of report: Initial Follow-up

Date of SAE reporting to Laboratories AAA: ⌊_⌋⌊_⌋⌊_⌋ dd/mm/yy

NB: The format of dates of the document is: dd/mm/yy

1. Patient Data

PATIENT (Initials):	**PATIENT** N°:	**SEXE*:**	**BIRTHDATE:**	**WEIGHT(kg):**
Last name ⌊_⌋⌊_⌋⌊_⌋ First name ⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋⌊_⌋	M ☐ F ☐	⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋.⌊_⌋ **HEIGHT (cm):** ⌊_⌋⌊_⌋⌊_⌋

2. Seriousness Criterion

SERIOUS CRITERIA:

☐ DEATH
☐ LIFE-THREATENING
☐ INVOLVED OR PROLONGED HOSPITALIZATION
☐ CONGENITAL ANOMALY
☐ PERSISTENT OR SIGNIFICANT DISABILITY OR INCAPACITY
☐ MEDICALLY SIGNIFICANT *precise*: _____

3. Relevant Medical History

4. Serious Adverse Event

Reaction start date ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Date of Hospitalization (if applicable) ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋

Description
Include a history of the event chronologically including: signs and characteristics, severity, dates and outcome of hospitalization and any other relevant information not captured elsewhere on the form

Chronological data relevant for the SAE:
Date of inclusion ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋
Date of first administration of study drug ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋

5. Non Serious event of special interest

☐ No
☐ Yes – Specify bellow

Use only in exceptional circumstances to indicate when a non-serious event of special interest (as defined by the protocol) is being reported:

6. Serious adverse Event Outcome

SERIOUS CRITERIA:	Date	
☐ FATAL	__/__/__	
☐ RESOLVED	__/__/__	
☐ RESOLVED WITH SEQUELAE	__/__/__	Sequelae : ____________________
☐ IMPROVED		
☐ PERSISTING		
☐ WORSENED		
☐ UNKNOW		

7. Study drug(s)

All study treatments specified in the protocol

Name	Dose	Batch Number	Frequency	Route	Start date	End date	Ongoing
					__/__/__	__/__/__	☐
					__/__/__	__/__/__	☐
					__/__/__	__/__/__	☐
					__/__/__	__/__/__	☐
					__/__/__	__/__/__	☐

Delay of occurrence after last dose intake: ___ ________ (time unit)

8. Action taken with study drug(s)

Name	No change	Temporarily interrupted		Ongoing	Dose adjusted (detail)	Unknow
		Date stopped	Date restarted	Date stopped		
	☐	__/__/__	__/__/__	__/__/__	☐	☐
	☐	__/__/__	__/__/__	__/__/__	☐	☐
	☐	__/__/__	__/__/__	__/__/__	☐	☐
	☐	__/__/__	__/__/__	__/__/__	☐	☐
	☐	__/__/__	__/__/__	__/__/__	☐	☐

In case of re-administration, did the reaction recur? ☐ Yes ☐ No

9. Concomitant treatment

Name	Daily dose	Route	Start date	End date	Ongoing	Indication
			⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐	
			⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐	
			⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐	
			⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐	
			⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐	

10. Corrective treatment (drug, procedure, etc.)

Name	Total daily dose	Start date	End date	Ongoing
		⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐
		⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐
		⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐
		⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐
		⌊_⌋⌊_⌋⌊_⌋	⌊_⌋⌊_⌋⌊_⌋	☐

11. Relevant Laboratory / Diagnostic Tests

Rq: Include laboratory values preceding the event

Details of additional tests can be completed on the Additional Laboratory / Diagnostic Tests form.

Additional form provided? ☐ No ☐ Yes (Number of extra-pages: ___)

Name of the test	Results (units)	Normal values / Reference ranges	Sample collection date	Result pending
			⌊_⌋⌊_⌋⌊_⌋	☐
			⌊_⌋⌊_⌋⌊_⌋	☐
			⌊_⌋⌊_⌋⌊_⌋	☐
			⌊_⌋⌊_⌋⌊_⌋	☐
			⌊_⌋⌊_⌋⌊_⌋	☐
			⌊_⌋⌊_⌋⌊_⌋	☐
			⌊_⌋⌊_⌋⌊_⌋	☐

12. Possible causes of the event

Check off all that apply		Specify
Pre-existing / underlying disease	☐	
Study treatment	☐	
Other treatment	☐	
Protocol-related procedure	☐	
Other (e.g. accident, new or intercurrent illness)	☐	

13. Investigator

SAE Form	Full name (in uppercase letters)	Function	Date	Signature
Filled out by:			⌊_⌋⌊_⌋⌊_⌋	
Validated by:			⌊_⌋⌊_⌋⌊_⌋	

14. AAA PV department

SAE Form	Full name (in uppercase letters)	Function	Date	Signature
Reception by:			⌊_⌋⌊_⌋⌊_⌋	
QC by:			⌊_⌋⌊_⌋⌊_⌋	

15. AAA Comments