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- (71) **Applicant (for all designated States except US):**
ATHERA BIOTECHNOLOGIES AB [SE/SE]; Fog-
devreten 2A, S-171 77 Stockholm (SE).
- (72) **Inventor; and**
- (75) **Inventor/Applicant (for US only):** **PETTERSSON, Knut**
[SE/SE]; Kungsladugårdsgatan 110, S-414 76 Göteborg
(SE).
- (74) **Agent:** **WRIGHT, Andrew, John**; Park View House, 58
The Ropewalk, Nottingham Nottinghamshire NG1 5DD
(GB).
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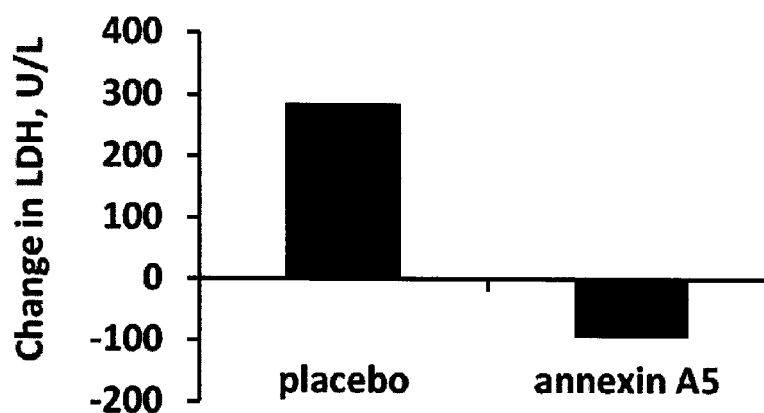
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- (54) **Title:** THERAPEUTIC AND PROPHYLACTIC METHODS, USES AND COMPOSITIONS COMPRISING ANEXIN A5

Fig 1

- (57) **Abstract:** The present invention provides a method for the prevention and/or reduction of peri- or postoperative complications following surgical intervention, such as complications following vascular surgery, especially peripheral vascular surgery, comprising administering a therapeutically effective amount of Annexin (A5) or a functional analogue or variant thereof to a patient in need of such treatment. Also provided is a pharmaceutical composition comprising a therapeutically effective amount of Annexin (A5) or a functional analogue or variant thereof for use in the prevention and/or reduction of peri- or postoperative complications following surgical intervention, such as complications following vascular surgery, especially peripheral vascular surgery.

THERAPEUTIC AND PROPHYLACTIC METHODS, USES AND COMPOSITIONS COMPRISING ANEXIN A5

Field of the Invention

The invention relates to novel methods and compositions for the prevention and/or
5 reduction of peri- and postoperative complications following surgical intervention, such as
complications following vascular surgery, especially peripheral vascular surgery.

Background to the Invention

10 The listing or discussion of an apparently prior-published document in this specification
should not necessarily be taken as an acknowledgement that the document is part of the
state of the art or is common general knowledge.

It is well known that surgical interventions frequently lead to complications. Not
15 surprisingly, such complications are more common after major and long lasting
interventions than after rapid and simple interventions. The status of the patient is also
important for the risk of complications. Interventions normally require some form of
anaesthesia, and the anaesthesia itself can lead to complications.

20 Peri- and post-operative complications can affect the organ or organs that are the target
for the intervention, but can also affect other organs. One example is the risk for deep
vein thrombosis that can be a result of orthopaedic surgery such as hip replacement
surgery (Duggan. Am J Cardiovasc Drugs, 2012. 12(1): p. 57-72). To make surgery
easier or even possible, it is common to stop blood flow to the target organ during the
25 intervention. This can result in target organ injury, but also in complications in other
organs. An example of this is lung injury following a blockade of intestinal blood flow
(Feinman. Am J Physiol Gastrointest Liver Physiol, 2010. 299(4): p. G833-43). The
mechanical trauma to a target organ during surgery can lead to cell death and direct
injury to the organ, but this can also result in damage to distant organs. One such
30 example is the release of myoglobin from skeletal muscle being injured during surgical
intervention that can lead to post surgical kidney injury.

The complications of surgical interventions range in severity from minor to very serious
complication, such as death. Post surgical complications also range from very frequent to
35 very infrequent. Examples of frequent complications are complications following
interventions aimed at restoring blood flow in limbs in patients with critical limb
ischemia due to peripheral artery disease which lead to death or amputation in up to 30

% of the patients within one month after surgery. In contrast, pituitary infarction following cardiac bypass surgery, which is a common form of surgery, has been described in only 4 patients (Zayour and Azar. *Endocr Pract*, 2006. 12(1): p. 59-62).

- 5 From the above, it becomes evident that preventive treatment that reduces the risk and incidence of peri- and post-operative complications following surgery, especially vascular surgery is at great need.

10 Annexin A5 is an endogenous protein that binds to charged phospholipids such as phosphatidylserine (PS) (Cederholm and Frostegard (2007) *Ann. NY Acad. Sci.*, 1108:96-103). Annexin A5 is a potent anti-thrombotic agent (Thiagarajan and Benedict (1997) *Circulation*, 96(7):2339-2347), and it is proposed that Annexin A5 by binding to exposed PS can form a 'protective shield' that can inhibit the effects of PS on thrombosis formation (Rand (2000) *Autoimmun.*, 15(2):107-111).

15

It has been shown that in addition to anti-platelet and anti-coagulant effects of Annexin A5, this protein and an analogue thereof, the Annexin A5 dimer diannexin, is effective in preventing reperfusion injury in the liver, and it improved the outcome of rat liver transplants (Shen et al. (2007) *Am. J. Transplant.*, 7(11):2463-2471.). Interestingly, in
20 these studies the treatments were associated with a reduced inflammatory activity in the hepatic endothelium, measured as reduced expression of adhesion molecules, that is Annexin A5 has anti-inflammatory effects. It was suggested that diannexin improved the survival of the liver transplants by an anti-thrombotic effect leading to maintained blood supply to the liver (Shen et al. (2007) *Am. J. Transplant.*, 7(11):2463-2471).

25

It has earlier been suggested that Annexin A5 can be used to stabilise atherosclerotic lesions in coronary arteries in patients, which should reduce the risk for myocardial infarction in these patients (WO 2005/099744).

- 30 Annexin A5 has further been suggested as a treatment of various vascular dysfunctions such as angina pectoris (WO 2009/077764) and for the prevention of restenosis (WO 2009/103977).

Summary of the invention

A first aspect of the invention provides a method for the prevention and/or the reduction of peri- and postoperative complications following surgical interventions comprising administering a therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof to a patient in need of such treatment.

Accordingly, the first aspect of the invention also provides a pharmaceutical composition comprising a therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof for use in the prevention and/or the reduction of peri- and postoperative complications following surgical interventions.

Put another way, the first aspect of the invention also provides for the use of Annexin A5 or a functional analogue or variant thereof in the manufacture of a medicament for the prevention and/or the reduction of peri- and postoperative complications following surgical interventions.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

These, and other, embodiments of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating various embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many substitutions, modifications, additions and/or rearrangements may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such substitutions, modifications, additions and/or rearrangements.

Description of the figures

Figure 1 shows changes in LDH during ischemia and reperfusion in an in vivo rat model, with or without Annexin A5 pre-treatment, as described in Example 1.

Figure 2 shows changes in lung inflammation following intestinal ischemia in an *in vivo* rat model, with or without Annexin A5 pre-treatment, as described in Example 2.

5 **Detailed description of the invention**

Here, the examples presented herein are designed to illustrate the utility of Annexin A5 to reduce the occurrence of postoperative complications following surgery, by *in vivo* testing in a rat model. Thus, Annexin A5 or functional analogues and/or variants thereof
10 present a new treatment modality that can reduce peri- and post-operative complications in patients undergoing surgical interventions, such as vascular surgery.

In a first aspect, the invention provides a method for the prevention and/or the reduction of peri- and post-operative complications following surgical intervention, said method
15 comprising administering a therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof to a patient in need of such treatment.

Put another way, the first aspect of the invention provides Annexin A5 or a functional analogue or variant thereof for use in the prevention and/or the reduction of peri- and
20 postoperative complications following surgical intervention.

The first aspect of the invention also provides for the use of Annexin A5 or a functional analogue or variant thereof in the manufacture of a medicament for the prevention and/or the reduction of peri- and postoperative complications following surgical interventions.

25 In one embodiment of the first aspect of the present invention, Annexin A5 or a functional analogue or variant thereof for is used for peri- or post-operative organ protection. Thus, the patient (preferably, a human patient) is treated to prevent, reduce or ameliorate damage to organs that occurs during or following a surgical intervention, including
30 anaesthesia. In one preferred embodiment the organ(s) to be protected are distal from the site of the surgical intervention. In a further preferred embodiment, the organ(s) to be protected are distal from the site of the surgical intervention and are also not directly affected by the surgical procedure. In this context, "directly effected" may include the effect of one, two or all three of ischemia, reperfusion and/or thrombosis as a result of
35 the surgery. Thus, in one embodiment, the organ(s) to be protected by the present invention do not experience any one or more, preferably all, of immediate cellular damage, ischemia, reperfusion and/or thrombosis as a result of the surgical intervention.

For example, Example 1 of the present application relates to the use of Annexin A5 to prevent liver and renal damage following a surgical intervention that causes ischemia and reperfusion in lower limbs; surgical interventions that causes ischemia and reperfusion in lower limbs do not have the effect of causing ischemia and reperfusion in the liver and the kidney, but nevertheless a surgical intervention to a lower limb can cause organ damage in the kidney and elsewhere. The present invention provides methods to prevent, reduce or ameliorate such damage that can occur during or following a surgical intervention performed on, or causing ischemia and/or reperfusion in, the lower limbs.

Likewise, Example 2 of the present application relates to the use of Annexin A5 to prevent lung damage following a surgical intervention that causes ischemia and reperfusion in the intestines; surgical interventions that causes ischemia and reperfusion in the intestine do not have the effect of causing ischemia and reperfusion in the lungs, but nevertheless a surgical intervention to the intestines can cause organ damage in the lungs. The present invention provides methods to prevent, reduce or ameliorate damage to the lungs that can occur during or following a surgical intervention performed on, or causing ischemia and/or reperfusion in, the intestines.

There are a large number of examples known in the art of surgical interventions in one part of the body causing indirect organ damage in other parts of the body. Prior to the present invention, there was no effective way to prevent the indirect organ damage that followed during, or after, some surgical interventions. If, for example, as a result of a surgical procedure performed on, or causing ischemia and/or reperfusion in, the intestines, the patient survived the initial ischemic event and post-operative indirect complications resulted which caused organ damage to the lungs, then the person skilled in the art would only be able to treat the organ damage to the lungs after the damage had occurred, for example by lung ventilation and/or anti-inflammatory drugs. If, for example, as a result of a surgical procedure performed on, or causing ischemia and/or reperfusion in, a lower limb, the patient survived the initial ischemic event and post-operative indirect complications resulted which caused organ damage to the kidney, then the person skilled in the art would only be able to treat the organ damage to the kidney after the damage had occurred, for example by haemodilution, dialysis or, if necessary, transplantation.

The aim of the present invention is to use Annexin A5 to treat/prevent this type of indirect organ damage following surgery/anaesthesia.

5 In one embodiment, the type of organ damage to be treated is not thrombosis and/or the downstream effects thereof. Thus, the claims of the present application are not intended to encompass the use of Annexin A5 to treat or prevent thrombosis in any organs.

10 Thus, in one embodiment, the first aspect of the present invention relates to the use of Annexin A5 or a functional analogue or variant thereof for peri- or postoperative organ protection in order to prevent organ damage in an organ wherein the patient is subjected to a surgical intervention (including anaesthesia) that is at a site that is distal from the organ that is to be protected from damage, and wherein the organ to be protected from damage does not experience one or more (including all) of ischemia, reperfusion and thrombosis, or any other direct effect, as a result of the surgical intervention.

15 For example, in one embodiment, the patient may be an elderly patient (for example, at an age about, or greater than, 50, 55, 60, 65, 70, 75, 80, 85, 90 or more years old) in whom even anaesthesia can sometimes be enough to cause organ damage (such as myocardial damage and infarction) at a site distal from the site of administration of the anaesthetic. Accordingly, in one aspect of the present invention, Annexin A5 or a
20 functional analogue or variant thereof can be used to prevent organ damage, such as myocardial damage and/or infarction, in an elderly patient who is treated, is to be treated, or has been treated with an anaesthetic.

25 In another exemplary embodiment, patients suffering from diabetes can experience critical limb ischemia, and can have a higher incidence rate of cardiac events than non-diabetic patients. Accordingly, in another aspect of the present invention, Annexin A5 or a functional analogue or variant thereof can be used to prevent organ damage, such as cardiac damage, in a patient with diabetes who critical limb ischemia, for example when
30 treated by a surgical intervention.

Accordingly, the first aspect of the invention also provides a pharmaceutical composition comprising a therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof for use in the prevention and/or reduction of peri- and postoperative
35 complications following surgical interventions.

Put another way, the first aspect of the invention also provides use of Annexin A5 or a functional analogue or variant thereof for the manufacture of a medicinal product for the prevention and/or reduction of peri- and postoperative complications following surgical interventions.

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The patient is typically a human patient. Alternatively, the patient may be a non-human animal, such as a domestic animal (for example, cat, dog, rabbit, cow, sheep, pig, mouse or other rodent).

10 Peri- and postoperative complications can occur after any form of surgical intervention. Different types of surgical interventions where Annexin A5 or a functional analogue or variant thereof can be used to prevent and/or reduce postoperative complications are exemplified by, but not limited to, cardiovascular surgery, such as cardiac surgery and cardiopulmonary bypass, vascular surgery, such as peripheral vascular surgery,
15 pulmonary surgery, digestive system surgery, such as gastrectomy and colectomy, obstetric surgery, such as caesarean sections, orthopaedic surgery, such as joint replacements, especially hip replacements, reconstructive surgery, transplantations, such as kidney transplantations, heart transplantations, liver transplantations and lung transplantations, as well as trauma surgery and amputations.

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As discussed above, peri- and postoperative complications are more common after major and long lasting interventions than after rapid and simple interventions. In an embodiment of the first aspect of the present invention the duration of the surgical intervention may be at least, or greater than 5, 10, 15 20, 30, 40, 50, 60, 90, 120, 150,
25 180, or 240 minutes.

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The surgical interventions where Annexin A5 or a functional analogue or variant thereof can be used according to the invention include interventions comprising restrictions in organ blood flow during part (such as, at least or greater than half), or all, of the duration of the intervention.

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The surgical interventions where Annexin A5 or a functional analogue or variant thereof can be used according to the invention further include interventions wherein the intervention causes immediate cellular damage (i.e. physical injury) to the target organ.

The peri- and postoperative complications that can be prevented and/or reduced using the methods and/or pharmaceutical compositions according to the present invention are

both complications arising in the organ being the target of the surgical intervention, as well as complications arising in other organs not being the target of the surgical intervention. As discussed above, the surgical intervention (including anaesthesia) is typically at a site that is distal from the target organ that is to be protected from peri- and postoperative damage, and preferably the organ that is to be protected does not experience one or more (including all) of ischemia, reperfusion and thrombosis, or any other direct effect, as a result of the surgical intervention.

The peri- and postoperative complications that can be prevented and/or reduced using the methods and/or pharmaceutical compositions according to the present invention are exemplified by, but not limited to, cardiovascular complications, such as myocardial infarction, congestive heart failure, stroke and TIA (transient ischemic attack), postoperative limb ischemia, kidney complications, such as renal dysfunction and nephritis, liver injury, gastrointestinal complications such as ischaemic intestinal disease, pulmonary complication such as pulmonary embolism.

Depending on the nature and location of the surgical intervention, the organ in which the peri- or postoperative complication is prevented and/or reduced in accordance with in an embodiment of the first aspect of the present invention may be selected from any one or more of the following organs –

- One or more organs in the abdomen or pelvis, such as adrenals; appendix; bladder; gallbladder; large intestine; small intestine; kidney; liver; pancreas; spleen; stomach; in the male pelvis the prostate and/or testes; in the female pelvis the ovaries and/or uterus;
- One or more organs in the thorax, such as heart; lung; esophagus; thymus; pleura;
- One or more organs in the head and/or neck, such as the brain (including brain basal ganglia; brain stem or part thereof e.g. medulla, midbrain, pons; cerebellum; cerebral cortex; hypothalamus; limbic system or part thereof e.g. amygdale);
- Eye;
- Pituitary;
- Thyroid and Parathyroids.

Peri- and post operative complications are well known to the skilled person and well characterised in the art. A peri- or postoperative complication that can be treated or

prevented in accordance with the present invention may, for example, include a partial or total loss of the measurable function of one or more organs. Means and methods for the measurement of organ function will vary from organ to organ, and are well known to those skilled in the art. Organ function, or organ damage can, for example, be established by the measurement of art-known markers for the function or damage of an organ of interest in samples obtained from the patient. For example, plasma levels of lactate dehydrogenase (LDH) and myoglobin may be used as a measurement of target organ injury; levels of aspartic acid aminotransferase (ASAT) are a suitable marker for liver injury (such as during ischemia and reperfusion); serum levels of cardiac specific troponins or ECG for cardiac injury; urine protein levels for the measurement of kidney injury; and white blood cell number in the lungs or functional lung capacity tests can be used to measure acute lung injury (such as following intestinal ischemia).

In a method and use according to the first aspect of the invention that is intended to prevent or reduce peri- and postoperative complications, the Annexin A5 or functional analogue or variant thereof is typically administered at the time of or shortly before the surgical intervention. For example, it may be administered at least 1 week, 6 days, 5 days, 4 days, 3 days, 2 days, 1 day, 12 hours, 8 hours, 4 hours, 2 hours, 1 hour, or 50, 40, 30, 20, 10, 5, 4, 3, 2, 1, or less than 1 minutes before the surgical intervention or at the time of surgical the intervention. Typically, administration of the Annexin A5 or functional analogue or variant thereof is continued after the surgical intervention, for example for at least 1 week, 2 weeks, 1 month, 2 months, 6 months, 1 year, 2 years, 5 years or 10 years after the intervention. Annexin A5 or functional analogue or variant thereof may be provided by repeated administration of suitable dosage forms, or by administration in a controlled release form, or as a combination of one or more forms of administration. It will be appreciated that the exact duration of continuation of administration of the Annexin A5 or functional analogue or variant thereof may be chosen to coincide with the degree of risk of peri- and postoperative complications at different times after the surgical intervention.

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In other embodiments of the first aspect of the invention, Annexin A5 or functional analogue or variant thereof is administered as a secondary prophylaxis, to prevent or reduce worsening of peri- and postoperative complications that has already occurred; or as a treatment, to completely or partially reverse peri- and postoperative complications that have already occurred.

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Typically, Annexin A5 or functional analogue or variant thereof is provided by repeat administration of suitable dosage forms. Typically, administration is continued for at least 1 week, 2 weeks, 1 month, 2 months, 6 months, 1 year, 2 years, 5 years or 10 years after the surgical intervention

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By administering a "therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof", we mean administering an amount which has a beneficial effect in preventing and/or reducing peri- and postoperative complications, or preventing and/or reducing the worsening of existing peri- and postoperative complications. Where
10 the Annexin A5 or a functional analogue or variant thereof prevents and/or reduces peri- and postoperative complications typically, peri- and postoperative complications are not seen following onset of administration, or are seen later than would be expected (i.e. compared to its development in the absence of the administration of Annexin A5 or functional analogue or variant thereof), or develops less quickly, or develops to a
15 reduced extent, or there is a combination of slower progression and reduced development.

Typically, postoperative complications would be seen at least, 1 hour, 6 hours, 12 hours, 1 day, 1 week, 2 weeks, 1 month, at least 3 months, 6 months, 1 year, 2 years, 5 years
20 or 10 years later than would have been expected in the absence of administration of Annexin A5 or a functional analogue or variant thereof. Typically, where the Annexin A5 or a functional analogue or variant thereof is administered to prevent and/or reduce existing postoperative complications, symptoms of postoperative complications disappear over time, such that postoperative complications is no longer seen at 10 years,
25 at 5 years, 2 years, 1 year, 6 months, 3 months, 1 month, 2 weeks, 1 week, 12 hours, 6 hours following administration.

The skilled person will appreciate that different endpoints may be appropriate to identify a therapeutic effect of administration of Annexin A5 or functional analogue or variant
30 thereof. For example, myocardial infarction and/or death are common postoperative complications following peripheral vascular surgery. Subjects administered Annexin A5 or functional analogue or variant thereof may be afflicted by myocardial infarction at lower incidence, and/or survive for longer time than would be expected in the absence of the administration of Annexin A5 or functional analogue or variant thereof.

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A further demonstration of the effect of administration of Annexin A5 or a functional analogue or variant thereof on postoperative complications following surgical

interventions can be determined by clinical trials, typically involving tens, hundreds, or thousands of patients (such as 10, 50, 100, 1000 or 10000 or values between these). Alternatively, animal tests may be used, such as those described below in relation to the testing of functional analogues and variants of Annexin A5, and in the Examples.

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The Annexin A5 or the functional analogue or variant thereof can be administered in conjunction with one or more further active agent(s), such as a thrombolytic therapeutic such as aspirin, clopidogrel, ticlopidin, tissue plasminogen activator, urokinase, or a bacterial enzyme such as streptokinase; an analgesic therapeutic such as an opiate, an anti-infective therapeutic such as a beta-lactam, a tetracycline, an amphenicol, or an aminoglycoside. The Annexin A5 or functional analogue or variant thereof can be co-administered with any of one or more further active agent(s), or it may be administered separately, simultaneously or sequentially with such agent(s). Typically, one or more of these agents is used to reduce the risk of thrombosis, which can occlude blood vessels, including stented blood vessels. Suitably, therapy is commenced with ticlopidin or clopidogrel before a revascularisation procedure involving stent deployment, and is continued for at least three, six or even twelve months after the procedure (Ong and Serruys, 2005). Typically, aspirin is administered concomitantly with ticlopidin or clopidogrel and then continued indefinitely. The use of aspirin together with clopidogrel or ticlopidin is referred to as dual antiplatelet therapy. Similar regimes of antithrombotic drug administration may be applied before and following other types of intervention.

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The Annexin A5 or the functional analogue or variant thereof can be administered parenterally, intravenously, intra-arterially, intra-peritoneally, intra-muscularly, or subcutaneously or locally.

25

The first aspect of the invention also provides a pharmaceutical composition comprising a therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof for use in the prevention and/or reduction of peri- and postoperative complications following surgical interventions. Put another way, the first aspect of the invention also provides use of Annexin A5 or a functional analogue or variant thereof for the manufacture of a medicinal product for the prevention and/or reduction of peri- and postoperative complications following surgical interventions.

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A pharmaceutical composition according to the first aspect of the invention may thus comprise Annexin A5 or a functional analogue or variant thereof in admixture with a pharmaceutically or veterinarily acceptable adjuvant, diluent or carrier, which will typically

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be selected with regard to the intended route of administration and standard pharmaceutical practice. The composition may be in the form of immediate-, delayed- or controlled-release applications. Preferably, the formulation is a unit dosage containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of the active ingredient.

5

The phrases "pharmaceutical or pharmacologically acceptable" refer to compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of such pharmaceutical compositions are known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack
10 Printing Company, 1990, incorporated herein by reference. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

15

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, salts, preservatives, drugs, drug stabilizers, excipients, disintegration agents, such like materials and combinations thereof, as would
20 be known to one of ordinary skill in the art. Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The pharmaceutical compositions according to the invention may, or may not, be
25 intended for, and, thus formulated in a manner suitable for, parenteral, intravenous, intra-arterial, intraperitoneal, intra-muscular or subcutaneous administration, or administration from a drug-eluting stent, or they may be administered by infusion techniques. Sterile injectable solutions may be prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients
30 enumerated above, as required, followed by sterilization. The pharmaceutical compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions may be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable pharmaceutical formulations
35 under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

A therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof for administration to a patient, such as a human patient, on the basis of a daily dosage level may be from 0.01 to 1000 mg of Annexin A5 or a functional analogue or variant thereof per adult (for example, from about 0.001 to 20 mg per kg of the patient's
5 body weight, such as 0.01 to 10 mg/kg, for example greater than 0.1 mg/kg and up to or less than 20, 10, 5, 4, 3 or 2 mg/kg, such as about 1 mg/kg), administered in single or divided doses.

The physician in any event will determine the actual dosage which will be most suitable
10 for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

15 For veterinary use, a compound of the invention is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

20 The Annexin A5 or functional analogue or variant thereof referred to in relation to the first aspect of the invention may be selected from:

- a) human Annexin A5 (SEQ ID NO:1);
- b) a mammalian orthologue of human Annexin A5;
- c) an allelic or genetic variant of a) or b);
- 25 d) a functional analogue of Annexin which is a protein which is more than 50%, 60%, 70%, 75%, such as more than 80%, 85%, more than 90%, or even more preferably more than 95% or 99% identical to human Annexin A5, SEQ ID NO:1;
- e) a dimer of, or fusion protein comprising, any of a), b), c) or d); and
- 30 f) a PEGylated variant of any of a), b), c), d) or e).

In particular embodiments, the functional analogue or variant of Annexin A5 according to the invention is more than 50%, 60%, 70%, 75%, such as more than 80%, 85%, more than 90%, or even more preferably more than 95% or 99% identical to human Annexin
35 A5, SEQ ID NO:1.

The percent identity between two amino acid sequences is determined as follows. First, an amino acid sequence is compared to, for example, SEQ ID NO:1 using the BLAST 2 Sequences (BI2seq) program from the stand-alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from the U.S. government's National Center for Biotechnology Information web site at ncbi.nlm.nih.gov. Instructions explaining how to use the BI2seq program can be found in the readme file accompanying BLASTZ. BI2seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of BI2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\BI2seq -i c:\seq1.txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences. Once aligned, the number of matches is determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences.

The percent identity is determined by dividing the number of matches by the length of the sequence set forth in an identified sequence followed by multiplying the resulting value by 100. For example, if a sequence is compared to the sequence set forth in SEQ ID NO:1 (the length of the sequence set forth in SEQ ID NO:1 is 320) and the number of matches is 288, then the sequence has a percent identity of 90 (i.e., $288 \div 320 * 100 = 90$) to the sequence set forth in SEQ ID NO:1.

Thus, a functional analogue or variant of Annexin A5 may be a protein wherein at one or more positions there have been amino acid insertions, deletions, or substitutions, either conservative or non-conservative, provided that such changes result in a protein whose basic properties to function in an equivalent manner to Annexin A5 have not significantly been changed. "Significantly" in this context means that one skilled in the art would say that the properties of the variant may still be different but would not be unobvious over the ones of the original protein.

By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

Such variants may be made using the methods of protein engineering and site-directed
5 mutagenesis which are well known in the art.

The functional analogue or variant of Annexin A5 according to the invention may be a dimer of Annexin A5 (such as DiAnnexin) or a functional analogue or variant thereof, or may be a PEGylated Annexin A5 or a functional analogue or variant thereof.
10 DiAnnexinA5 and PEGylated AnnexinA5 are disclosed in WO 02/067857.

PEGylation is a method well known to those skilled in the art wherein a polypeptide or peptidomimetic compound (for the purposes of the present invention, Annexin A5 or the functional analogue or variant) is modified such that one or more polyethylene glycol (PEG)
15 molecules are covalently attached to the side chain of one or more amino acids or derivatives thereof. It is one of the most important molecule altering structural chemistry techniques (MASC). Other MASC techniques may be used; such techniques may improve the pharmacodynamic properties of the molecule, for example extending its half-life *in vivo*. A PEG-protein conjugate is formed by first activating the PEG moiety so that it will react
20 with, and couple to, the protein or peptidomimetic compound of the invention. PEG moieties vary considerably in molecular weight and conformation, with the early moieties (monofunctional PEGs; mPEGs) being linear with molecular weights of 12kDa or less, and later moieties being of increased molecular weights. PEG2, a recent innovation in PEG technology, involves the coupling of a 30kDa (or less) mPEG to a lysine amino acid
25 (although PEGylation can be extended to the addition of PEG to other amino acids) that is further reacted to form a branched structure that behaves like a linear mPEG of much greater molecular weight (Kozlowski et al., 2001). Methods that may be used to covalently attach the PEG molecules to polypeptides are further described in Roberts et al. (2002) *Adv Drug Deliv Rev*, 54, 459 – 476; Bhadra et al. (2002) *Pharmazie* 57, 5 – 29; Kozlowski et al.
30 (2001) *J Control Release* 72, 217 – 224; and Veronese (2001) *Biomaterials* 22, 405 – 417 and references referred to therein.

The advantages of PEGylation include reduced renal clearance which, for some products, results in a more sustained adsorption after administration as well as restricted distribution,
35 possibly leading to a more constant and sustained plasma concentrations and hence an increase in clinical effectiveness (Harris et al. (2001) *Clin Pharmacokinet* 40, 539 – 551). Further advantages can include reduced immunogenicity of the therapeutic compound

(Reddy (2001) *Ann Pharmacother*, 34, 915 – 923), and lower toxicity (Kozlowski et al. (2001), *Biodrugs* 15, 419 – 429).

5 The functional analogue or variant of Annexin A5 can be a fusion protein comprising the sequence of Annexin A5 or a variant thereof. Thus, for example, Annexin A5 or a variant thereof can be fused to one or more fusion partner polypeptide sequence(s) so as to extend the half-life of the molecule within a patient's circulatory system and/or add further functionality to the molecule.

10 A "functional" analogue or variant of Annexin A5 may be capable of binding to phosphatidylserine on a biological membrane, preferably to a level that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99% or about 100% of that displayed by human Annexin A5 (SEQ ID NO:1) under the same conditions. A suitable method for measuring Annexin A5 binding to phosphatidylserine on a biological membrane is known in
15 the art (Vermes et al. (1995) *J Immunol Methods*, **184**(1): p. 39-51).

A "functional" analogue or variant of Annexin A5 may, additionally, or alternatively, possess at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99% or about 100% of the therapeutic activity human Annexin A5 (SEQ ID NO:1) when used at the same (i.e.
20 molar equivalent) dosage, for prophylaxis or treatment of restenosis in accordance with the first aspect of the invention. In this context, the therapeutic activity of a "functional" analogue or variant of Annexin A5 may be determined, compared to that of human Annexin A5 (SEQ ID NO:1), by comparing the ability of a molar equivalent amount of the functional analogue or variant and of human Annexin A5 to treat or provide prophylaxis for restenosis
25 as described above, and/or by animal tests, such as using the methodology set out in the following examples.

Examples

30 The following examples are included to demonstrate particular embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the
35 present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1: Rat hind limb surgery model

The aim of the experiments described in this example is to perform a surgical intervention in rats in a peripheral organ to which blood flow is stopped during the surgery.

For this purpose, rats are anaesthetised and a ligature is placed around the lower abdominal aorta. The threads are passed through a PE50 catheter, and the blood flow is occluded by pushing the threads up through the catheter so the aorta becomes clamped, and blood flow to the hind limbs is stopped. The suture is then fixed in the closed position by clamping the catheter. In this experiment, there is no principal intervention made, but during the occlusion period, the left femoral artery is exposed and gently clamped, to mimic a trauma obtained during surgery. Forty-five minutes after the stopping of blood flow, the aorta is again opened and blood flow re-instituted.

This experimental protocol is performed as an example of a reproducible surgical intervention. Blood samples are taken from an indwelling catheter before blood flow to the hind limbs are stopped, and 180 minutes after blood flow is re-instituted. Rats are administered intravenously with Annexin A5 in a dose of 1 mg/kg or placebo at least 10 minutes prior to anaesthesia.

Plasma levels of lactate dehydrogenase (LDH) and myoglobin are used as measured as of target organ injury. These proteins are intracellular, but are released from muscle cells that are injured. It is also well known that myoglobin is a common cause of renal failure, so the level of myoglobin is also a measure of the risk for a complication in a non-target organ.

In all animals, plasma levels were quite high already at time 0, most likely reflecting that anaesthesia and surgery itself is traumatic and associated to tissue injury. In sham-operated animals (that is animals that had the ligature placed around the aorta, but they were not subjected to ischemia) there was a marked drop during the experimental period. However, as is seen in Figure 1, in untreated (placebo) animals the plasma LDH levels continued to increase, whereas if the animals were administered annexin A5 prior to ischemia, there was instead a slight reduction in LDH levels.

Interestingly, we also found an increase in aspartic acid aminotransferase (ASAT), a marker for liver injury during ischemia and reperfusion (but not in the sham operated

rats), indicating that the procedure caused liver injury. There was an increase in both placebo and annexin A5 treated rats, but annexin A5 treatment reduced the increase. Further, major increase in myoglobin levels were found in 25 % of the placebo treated rats, but in no annexin A5 treated rat.

5

This experiment shows that Annexin A5 can limit peri- and postoperative complications.

Example 2.

10 Intestinal ischemia/reperfusion (I/R) injury is a grave condition resulting from acute mesenteric ischemia, haemorrhagic, traumatic or septic shock, or severe burns and some surgical procedures including small bowel transplantation and abdominal aortic surgery . Multiple organ failure, including acute lung injury (ALI) and its severe form acute respiratory distress syndrome (ARDS), is a common complication of intestinal I/R
15 injuries and contributes to its high mortality rate (Ding R et al (2012), Inflammation 35(1):158-66).

This example tests the hypothesis that Annexin A5 can prevent or reduce the injury in lungs following intestinal surgery. Acute lung injury was induced by intestinal ischemia-reperfusion. Briefly, Sprague-Dawley rats (approximately 250 g) were anesthetized, and
20 a midline laparotomy performed and the superior mesenteric artery identified and occluded below the celiac trunk with an arterial microclamp. Intestinal ischemia is confirmed by paleness of the jejunum and ileum. After 45 min of ischemia, reperfusion is initiated by removal of the clamp and confirmed by the colour recovery of the intestine.

25 Pre-warmed (37°C) saline (0.5 ml) is instilled into the peritoneal cavity before closed with a suture. The animals are sacrificed 2 hours later by exsanguinations. The rats are administered intravenously with 1 mg/kg of annexin A5 or placebo one hour before ischemia.

30 At termination the lung tissue was isolated and fixed in formalin solution for further processing and histopathological analyses All samples were processed from formalin through graded alcohols, xylene to paraffin wax and subsequently embedded in paraffin wax. 5µm sections were cut from sample and placed onto Superfrost Plus slides. They were stained to demonstrate white blood cells and lymphocytes using an antibody,
35 CD45, in an immunohistochemical (IHC) assay and subsequently digitally scanned by OracleBio. The scanned image was then analysed using Definen's Tissue Studio 3.0 software. Image analysis showed that annexin A5 treatment reduced the number of

white cells (figure 2), showing that annexin A5 reduced acute lung injury following intestinal ischemia.

References

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference:

- 10 PCT Appln. WO 02/067857
PCT Appln. WO 2005/099744
PCT Appln WO 2009/077764)
PCT Appln WO 2009/103977.
- 15 Bhadra *et al.* (2002) *Pharmazie* 57, 5 – 29
Cederholm and Frostegard (2007) *Ann. NY Acad. Sci.*, 1108:96-103.
Cederholm *et al.* (2005) *Arterioscler. Thromb. Vasc. Biol.*, 25(1):198-203.
Ding R *et al* (2012), *Inflammation* 35(1):158-66
Duggan. *Am J Cardiovasc Drugs*, 2012. 12(1): p. 57-72.
- 20 Feinman. *Am J Physiol Gastrointest Liver Physiol*, 2010. 299(4): p. G833-43.
Harris *et al.* (2001) *Clin Pharmacokinet* 40, 539 – 551
Kozlowski *et al.* (2001) *J Control Release* 72, 217 – 224
Kozlowski *et al.* (2001), *Biodrugs* 15, 419 – 429
Ong and Serruys (2005) *Curr Issues Cardiol* 32: 372-377
- 25 Rand (2000) *Autoimmun.*, 15(2):107-111.
Reddy (2001) *Ann Pharmacother*, 34, 915 – 923
Roberts *et al.* (2002) *Adv Drug Deliv Rev*, 54, 459 – 476
Shen *et al.* (2007) *Am. J. Transplant.*, 7(11):2463-2471.
Smith Jr. *et al.* (2006) *J. Am. Coll. Cardiol.*, 47(1):216-235.
- 30 Teoh *et al.* (2007) *Gastroenterology*, 133(2):632-646.
Thiagarajan and Benedict (1997) *Circulation*, 96(7):2339-2347.
Vermes *et al.* (1995) *J Immunol Methods*, 184(1): p. 39-51
Veronese (2001) *Biomaterials* 22, 405 – 417
Vogt *et al.* (2004) *European Heart Journal* 25: 1330-40.
- 35 Zayour and Azar. *Endocr Pract*, 2006. 12(1): p. 59-62.
Zhang *et al.* (2004) *Arterioscler. Thromb. Vasc. Biol.*, 24(12):2277-2283.

CLAIMS

1. A method for the prevention and/or reduction of peri- and postoperative complications following surgical intervention, said method comprising administering a therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof to a patient in need of such treatment.
2. The method according to claim 1, wherein the surgical intervention causes immediate cellular damage, ischemia and/or reperfusion injury to, and/or thrombosis in, a target organ.
3. The method according to claim 1, wherein the surgical intervention comprises orthopaedic surgery such as joint replacements.
4. The method according to claim 1, wherein the surgical intervention comprises restrictions in organ blood flow during the surgical intervention.
5. The method according to claim 4, wherein the surgical intervention comprises peripheral vascular surgery.
6. The method according to claim 4, wherein the surgical intervention comprises cardiac surgery.
7. The method according to any of claims 1 to 6, wherein the peri- or postoperative complication is a complication of an organ being the target of the surgical intervention.
8. The method according to any of claims 1 to 6, preferably Claim 2, wherein the peri- or postoperative complication is a complication of an organ not being the target of the surgical intervention, and preferably wherein the surgical intervention does not cause immediate cellular damage, ischemia and/or reperfusion injury to, and/or thrombosis in, the organ in which the peri- or postoperative complication is prevented and/or reduced.
9. The method according to any of claims 1 to 8, wherein the peri- or postoperative complication is selected from a renal complication, a pulmonary complication, and a gastrointestinal complication,
10. The method according to any of claims 1 to 8, wherein the peri- or postoperative complication is a cardiovascular complication.

11. The method according to claim 10, wherein the peri- or postoperative complication is selected from myocardial infarction, congestive heart disease, stroke, TIA, and postoperative limb ischemia.

12. The method of any one of the preceding claims wherein the therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof is administered parenterally, intravenously, intra-arterially, intra-peritoneally, intramuscularly, subcutaneously or is administered locally from a drug eluting stent.

13. The method of any one of the preceding claims wherein the Annexin A5 or the functional analogue or variant thereof is administered in conjunction with a thrombolytic therapeutic such as aspirin, clopidogrel, ticlopidin, tissue plasminogen activator, urokinase, or a bacterial enzyme.

14. The method of any one of the preceding claims wherein the Annexin A5 or the functional analogue or variant thereof is selected from:

- a) human Annexin A5 (SEQ ID NO:1);
- b) a mammalian orthologue of human Annexin A5;
- c) an allelic or genetic variant of a) or b);
- d) a functional analogue of Annexin which is a protein which is more than 50%, more than 75%, such as more than 80%, more than 90%, or even more preferably more than 95% identical to human Annexin A5, SEQ ID NO:1;
- e) a dimer of, or fusion protein comprising, any of a), b), c) or d); and
- f) a PEGylated variant of any of a), b), c), d) or e).

15. A pharmaceutical composition comprising a therapeutically effective amount of Annexin A5 or a functional analogue or variant thereof for use in the prevention and/or reduction of peri- and postoperative complications following surgical intervention.

16. Use of Annexin A5 or a functional analogue or variant thereof in the manufacture of a medicament for the prevention and/or reduction of peri- and postoperative complications following surgical intervention.

17. The pharmaceutical composition according to claim 15, or the use according to Claim 16, wherein the surgical intervention causes immediate cellular damage, ischemia and/or reperfusion injury to, and/or thrombosis in, a target organ.
- 5 18. The pharmaceutical composition according to claim 15, or the use according to Claim 16, wherein the surgical intervention comprises orthopaedic surgery, such as joint replacement.
- 10 19. The pharmaceutical composition according to claim 15, or the use according to Claim 16, wherein the surgical intervention comprises restrictions in organ blood flow during the surgical intervention.
20. The pharmaceutical composition or use according to claim 19, wherein the surgical intervention comprises peripheral vascular surgery.
- 15 21. The pharmaceutical composition or use according to claim 19, wherein the surgical intervention comprises cardiac surgery.
- 20 22. The pharmaceutical composition according to any of claims 15 or 17 to 21, or the use according to any of Claims 16 to 21, wherein the peri- or postoperative complication is a complication of an organ being the target of the surgical intervention.
- 25 23. The pharmaceutical composition according to any of claims 15 or 17 to 21, preferably Claim 17, or the use according to any of Claims 16 to 21, preferably Claim 17, wherein the peri- or postoperative complication is a complication of an organ not being the target of the surgical intervention, and preferably wherein the surgical intervention does not cause immediate cellular damage, ischemia and/or reperfusion injury to, and/or thrombosis in, the organ in which the peri- or postoperative complication is prevented and/or reduced.
- 30 24. The pharmaceutical composition according to any of claims 15 or 17 to 23, or the use according to any of Claims 16 to 23, wherein the peri- or postoperative complication is a complication is selected from a renal complication, a pulmonary complication, a gastrointestinal complication.
- 35 25. The pharmaceutical composition according to any of claims 15 or 17 to 23, or the use according to any of Claims 16 to 23, wherein the peri- or postoperative complication is a cardiovascular complication.

26. The pharmaceutical composition or use according to claim 25, wherein the peri- or postoperative complication is selected from myocardial infarction, congestive heart disease, stroke, TIA, and postoperative limb ischemia.

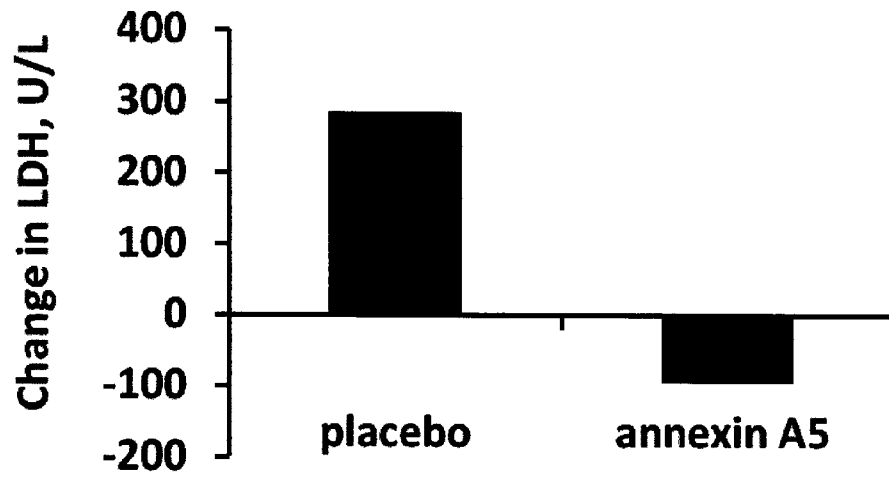
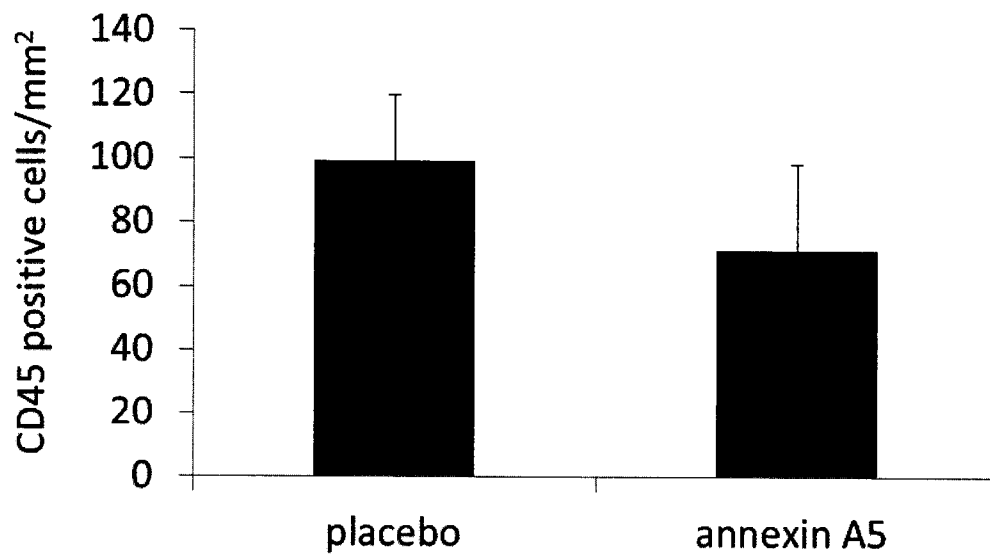
5

27. The pharmaceutical composition according to any of claims 15 or 17 to 26, or the use according to any of Claims 16 to 26, intended for parenteral, intravenous, intra-arterial, intra-peritoneal, intra-muscular or subcutaneous administration, or local administration from a drug eluting stent.

10

28. The pharmaceutical composition according to any of claims 15 or 17 to 27, or the use according to any of Claims 16 to 27, wherein the Annexin A5 or the functional analogue or variant thereof is selected from:

- 15 a) human Annexin A5 (SEQ ID NO:1);
b) a mammalian orthologue of human Annexin A5;
c) an allelic or genetic variant of a) or b);
d) a functional analogue of Annexin which is a protein which is more than 50%, more than 75%, such as more than 80%, more than 90%, or even more
20 preferably more than 95% identical to human Annexin A5, SEQ ID NO:1;
e) a dimer of, or a fusion protein comprising, any of a), b), c) or d); and
f) a PEGylated variant of any of a), b), c), d) or e).

Fig 1**Fig 2**

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/056370A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47 A61K38/17
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TEOH ET AL: "Diannexin, a Novel Annexin V Homodimer, Provides Prolonged Protection Against Hepatic Ischemia-Reperfusion Injury in Mice", GASTROENTEROLOGY, ELSEVIER, PHILADELPHIA, PA, vol. 133, no. 2, 3 August 2007 (2007-08-03), pages 632-646, XP022198888, ISSN: 0016-5085, DOI: 10.1053/J.GASTRO.2007.05.027 cited in the application the whole document ----- -/--	1-28



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 June 2012

Date of mailing of the international search report

04/07/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Kools, Patrick

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/056370

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOLSKI MICHAL ET AL: "Diannexin treatment decreases ischemia-reperfusion injury at the endothelial cell level of the microvascular bed in muscle flaps.", ANNALS OF PLASTIC SURGERY NOV 2009 LNKD-PUBMED:19806047, vol. 63, no. 5, November 2009 (2009-11), pages 564-571, XP008153086, ISSN: 1536-3708 the whole document	1-28
A	----- CEDERHOLM ANNA ET AL: "Annexin A5 as a novel player in prevention of atherothrombosis in SLE and in the general population", NEW YORK ACADEMY OF SCIENCES. ANNALS, WILEY-BLACKWELL PUBLISHING, INC, US, vol. 1108, 1 June 2007 (2007-06-01), pages 96-103, XP008107390, ISSN: 0077-8923, DOI: 10.1196/ANNALS.1422.011 [retrieved on 2007-08-29] cited in the application abstract	1-28
A	----- WO 2009/103977 A1 (ATHERA BIOTECHNOLOGIES AB [SE]; WRIGHT ANDREW [GB]; PETTERSSON KNUT [S]) 27 August 2009 (2009-08-27) cited in the application the whole document	1-28
X,P	----- KIMBERLEY E. WEVER ET AL: "Diannexin Protects against Renal Ischemia Reperfusion Injury and Targets Phosphatidylserines in Ischemic Tissue", PLOS ONE, vol. 6, no. 8, 30 August 2011 (2011-08-30), page E24276, XP55031079, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0024276 the whole document	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/056370

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009103977 A1	27-08-2009	AU 2009216543 A1	27-08-2009
		CA 2715859 A1	27-08-2009
		CN 101969978 A	09-02-2011
		EP 2254589 A1	01-12-2010
		JP 2011512393 A	21-04-2011
		KR 20100122501 A	22-11-2010
		US 2010331970 A1	30-12-2010
		WO 2009103977 A1	27-08-2009

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2012/056370

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)

☒

 on paper
 - ☒

 in electronic form
 - b. (time)

☒

 in the international application as filed
 - ☐

 together with the international application in electronic form
 - ☒

 subsequently to this Authority for the purpose of search
2.

☒

 In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments: