

Anti-Adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock

5 Field of the invention

Subject matter of the present invention is an anti-Adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said patient is characterized by having a level of dipeptidyl peptidase 3 (DPP3) in a sample of bodily fluid below a threshold and said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal part (amino acid 1-21) of ADM: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

Background

Dipeptidyl peptidase 3 – also known as Dipeptidyl aminopeptidase III, Dipeptidyl arylamidase III, Dipeptidyl peptidase III, Enkephalinase B or red cell angiotensinase; short name: DPP3, DPPIII – is a metallopeptidase that removes dipeptides from physiologically active peptides, such as enkephalins and angiotensins. DPP3 was first identified and its activity measured in extracts of purified bovine anterior pituitary by Ellis & Nuenke 1967. The enzyme, which is listed as EC 3.4.14.4, has a molecular mass of about 83 kDa and is highly conserved in procaryotes and eucaryotes (*Prajapati & Chauhan 2011*). The amino acid sequence of the human variant is depicted in SEQ ID NO 1. Dipeptidyl peptidase III is a mainly cytosolic peptidase which is ubiquitously expressed. Despite lacking a signal sequence, a few studies reported membranous activity (*Lee & Snyder 1982*).

DPP3 is a zinc-depending exo-peptidase belonging to the peptidase family M49. It has a broad substrate specificity for oligopeptides from three/ four to ten amino acids of various compositions and is also capable of cleaving after proline. DPP3 is known to hydrolyze dipeptides from the N-terminus of its substrates, including angiotensin II, III and IV; Leu- and Met-enkephalin; endomorphin 1 and 2. The metallopeptidase DPP3 has its activity optimum at pH 8.0-9.0 and can be activated by addition of divalent metal ions, such as Co²⁺ and Mg²⁺.

Structural analysis of DPP3 revealed the catalytic motifs HELLGH (hDPP3 450-455) and EECRAE (hDPP3 507-512), as well as following amino acids, that are important for substrate binding and hydrolysis: Glu316, Tyr, 318, Asp366, Asn391, Asn394, His568, Arg572, Arg577, Lys666 and Arg669 (*Prajapati & Chauhan 2011; Kumar et al. 2016*; numbering refers to the sequence of human DPP3, see SEQ ID NO. 1). Considering all known amino acids or sequence regions that are involved in substrate

binding and hydrolysis, the active site of human DPP3 can be defined as the area between amino acids 316 and 669.

The most prominent substrate of DPP3 is angiotensin II (Ang II), the main effector of the renin-angiotensin system (RAS). The RAS is activated in cardiovascular diseases (*Dostal et al. 1997. J Mol Cell Cardiol*;29:2893-902; *Roks et al. 1997. Heart Vessels. Suppl 12:119-24*), sepsis, and septic shock (*Corrêa et al. 2015. Crit Care 2015;19:98*). Ang II, in particular, has been shown to modulate many cardiovascular functions including the control of blood pressure and cardiac remodeling.

Recently, two assays were generated, characterized, and validated to specifically detect DPP3 in human bodily fluids (e.g., blood, plasma, serum): a luminescence immunoassay (LIA) to detect DPP3 protein concentration and an enzyme capture activity assay (ECA) to detect specific DPP3 activity (*Rehfeld et al. 2019. JALM 3(6): 943-953*). A washing step removes all interfering substances before the actual detection of DPP3 activity is performed. Both methods are highly specific and allow the reproducible detection of DPP3 in blood samples.

Circulating DPP3 levels were shown to be increased in cardiogenic shock patients and were associated with an increased risk of short-term mortality and severe organ dysfunction (*Deaniau et al. 2019. Eur J Heart Fail. in press*). Moreover, DPP3 measured at inclusion discriminated cardiogenic shock patients who did develop refractory shock vs. non-refractory shock and a DPP3 concentration ≥ 59.1 ng/mL was associated with a greater risk of death (*Takagi et al. Eur J Heart Fail. in press*).

The peptide adrenomedullin (ADM) was described for the first time in 1993 (*Kitamura et al., 1993. Biochem Biophys Res Comm 192 (2): 553-560*) as a novel hypotensive peptide comprising 52 amino acids, which had been isolated from a human pheochromocytoma cell line (SEQ ID No.: 20). In the same year, cDNA coding for a precursor peptide comprising 185 amino acids and the complete amino acid sequence of this precursor peptide were also described. The precursor peptide, which comprises, inter alia, a signal sequence of 21 amino acids at the N-terminus, is referred to as "pre-proadrenomedullin" (pre-proADM). In the present description, all amino acid positions specified usually relate to the pre-proADM, which comprises the 185 amino acids. The peptide adrenomedullin (ADM) is a peptide which comprises 52 amino acids (SEQ ID No: 20) and which comprises the amino acids 95 to 146 of pre-proADM, from which it is formed by proteolytic cleavage. To date, substantially only a few fragments of the peptide fragments formed in the cleavage of the pre-proADM have been more exactly investigated, in particular the physiologically active peptides ADM and "PAMP", a peptide comprising 20 amino acids (22-41), which follows the 21 amino acids of the signal peptide in pre-

proADM. The discovery and characterization of ADM in 1993 triggered intensive research activity, the results of which have been summarized in various review articles, in the context of the present description, reference being made in particular to the articles to be found in an issue of "Peptides" devoted to ADM in particular (*Takahashi 2001. Peptides 22: 1691; Eto 2001. Peptides 22: 1693-1711*).

5 A further review is Hinson et al. 2000 (*Hinson et al. 2000. Endocrine Reviews 21(2):138-167*). In the scientific investigations to date, it has been found, inter alia, that ADM may be regarded as a polyfunctional regulatory peptide. It is released into the circulation in an inactive form extended by glycine (*Kitamura et al. 1998. Biochem Biophys Res Commun 244(2): 551-555*). There is also a binding protein (*Pio et al. 2001. The Journal of Biological Chemistry 276(15): 12292-12300*), which is specific
10 for ADM and probably likewise modulates the effect of ADM. Those physiological effects of ADM as well as of PAMP, which are of primary importance in the investigations to date, were the effects influencing blood pressure.

Hence, ADM is an effective vasodilator, and thus it is possible to associate the hypotensive effect with
15 the particular peptide segments in the C-terminal part of ADM. It has furthermore been found that the above-mentioned physiologically active peptide PAMP formed from pre-proADM likewise exhibits a hypotensive effect, even if it appears to have an action mechanism differing from that of ADM (in addition to the above mentioned review articles *Eto et al. 2001* and *Hinson et al. 2000* see also *Kuwasaki et al. 1997. FEBS Lett 414(1): 105-110; Kuwasaki et al. 1999. Ann. Clin. Biochem. 36: 622-628; Tsuruda et al. 2001 Life Sci. 69(2): 239-245 and EP-A2 0 622 458*).
20 It has furthermore been found, that the concentrations of ADM, which can be measured in the circulation and other biological liquids, are in a number of pathological states, significantly above the concentrations found in healthy control subjects. Thus, the ADM level in patients with congestive heart failure, myocardial infarction, kidney diseases, hypertensive disorders, diabetes mellitus, in the acute
25 phase of shock and in sepsis and septic shock are significantly increased, although to different extents. The PAMP concentrations are also increased in some of said pathological states, but the plasma levels are lower relative to ADM (*Eto 2001. Peptides 22: 1693-1711*). It was reported that unusually high concentrations of ADM are observed in sepsis, and the highest concentrations in septic shock (*Eto 2001. Peptides 22: 1693-1711; Hirata et al. Journal of Clinical Endocrinology and Metabolism 81(4): 1449-1453; Ehlenz et al. 1997. Exp Clin Endocrinol Diabetes 105: 156-162; Tomoda et al. 2001. Peptides 22: 1783-1794; Ueda et al. 1999. Am. J. Respir. Crit. Care Med. 160: 132-136 and Wang et al. 2001. Peptides 22: 1835-1840*).

Plasma concentrations of ADM are elevated in patients with heart failure and correlate with disease
35 severity (*Hirayama et al. 1999. J Endocrinol 160: 297-303; Yu et al. 2001. Heart 86: 155-160*). High

plasma ADM is an independent negative prognostic indicator in these subjects (*Poyner et al. 2002. Pharmacol Rev 54: 233-246*).

WO2004/097423 describes the use of an antibody against adrenomedullin for diagnosis, prognosis, and treatment of cardiovascular disorders. Treatment of diseases by blocking the ADM receptor are also described in the art, (e.g. WO2006/027147, PCT/EP2005/012844) said diseases may be sepsis, septic shock, cardiovascular diseases, infections, dermatological diseases, endocrinological diseases, metabolic diseases, gastroenterological diseases, cancer, inflammation, hematological diseases, respiratory diseases, muscle skeleton diseases, neurological diseases, urological diseases.

It is reported for the early phase of sepsis that ADM improves heart function and the blood supply in liver, spleen, kidney and small intestine. Anti-ADM-neutralizing antibodies neutralize the before mentioned effects during the early phase of sepsis (*Wang et al. 2001. Peptides 22: 1835-1840*).

For other diseases blocking of ADM may be beneficial to a certain extent. However, it might also be detrimental if ADM is totally neutralized, as a certain amount of ADM may be required for several physiological functions. In many reports it was emphasized, that the administration of ADM may be beneficial in certain diseases. In contrast thereto, in other reports ADM was reported as being life threatening when administered in certain conditions.

WO2013/072510 describes a non-neutralizing anti-ADM antibody for use in therapy of a severe chronical or acute disease or acute condition of a patient for the reduction of the mortality risk for said patient.

WO2013/072511 describes a non-neutralizing anti-ADM antibody for use in therapy of a chronical or acute disease or acute condition of a patient for prevention or reduction of organ dysfunction or organ failure.

WO2013/072512 describes a non-neutralizing anti-ADM antibody that is an ADM stabilizing antibody that enhances the half-life ($t_{1/2}$ half retention time) of adrenomedullin in serum, blood, plasma. This ADM stabilizing antibody blocks the bioactivity of ADM to less than 80 %.

WO2013/072513 describes a non-neutralizing anti-ADM antibody for use in therapy of an acute disease or condition of a patient for stabilizing the circulation.

WO2013/072514 describes a non-neutralizing anti-ADM antibody for regulating the fluid balance in a patient having a chronic or acute disease or acute condition.

WO2017/182561 describes methods for determining the total amount or active DPP3 in a sample of a patient for the diagnosis of a disease related to necrotic processes. It further describes a method of treatment of necrosis-related diseases by antibodies directed to DPP3.

10

Although a large number of markers were known in the prior art to be connected with shock, nothing in particular suggested DPP3 as a marker of shock.

Examples for such biomarkers include MR-proADM, lactate, C-Reactive Protein (CRP) and procalcitonin (PCT) (Ana Maria Navio Serano,¹ Joaquín Valle Alonso,^{2,*} Gustavo Rene Piñero,³ Alejandro Rodriguez Camacho,⁴ Josefa Soriano Benet,⁵ and Manuel Vaquero⁶ Bull Emerg Trauma. 2019 Jul; 7(3): 232–239.) and pentraxin 3, heparin-binding protein, soluble triggering receptor, PARK7 and IL-8 cited in a recent review (Charalampos Pierrakos, Dimitrios Velissaris, Max Bisdorff, John C. Marshall & Jean-Louis Vincent Critical Care volume 24, Article number: 287 (2020) Biomarkers of sepsis: time for a reappraisal).

Therefore, it is the surprising finding of the present invention, that in patients with shock the level of DPP3 in a bodily fluid sample is to be used for the guidance or monitoring of therapy with an anti-ADM antibody. Moreover, the results of the present invention clearly show, that patients with shock will have most benefit of a therapy with an anti-ADM antibody if the level of DPP3 in a bodily fluid sample is below a threshold.

25

Description of the invention

Subject-matter of the present invention is an anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said patient is characterized by having a level of DPP3 in a sample of bodily fluid below a threshold and said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal part (amino acid 1-21) of ADM: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

One embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said shock is selected from the group comprising shock due to hypovolemia, cardiogenic shock, obstructive shock and distributive shock, in particular cardiogenic shock or septic shock.

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein

- in case of cardiogenic shock said patient may have suffered an acute coronary syndrome (e.g. acute myocardial infarction) or wherein said patient has heart failure (e.g. acute decompensated heart failure), myocarditis, arrhythmia, cardiomyopathy, valvular heart disease, aortic dissection with acute aortic stenosis, traumatic chordal rupture or massive pulmonary embolism, or
- in case of hypovolemic shock said patient may have suffered a hemorrhagic disease including gastrointestinal bleed, trauma, vascular etiologies (e.g. ruptured abdominal aortic aneurysm, tumor eroding into a major blood vessel) and spontaneous bleeding in the setting of anticoagulant use or a non-hemorrhagic disease including vomiting, diarrhea, renal loss, skin losses/insensible losses (e.g. burns, heat stroke) or third-space loss in the setting of pancreatitis, cirrhosis, intestinal obstruction, trauma, or
- in case of obstructive shock said patient may have suffered a cardiac tamponade, tension pneumothorax, pulmonary embolism or aortic stenosis, or
- in case of distributive shock said patient may have septic shock, neurogenic shock, anaphylactic shock or shock due to adrenal crisis.

One preferred embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said threshold of DPP3 in a sample of bodily fluid of said patient is between 20 and 120 ng/mL, more preferred between 30 and 80 ng/mL, more preferred between 40 and 90 ng/mL, even more preferred between 40 and 60 ng/mL, most preferred said threshold is 50 ng/mL.

One specific embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein the level of DPP3 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to DPP3.

5

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein either the level of DPP3 protein and/or the level of active DPP3 is determined and compared to a predetermined threshold.

10

One embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said patient is additionally characterized by having a level of ADM-NH₂ above a threshold.

The level of ADM -NH₂ is measured in order to identify patients in shock.

15

One preferred embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said threshold of ADM-NH₂ in a sample of bodily fluid of said patient is between 40 and 100 pg/mL, more preferred between 50 and 90 pg/mL, even more preferred between 60 and 80 pg/mL, most preferred said threshold is 70 pg/mL.

20

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein the level of ADM-NH₂ is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to ADM-NH₂.

25

Another preferred embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein the sample of bodily fluid of said patient is selected from the group of blood, serum, plasma, urine, cerebrospinal fluid (CSF), and saliva.

30

Another specific embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold recognizes and binds to the N-terminal end (amino acid 1) of ADM-Gly and/ or ADM-NH₂.

35

A further embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said antibody, antibody fragment or non-Ig scaffold does not bind to the C-terminal portion of ADM, having the sequence amino acid 43-52 of ADM: PRSKISPQGY-NH₂ (SEQ ID NO: 24).

5

One embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said antibody or fragment or scaffold blocks the bioactivity of ADM not more than 80 %, preferably not more than 50%.

10

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said antibody or fragment is a monoclonal antibody or fragment that binds to ADM or an antibody fragment thereof, wherein the heavy chain comprises the sequences:

15

CDR1: SEQ ID NO: 1

GYTFSTRYW

CDR2: SEQ ID NO: 2

20

ILPGSGST

CDR3: SEQ ID NO: 3

TEGYEYDGFY

25

and wherein the light chain comprises the sequences:

CDR1: SEQ ID NO: 4

QSIVYSNGNTY

30

CDR2:

RVS

CDR3: SEQ ID NO: 5

FQGSHIPYT.

- 5 Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said antibody or fragment comprises a sequence selected from the group comprising as a VH region:

SEQ ID NO: 6 (AM-VH-C)

10 QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGSTNYNE
KFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFQDYWGQGTTTLTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
TVPSSSLGTQTYICNVNHNKPSNTKVDKRVEPK

15 SEQ ID NO: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCTEGYEYDGFQDYWGQGTTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHNKPSNTKVDKRVEPK

20

SEQ ID NO: 8 (AM-VH2-E40)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCTEGYEYDGFQDYWGQGTTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
25 VTVPSSSLGTQTYICNVNHNKPSNTKVDKRVEPK

SEQ ID NO: 9 (AM-VH3-T26-E55)

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCTEGYEYDGFQDYWGQGTTTVTVSSASTKG
30 PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHNKPSNTKVDKRVEPK

SEQ ID NO: 10 (AM-VH4-T26-E40-E55)

QVQLVQSGAEVKKPGSSVKV SCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFYWGQGTTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
5 VTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

and comprises a sequence selected from the group comprising the following sequence as a VL region:

SEQ ID NO: 11 (AM-VL-C)

10 DVLLSQTPLSLPVS LGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

15 SEQ ID NO: 12 (AM-VL1)

DVVMQTQSPLSLPVT LGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

20

SEQ ID NO: 13 (AM-VL2-E40)

DVVMQTQSPLSLPVT LGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
25 LKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC.

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said antibody or fragment comprises the following sequence as a heavy chain:

30

SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
 KFQGRVTITADTSTSTAYMELSSLRSEDNAVYYCTEGYEYDGFQDYWGQGTTVTVSSASTKGP
 SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
 TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
 5 LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
 DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
 SDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNH
 YTQKSLSLSPGK

- 10 or a sequence that is > 95% identical to it,
and comprises the following sequence as a light chain:

SEQ ID NO: 33

15 DVVLTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNRFSQVP
 DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
 LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
 EKHKVYACEVTHQGLSPVTKSFNRGEC

- 20 or a sequence that is > 95% identical to it.

Subject-matter of the present application is also a pharmaceutical formulation for use in therapy or prevention of shock of a patient comprising an anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold.

25

One embodiment of the present application relates to a pharmaceutical formulation for use in therapy or prevention of shock of a patient, wherein said pharmaceutical formulation is a solution, preferably a ready-to-use solution.

- 30 Another embodiment of the present application relates to a pharmaceutical formulation for use in therapy or prevention of shock of a patient, wherein said pharmaceutical formulation is in a freeze-dried state.

- 35 One embodiment of the present application relates to a pharmaceutical formulation for use in therapy or prevention of shock of a patient, wherein said pharmaceutical formulation is administered intramuscular.

One preferred embodiment of the present application relates to a pharmaceutical formulation for use in therapy or prevention of shock of a patient, wherein said pharmaceutical formulation is administered intra-vascular.

- 5 Another embodiment of the present application relates to an pharmaceutical formulation for use in therapy or prevention of shock of a patient, wherein said pharmaceutical formulation is administered via infusion.

Another specific embodiment of the present application relates to a pharmaceutical formulation for use
10 in therapy or prevention of shock of a patient, wherein said pharmaceutical formulation is to be administered systemically.

Another embodiment of the present application relates to a method of therapy or prevention of shock in a patient, the method comprising administering an anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold to said patient, the method comprising
15 the steps:

- determining the level of DPP3 in a sample of bodily fluid of said subject,
- comparing said level of determined DPP3 to a pre-determined threshold, and
wherein said patient is treated if said determined level of DPP3 is below said pre-determined
20 threshold, and
wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal part (aa 1-21) of ADM: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

25 Another specific embodiment of the present application relates to a method of therapy or prevention of shock in a patient, the method comprising administering an anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold to said patient, wherein the method is additionally comprising the steps of

- determining the level of ADM-NH₂ in a sample of bodily fluid of said subject,
- 30 • comparing said level of ADM-NH₂ to a pre-determined threshold,
wherein said patient is treated if said determined level of ADM-NH₂ is above said pre-determined threshold level.

Subject-matter of the present invention is an anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a
35 patient, wherein said patient is characterized by having a level of DPP3 in a sample of bodily fluid below a threshold and said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal part (amino acid 1-21) of ADM: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

One embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein said shock is selected from the group comprising shock due to hypovolemia, cardiogenic shock, obstructive shock and distributive shock, in particular cardiogenic shock or septic shock.

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein

- in case of cardiogenic shock said patient may have suffered an acute coronary syndrome (e.g. acute myocardial infarction) or wherein said patient has heart failure (e.g. acute decompensated heart failure), myocarditis, arrhythmia, cardiomyopathy, valvular heart disease, aortic dissection with acute aortic stenosis, traumatic chordal rupture or massive pulmonary embolism, or
- in case of hypovolemic shock said patient may have suffered a hemorrhagic disease including gastrointestinal bleed, trauma, vascular etiologies (e.g. ruptured abdominal aortic aneurysm, tumor eroding into a major blood vessel) and spontaneous bleeding in the setting of anticoagulant use or a non-hemorrhagic disease including vomiting, diarrhea, renal loss, skin losses/insensible losses (e.g. burns, heat stroke) or third-space loss in the setting of pancreatitis, cirrhosis, intestinal obstruction, trauma, or
- in case of obstructive shock said patient may have suffered a cardiac tamponade, tension pneumothorax, pulmonary embolism or aortic stenosis, or
- in case of distributive shock said patient may have septic shock, neurogenic shock, anaphylactic shock or shock due to adrenal crisis.

One preferred embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein said threshold of DPP3 in a sample of bodily fluid of said patient is between 20 and 120 ng/mL, more preferred between 30 and 80 ng/mL, more preferred between 40 and 90 ng/mL, even more preferred between 40 and 60 ng/mL, most preferred said threshold is 50 ng/mL.

30

One specific embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein the level of DPP3 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to DPP3.

35

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein either the level of DPP3 protein and/or the level of active DPP3 is determined and compared to a predetermined threshold.

5

One embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein said patient is additionally characterized by having a level of ADM-NH₂ above a threshold.

- 10 One preferred embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein said threshold of ADM-NH₂ in a sample of bodily fluid of said patient is between 40 and 100 pg/mL, more preferred between 50 and 90 pg/mL, even more preferred between 60 and 80 pg/mL, most preferred said threshold is 70 pg/mL.

15

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein the level of ADM-NH₂ is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to ADM-NH₂.

20

Another preferred embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein the sample of bodily fluid of said patient is selected from the group of blood, serum, plasma, urine, cerebrospinal fluid (CSF), and saliva.

25

In a preferred embodiment, the bio-ADM is measured from plasma. It is however typical in the technical lifecycle improvement of measurement of analytes that possibilities exist to measure such analytes in other – at least blood-based – matrices, not only plasma. For instance, in case of bio-ADM, another technology has been developed, which uses whole (EDTA-) blood called IB10 sphingotest® bio-ADM (<https://www.nexus-dx.com/wp-content/uploads/2020/07/bio-ADM-IFU-REV-A.pdf>). The IB10 sphingotest® bio-ADM® is a rapid point-of-care (POC) immunoassay for the in vitro quantitative determination of human amidated adrenomedullin peptide (1-52), in the following referred as bioactive adrenomedullin (bio-ADM®), in human EDTA whole blood and plasma.

30

- 35 Another specific embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a

patient, wherein said anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold recognizes and binds to the N-terminal end (amino acid 1) of ADM-Gly and/ or ADM-NH₂.

5 A further embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein said antibody, antibody fragment or non-Ig scaffold does not bind to the C-terminal portion of ADM, having the sequence amino acid 43-52 of ADM: PRSKISPQGY-NH₂ (SEQ ID NO: 24).

10 One embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein said antibody or fragment or scaffold blocks the bioactivity of ADM not more than 80 %, preferably not more than 50%.

15 Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein said antibody or fragment is a monoclonal antibody or fragment that binds to ADM or an antibody fragment thereof, wherein the heavy chain comprises the sequences:

CDR1: SEQ ID NO: 1

20 GYTFSRYW

CDR2: SEQ ID NO: 2

ILPGSGST

25 CDR3: SEQ ID NO: 3

TEGYEYDGFY

and wherein the light chain comprises the sequences:

CDR1: SEQ ID NO: 4

30 QSIVYSNGNTY

CDR2:

RVS

CDR3: SEQ ID NO: 5

FQGSHIPYT.

5

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient, wherein said antibody or fragment comprises a sequence selected from the group comprising as a VH region:

10

SEQ ID NO: 6 (AM-VH-C)

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGSTNYNE
KFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFYWGQGTTTLTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
15 TVPSSSLGTQTYICNVNHNKPSNTKVDKRVEPK

SEQ ID NO: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCTEGYEYDGFYWGQGTTTVTVSSASTKG
20 PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHNKPSNTKVDKRVEPK

SEQ ID NO: 8 (AM-VH2-E40)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPQGLEWMGRILPGSGSTNYA
25 QKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCTEGYEYDGFYWGQGTTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHNKPSNTKVDKRVEPK

SEQ ID NO: 9 (AM-VH3-T26-E55)

30 QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCTEGYEYDGFYWGQGTTTVTVSSASTKG

PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 10 (AM-VH4-T26-E40-E55)

5 QVQLVQSGAEVKKPGSSVKV SCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFYWGQGTITVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

and comprises a sequence selected from the group comprising the following sequence as a VL region:

10 SEQ ID NO: 11 (AM-VL-C)

DVLLSQTPSLPVS LGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

15

SEQ ID NO: 12 (AM-VL1)

DVVMQTQSPLSLPVT LGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
20 EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 13 (AM-VL2-E40)

DVVMQTQSPLSLPVT LGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSGVP
25 DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC.

Another embodiment of the present application relates to an anti-ADM antibody or anti-ADM antibody
30 fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient,
wherein said antibody or fragment comprises the following sequence as a heavy chain:

SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
 KFQGRVTITADTSTSTAYMELSSLRSEDVAVYYCTEGYEDGFDYWGQGTTVTVSSASTKGP
 SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
 5 TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
 LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
 DWLNGKEYCKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
 SDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNH
 YTQKSLSLSPGK

10

or a sequence that is > 95% identical to it,
 and comprises the following sequence as a light chain:

SEQ ID NO: 33

15

DVVLTSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNRFSGVP
 DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
 LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADY
 EKHKVYACEVTHQGLSSPVTKSFNRGEC

20

or a sequence that is > 95% identical to it.

Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or
 anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in
 a patient according to claims 1-24, wherein the anti-adrenomedullin (ADM) antibody or anti-ADM
 25 antibody fragment or anti-ADM non-Ig scaffold binds to the N-terminal part (amino acid 1-10) of ADM:
 YRQSMNNFQG (SEQ ID No. 26).

Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an
 anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold for use in therapy, wherein said
 30 antibody or fragment or scaffold exhibits a binding affinity to ADM of at least 10^{-7} M by label-free
 surface plasmon resonance using a Biacore 2000 system.

Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or anti-
 ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a
 35 patient, wherein the anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold

exhibits an affinity towards human ADM is between 1×10^{-9} to 3×10^{-9} by label-free surface plasmon resonance using a Biacore 2000 system.

Another embodiment of the present application relates to an anti -adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein the anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is an IgG1 antibody.

Subject-matter of the present application is also a pharmaceutical formulation for use in the treatment or prevention of shock of a patient comprising an anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold.

One embodiment of the present application relates to a pharmaceutical formulation for use in the treatment or prevention of shock of a patient, wherein said pharmaceutical formulation is a solution, preferably a ready-to-use solution.

Another embodiment of the present application relates to a pharmaceutical formulation for use in the treatment or prevention of shock of a patient, wherein said pharmaceutical formulation is in a freeze-dried state.

One embodiment of the present application relates to a pharmaceutical formulation for use in the treatment or prevention of shock of a patient, wherein said pharmaceutical formulation is administered intra-muscular.

One preferred embodiment of the present application relates to a pharmaceutical formulation for use in the treatment or prevention of shock of a patient, wherein said pharmaceutical formulation is administered intra-vascular.

Another embodiment of the present application relates to a pharmaceutical formulation for use in the treatment or prevention of shock of a patient, wherein said pharmaceutical formulation is administered via infusion.

Another specific embodiment of the present application relates to a pharmaceutical formulation for use in the treatment or prevention of shock of a patient, wherein said pharmaceutical formulation is to be administered systemically.

Another embodiment of the present application relates to a method of treatment or prevention of shock in a patient, the method comprising administering an anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold to said patient, the method comprising the steps:

- 5 • determining the level of DPP3 in a sample of bodily fluid of said subject,
- comparing said level of determined DPP3 to a pre-determined threshold, and
 wherein said patient is treated if said determined level of DPP3 is below said pre-determined threshold, and
 wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to
10 the N-terminal part (aa 1-21) of ADM: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

Another specific embodiment of the present application relates to a method of treatment or prevention of shock in a patient, the method comprising administering an anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold to said patient, wherein the

15 method is additionally comprising the steps of

- determining the level of ADM-NH₂ in a sample of bodily fluid of said subject,
- comparing said level of ADM-NH₂ to a pre-determined threshold,
 wherein said patient is treated if said determined level of ADM-NH₂ is above said
 pre-determined threshold level.

20

In one embodiment of the invention either the level of DPP3 protein and/or the level of active DPP3 is determined and compared to a threshold level.

25 In a specific embodiment of the invention a threshold of DPP3 in a sample of bodily fluid of said patient is between 20 and 120 ng/mL, more preferred between 40 and 90 ng/mL, more preferred between 30 and 80 ng/mL, even more preferred between 40 and 60 ng/mL, most preferred said threshold is 50 ng/mL.

30 In a specific embodiment of the invention a threshold for the level of DPP3 is the 5fold median concentration, preferably the 4fold median concentration, more preferred the 3fold median concentration, most preferred the 2fold median concentration of a normal healthy population.

The level of DPP3 as the amount of DPP3 protein and/ or DPP3 activity in a sample of bodily fluid of said subject may be determined by different methods, e.g. immunoassays, activity assays, mass spectrometric methods etc.

According to the present invention, any types of binding assays (immunoassays and analogous assays, which use other types of antigen-specific binders instead of antibodies), and b) DPP3 enzyme activity assays, which are specific for DPP3 by specifically capturing DPP3 from a sample using a specific binder (anti-DPP3 antibody or other type of binder) prior to determination of enzyme activity).

5

DPP3 activity can be measured by detection of cleavage products of DPP3 specific substrates. Known peptide hormone substrates include Leu-enkephalin, Met-enkephalin, endomorphin 1 and 2, valorphin, β -casomorphin, dynorphin, proctolin, ACTH (Adrenocorticotrophic hormone) and MSH (melanocyte-stimulating hormone; *Abramić et al. 2000, Baršun et al. 2007, Dhanda et al. 2008*). The cleavage of mentioned peptide hormones as well as other untagged oligopeptides (e.g. Ala-Ala-Ala-Ala, *Dhanda et al. 2008*) can be monitored by detection of the respective cleavage products. Detection methods include, but are not limited to, HPLC analysis (e.g. *Lee & Snyder 1982*), mass spectrometry (e.g. *Abramić et al. 2000*), H1-NMR analysis (e.g. *Vandenberg et al. 1985*), capillary zone electrophoresis (CE; e.g. *Baršun et al. 2007*), thin layer chromatography (e.g. *Dhanda et al. 2008*) or reversed phase chromatography (e.g. *Mazocco et al. 2006*).

Detection of fluorescence due to hydrolysis of fluorogenic substrates by DPP3 is a standard procedure to monitor DPP3 activity. Those substrates are specific di- or tripeptides (Arg-Arg, Ala-Ala, Ala-Arg, Ala-Phe, Asp-Arg, Gly-Ala, Gly-Arg, Gly-Phe, Leu-Ala, Leu-Gly, Lys-Ala, Phe-Arg, Suc-Ala-Ala-Phe) coupled to a fluorophore. Fluorophores include but are not limited to β -naphthylamide (2-naphthylamide, β NA, 2NA), 4-methoxy- β -naphthylamide (4-methoxy-2-naphthylamide) and 7-amido-4-methylcoumarin (AMC, MCA; *Abramić et al. 2000, Ohkubo et al. 1999*). Cleavage of these fluorogenic substrates leads to the release of fluorescent β -naphthylamine or 7-amino-4-methylcoumarin respectively. In a liquid phase assay or an ECA substrate and DPP3 are incubated in for example a 96 well plate format and fluorescence is measured using a fluorescence detector (*Ellis & Nuenke 1967*). Additionally, DPP3 carrying samples can be immobilized and divided on a gel by electrophoresis, gels stained with fluorogenic substrate (e.g. Arg-Arg- β NA) and Fast Garnet GBC and fluorescent protein bands detected by a fluorescence reader (*Ohkubo et al. 1999*). The same peptides (Arg-Arg, Ala-Ala, Ala-Arg, Ala-Phe, Asp-Arg, Gly-Ala, Gly-Arg, Gly-Phe, Leu-Ala, Leu-Gly, Lys-Ala, Phe-Arg, Suc-Ala-Ala-Phe) can be coupled to chromophores, such as p-nitroanilide diacetate. Detection of color change due to hydrolysis of chromogenic substrates can be used to monitor DPP3 activity.

Another option for the detection of DPP3 activity is a Protease-Glo™ Assay (commercially available at Promega). In this embodiment of said method DPP3 specific di- or tripeptides (Arg-Arg, Ala-Ala, Ala-Phe, Ala-Phe, Asp-Arg, Gly-Ala, Gly-Arg, Gly-Phe, Leu-Ala, Leu-Gly, Lys-Ala, Phe-Arg, Suc-Ala-Ala-Phe) are coupled to aminoluciferin. Upon cleavage by DPP3,

aminoluciferin is released and serves as a substrate for a coupled luciferase reaction that emits detectable luminescence.

In a preferred embodiment DPP3 activity is measured by addition of the fluorogenic substrate Arg-Arg- β NA and monitoring fluorescence in real time.

In a specific embodiment of said method for determining active DPP3 in a bodily fluid sample of a subject said capture binder reactive with DPP3 is immobilized on a solid phase.

The test sample is passed over the immobile binder, and DPP3, if present, binds to the binder and is itself immobilized for detection. A substrate may then be added, and the reaction product may be detected to indicate the presence or amount of DPP3 in the test sample. For the purposes of the present description, the term "solid phase" may be used to include any material or vessel in which or on which the assay may be performed and includes, but is not limited to: porous materials, nonporous materials, test tubes, wells, slides, agarose resins (e.g. Sepharose from GE Healthcare Life Sciences), magnetic particulates (e.g. DynabeadsTM or PierceTM magnetic beads from Thermo Fisher Scientific), etc.

In another embodiment of the invention, the level of DPP3 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to DPP3.

In another preferred embodiment of the invention, said capture binder for determining the level of DPP3 may be selected from the group of antibody, antibody fragment or non-IgG scaffold.

In a specific embodiment of the invention, said capture binder is an antibody.

The amount of DPP3 protein and/ or DPP3 activity in a sample of bodily fluid of said subject may be determined for example by one of the following methods:

1. Luminescence immunoassay for the quantification of DPP3 protein concentrations (LIA) (*Rehfeld et al., 2019 JALM 3(6): 943-953*).

The LIA is a one-step chemiluminescence sandwich immunoassay that uses white high-binding polystyrene microtiter plates as solid phase. These plates are coated with monoclonal anti-DPP3 antibody AK2555 (capture antibody). The tracer anti-DPP3 antibody AK2553 is labeled with MA70-acridinium-NHS-ester and used at a concentration of 20 ng per well. Twenty microliters of samples (e.g. serum, heparin-plasma, citrate-plasma or EDTA-plasma derived from patients' blood) and

calibrators are pipetted into coated white microtiter plates. After adding the tracer antibody AK2553, the microtiter plates are incubated for 3 h at room temperature and 600 rpm. Unbound tracer is then removed by 4 washing steps (350 μ L per well). Remaining chemiluminescence is measured for 1s per well by using a microtiter plate luminometer. The concentration of DPP3 is determined with a 6-point calibration curve. Calibrators and samples are preferably run in duplicate.

2. Enzyme capture activity assay for the quantification of DPP3 activity (ECA) (*Rehfeld et al., 2019 JALM 3(6): 943-953*).

The ECA is a DPP3-specific activity assay that uses black high-binding polystyrene microtiter plates as solid phase. These plates are coated with monoclonal anti-DPP3 antibody AK2555 (capture antibody). Twenty microliters of samples (e.g. serum, heparin-plasma, citrate-plasma, EDTA-plasma, cerebrospinal fluid and urine) and calibrators are pipetted into coated black microtiter plates. After adding assay buffer (200 μ L), the microtiter plates are incubated for 2 h at 22°C and 600 rpm. DPP3 present in the samples is immobilized by binding to the capture antibody. Unbound sample components are removed by 4 washing steps (350 μ L per well). The specific activity of immobilized DPP3 is measured by the addition of the fluorogenic substrate, Arg-Arg- β -Naphthylamide (Arg2- β NA), in reaction buffer followed by incubation at 37 °C for 1 h. DPP3 specifically cleaves Arg2- β NA into Arg-Arg dipeptide and fluorescent β -naphthylamine. Fluorescence is measured with a fluorometer using an excitation wavelength of 340 nm and emission is detected at 410 nm. The activity of DPP3 is determined with a 6-point calibration curve. Calibrators and samples are preferably run in duplicates.

3. Liquid-phase assay for the quantification of DPP3 activity (LAA) (modified from *Jones et al., Analytical Biochemistry, 1982*).

The LAA is a liquid phase assay that uses black non-binding polystyrene microtiter plates to measure DPP3 activity. Twenty microliter of samples (e.g. serum, heparin-plasma, citrate-plasma) and calibrators are pipetted into non-binding black microtiter plates. After addition of fluorogenic substrate, Arg2- β NA, in assay buffer (200 μ L), the initial β NA fluorescence (T=0) is measured in a fluorimeter using an excitation wavelength of 340 nm and emission is detected at 410 nm. The plate is then incubated at 37 °C for 1 hour. The final fluorescence of (T=60) is measured. The difference between final and initial fluorescence is calculated. The activity of DPP3 is determined with a 6-point calibration curve. Calibrators and samples are preferably run in duplicates.

In a specific embodiment an assay is used for determining the level of DPP3, wherein the assay sensitivity of said assay is able to quantify the DPP3 of healthy subjects and is < 20 ng/ml, preferably < 30 ng/ml and more preferably < 40 ng/ml.

4. Another immunoassay method for measuring DPP3 from a plasma of whole blood sample is available, IB10 sphingotest® DPP3 (https://www.nexus-dx.com/wp-content/uploads/2020/11/DPP3-022-00072-IFU-REV-B_8x11.pdf).

5 The IB10 sphingotest® DPP3 is a rapid point-of-care (POC) immunoassay for the in vitro quantitative determination of Dipeptidyl Peptidase 3 (DPP3) in human EDTA whole blood and plasma. The Nexus IB10 immunochemistry system combines chemistry with microfluidics and centrifugal flow to rapidly prepare a cell free plasma from whole blood that can then be moved through a channel to rehydrate, solubilize and mix with freeze dried immunoconjugates

10 In a specific embodiment, said binder exhibits a binding affinity to DPP3 of at least 10^7 M^{-1} , preferred 10^8 M^{-1} , more preferred affinity is greater than 10^9 M^{-1} , most preferred greater than 10^{10} M^{-1} . A person skilled in the art knows that it may be considered to compensate lower affinity by applying a higher dose of compounds and this measure would not lead out-of-the-scope of the invention.

15 In another embodiment of the invention, said sample of bodily fluid is selected from the group of whole blood, plasma, and serum.

Mature ADM, bio-ADM and ADM-NH₂ is used synonymously throughout this application and is a molecule according to SEQ ID No.: 20.

20

A bodily fluid according to the present invention is in one particular embodiment a blood sample. A blood sample may be selected from the group comprising whole blood, serum and plasma. In a specific embodiment of the method said sample is selected from the group comprising human citrate plasma, heparin plasma and EDTA plasma.

25

In a specific embodiment an assay is used for determining the level ADM-NH₂, wherein the assay sensitivity of said assay is able to quantify the mature ADM-NH₂ of healthy subjects and is $< 70 \text{ pg/ml}$, preferably $< 40 \text{ pg/ml}$ and more preferably $< 10 \text{ pg/ml}$.

30 In a specific embodiment of the invention the threshold for ADM-NH₂ is between 40 and 100 pg/mL, more preferred between 50 and 90 pg/mL, even more preferred between 60 and 80, most preferred a threshold of 70 pg/ml is applied.

In a specific embodiment of the invention a threshold for plasma ADM-NH₂ is the 5fold median concentration, preferably the 4fold median concentration, more preferred the 3fold median concentration, most preferred the 2fold median concentration of a normal healthy population.

- 5 In a specific embodiment, said binder exhibits a binding affinity to ADM-NH₂ of at least 10⁷ M⁻¹, preferred 10⁸ M⁻¹, preferred affinity is greater than 10⁹ M⁻¹, most preferred greater than 10¹⁰ M⁻¹. A person skilled in the art knows that it may be considered to compensate lower affinity by applying a higher dose of compounds and this measure would not lead out-of-the-scope of the invention.
- 10 To determine the affinity of the antibodies to Adrenomedullin, the kinetics of binding of Adrenomedullin to immobilized antibody was determined by means of label-free surface plasmon resonance using a Biacore 2000 system (GE Healthcare Europe GmbH, Freiburg, Germany). Reversible immobilization of the antibodies was performed using an anti-mouse Fc antibody covalently coupled in high density to a CM5 sensor surface according to the manufacturer's instructions (mouse antibody
- 15 capture kit; GE Healthcare), (*Lorenz et al. 2011. Antimicrob Agents Chemother. 55 (1): 165–173*).

In a specific embodiment, said binder is selected from the group comprising an antibody or an antibody fragment or a non-Ig scaffold binding to ADM-NH₂.

- 20 In a specific embodiment an assay is used for determining the level of ADM-NH₂, wherein such assay is a sandwich assay, preferably a fully automated assay.

- In one embodiment such assay for determining the level of the biomarkers (DPP3 and/ or ADM-NH₂) is a sandwich immunoassay using any kind of detection technology including but not restricted to
- 25 enzyme label, chemiluminescence label, electrochemiluminescence label, preferably a fully automated assay. In one embodiment of the diagnostic method such an assay is an enzyme labeled sandwich assay. Examples of automated or fully automated assay comprise assays that may be used for one of the following systems: Roche Elecsys®, Abbott Architect®, Siemens Centaur®, Brahms Kryptor®, BiomerieuxVidas®, Alere Triage®.

30

A variety of immunoassays are known and may be used for the assays and methods of the present invention, these include: radioimmunoassays ("RIA"), homogeneous enzyme-multiplied immunoassays ("EMIT"), enzyme linked immunoadsorbent assays ("ELISA"), apoenzyme reactivation immunoassay ("ARIS"), dipstick immunoassays and immuno-chromatography assays.

In one embodiment of the invention such an assay is a sandwich immunoassay using any kind of detection technology including but not restricted to enzyme label, chemiluminescence label, electrochemiluminescence label, preferably a fully automated assay. In one embodiment of the invention
5 such an assay is an enzyme labeled sandwich assay. Examples of automated or fully automated assay comprise assays that may be used for one of the following systems: Roche Elecsys®, Abbott Architect®, Siemens Centauer®, Brahms Kryptor®, Biomerieux Vidas®, Alere Triage®.

In one embodiment of the invention it may be a so-called POC-test (point-of-care) that is a test
10 technology, which allows performing the test within less than 1 hour near the patient without the requirement of a fully automated assay system. One example for this technology is the immunochromatographic test technology.

In a preferred embodiment said label is selected from the group comprising chemiluminescent label,
15 enzyme label, fluorescence label, radioiodine label.

The assays can be homogenous or heterogeneous assays, competitive and non-competitive assays. In one embodiment, the assay is in the form of a sandwich assay, which is a non-competitive immunoassay, wherein the molecule to be detected and/or quantified is bound to a first
20 antibody and to a second antibody. The first antibody may be bound to a solid phase, *e.g.* a bead, a surface of a well or other container, a chip or a strip, and the second antibody is an antibody which is labeled, *e.g.* with a dye, with a radioisotope, or a reactive or catalytically active moiety. The amount of labeled antibody bound to the analyte is then measured by an appropriate method. The general composition and procedures involved with “sandwich assays” are well-established and known to the
25 skilled person (*The Immunoassay Handbook*, Ed. David Wild, Elsevier LTD, Oxford; 3rd ed. (May 2005), ISBN-13: 978-0080445267; Hultschig C *et al.*, *Curr Opin Chem Biol.* 2006 Feb;10(1):4-10. PMID: 16376134).

In another embodiment the assay comprises two capture molecules, preferably antibodies which are
30 both present as dispersions in a liquid reaction mixture, wherein a first labelling component is attached to the first capture molecule, wherein said first labelling component is part of a labelling system based on fluorescence- or chemiluminescence-quenching or amplification, and a second labelling component of said marking system is attached to the second capture molecule, so that upon binding of both capture molecules to the analyte a measurable signal is generated that allows for the detection of the formed
35 sandwich complexes in the solution comprising the sample.

In another embodiment, said labeling system comprises rare earth cryptates or rare earth chelates in combination with fluorescence dye or chemiluminescence dye, in particular a dye of the cyanine type.

In the context of the present invention, fluorescence based assays comprise the use of dyes, which may for instance be selected from the group comprising FAM (5-or
5 6-carboxyfluorescein), VIC, NED, Fluorescein, Fluorescein isothiocyanate (FITC), IRD-700/800, Cyanine dyes, such as CY3, CY5, CY3.5, CY5.5, Cy7, Xanthen, 6-Carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), TET, 6-Carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE), N,N,N',N'-Tetramethyl-6-carboxyrhodamine (TAMRA), 6-Carboxy-X-rhodamine (ROX), 5-Carboxyrhodamine-6G (R6G5), 6-carboxyrhodamine-6G (RG6), Rhodamine, Rhodamine Green,
10 Rhodamine Red, Rhodamine 110, BODIPY dyes, such as BODIPY TMR, Oregon Green, Coumarines such as Umbelliferone, Benzimides, such as Hoechst 33258; Phenanthridines, such as Texas Red, Yakima Yellow, Alexa Fluor, PET, Ethidiumbromide, Acridinium dyes, Carbazol dyes, Phenoxazine dyes, Porphyrine dyes, Polymethin dyes, and the like.

15 In the context of the present invention, chemiluminescence based assays comprise the use of dyes, based on the physical principles described for chemiluminescent materials in (*Kirk-Othmer, Encyclopedia of chemical technology, 4th ed., executive editor, J. I. Kroschwitz; editor, M. Howe-Grant, John Wiley & Sons, 1993, vol.15, p. 518-562, incorporated herein by reference, including citations on pages 551-562*). Preferred chemiluminescent dyes are acridiniumesters.

20

As mentioned herein, an "assay" or "diagnostic assay" can be of any type applied in the field of diagnostics. Such an assay may be based on the binding of an analyte to be detected to one or more capture probes with a certain affinity. Concerning the interaction between capture molecules and target molecules or molecules of interest, the affinity constant is preferably greater than 10^8 M^{-1} .

25 In a specific embodiment at least one of said two binders is labeled in order to be detected.

The ADM-NH₂ levels of the present invention have been determined with the described ADM-NH₂ assay (*Weber et al. 2017. JALM 2(2):1-4*). The DPP3 levels of the present invention have been determined with the described DPP3-assays as outlined in the examples (*Rehfeld et al. 2019. JALM*
30 *3(6): 943-953*). The mentioned threshold values above might be different in other assays, if these have been calibrated differently from the assay systems used in the present invention. Therefore, the mentioned cut-off values above shall apply for such differently calibrated assays accordingly, taking into account the differences in calibration. One possibility of quantifying the difference in calibration is a method comparison analysis (correlation) of the assay in question with the respective biomarker assay
35 used in the present invention by measuring the respective biomarker (e.g. bio-ADM, DPP3) in samples

using both methods. Another possibility is to determine with the assay in question, given this test has sufficient analytical sensitivity, the median biomarker level of a representative normal population, compare results with the median biomarker levels as described in the literature and recalculate the calibration based on the difference obtained by this comparison. With the calibration used in the present invention, samples from normal (healthy) subjects have been measured: median plasma bio-ADM (mature ADM-NH₂) was 24.7 pg/ml, the lowest value 11 pg/ml and the 99th percentile 43 pg/ml (*Marino et al. 2014, Critical Care 18:R34*). With the calibration used in the present invention, samples from 5,400 normal (healthy) subjects (swedish single-center prospective population-based Study (MPP-RES)) have been measured: median (interquartile range) plasma DPP3 was 14.5 ng/ml (11.3 ng/ml – 19 ng/ml).

In the present invention, the level of ADM –NH₂ is therefore measured in order to identify patients having an increased risk of running into shock.

In a specific embodiment of the invention, said shock is selected from the group comprising shock due to hypovolemia, cardiogenic shock, obstructive shock and distributive shock.

In another specific embodiment of the invention, said shock is selected from the group comprising shock due to hypovolemia, cardiogenic shock, obstructive shock and distributive shock, in particular cardiogenic or septic shock.

In a specific embodiment of the invention, said shock is selected from the group comprising:

- in case of cardiogenic shock said patient has suffered an acute coronary syndrome (e.g. acute myocardial infarction) or has heart failure (e.g. acute decompensated heart failure), myocarditis, arrhythmia, cardiomyopathy, valvular heart disease, aortic dissection with acute aortic stenosis, traumatic chordal rupture or massive pulmonary embolism, or
- in case of hypovolemic shock said patient may have suffered a hemorrhagic disease including gastrointestinal bleed, trauma, vascular etiologies (e.g. ruptured abdominal aortic aneurysm, tumor eroding into a major blood vessel) and spontaneous bleeding in the setting of anticoagulant use or a non-hemorrhagic disease including vomiting, diarrhea, renal loss, skin losses/insensible losses (e.g. burns, heat stroke) or third-space loss in the setting of pancreatitis, cirrhosis, intestinal obstruction, trauma, or
- in case of obstructive shock said patient may have suffered a cardiac tamponade, tension pneumothorax, pulmonary embolism or aortic stenosis, or

- in case of distributive shock said patient has septic shock, neurogenic shock, anaphylactic shock or shock due to adrenal crisis.

Shock is characterized by decreased oxygen delivery and/or increased oxygen consumption or inadequate oxygen utilization leading to cellular and tissue hypoxia. It is a life-threatening condition of circulatory failure and most commonly manifested as hypotension (systolic blood pressure less than 90 mm Hg or MAP less than 65 mmHg). Shock is divided into four main types based on the underlying cause: hypovolemic, cardiogenic, obstructive, and distributive shock (*Vincent and De Backer 2014. N. Engl. J. Med. 370(6): 583*).

10

Hypovolemic shock is characterized by decreased intravascular volume and can be divided into two broad subtypes: hemorrhagic and non-hemorrhagic. Common causes of hemorrhagic hypovolemic shock include gastrointestinal bleed, trauma, vascular etiologies (e.g. ruptured abdominal aortic aneurysm, tumor eroding into a major blood vessel) and spontaneous bleeding in the setting of anticoagulant use. Common causes of non-hemorrhagic hypovolemic shock include vomiting, diarrhea, renal loss, skin losses/insensible losses (e.g. burns, heat stroke) or third-space loss in the setting of pancreatitis, cirrhosis, intestinal obstruction, trauma. For review see *Koya and Paul 2018. Shock. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019-2018 Oct 27*.

Cardiogenic shock (CS) is defined as a state of critical endorgan hypoperfusion due to reduced cardiac output. Notably, CS forms a spectrum that ranges from mild hypoperfusion to profound shock. Established criteria for the diagnosis of CS are: (i) systolic blood pressure, ≤ 90 mmHg for >30 min or vasopressors required to achieve a blood pressure ≥ 90 mmHg; (ii) pulmonary congestion or elevated left-ventricular filling pressures; (iii) signs of impaired organ perfusion with at least one of the following criteria: (a) altered mental status; (b) cold, clammy skin; (c) oliguria (< 0.5 mL/kg/h or <30 mL/h); (d) increased serum-lactate (*Reynolds and Hochman 2008. Circulation 117: 686–697*). Acute myocardial infarction (AMI) with subsequent ventricular dysfunction is the most frequent cause of CS accounting for approximately 80% of cases. Mechanical complications such as ventricular septal (4%) or free wall rupture (2%), and acute severe mitral regurgitation (7%) are less frequent causes of CS after AMI. (*Hochman et al. 2000. J Am Coll Cardiol 36: 1063–1070*). Non-AMI-related CS may be caused by decompensated valvular heart disease, acute myocarditis, arrhythmias, etc. with heterogeneous treatment options. This translates in 40 000 to 50 000 patients per year in the USA and 60 000 to 70 000 in Europe. Despite advances in treatment mainly by early revascularization with subsequent mortality reduction, CS remains the leading cause of death in AMI with mortality rates still approaching 40–50% according to recent registries and randomized trials (*Goldberg et al. 2009. Circulation 119: 1211–1219*).

Obstructive shock is due to a physical obstruction of the great vessels or the heart itself. Several conditions can result in this form of shock (e.g. cardiac tamponade, tension pneumothorax, pulmonary

embolism, aortic stenosis). For review see *Koya and Paul 2018. Shock. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019-2018 Oct 27.*

According to the cause, there are four types of distributive shock: neurogenic shock (decreased sympathetic stimulation leading to decreased vasal tone), anaphylactic shock, septic shock and shock due to adrenal crisis. In addition to sepsis, distributive shock can be caused by systemic inflammatory response syndrome (SIRS) due to conditions other than infection such as pancreatitis, burns or trauma. Other causes include, toxic shock syndrome (TSS), anaphylaxis (a sudden, severe allergic reaction), adrenal insufficiency (acute worsening of chronic adrenal insufficiency, destruction or removal of the adrenal glands, suppression of adrenal gland function due to exogenous steroids, hypopituitarism and metabolic failure of hormone production), reactions to drugs or toxins, heavy metal poisoning, hepatic (liver) insufficiency and damage to the central nervous system. For review see *Koya and Paul 2018. Shock. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019-2018 Oct 27.*

Refractory shock has been defined as requirement of noradrenaline infusion of $>0.5 \mu\text{g/kg/min}$ despite adequate volume resuscitation. Mortality in these patients may be as high as 94% and the assessment and management of these patients requires a much more aggressive approach for survival. The term „refractory shock” is used when the tissue perfusion cannot be restored with the initial corrective measures employed (e.g. vasopressors) and may therefore be referred to as „high vasopressor-dependent“ or „vasopressor-resistant“ shock (*Udupa and Shetty 2018. Indian J Respir Care 7: 67-72*). Patients with refractory shock may have features of inadequate perfusion such as hypotension (mean arterial blood pressure $<65 \text{ mmHg}$), tachycardia, cold peripheries, prolonged capillary refill time, and tachypnea consequent to the hypoxia and acidosis. Fever may be seen in septic shock. Other signs of hypoperfusion such as altered sensorium, hyperlactatemia, and oliguria may also be seen. These well-known signs of shock are not helpful in identifying whether the problem is at the pump (heart) or circuitry (vessels and tissues). Different types of shock can coexist, and all forms of shock can become refractory, as evidenced by unresponsiveness to high-dose vasopressors (*Udupa and Shetty 2018. Indian J Respir Care 7: 67-72*).

Septic shock is a potentially fatal medical condition that occurs when sepsis, which is organ injury or damage in response to infection, leads to dangerously low blood pressure and abnormalities in cellular metabolism. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) defines septic shock as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone. Patients with septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L ($>18 \text{ mg/dL}$) in the absence of hypovolemia. This combination is associated with hospital mortality rates greater than 40% (*Singer et al. 2016. JAMA. 315 (8): 801–10*). The primary infection is most commonly caused by bacteria, but also may be by fungi, viruses or parasites. It may be located in any part of the body, but

most commonly in the lungs, brain, urinary tract, skin or abdominal organs. It can cause multiple organ dysfunction syndrome (formerly known as multiple organ failure) and death. Frequently, people with septic shock are cared for in intensive care units. It most commonly affects children, immunocompromised individuals, and the elderly, as their immune systems cannot deal with infection as effectively as those of healthy adults. The mortality rate from septic shock is approximately 25–50%.

The term “prevention” or any grammatical variation thereof (e.g., prevent, preventing, and prevention etc.), as used herein, includes but is not limited to, delaying the onset of symptoms, preventing relapse to a disease, increasing latency between symptomatic episodes, or a combination thereof. Prevention, as used herein, does not require the complete absence of symptoms.

The efficacy of non-neutralizing antibody targeted against the N-terminus of ADM was investigated in a survival study in CLP-induced sepsis in mice. Pre-treatment with the non-neutralizing antibody resulted in decreased catecholamine infusion rates, kidney dysfunction, and ultimately improved survival (*Struck et al. 2013. Intensive Care Med Exp 1(1):22; Wagner et al. 2013. Intensive Care Med Exp 1(1):21*).

Due to these positive results, a humanized version of an N-terminal anti-ADM antibody, named Adrecizumab, has been developed for further clinical development. Beneficial effects of Adrecizumab on vascular barrier function and survival were recently demonstrated in preclinical models of systemic inflammation and sepsis (*Geven et al. 2018. Shock 50(6):648-654*). In this study, pre-treatment with Adrecizumab attenuated renal vascular leakage in endotoxemic rats as well as in mice with CLP-induced sepsis, which coincided with increased renal expression of the protective peptide Ang-1 and reduced expression of the detrimental peptide vascular endothelial growth factor. Also, pre-treatment with Adrecizumab improved 7-day survival in CLP-induced sepsis in mice from 10 to 50% for single and from 0 to 40% for repeated dose administration. Moreover, in a phase I study, excellent safety and tolerability was demonstrated (see Example 6): no serious adverse events were observed, no signal of adverse events occurring more frequently in Adrecizumab-treated subjects was detected and no relevant changes in other safety parameters were found (*Geven et al. 2017. Intensive Care Med Exp 5 (Suppl 2): 0427*). Of particular interest is the proposed mechanism of action of Adrecizumab. Both animal and human data reveal a potent, dose-dependent increase of circulating ADM following administration of this antibody. Based on pharmacokinetic data and the lack of an increase in MR-proADM (an inactive peptide fragment derived from the same prohormone as ADM), the higher circulating ADM levels cannot be explained by an increased production.

A mechanistic explanation for this increase could be that the excess of antibody in the circulation may drain ADM from the interstitium to the circulation, since ADM is small enough to cross the endothelial barrier, whereas the antibody is not (*Geven et al. 2018. Shock. 50(2):132-140 and Voors et al (J. Eur J*

Heart Fail. 2019 Feb;21(2):163-171)). In addition, binding of the antibody to ADM leads to a prolongation of ADM's half-life. Even though NT-ADM antibodies partially inhibit ADM-mediated signalling, a large increase of circulating ADM results in an overall "net" increase of ADM activity in the blood compartment, where it exerts beneficial effects on ECs (predominantly barrier stabilization),
5 whereas ADMs detrimental effects on VSMCs (vasodilation) in the interstitium are reduced.

The invention is not limited to the use of Adrecizumab specifically. There is no reason to doubt that what is true for Adrecizumab will also be true for antibodies sharing main essential features (in particular affinity and epitope specificity) Antibodies that target the same region must be expected to have the
10 same technical effect, provided they have the same affinity and same or very comparable structural features (size, shape,...).

Throughout the specification the "antibodies", or "antibody fragments" or "non-Ig scaffolds" in accordance with the invention are capable to bind ADM, and thus are directed against ADM, and thus
15 can be referred to as "anti-ADM antibodies", "anti-ADM antibody fragments", or "anti-ADM non-Ig scaffolds".

The term "antibody" generally comprises monoclonal and polyclonal antibodies and binding fragments thereof, in particular Fc-fragments as well as so called "single-chain-antibodies" (Bird et al. 1988),
20 chimeric, humanized, in particular CDR-grafted antibodies, and dia or tetrabodies (Holliger et al. 1993). Also comprised are immunoglobulin-like proteins that are selected through techniques including, for example, phage display to specifically bind to the molecule of interest contained in a sample. In this context the term "specific binding" refers to antibodies raised against the molecule of interest or a fragment thereof. An antibody is considered to be specific, if its affinity towards the molecule of interest
25 or the aforementioned fragment thereof is at least preferably 50-fold higher, more preferably 100-fold higher, most preferably at least 1000-fold higher than towards other molecules comprised in a sample containing the molecule of interest. It is well known in the art how to make antibodies and to select antibodies with a given specificity.

30 In one embodiment of the invention the anti-Adrenomedullin (ADM) antibody or anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold is monospecific.

Monospecific anti-adrenomedullin (ADM) antibody or monospecific anti-adrenomedullin antibody fragment or monospecific anti-ADM non-Ig scaffold means that said antibody or antibody fragment or

non-Ig scaffold binds to one specific region encompassing at least 5 amino acids within the target ADM. Monospecific anti-Adrenomedullin (ADM) antibody or monospecific anti-adrenomedullin antibody fragment or monospecific anti-ADM non-Ig scaffold are anti-adrenomedullin (ADM) antibodies or anti-adrenomedullin antibody fragments or anti-ADM non-Ig scaffolds that all have affinity for the same antigen. Monoclonal antibodies are monospecific, but monospecific antibodies may also be produced by other means than producing them from a common germ cell.

Said anti-ADM antibody or antibody fragment binding to ADM or non-Ig scaffold binding to ADM may be a non-neutralizing anti-ADM antibody or antibody fragment binding to ADM or non-Ig scaffold binding to ADM.

In a specific embodiment said anti-ADM antibody, anti-ADM antibody fragment or anti-ADM non-Ig scaffold is a non-neutralizing antibody, fragment or non-Ig scaffold. A neutralizing anti-ADM antibody, anti-ADM antibody fragment or anti-ADM non-Ig scaffold would block the bioactivity of ADM to nearly 100%, to at least more than 90%, preferably to at least more than 95%.

In contrast, a non-neutralizing anti-ADM antibody, or anti-ADM antibody fragment or anti-ADM non-Ig scaffold blocks the bioactivity of ADM less than 100%, preferably to less than 95%, preferably to less than 90%, more preferred to less than 80 % and even more preferred to less than 50 %. This means that bioactivity of ADM is reduced to less than 100%, to 95 % or less but not more, to 90 % or less but not more, to 80 % or less but not more, to 50 % or less but not more. This means that the residual bioactivity of ADM bound to the non-neutralizing anti-ADM antibody, or anti-ADM antibody fragment or anti-ADM non-Ig scaffold would be more than 0%, preferably more than 5 %, preferably more than 10 %, more preferred more than 20 %, more preferred more than 50 %.

25

In this context (a) molecule(s), being it an antibody, or an antibody fragment or a non-Ig scaffold with “non-neutralizing anti-ADM activity”, collectively termed here for simplicity as “non-neutralizing” anti-ADM antibody, antibody fragment, or non-Ig scaffold, that *e.g.* blocks the bioactivity of ADM to less than 80 %, is defined as

- 30 - a molecule or molecules binding to ADM, which upon addition to a culture of an eukaryotic cell line, which expresses functional human recombinant ADM receptor composed of CRLR (calcitonin receptor like receptor) and RAMP3 (receptor-activity modifying protein 3), reduces the amount of cAMP produced by the cell line through the action of parallel added human synthetic ADM peptide, wherein said added human synthetic ADM is added
- 35 in an amount that in the absence of the non-neutralizing antibody to be analyzed, leads to

half-maximal stimulation of cAMP synthesis, wherein the reduction of cAMP by said molecule(s) binding to ADM takes place to an extent, which is not more than 80%, even when the non-neutralizing molecule(s) binding to ADM to be analyzed is added in an amount, which is 10-fold more than the amount, which is needed to obtain the maximal reduction of cAMP synthesis obtainable with the non-neutralizing antibody to be analyzed.

The same definition applies to the other ranges; 95%, 90%, 50% etc.

An antibody or fragment according to the present invention is a protein including one or more polypeptides substantially encoded by immunoglobulin genes that specifically binds an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha (IgA), gamma (IgG₁, IgG₂, IgG₃, IgG₄), delta (IgD), epsilon (IgE) and mu (IgM) constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin light chains are generally about 25 Kd or 214 amino acids in length.

Full-length immunoglobulin heavy chains are generally about 50 Kd or 446 amino acid in length. Light chains are encoded by a variable region gene at the NH₂-terminus (about 110 amino acids in length) and a kappa or lambda constant region gene at the COOH-terminus. Heavy chains are similarly encoded by a variable region gene (about 116 amino acids in length) and one of the other constant region genes.

The basic structural unit of an antibody is generally a tetramer that consists of two identical pairs of immunoglobulin chains, each pair having one light and one heavy chain. In each pair, the light and heavy chain variable regions bind to an antigen, and the constant regions mediate effector functions. Immunoglobulins also exist in a variety of other forms including, for example, Fv, Fab, and (Fab')₂, as well as bifunctional hybrid antibodies and single chains (e.g., Lanzavecchia et al. 1987. Eur. J. Immunol. 17:105; Huston et al. 1988. Proc. Natl. Acad. Sci. U.S.A., 85:5879-5883; Bird et al. 1988. Science 242:423-426; Hood et al. 1984, Immunology, Benjamin, N.Y., 2nd ed.; Hunkapiller and Hood 1986. Nature 323:15-16). An immunoglobulin light or heavy chain variable region includes a framework region interrupted by three hypervariable regions, also called complementarity determining regions (CDR's) (see, Sequences of Proteins of Immunological Interest, E. Kabat et al. 1983, U.S. Department of Health and Human Services). As noted above, the CDRs are primarily responsible for binding to an epitope of an antigen. An immune complex is an antibody, such as a monoclonal antibody, chimeric antibody, humanized antibody or human antibody, or functional antibody fragment, specifically bound to the antigen.

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody can be joined to human constant segments, such as kappa and gamma 1 or gamma 3. In one example, a therapeutic chimeric antibody is thus a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody, although other mammalian species can be used, or the variable region can be produced by molecular techniques. Methods of making chimeric antibodies are well known in the art, *e.g.*, see U.S. Patent No. 5,807,715. A "humanized" immunoglobulin is an immunoglobulin including a human framework region and one or more CDRs from a non-human (such as a mouse, rat, or synthetic) immunoglobulin. The non-human immunoglobulin providing the CDRs is termed a "donor" and the human immunoglobulin providing the framework is termed an "acceptor." In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they must be substantially identical to human immunoglobulin constant regions, *i.e.*, at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of natural human immunoglobulin sequences. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. A humanized antibody binds to the same antigen as the donor antibody that provides the CDR's. The acceptor framework of a humanized immunoglobulin or antibody may have a limited number of substitutions by amino acids taken from the donor framework. Humanized or other monoclonal antibodies can have additional conservative amino acid substitutions, which have substantially no effect on antigen binding or other immunoglobulin functions. Exemplary conservative substitutions are those such as gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr. Humanized immunoglobulins can be constructed by means of genetic engineering (*e.g.*, see U.S. Patent No. 5,585,089). A human antibody is an antibody wherein the light and heavy chain genes are of human origin. Human antibodies can be generated using methods known in the art. Human antibodies can be produced by immortalizing a human B cell secreting the antibody of interest. Immortalization can be accomplished, for example, by EBV infection or by fusing a human B cell with a myeloma or hybridoma cell to produce a trioma cell. Human antibodies can also be produced by phage display methods (see, *e.g.* WO91/17271; WO92/001047; WO92/20791), or selected from a human combinatorial monoclonal antibody library (see the Morphosys website). Human antibodies can also be prepared by using transgenic animals carrying a human immunoglobulin gene (for example, see WO93/12227; WO 91/10741).

Thus, the anti-ADM antibody may have the formats known in the art. Examples are human antibodies, monoclonal antibodies, humanized antibodies, chimeric antibodies, CDR-grafted antibodies. In a preferred embodiment antibodies according to the present invention are recombinantly produced

antibodies as *e.g.* IgG, a typical full-length immunoglobulin, or antibody fragments containing at least the F-variable domain of heavy and/or light chain as *e.g.* chemically coupled antibodies (fragment antigen binding) including but not limited to Fab-fragments including Fab minibodies, single chain Fab antibody, monovalent Fab antibody with epitope tags, *e.g.* Fab-V5Sx2; bivalent Fab (mini-antibody) dimerized with the CH3 domain; bivalent Fab or multivalent Fab, *e.g.* formed via multimerization with the aid of a heterologous domain, *e.g.* via dimerization of dHLX domains, *e.g.* Fab-dHLX-FSx2; F(ab')₂-fragments, scFv-fragments, multimerized multivalent or/and multi-specific scFv-fragments, bivalent and/or bispecific diabodies, BITE[®] (bispecific T-cell engager), trifunctional antibodies, polyvalent antibodies, *e.g.* from a different class than G; single-domain antibodies, *e.g.* nanobodies derived from camelid or fish immunoglobulins and numerous others.

In addition to anti-ADM antibodies other biopolymer scaffolds are well known in the art to complex a target molecule and have been used for the generation of highly target specific biopolymers. Examples are aptamers, spiegelmers, anticalins and conotoxins. For illustration of antibody formats please see Fig. 1a, 1b and 1c.

In a preferred embodiment the anti-ADM antibody format is selected from the group comprising Fv fragment, scFv fragment, Fab fragment, scFab fragment, F(ab)₂ fragment and scFv-Fc Fusion protein. In another preferred embodiment the antibody format is selected from the group comprising scFab fragment, Fab fragment, scFv fragment and bioavailability optimized conjugates thereof, such as PEGylated fragments. One of the most preferred formats is the scFab format.

Non-Ig scaffolds may be protein scaffolds and may be used as antibody mimics as they are capable to bind to ligands or antigens. Non-Ig scaffolds may be selected from the group comprising tetranectin-based non-Ig scaffolds (*e.g.* described in US 2010/0028995), fibronectin scaffolds (*e.g.* described in EP 1 266 025; lipocalin-based scaffolds (*e.g.* described in WO 2011/154420); ubiquitin scaffolds (*e.g.* described in WO 2011/073214), transferrin scaffolds (*e.g.* described in US 2004/0023334), protein A scaffolds (*e.g.* described in EP 2 231 860), ankyrin repeat based scaffolds (*e.g.* described in WO 2010/060748), microproteins preferably microproteins forming a cysteine knot) scaffolds (*e.g.* described in EP 2314308), Fyn SH3 domain based scaffolds (*e.g.* described in WO 2011/023685) EGFR-A-domain based scaffolds (*e.g.* described in WO 2005/040229) and Kunitz domain based scaffolds (*e.g.* described in EP 1 941 867).

In one embodiment of the invention anti-ADM antibodies according to the present invention may be produced as outlined in Example 1 by synthesizing fragments of ADM as antigens. Thereafter, binder to said fragments are identified using the below described methods or other methods as known in the art.

5

Humanization of murine antibodies may be conducted according to the following procedure:

For humanization of an antibody of murine origin the antibody sequence is analyzed for the structural interaction of framework regions (FR) with the complementary determining regions (CDR) and the antigen. Based on structural modelling an appropriate FR of human origin is selected and the murine CDR sequences are transplanted into the human FR. Variations in the amino acid sequence of the CDRs or FRs may be introduced to regain structural interactions, which were abolished by the species switch for the FR sequences. This recovery of structural interactions may be achieved by random approach using phage display libraries or via directed approach guided by molecular modelling (*Almagro and Fransson 2008. Humanization of antibodies. Front Biosci. 2008 Jan 1;13:1619-33*).

15

In a preferred embodiment the ADM antibody format is selected from the group comprising Fv fragment, scFv fragment, Fab fragment, scFab fragment, F(ab)₂ fragment and scFv-Fc Fusion protein. In another preferred embodiment the antibody format is selected from the group comprising scFab fragment, Fab fragment, scFv fragment and bioavailability optimized conjugates thereof, such as PEGylated fragments. One of the most preferred formats is scFab format.

20

In another preferred embodiment, the anti-ADM antibody, anti-ADM antibody fragment, or anti-ADM non-Ig scaffold is a full-length antibody, antibody fragment, or non-Ig scaffold.

In a preferred embodiment the anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to an epitope of at least 5 amino acids in length contained in ADM.

25

In a more preferred embodiment, the anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to an epitope of at least 4 amino acids in length contained in ADM.

30

In one specific embodiment of the invention the anti-ADM antibody or anti-ADM antibody fragment binding to adrenomedullin or anti-ADM non-Ig scaffold binding to adrenomedullin is provided for use in therapy or prevention of shock in a patient, wherein said antibody or fragment or scaffold is not ADM-binding-Protein-1 (complement factor H).

5

In one specific embodiment of the invention the anti-Adrenomedullin (ADM) antibody or anti-ADM antibody fragment binding to adrenomedullin or anti-ADM non-Ig scaffold binding to adrenomedullin is provided for use in therapy or prevention of shock in a patient, wherein said antibody or fragment or scaffold binds to a region of preferably at least 4, or at least 5 amino acids within the sequence of amino acid 1-21 of mature human ADM: YRQSMNNFQGLRSFGCRFGTC SEQ ID No.: 22.

10

In a preferred embodiment of the present invention said anti-ADM antibody or anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold binds to a region or epitope of ADM that is located in the N-terminal part (amino acid 1-21) of adrenomedullin.

15

In another preferred embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold recognizes and binds to a region or epitope within amino acids 1-14 of adrenomedullin: YRQSMNNFQGLRSF (SEQ ID No.: 25) that means to the N-terminal part (amino acid 1-14) of adrenomedullin.

20

In another preferred embodiment said anti-ADM-antibody or anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold recognizes and binds to a region or epitope within amino acids 1-10 of adrenomedullin: YRQSMNNFQG (SEQ ID No.: 26); that means to the N-terminal part (amino acid 1-10) of adrenomedullin.

25

In another preferred embodiment said anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold recognizes and binds to a region or epitope within amino acids 1-6 of adrenomedullin: YRQSMN (SEQ ID No.: 27); that means to the N-terminal part (amino acid 1-6) of adrenomedullin. As stated above said region or epitope comprises preferably at least 4 or at least 5 amino acids in length.

30

In another preferred embodiment said anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold recognizes and binds to the N-terminal end (amino acid 1) of adrenomedullin. N-terminal end means that the amino acid 1, that is "Y" of SEQ ID No. 20, 22 or 23, respectively and is

mandatory for binding. The antibody or fragment or scaffold would neither bind N-terminal extended nor N-terminal modified Adrenomedullin nor N-terminal degraded adrenomedullin. This means in another preferred embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold binds only to a region within the sequence of mature ADM if the N-terminal end of ADM is free. In said embodiment the anti-ADM antibody or anti-ADM antibody fragment or non-Ig scaffold would not bind to a region within the sequence of mature ADM if said sequence is *e.g.* comprised within pro-ADM.

For the sake of clarity, the numbers in brackets for specific regions of ADM like “N-terminal part (amino acid 1-21)” is understood by a person skilled in the art that the N-terminal part of ADM consists of amino acids 1-21 of the mature ADM sequence.

In another specific embodiment pursuant to the invention the herein provided anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold does not bind to the C-terminal portion of ADM, *i.e.* the amino acid 43 – 52 of ADM: PRSKISPQGY-NH₂ (SEQ ID No.: 24).

An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies. For example, the epitope is the specific piece of the antigen to which an antibody binds. The part of an antibody that binds to the epitope is called a paratope. The epitopes of protein antigens are divided into two categories, conformational epitopes and linear epitopes, based on their structure and interaction with the paratope.

Conformational and linear epitopes interact with the paratope based on the 3-D conformation adopted by the epitope, which is determined by the surface features of the involved epitope residues and the shape or tertiary structure of other segments of the antigen. A conformational epitope is formed by the 3-D conformation adopted by the interaction of discontinuous amino acid residues. A linear or a sequential epitope is an epitope that is recognized by antibodies by its linear sequence of amino acids, or primary structure and is formed by the 3-D conformation adopted by the interaction of contiguous amino acid residues.

In one specific embodiment it is preferred to use an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold according to the present invention, wherein said anti-ADM antibody or said anti-ADM antibody fragment or anti-ADM non-Ig scaffold leads to an increase of the ADM level or ADM immunoreactivity in serum, blood, plasma of at least 10 %, preferably at least 50 %, more preferably >50 %, most preferably >100%.

In one specific embodiment it is preferred to use an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold according to the present invention, wherein said anti-ADM antibody or said anti-ADM antibody fragment or anti-ADM non-Ig scaffold is an ADM stabilizing antibody or an ADM stabilizing antibody fragment or an ADM stabilizing non-Ig scaffold that enhances the half-life (t_{1/2}; half retention time) of adrenomedullin in serum, blood, plasma at least 10 %, preferably at least 50 %, more preferably >50 %, most preferably >100%.

The half-life (half retention time) of ADM may be determined in human serum, blood or plasma in absence and presence of an ADM stabilizing antibody or an ADM stabilizing antibody fragment or an ADM stabilizing non-Ig scaffold, respectively, using an immunoassay for the quantification of ADM.

The following steps may be conducted:

- ADM may be diluted in human citrate plasma in absence and presence of an ADM stabilizing antibody or an adrenomedullin stabilizing antibody fragment or an adrenomedullin stabilizing non-Ig scaffold, respectively, and may be incubated at 24 °C.
- Aliquots are taken at selected time points (*e.g.* within 24 hours) and degradation of ADM may be stopped in said aliquots by freezing at -20 °C.
- The quantity of ADM may be determined by a hADM immunoassay directly, if the selected assay is not influenced by the stabilizing antibody. Alternatively, the aliquot may be treated with denaturing agents (like HCl) and, after clearing the sample (*e.g.* by centrifugation) the pH can be neutralized and the ADM-quantified by an ADM immunoassay. Alternatively, non-immunoassay technologies (*e.g.* RP-HPLC) can be used for ADM-quantification.
- The half-life of ADM is calculated for ADM incubated in absence and presence of an ADM stabilizing antibody or an adrenomedullin stabilizing antibody fragment or an adrenomedullin stabilizing non-Ig scaffold, respectively.
- The enhancement of half-life is calculated for the stabilized ADM in comparison to ADM that has been incubated in absence of an ADM stabilizing antibody or an adrenomedullin stabilizing antibody fragment or an adrenomedullin stabilizing non-Ig scaffold.

A two-fold increase of the half-life of ADM is an enhancement of half-life of 100%.

Half-life (half retention time) is defined as the period over which the concentration of a specified chemical or drug takes to fall to half its baseline concentration in the specified fluid or blood.

5 An assay that may be used for the determination of the half-life (half retention time) of adrenomedullin in serum, blood, plasma is described in Example 3.

In a preferred embodiment said anti-ADM antibody, anti-ADM antibody fragment or anti-ADM non-Ig scaffold is a non-neutralizing antibody, fragment or scaffold. A neutralizing anti-ADM antibody, anti-ADM antibody fragment or anti-ADM non-Ig scaffold would block the bioactivity of ADM to nearly
10 100%, to at least more than 90%, preferably to at least more than 95%. In other words, this means that said non-neutralizing anti-ADM antibody, anti-ADM antibody fragment or anti-ADM non-Ig scaffold blocks the bioactivity of ADM to less than 100 %, preferably less than 95% preferably less than 90%.
In an embodiment wherein said non-neutralizing anti-ADM antibody, anti-ADM antibody fragment or anti-ADM non-Ig scaffold blocks the bioactivity of ADM to less than 95% an anti-ADM antibody, anti-
15 ADM antibody fragment or anti-ADM non-Ig scaffold that would block the bioactivity of ADM to more than 95 % would be outside of the scope of said embodiment. This means in one embodiment that the bioactivity is reduced to 95 % or less but not more, preferably to 90 % or less, more preferably to 80 % or less, more preferably to 50 % or less but not more.

20 In one embodiment of the invention the non-neutralizing antibody is an antibody binding to a region of at least 5 amino acids within the sequence of amino acid 1-21 of mature human ADM (SEQ ID No.: 14), or an antibody binding to a region of at least 5 amino acids within the sequence of amino acid 1-19 of mature murine ADM (SEQ ID No.: 17).

25 In another preferred embodiment of the invention the non-neutralizing antibody is an antibody binding to a region of at least 4 amino acids within the sequence of amino acid 1-21 of mature human ADM (SEQ ID No.: 14), or an antibody binding to a region of at least 5 amino acids within the sequence of amino acid 1-19 of mature murine ADM (SEQ ID No.: 17).

30 In a specific embodiment according to the present invention a non-neutralizing anti-ADM antibody or anti-ADM antibody fragment or ADM non-Ig scaffold is used, wherein said anti-ADM antibody or an anti-ADM antibody fragment blocks the bioactivity of ADM to less than 80 %, preferably less than 50% (of baseline values). It has to be understood that said limited blocking of the bioactivity (meaning reduction of the bioactivity) of ADM occurs even at excess concentration of the antibody, fragment or

scaffold, meaning an excess of the antibody, fragment or scaffold in relation to ADM. Said limited blocking is an intrinsic property of the ADM binder itself in said specific embodiment. This means that said antibody, fragment or scaffold has a maximal inhibition of 80% or 50% respectively. In a preferred embodiment said anti-ADM antibody, anti-ADM antibody fragment or anti-ADM non-Ig scaffold
5 would block the bioactivity / reduce the bioactivity of anti-ADM to at least 5 %. The stated above means that approximately 20% or 50% or even 95% residual ADM bioactivity remains present, respectively.

Thus, in accordance with the present invention the provided anti-ADM antibodies, anti-ADM antibody fragments, and anti-ADM non-Ig scaffolds do not neutralize the respective ADM bioactivity.

10

The bioactivity is defined as the effect that a substance takes on a living organism or tissue or organ or functional unit *in vivo* or *in vitro* (e.g. in an assay) after its interaction. In case of ADM bioactivity this may be the effect of ADM in a human recombinant ADM receptor cAMP functional assay. Thus, according to the present invention bioactivity is defined via an ADM receptor cAMP functional assay.
15 The following steps may be performed in order to determine the bioactivity of ADM in such an assay:

- Dose response curves are performed with ADM in said human recombinant ADM receptor cAMP functional assay.
- The ADM concentration of half-maximal cAMP stimulation may be calculated.
- At constant half-maximal cAMP-stimulating ADM concentrations dose response curves (up to
20 100µg/ml final concentration) are performed by an ADM stabilizing antibody or ADM stabilizing antibody fragment or ADM stabilizing non-Ig scaffold, respectively.

A maximal inhibition in said ADM bioassay of 50% means that said anti-ADM antibody or said anti-ADM antibody fragment or said anti-ADM non-Ig scaffold, respectively, blocks the bioactivity of ADM
25 to 50% of baseline values. A maximal inhibition in said ADM bioassay of 80% means that said anti-ADM antibody or said anti-adrenomedullin antibody fragment or said anti-adrenomedullin non-Ig scaffold, respectively, blocks the bioactivity of ADM to 80%. This is in the sense of blocking the ADM bioactivity to not more than 80%. This means approximately 20% residual ADM bioactivity remains present.

30

However, by the present specification and in the above context the expression “blocks the bioactivity of ADM” in relation to the herein disclosed anti-ADM antibodies, anti-ADM antibody fragments, and anti-ADM non-Ig scaffolds should be understood as mere decreasing the bioactivity of ADM from 100% to 20% remaining ADM bioactivity at maximum, preferably decreasing the ADM bioactivity from 100%

to 50% remaining ADM bioactivity; but in any case there is ADM bioactivity remaining that can be determined as detailed above.

The bioactivity of ADM may be determined in a human recombinant Adrenomedullin receptor cAMP
5 functional assay (Adrenomedullin Bioassay) according to Example 2.

In a preferred embodiment a modulating anti-ADM antibody or a modulating anti-ADM antibody fragment or a modulating anti-ADM non-Ig scaffold is used in therapy or prevention of shock in a patient.

10

A “modulating” anti-ADM antibody or a modulating anti-ADM antibody fragment or a modulating anti-ADM non-Ig scaffold is an antibody or antibody fragment or non-Ig scaffold that enhances the half-life ($t_{1/2}$ half retention time) of adrenomedullin in serum, blood, plasma at least 10 %, preferably at least, 50 %, more preferably >50 %, most preferably >100% and blocks the bioactivity of ADM to less than 80
15 %, preferably less than 50 % and said anti-ADM antibody, anti-ADM antibody fragment or anti-ADM non-Ig scaffold would block the bioactivity of ADM to at least 5 %. These values related to half-life and blocking of bioactivity have to be understood in relation to the before-mentioned assays in order to determine these values. This is in the sense of blocking the ADM bioactivity of not more than 80 % or not more than 50 %, respectively.

20

Such a modulating anti-ADM antibody or modulating anti-ADM antibody fragment or a modulating anti-ADM non-Ig scaffold offers the advantage that the dosing of the administration is facilitated. The combination of partially blocking or partially reducing ADM bioactivity and increase of the in vivo half-life (increasing the ADM bioactivity) leads to beneficial simplicity of anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold dosing. In a situation of excess of endogenous ADM (maximal stimulation, late sepsis phase, shock, hypodynamic phase) the activity lowering effect is the major impact of the antibody or fragment or scaffold, limiting the (negative) effect of ADM. In case of low or normal endogenous ADM concentrations, the biological effect of anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is a combination of lowering (by partially
25 blocking) and increase by increasing the ADM half-life. Thus, the non-neutralizing and modulating anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold acts like an ADM bioactivity buffer in order to keep the bioactivity of ADM within a certain physiological range.

30

In a specific embodiment of the invention the antibody is a monoclonal antibody or a fragment thereof. In one embodiment of the invention the anti-ADM antibody or the anti-ADM antibody fragment is a human or humanized antibody or derived therefrom. In one specific embodiment one or more (murine) CDR's are grafted into a human antibody or antibody fragment.

5

Subject matter of the present invention in one aspect is a human or humanized CDR-grafted antibody or antibody fragment thereof that binds to ADM, wherein the human or humanized CDR-grafted antibody or antibody fragment thereof comprises an antibody heavy chain (H chain) comprising:

GYTFSRYW (SEQ ID No.:1),

10 ILPGSGST (SEQ ID No.: 2) and/or

TEGYEYDGFY (SEQ ID No.: 3)

and/or further comprises an antibody light chain (L chain) comprising:

QSIVYSNGNTY (SEQ ID No.: 4),

RVS (not part of the Sequencing Listing) and/or

15 FQGSHPYT (SEQ ID No.: 5).

In one specific embodiment of the invention subject matter of the present invention is a human or humanized monoclonal antibody that binds to ADM or an antibody fragment thereof that binds to ADM wherein the heavy chain comprises at least one CDR selected from the group comprising:

20 GYTFSRYW (SEQ ID No.:1),

ILPGSGST (SEQ ID No.: 2),

TEGYEYDGFY (SEQ ID No.: 3)

and wherein the light chain comprises at least one CDR selected from the group comprising:

QSIVYSNGNTY (SEQ ID No.: 4),

25 RVS (not part of the Sequencing Listing),

FQGSHPYT (SEQ ID No.: 5).

In a more specific embodiment of the invention subject matter of the invention is a human monoclonal antibody that binds to ADM or an antibody fragment thereof that binds to ADM wherein the heavy chain comprises the sequences:

30 GYTFSRYW (SEQ ID No.:1),

ILPGSGST (SEQ ID No.: 2),

TEGYEYDGFYD (SEQ ID No.: 3)

and wherein the light chain comprises the sequences:

QSIVYSNGNTY (SEQ ID No.: 4),

RVS (not part of the Sequencing Listing),

5 FQGSHPYT (SEQ ID No.: 5).

In a very specific embodiment, the anti-ADM antibody has a sequence selected from the group comprising: SEQ ID No. 6, 7, 8, 9, 10, 11, 12, 13, 32 and 33.

- 10 The anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold according to the present invention exhibits an affinity towards human ADM in such that affinity constant is greater than 10^{-7} M, preferred 10^{-8} M, preferred affinity is greater than 10^{-9} M, most preferred higher than 10^{-10} M. A person skilled in the art knows that it may be considered to compensate lower affinity by applying a higher dose of compounds and this measure would not lead out-of-the-scope of the invention. The
- 15 affinity constants may be determined according to the method as described in Example 1.

Subject matter of the present invention is a human or humanized monoclonal antibody or fragment that binds to ADM or an antibody fragment thereof for use in therapy or prevention of shock in a patient according to the present invention, wherein said antibody or fragment comprises a sequence selected

20 from the group comprising:

SEQ ID NO: 6 (AM-VH-C)

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPBGHGLEWIGELPGSGSTNYNE
KFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFYWGQGTTLTVSSASTKGP
25 SVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSGSTNYA
30 QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFYWGQGTTLTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 8 (AM-VH2-E40)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFQDYWGQGTTTVTVSSASTKG
5 PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 9 (AM-VH3-T26-E55)

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGSTNYA
10 QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFQDYWGQGTTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 10 (AM-VH4-T26-E40-E55)

15 QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFQDYWGQGTTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

20 SEQ ID NO: 11 (AM-VL-C)

DVLLSQTPSLPVS LGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFS GVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

25

SEQ ID NO: 12 (AM-VL1)

DVVMQTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDS GVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
30 EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 13 (AM-VL2-E40)

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC.

5

Another embodiment of the invention relates to a human or humanized monoclonal antibody or fragment that binds to ADM or an antibody fragment thereof for use in therapy or prevention of shock in a patient, wherein said antibody or fragment comprises the following sequence as a heavy chain:

10 SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
KFQGRVTITADTSTSTAYMELSSLRSEDVAVYYCTEGYEYDGFYWGQGTTVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
15 LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFS_cSVMHEALHNHY
TQKSLSLSPGK

20 and comprises the following sequence as a light chain:

SEQ ID NO: 33

DVVLTSQPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
25 LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC.

In a specific embodiment of the invention the antibody comprises the following sequence as a heavy chain:

30 SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
KFQGRVTITADTSTSTAYMELSSLRSEDVAVYYCTEGYEYDGFYWGQGTTVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV

TVPSSSLGTQTYICNVNHHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
 LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
 DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
 SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
 5 YTQKSLSLSPGK

or a sequence that is > 95% identical to it, preferably > 98%, preferably > 99% and comprises the following sequence as a light chain:

10 SEQ ID NO: 33
 DVVLTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNRFSQVP
 DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
 LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADY
 EKHKVYACEVTHQGLSSPVTKSFNRGEC

15

or a sequence that is > 95% identical to it, preferably > 98%, preferably > 99%.

To assess the identity between two amino acid sequences, a pairwise alignment is performed. Identity defines the percentage of amino acids with a direct match in the alignment.

20

In a specific embodiment of the invention the antibody comprises the following sequence as a heavy chain:

SEQ ID NO: 32
 QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
 25 KFQGRVTITADTSTSTAYMELSSLRSEDVAVYYCTEGYEYDGFYWGQGTITVTVSSASTKGP
 SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
 TVPSSSLGTQTYICNVNHHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
 LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
 DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
 30 SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
 YTQKSLSLSPGK

or a sequence that comprises CDR-sequences 100% identical to SEQ ID No.: 1, SEQ ID No.: 2 and/or SEQ ID No.: 3 and is > 95% identical to SEQ ID NO: 32, preferably > 98%, preferably > 99% and comprises the following sequence as a light chain:

5 SEQ ID NO: 33

DVVLTSQSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

10

or a sequence that comprises CDR-sequences 100% identical to SEQ ID No.: 4 and/or SEQ ID No.: 5 and is > 95% identical to SEQ ID NO: 33, preferably > 98%, preferably > 99%.

15 In embodiments of the present invention, the anti-ADM antibody or anti-ADM antibody fragment for use in the treatment or prevention of shock in a patient, may be administered in a dose of at least 0.5 mg / Kg body weight, particularly at least 1.0 mg/kg body weight, more particularly, from 1.0 to 20.0 mg/kg body weight, e.g., from 2.0 to 10 mg/kg body weight, from 2.0 to 8.0 mg/kg body weight, or from 2.0 to 5.0 mg/kg body weight.

20 The term "pharmaceutical formulation" as used herein refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

25 The present invention also relates to a pharmaceutical formulation comprising a therapeutically effective dose of the active ingredient, in combination with at least one pharmaceutically acceptable excipient.

30 "Pharmaceutically acceptable excipient" refers to an excipient that does not produce an adverse, allergic or other untoward reaction when administered to a subject. It includes in addition to a therapeutic protein, carriers, various diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The characteristics of the carrier will depend on the route of administration.

Subject matter of the present invention is a pharmaceutical formulation for use in therapy or prevention of shock in a patient comprising an antibody or fragment or scaffold according to the present invention.

5 Subject matter of the present invention is a pharmaceutical formulation for use in therapy or prevention of shock in a patient comprising an antibody or fragment or scaffold according to the present invention wherein said shock is selected from the group comprising shock due to hypovolemia, cardiogenic shock, obstructive shock and distributive shock, in particular cardiogenic shock or septic shock.

10 Subject matter of the present invention is a pharmaceutical formulation for use in therapy or prevention of shock in a patient according to the present invention, wherein said pharmaceutical formulation is a solution, preferably a ready-to-use solution.

15 Subject matter of the present invention is a pharmaceutical formulation for use in therapy or prevention of shock in a patient according to the present invention, wherein said pharmaceutical formulation is in a freeze-dried state.

Subject matter of the present invention is a pharmaceutical formulation for use in therapy or prevention of shock in a patient according to the present invention, wherein said pharmaceutical formulation is administered intra-muscular.

20 Subject matter of the present invention is a pharmaceutical formulation for use in intervention and therapy of congestion in a patient according to the present invention, wherein said pharmaceutical formulation is administered intra-vascular.

25 Subject matter of the present invention is a pharmaceutical formulation for use in intervention and therapy of congestion in a patient according to the present invention, wherein said pharmaceutical formulation is administered via infusion.

30 Subject matter of the present invention is a pharmaceutical formulation for use in therapy or prevention of shock in a patient according to the present invention, wherein said pharmaceutical formulation is to be administered systemically.

With the above context, the following consecutively numbered embodiments provide further specific aspects of the invention:

1. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient, wherein said patient is characterized by having a level of dipeptidyl peptidase 3 (DPP3) in a sample of bodily fluid below a threshold and said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal part (amino acid 1-21) of ADM: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).
2. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiment 1, wherein said shock is selected from the group comprising shock due to hypovolemia, cardiogenic shock, obstructive shock and distributive shock, in particular cardiogenic shock or septic shock.
3. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiment 1 and 2, wherein
 - in case of cardiogenic shock said patient may have suffered an acute coronary syndrome (e.g. acute myocardial infarction) or wherein said patient has heart failure (e.g. acute decompensated heart failure), myocarditis, arrhythmia, cardiomyopathy, valvular heart disease, aortic dissection with acute aortic stenosis, traumatic chordal rupture or massive pulmonary embolism, or
 - in case of hypovolemic shock said patient may have suffered a hemorrhagic disease including gastrointestinal bleed, trauma, vascular etiologies (e.g. ruptured abdominal aortic aneurysm, tumor eroding into a major blood vessel) and spontaneous bleeding in the setting of anticoagulant use or a non-hemorrhagic disease including vomiting, diarrhea, renal loss, skin losses/insensible losses (e.g. burns, heat stroke) or third-space loss in the setting of pancreatitis, cirrhosis, intestinal obstruction, trauma, or
 - in case of obstructive shock said patient may have suffered a cardiac tamponade, tension pneumothorax, pulmonary embolism or aortic stenosis, or
 - in case of distributive shock said patient may have septic shock, neurogenic shock, anaphylactic shock or shock due to adrenal crisis.
4. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 3,

wherein said threshold of DPP3 in a sample of bodily fluid of said patient is between 20 and 120 ng/mL, more preferred between 30 and 90 ng/mL, more preferred between 30 and 80 ng/mL, even more preferred between 40 and 60 ng/mL, most preferred said threshold is 50 ng/mL.

- 5 5. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiment 4, wherein said threshold of DPP3 in a sample of bodily fluid of said patient is between 40 and 60 ng/mL.
- 10 6. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 5, wherein the bodily fluid is selected from whole blood, plasma and serum.
- 15 7. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiment 6, wherein the bodily fluid is plasma.
- 20 8. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 7, wherein the level of DPP3 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to DPP3.
- 25 9. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiment 8, wherein the capture binder is an antibody.
- 30 10. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment or prevention of shock in a patient according to embodiments 1 to 9, wherein either the level of DPP3 protein and/or the level of active DPP3 is determined and compared to a predetermined threshold.
- 35 11. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 10, wherein said patient is additionally characterized by having a level of ADM-NH2 above a threshold.

12. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 11, wherein said threshold of ADM-NH₂ in a sample of bodily fluid of said patient is between 40 and 100 pg/mL, more preferred between 50 and 90 pg/mL, even more preferred between 60 and 80 pg/mL, most preferred said threshold is 70 pg/mL.
13. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiment 12, wherein said threshold of ADM-NH₂ in a sample of bodily fluid of said patient is 70 pg/mL.
14. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 13, wherein the bodily fluid is selected from whole blood, plasma and serum.
15. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiment 14, wherein the bodily fluid is plasma.
16. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 11-15, wherein the level of ADM-NH₂ is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to ADM-NH₂.
17. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiment 16, wherein the capture binder is an antibody.
18. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 17, wherein the sample of bodily fluid of said patient is selected from the group of blood, serum, plasma, urine, cerebrospinal fluid (CSF), and saliva.
19. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 18, wherein said anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold recognizes and binds to the N-terminal end (amino acid 1) of ADM-Gly and/or ADM-NH₂.

20. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 19, wherein said antibody, antibody fragment or non-Ig scaffold does not bind to the C-terminal portion of ADM, having the sequence amino acid 43-52 of ADM: PRSKISPQGY-NH₂ (SEQ ID NO: 24).

21. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 20, wherein said antibody or fragment or scaffold blocks the bioactivity of ADM not more than 80 %, preferably not more than 50%.

22. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 21, wherein said antibody or fragment is a monoclonal antibody or fragment that binds to ADM or an antibody fragment thereof, wherein the heavy chain comprises the sequences:

CDR1: SEQ ID NO: 1

GYTFSTRYW

CDR2: SEQ ID NO: 2

ILPGSGST

CDR3: SEQ ID NO: 3

TEGYEYDGFY

and wherein the light chain comprises the sequences:

CDR1: SEQ ID NO: 4

QSIVYSNGNTY

CDR2:

RVS

CDR3: SEQ ID NO: 5

FQGSHPYT.

23. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to 22, wherein said antibody or fragment comprises a sequence selected from the group comprising as a VH region:

SEQ ID NO: 6 (AM-VH-C)

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGST
NYNEKFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFQDYWGQGTTTLT
10 SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSGS
TNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFQDYWGQGTTVT
15 VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 8 (AM-VH2-E40)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGS
TNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFQDYWGQGTTVT
20 VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 9 (AM-VH3-T26-E55)

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGS
TNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFQDYWGQGTTVT
25 VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 10 (AM-VH4-T26-E40-E55)

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGS
TNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFQDYWGQGTTVT
30 VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

and comprises a sequence selected from the group comprising the following sequence as a VL region:

SEQ ID NO: 11 (AM-VL-C)

DVLLSQTPSLPVS LGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYS
5 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 12 (AM-VL1)

DVVMQTQSPSLPVT LGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRD
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYS
10 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 13 (AM-VL2-E40)

DVVMQTQSPSLPVT LGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRD
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYS
15 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

24. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig
scaffold for use in therapy or prevention of shock in a patient according to embodiments 1 to
23, wherein said antibody or fragment comprises the following sequence as a heavy chain:

20

SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTN
YNQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCTEGYEYDGFYWGQGTITVTVSS
25 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ
30 QGNVFSCSVMH EALHNHYTQKSLSLSPGK

or a sequence that is > 95% identical to it,
and comprises the following sequence as a light chain:

SEQ ID NO: 33

DVVLTSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNRFSG
 VPDRFSGSGSGTDFTLKISRVEAEDVGVIYCFQGGSHIPYTFGGGKLEIKRTVAAPSVFIFP
 5 PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL
 TLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

or a sequence that is > 95% identical to it.

25. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig
 10 scaffold for use in therapy or prevention of shock in a patient according to embodiments 1-24,
 wherein the anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-
 ADM non-Ig scaffold binds to the N-terminal part (amino acid 1-10) of ADM: YRQSMNNFQG
 (SEQ ID No. 26).
- 15 26. Anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-
 ADM non-Ig scaffold for use in therapy according to any of embodiment 25, wherein said
 antibody or fragment or scaffold exhibits a binding affinity to ADM of at least 10^{-7} M by label-
 free surface plasmon resonance using a Biacore 2000 system.
- 20 27. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig
 scaffold for use in therapy or prevention of shock in a patient according to embodiment 26,
 wherein the anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold
 exhibits an affinity towards human ADM is between 1×10^{-9} to 3×10^{-9} by label-free surface
 plasmon resonance using a Biacore 2000 system.
- 25 28. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig
 scaffold for use in therapy or prevention of shock in a patient according to embodiments 25 to
 27, wherein the anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig
 scaffold is an IgG1 antibody.
- 30 29. Pharmaceutical formulation for use in therapy or prevention of shock of a patient comprising
 an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-
 Ig scaffold according to any of embodiments 1 -28.

30. Pharmaceutical formulation for use in therapy or prevention of shock of a patient according to embodiment 29, wherein said pharmaceutical formulation is a solution, preferably a ready-to-use solution.
- 5 31. Pharmaceutical formulation for use in therapy or prevention of shock of a patient according to embodiments 29 and 30, wherein said pharmaceutical formulation is in a freeze-dried state.
32. Pharmaceutical formulation for use in therapy or prevention of shock of a patient according to
10 embodiments 29 and 31, wherein said pharmaceutical formulation is administered intramuscular.
33. Pharmaceutical formulation for use in therapy or prevention of shock of a patient according to
15 embodiments 29 and 32, wherein said pharmaceutical formulation is administered intravascular.
34. Pharmaceutical formulation for use in therapy or prevention of shock of a patient according to
20 embodiments 29 and 33, wherein said pharmaceutical formulation is administered via infusion.
35. Pharmaceutical formulation for use in therapy or prevention of shock of a patient according to
embodiments 29 and 34, wherein said pharmaceutical formulation is to be administered systemically.

FIGURE DESCRIPTION**Fig. 1a:**

Illustration of antibody formats – Fv and scFv-Variants.

5

Fig. 1b:

Illustration of antibody formats – heterologous fusions and bifunctional antibodies.

Fig. 1c:

10 Illustration of antibody formats – bivalent antibodies and bispecific antibodies.

Fig. 2:

- a: Dose response curve of human ADM. Maximal cAMP stimulation was adjusted to 100% activation.
- 15 b: Dose/ inhibition curve of human ADM 22-52 (ADM-receptor antagonist) in the presence of 5.63nM hADM.
- c: Dose/ inhibition curve of CT-H in the presence of 5.63 nM hADM.
- d: Dose/ inhibition curve of MR-H in the presence of 5.63 nM hADM.
- e: Dose/ inhibition curve of NT-H in the presence of 5.63 nM hADM.
- 20 f: Dose response curve of mouse ADM. Maximal cAMP stimulation was adjusted to 100% activation.
- g: Dose/ inhibition curve of human ADM 22-52 (ADM-receptor antagonist) in the presence of 0,67 nM mADM.
- h: Dose/ inhibition curve of CT-M in the presence of 0,67 nM mADM.
- 25 i: Dose/ inhibition curve of MR-M in the presence of 0,67 nM mADM.
- j: Dose/ inhibition curve of NT-M in the presence of 0,67 nM mADM.
- k: Shows the inhibition of ADM by F(ab)₂ NT-M and by Fab NT-M.
- l: shows the inhibition of ADM by F(ab)₂ NT-M and by Fab NT-M.

30 **Fig. 3:**

This figure shows a typical hADM dose/ signal curve. And an hADM dose signal curve in the presence of 100 µg/mL antibody NT-H.

5 **Fig. 4:**

This figure shows the stability of hADM in human plasma (citrate) in absence and in the presence of NT-H antibody.

Fig. 5:

10 Alignment of the Fab with homologous human framework sequences.

Fig. 6: ADM-concentration in healthy human subjects after NT-H application at different doses up to 60 days.

15 **Fig. 7:** Kaplan-Meier survival plots in relation to low (< 40.5 ng/mL) and high (≥ 40.5 ng/mL) DPP3 concentrations. (A) 7-day survival of patients with sepsis in relation to DPP3 plasma concentration; (B) 7-day survival of patients with cardiogenic shock in relation to DPP3 plasma concentrations; (C) 7-day survival of patients with septic shock in relation to DPP3 plasma concentration.

20 **Fig. 8:** Kaplan-Meier survival plot for all patients (14-day mortality of patients treated with placebo (Plac) or the N-terminal ADM antibody Adrecizumab (Adz)

Fig. 9: Kaplan-Meier survival plot for patients with DPP3 < 50 ng/mL (14-day mortality of patients treated with placebo (Plac) or the N-terminal ADM antibody Adrecizumab (Adz)

25

Fig. 10: Kaplan-Meier survival plot for patients with DPP3 > 50 ng/mL (14-day mortality of patients treated with placebo (Plac) or the N-terminal ADM antibody Adrecizumab (Adz)

30 **Fig. 11:** Kaplan Meier Plots for the Adrecizumab treatment effect on 28-day mortality in patients with high and low pre-dose bio-ADM concentrations. Investigated populations: PP and PP/DPP3 ≤ 70 . **Fig. 11a** shows PP/DPP3 ≤ 70 - Patients with bio-ADM between 70 and 182 pg/mL (below median and **Fig**

11b shows patients with bio-ADM above 182 pg/mL (above median). Calculated HR with 95% CI and log rank p-values (2-sided) are as follows: PP below median: HR=0.630 [0.276 -1.44], log rank p=0.270; PP above median: HR=0.870 [0.499 -1.52], log rank p=0.634; PP/DPP3 $\leq$ 70 below median: HR=0.607 [0.242 -1.52], log rank p=0.284; PP/DPP3 $\leq$ 70 above median: HR=0.623 [0.327-1.19], log rank p=0.151.

EXAMPLES

Example 1 - Generation of Antibodies and determination of their affinity constants

5

Several human and murine antibodies were produced and their affinity constants were determined (see tables 1 and 2). It should be emphasized that the antibodies, antibody fragments and non-Ig scaffolds of the example portion in accordance with the invention are binding to ADM, and thus should be considered as anti-ADM antibodies/ antibody fragments/ non-Ig scaffolds.

10

Peptides / conjugates for Immunization:

Peptides for immunization were synthesized, see Table 1, (JPT Technologies, Berlin, Germany) with an additional N-terminal Cystein (if no Cystein is present within the selected ADM-sequence) residue for conjugation of the peptides to Bovine Serum Albumin (BSA). The peptides were covalently linked to BSA by using Sulfolink-coupling gel (Perbio-science, Bonn, Germany). The coupling procedure was performed according to the manual of Perbio.

15

Mouse monoclonal antibody production:

A Balb/c mouse was immunized with 100µg Peptide-BSA-Conjugate at day 0 and 14 (emulsified in 100µl complete Freund's adjuvant) and 50µg at day 21 and 28 (in 100µl incomplete Freund's adjuvant). Three days before the fusion experiment was performed, the animal received 50µg of the conjugate dissolved in 100µl saline, given as one intraperitoneal and one intra-venous injection. Splenocytes from the immunized mouse and cells of the myeloma cell line SP2/0 were fused with 1ml 50% polyethylene glycol for 30s at 37°C. After washing, the cells were seeded in 96-well cell culture plates. Hybrid clones were selected by growing in HAT medium [RPMI 1640 culture medium supplemented with 20% fetal calf serum and HAT-Supplement]. After two weeks the HAT medium is replaced with HT Medium for three passages followed by returning to the normal cell culture medium. The cell culture supernatants were primary screened for antigen specific IgG antibodies three weeks after fusion. The positive tested microcultures were transferred into 24-well plates for propagation. After retesting, the selected cultures were cloned and re-cloned using the limiting-dilution technique and the isotypes were determined (see also Lane, R.D. 1985. J. Immunol. Meth. 81: 223-228; Ziegler et al. 1996. Horm. Metab. Res. 28: 11-15).

20

25

30

Antibodies were produced via standard antibody production methods (*Marx et al., 1997. Monoclonal Antibody Production, ATLA 25, 121*) and purified via Protein A. The antibody purities were > 95% based on SDS gel electrophoresis analysis.

5 Human Antibodies:

Human Antibodies were produced by means of phage display according to the following procedure: The human naive antibody gene libraries HAL7/8 were used for the isolation of recombinant single chain F-Variable domains (scFv) against adrenomedullin peptide. The antibody gene libraries were screened with a panning strategy comprising the use of peptides containing a biotin tag linked via two different
10 spacers to the adrenomedullin peptide sequence. A mix of panning rounds using non-specifically bound antigen and streptavidin bound antigen were used to minimize background of non-specific binders. The eluted phages from the third round of panning have been used for the generation of monoclonal scFv expressing E. coli strains. Supernatant from the cultivation of these clonal strains has been directly used for an antigen ELISA testing (see also *Hust et al., 2011. Journal of Biotechnology 152, 159–170; Schütte
15 et al., 2009. PLoS One 4, e6625*). Positive clones have been selected based on positive ELISA signal for antigen and negative for streptavidin coated micro titer plates. For further characterizations the scFv open reading frame has been cloned into the expression plasmid pOPE107 (*Hust et al., J. Biotechnol. 2011*), captured from the culture supernatant via immobilized metal ion affinity chromatography and purified by a size exclusion chromatography.

20

Affinity Constants: To determine the affinity of the antibodies to ADM, the kinetics of binding of ADM to immobilized antibody was determined by means of label-free surface plasmon resonance using a Biacore 2000 system (GE Healthcare Europe GmbH, Freiburg, Germany). Reversible immobilization of the antibodies was performed using an anti-mouse Fc antibody covalently coupled in high density to
25 a CM5 sensor surface according to the manufacturer's instructions (mouse antibody capture kit; GE Healthcare). (*Lorenz et al., 2011. Antimicrob Agents Chemother. 55(1): 165–173*).

The monoclonal antibodies were raised against the below depicted ADM regions of human and murine ADM, respectively. The following table represents a selection of obtained antibodies used in further experiments. Selection was based on target region:

30

Table 1: immunization peptides

Sequence Number	Antigen/Immunogen	ADM Region	Designation	Affinity constants Kd (M)

SEQ ID: 14	YRQSMNNFQGLRSFGCRFGTC	1-21	NT-H	5.9×10^{-9}
SEQ ID: 15	CTVQKLAHQIYQ	21-32	MR-H	2×10^{-9}
SEQ ID: 16	CAPRSKISPQGY-NH ₂	C-42-52	CT-H	1.1×10^{-9}
SEQ ID: 17	YRQSMNQGSRSNGCRFGTC	1-19	NT-M	3.9×10^{-9}
SEQ ID: 18	CTFQKLAHQIYQ	19-31	MR-M	4.5×10^{-10}
SEQ ID: 19	CAPRNKISPQGY-NH ₂	C-40-50	CT-M	9×10^{-9}

The following is a list of further obtained monoclonal antibodies:

Table 2:

Target	Source	Clone number	Affinity (M)	max inhibition bioassay (%) (see example 2)
NT-M	Mouse	ADM/63	5.8×10^{-9}	45
	Mouse	ADM/364	2.2×10^{-8}	48
	Mouse	ADM/365	3.0×10^{-8}	
	Mouse	ADM/366	1.7×10^{-8}	
	Mouse	ADM/367	1.3×10^{-8}	
	Mouse	ADM/368	1.9×10^{-8}	
	Mouse	ADM/369	2.0×10^{-8}	
	Mouse	ADM/370	1.6×10^{-8}	
	Mouse	ADM/371	2.0×10^{-8}	
	Mouse	ADM/372	2.5×10^{-8}	
	Mouse	ADM/373	1.8×10^{-8}	
	Mouse	ADM/377	1.5×10^{-8}	
	Mouse	ADM/378	2.2×10^{-8}	
	Mouse	ADM/379	1.6×10^{-8}	
	Mouse	ADM/380	1.8×10^{-8}	
	Mouse	ADM/381	2.4×10^{-8}	

	Mouse	ADM/382	1.6×10^{-8}	
	Mouse	ADM/383	1.8×10^{-8}	
	Mouse	ADM/384	1.7×10^{-8}	
	Mouse	ADM/385	1.7×10^{-8}	
	Mouse	ADM/403	1.2×10^{-8}	
	Mouse	ADM/395	1.2×10^{-8}	
	Mouse	ADM/396	3.0×10^{-8}	
	Mouse	ADM/397	1.5×10^{-8}	
MR-M	Mouse	ADM/38	4.5×10^{-10}	68
MR-M	Mouse	ADM/39	5.9×10^{-9}	72
CT-M	Mouse	ADM/65	9.0×10^{-9}	100
CT-M	Mouse	ADM/66	1.6×10^{-8}	100
NT-H	Mouse	ADM/33	5.9×10^{-8}	38
NT-H	Mouse	ADM/34	1.6×10^{-8}	22
MR-H	Mouse	ADM/41	1.2×10^{-8}	67
MR-H	Mouse	ADM/42	$< 1 \times 10^{-8}$	
MR-H	Mouse	ADM/43	2.0×10^{-9}	73
MR-H	Mouse	ADM/44	$< 1 \times 10^{-8}$	
CT-H	Mouse	ADM/15	$< 1 \times 10^{-8}$	
CT-H	Mouse	ADM/16	1.1×10^{-9}	100
CT-H	Mouse	ADM/17	3.7×10^{-9}	100
CT-H	Mouse	ADM/18	$< 1 \times 10^{-8}$	
hADM	Phage display	ADM/A7	$< 1 \times 10^{-8}$	
	Phage display	ADM/B7	$< 1 \times 10^{-8}$	
	Phage display	ADM/C7	$< 1 \times 10^{-8}$	
	Phage display	ADM/G3	$< 1 \times 10^{-8}$	
	Phage display	ADM/B6	$< 1 \times 10^{-8}$	
	Phage display	ADM/B11	$< 1 \times 10^{-8}$	

	Phage display	ADM/D8	$<1 \times 10^{-8}$	
	Phage display	ADM/D11	$<1 \times 10^{-8}$	
	Phage display	ADM/G12	$<1 \times 10^{-8}$	

Generation of antibody fragments by enzymatic digestion: The generation of Fab and F(ab)₂ fragments was done by enzymatic digestion of the murine full-length antibody NT-M. Antibody NT-M was digested using a) the pepsin-based F(ab)₂ Preparation Kit (Pierce 44988) and b) the papain-based Fab Preparation Kit (Pierce 44985). The fragmentation procedures were performed according to the instructions provided by the supplier. Digestion was carried out in case of F(ab)₂-fragmentation for 8h at 37°C. The Fab-fragmentation digestion was carried out for 16 h, respectively.

Procedure for Fab Generation and Purification: The immobilized papain was equilibrated by washing the resin with 0.5 ml of Digestion Buffer and centrifuging the column at 5000 × g for 1 minute. The buffer was discarded afterwards. The desalting column was prepared by removing the storage solution and washing it with digestion buffer, centrifuging it each time afterwards at 1000 × g for 2 minutes. 0.5ml of the prepared IgG sample where added to the spin column tube containing the equilibrated Immobilized Papain. Incubation time of the digestion reaction was done for 16h on a tabletop rocker at 37°C. The column was centrifuged at 5000 × g for 1 minute to separate digest from the Immobilized Papain. Afterwards the resin was washed with 0.5ml PBS and centrifuged at 5000 × g for 1 minute. The wash fraction was added to the digested antibody that the total sample volume was 1.0ml. The NAb Protein A Column was equilibrated with PBS and IgG Elution Buffer at room temperature. The column was centrifuged for 1 minute to remove storage solution (contains 0.02% sodium azide) and equilibrated by adding 2ml of PBS, centrifuge again for 1 minute and the flow-through discarded. The sample was applied to the column and resuspended by inversion. Incubation was done at room temperature with end-over-end mixing for 10 minutes. The column was centrifuged for 1 minute, saving the flow-through with the Fab fragments. (References: *Coulter and Harris 1983. J. Immunol. Meth. 59, 199-203.; Lindner et al. 2010. Cancer Res. 70, 277-87; Kaufmann et al. 2010. PNAS. 107, 18950-5.; Chen et al. 2010. PNAS. 107, 14727-32; Uysal et al. 2009 J. Exp. Med. 206, 449-62; Thomas et al. 2009. J. Exp. Med. 206, 1913-27; Kong et al. 2009 J. Cell Biol. 185, 1275-840).*

Procedure for generation and purification of F(ab')₂ Fragments: The immobilized Pepsin was equilibrated by washing the resin with 0.5 ml of Digestion Buffer and centrifuging the column at 5000 × g for 1 minute. The buffer was discarded afterwards. The desalting column was prepared by removing the storage solution and washing it with digestion buffer, centrifuging it each time afterwards at 1000 × g for 2 minutes. 0.5ml of the prepared IgG sample where added to the spin column tube containing the

equilibrated Immobilized Pepsin. Incubation time of the digestion reaction was done for 16h on a tabletop rocker at 37°C. The column was centrifuged at $5000 \times g$ for 1 minute to separate digest from the Immobilized Papain. Afterwards the resin was washed with 0.5 mL PBS and centrifuged at $5000 \times g$ for 1 minute. The wash fraction was added to the digested antibody that the total sample volume was 1.0ml. The NAb Protein A Column was equilibrated with PBS and IgG Elution Buffer at room temperature. The column was centrifuged for 1 minute to remove storage solution (contains 0.02% sodium azide) and equilibrated by adding 2 mL of PBS, centrifuge again for 1 minute and the flow-through discarded. The sample was applied to the column and resuspended by inversion. Incubation was done at room temperature with end-over-end mixing for 10 minutes. The column was centrifuged for 1 minute, saving the flow-through with the Fab fragments. (References: *Mariani et al. 1991. Mol Immunol. 28: 69-77; Beale 1987. Exp Comp Immunol 11:287-96; Ellerson et al. 1972. FEBS Letters 24(3):318-22; Kerbel and Elliot 1983. Meth Enzymol 93:113-147; Kulkarni et al. 1985. Cancer Immunol Immunotherapy 19:211-4; Lamoyi 1986. Meth Enzymol 121:652-663; Parham et al. 1982. J Immunol Meth 53:133-73; Raychaudhuri et al. 1985. Mol Immunol 22(9):1009-19; Rousseaux et al. 1980. Mol Immunol 17:469-82; Rousseaux et al. 1983. J Immunol Meth 64:141-6; Wilson et al. 1991. J Immunol Meth 138:111-9).*

NT-H-Antibody Fragment Humanization: The antibody fragment was humanized by the CDR-grafting method (*Jones et al. 1986. Nature 321, 522-525*).

20

The following steps were done to achieve the humanized sequence:

Total RNA extraction: Total RNA was extracted from NT-H hybridomas using the Qiagen kit. First-round RT-PCR: QIAGEN® OneStep RT-PCR Kit (Cat No. 210210) was used. RT-PCR was performed with primer sets specific for the heavy and light chains. For each RNA sample, 12 individual heavy chain and 11 light chain RT-PCR reactions were set up using degenerate forward primer mixtures covering the leader sequences of variable regions. Reverse primers are located in the constant regions of heavy and light chains. No restriction sites were engineered into the primers.

Reaction Setup: 5x QIAGEN® OneStep RT-PCR Buffer 5.0 µl, dNTP Mix (containing 10 mM of each dNTP) 0.8 µl, Primer set 0.5 µl, QIAGEN® OneStep RT-PCR Enzyme Mix 0.8 µl, Template RNA 2.0 µl, RNase-free water to 20.0 µl, Total volume 20.0 µl PCR condition: Reverse transcription: 50°C, 30 min; Initial PCR activation: 95°C, 15 min Cycling: 20 cycles of 94°C, 25 sec; 54°C, 30 sec; 72°C, 30 sec; Final extension: 72°C, 10 min Second-round semi-nested PCR: The RT-PCR products from the first-round reactions were further amplified in the second-round PCR. 12

individual heavy chain and 11 light chain RT-PCR reactions were set up using semi-nested primer sets specific for antibody variable regions.

Reaction Setup: 2x PCR mix 10 µl; Primer set 2 µl; First-round PCR product 8 µl; Total volume 20 µl;

- 5 Hybridoma Antibody Cloning Report PCR condition: Initial denaturing of 5 min at 95°C; 25 cycles of 95°C for 25 sec, 57°C for 30 sec, 68°C for 30 sec; Final extension is 10 min 68°C.

- 10 After PCR was finished, PCR reaction samples were run onto agarose gel to visualize DNA fragments amplified. After sequencing more than 15 cloned DNA fragments amplified by nested RT-PCR, several mouse antibody heavy and light chains have been cloned and appear correct. Protein sequence alignment and CDR analysis identifies one heavy chain and one light chain. After alignment with homologous human framework sequences the resulting humanized sequence for the variable heavy chain is the following: see figure 5. As the amino acids on positions 26, 40 and 55 in the variable heavy chain and
15 amino acid on position 40 in the variable light are critical to the binding properties, they may be reverted to the murine original. The resulting candidates are depicted below. (*Padlan 1991. Mol. Immunol. 28, 489–498; Harris and Bajorath. 1995. Protein Sci. 4, 306–310*).

- 20 Annotation for the antibody fragment sequences (SEQ ID No.: 6-13; 32 and 33): bold and underlined are the CDR 1, 2, 3 in italic are constant regions; hinge regions are highlighted with bold letters; framework point mutation have a grey letter-background.

SEQ ID No.: 6 (AM-VH-C)

- 25 QVQLQQSGAELMKPGASVKISCKAT**GYTFSRYWIEWVKQRP**GHGLEWIGE**ILPGSGST**NYN
EKFKGKATITADTSSNTAYMQLSSLTSEDSAVYYC**TEGYEYDGF**FDYWGQGTTITVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGT
QTYICNVNHKPSNTKVDKRV**EPK**

SEQ ID No.: 7 (AM-VH1)

- 30 QVQLVQSGAEVKKPGSSVKVSKKAS**GYTFSRYWISWVRQAPGQ**GLEWMGRIL**LP**SGSGSTNY
AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYC**TEGYEYDGF**FDYWGQGTTITVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKRV**EPK**

SEQ ID No.: 8 (AM-VH2-E40)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGSTNY
AQKFQGRVTITADESTSTAYMELSSLRSED~~TAVYYC~~TEGYEYDGFDYWGQGTTVTVSSASTKGPSV
5 FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKRV**EPK**

SEQ ID No.: 9 (AM-VH3-T26-E55)

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGSTNY
10 AQKFQGRVTITADESTSTAYMELSSLRSED~~TAVYYC~~TEGYEYDGFDYWGQGTTVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKRV**EPK**

SEQ ID No.: 10 (AM-VH4-T26-E40-E55)

15 QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTNY
AQKFQGRVTITADESTSTAYMELSSLRSED~~TAVYYC~~TEGYEYDGFDYWGQGTTVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKRV**EPK**

20 SEQ ID No.: 11 (AM-VL-C)

DVLLSQTPSLPVS LGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFS GVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC

25

SEQ ID No.: 12 (AM-VL1)

DVVMQTSPSLPVTLGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDSGV
PDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKA
30 DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No.: 13 (AM-VL2-E40)

DVVMTQSPSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSGV
 PDRFSGSGSGTDFTLKISRVEAEDVGVYYCFOGSHIPYTFGQGGTKLEIKRTVAAPSVFIFPPSD
 EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKA
 5 DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 32 (Adrecizumab heavy chain)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYN
 QKFQGRVTITADTSTSTAYMELSSLRSEDAVYYCTEGYEYDGFWDYWGQGTTVTVSSASTK
 10 GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
 VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
 DTLNISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
 HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
 FYPSDIAVEWESNGQPENNYKTTTPVLD
 15 SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 33 (Adrecizumab light chain)

DVVLTSQSPSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQSPRLLIYRVSNRFSGV
 DRFSGSGSGTDFTLKISRVEAEDVGVYYCFOGSHIPYTFGGGGTKLEIKRTVAAPSVFIFPPSDE
 20 QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKAD
 YEKHKVYACEVTHQGLSSPVTKSFNRGEC

Example 2 - Effect of selected anti-ADM-antibodies on anti-ADM-bioactivity

- 25 The effect of selected ADM-antibodies on ADM-bioactivity was tested in a human recombinant Adrenomedullin receptor cAMP functional assay (Adrenomedullin Bioassay).

Testing of antibodies targeting human or mouse adrenomedullin in human recombinant Adrenomedullin receptor cAMP functional assay (Adrenomedullin Bioassay)

30

Materials: Cell line CHO-K1, Receptor Adrenomedullin (CRLR + RAMP3), Receptor Accession Number Cell line: CRLR: U17473; RAMP3: AJ001016

CHO-K1 cells expressing human recombinant adrenomedullin receptor (FAST-027C) grown prior to the test in media without antibiotic were detached by gentle flushing with PBS-EDTA (5 mM EDTA), recovered by centrifugation and resuspended in assay buffer (KRH: 5 mM KCl, 1.25 mM MgSO₄, 124 mM NaCl, 25 mM HEPES, 13.3 mM Glucose, 1.25 mM KH₂PO₄, 1.45 mM CaCl₂, 0.5 g/l BSA).

Dose response curves were performed in parallel with the reference agonists (hADM or mADM).

Antagonist test (96well): For antagonist testing, 6 µl of the reference agonist (human (5.63 nM) or mouse (0.67 nM) adrenomedullin) was mixed with 6 µl of the test samples at different antagonist dilutions; or with 6 µl buffer. After incubation for 60 min at room temperature, 12 µl of cells (2,500 cells/well) were added. The plates were incubated for 30 min at room temperature. After addition of the lysis buffer, percentage of DeltaF will be estimated, according to the manufacturer specification, with the HTRF kit from Cis-Bio International (cat n°62AM2 PEB) hADM 22-52 was used as reference antagonist.

15 Antibodies testing cAMP-HTRF assay

The anti-h-ADM antibodies (NT-H, MR-H, CT-H) were tested for antagonist activity in human recombinant adrenomedullin receptor (FAST-027C) cAMP functional assay in the presence of 5.63 nM Human ADM 1-52, at the following final antibody concentrations: 100 µg/ml, 20 µg/ml, 4 µg/ml, 0.8 µg/ml, 0.16 µg/ml.

20

The anti-m-ADM antibodies (NT-M, MR-M, CT-M) were tested for antagonist activity in human recombinant ADM receptor (FAST-027C) cAMP functional assay in the presence of 0.67 nM Mouse ADM 1-50, at the following final antibody concentrations: 100 µg/ml, 20 µg/ml, 4 µg/ml, 0.8 µg/ml, 0.16 µg/ml. Data were plotted relative inhibition vs. antagonist concentration (see figures 2 a to 2 l).

25 The maximal inhibition by the individual antibody is given in table 3.

Table 3: maximal inhibition of bio-ADM activity

Antibody	Maximal inhibition of ADM bioactivity (ADM-Bioassay) (%)
NT-H	38
MR-H	73
CT-H	100
NT-M FAB	26

NT-M FAB2	28
NT-M	45
MR-M	66
CT-M	100
Non specific mouse IgG	0

Example 3 - Stabilization of hADM by the anti-ADM antibody

The stabilizing effect of human ADM by human ADM antibodies was tested using a hADM immunoassay.

Immunoassay for the quantification of human Adrenomedullin

The technology used was a sandwich coated tube luminescence immunoassay, based on Acridinium ester labelling.

10

Labelled compound (tracer): 100µg (100 µl) CT-H (1mg/ml in PBS, pH 7.4, AdrenoMed AG Germany) was mixed with 10µl Acridinium NHS-ester (1mg/ ml in acetonitrile, InVent GmbH, Germany) (EP 0353971) and incubated for 20min at room temperature. Labelled CT-H was purified by Gel-filtration HPLC on Bio-Sil® SEC 400-5 (Bio-Rad Laboratories, Inc., USA) The purified CT-H was diluted in (300 mmol/L potassium phosphate, 100 mmol/L NaCl, 10 mmol/L Na-EDTA, 5 g/L Bovine Serum Albumin, pH 7.0). The final concentration was approx. 800.000 relative light units (RLU) of labelled compound (approx. 20ng labeled antibody) per 200 µL. Acridiniumester chemiluminescence was measured by using an AutoLumat LB 953 (Berthold Technologies GmbH & Co. KG).

Solid phase: Polystyrene tubes (Greiner Bio-One International AG, Austria) were coated (18h at room temperature) with MR-H (AdrenoMed AG, Germany) (1.5 µg MR-H/0.3 mL 100 mmol/L NaCl, 50 mmol/L TRIS/HCl, pH 7.8). After blocking with 5% bovine serum albumin, the tubes were washed with PBS, pH 7.4 and vacuum dried.

Calibration: The assay was calibrated, using dilutions of hADM (BACHEM AG, Switzerland) in 250 mmol/L NaCl, 2 g/L Triton X-100, 50 g/L Bovine Serum Albumin, 20 tabs/L Protease Inhibitor Cocktail (Roche Diagnostics AG, Switzerland).

25

hADM Immunoassay: 50 µl of sample (or calibrator) was pipetted into coated tubes, after adding labeled CT-H (200µl), the tubes were incubated for 4h at 4°C. Unbound tracer was removed by washing 5 times (each 1ml) with washing solution (20mM PBS, pH 7.4, 0.1 % Triton X-100).

5

Tube-bound chemiluminescence was measured by using the LB 953: Figure 3 shows a typical hADM dose/ signal curve. And an hADM dose signal curve in the presence of 100 µg/mL antibody NT-H. NT-H did not affect the described hADM immunoassay.

- 10 Stability of human Adrenomedullin: Human ADM was diluted in human Citrate plasma (final concentration 10 nM) and incubated at 24 °C. At selected time points, the degradation of hADM was stopped by freezing at -20 °C. The incubation was performed in absence and presence of NT-H (100 µg/ml). The remaining hADM was quantified by using the hADM immunoassay described above.
- 15 Figure 4 shows the stability of hADM in human plasma (citrate) in absence and in the presence of NT-H antibody. The half-life of hADM alone was 7.8 h and in the presence of NT-H, the half-life was 18.3 h. (2.3 times higher stability).

Example 4 - Sepsis Mortality

20

a) Early treatment of sepsis

- Animal model: 12-15 week-old male C57Bl/6 mice (Charles River Laboratories, Germany) were used for the study. Peritonitis had been surgically induced under light isofluran anesthesia. Incisions were made into the left upper quadrant of the peritoneal cavity (normal location of the cecum). The cecum was exposed and a tight ligature was placed around the cecum with sutures distal to the insertion of the small bowel. One puncture wound was made with a 24-gauge needle into the cecum and small amounts of cecal contents were expressed through the wound. The cecum was replaced into the peritoneal cavity and the laparotomy site was closed. Finally, animals were returned to their cages with free access to food and water. 500µl saline were given s.c. as fluid replacement.
- 25
- 30

Application and dosage of the compound (NT-M, MR-M, CT-M): Mice were treated immediately after CLP (early treatment). CLP is the abbreviation for cecal ligation and puncture (CLP).

Study groups: Three compounds were tested versus: vehicle and versus control compound treatment. Each group contained 5 mice for blood drawing after 1 day for BUN (serum blood urea nitrogen test) determination. Ten further mice per each group were followed over a period of 4 days.

5

Group Treatment (10µl/ g bodyweight) dose/ Follow-Up:

1 NT-M, 0.2 mg/ml survival over 4 days

2 MR-M, 0.2 mg/ml survival over 4 days

3 CT-M, 0.2 mg/ml survival over 4 days

10 4 non-specific mouse IgG, 0.2 mg/ml survival over 4 days

5 control - PBS 10µl/g bodyweight survival over 4 days

Clinical chemistry: Blood urea nitrogen (BUN) concentrations for renal function were measured baseline and day 1 after CLP. Blood samples were obtained from the cavernous sinus with a capillary under light ether anaesthesia. Measurements were performed by using an AU 400 Olympus Multianalyser. The 4-day mortality and the average BUN concentrations are given in table 4.

15

Table 4: 4-day mortality and BUN concentrations

4-day mortality	survival (%)	BUN pre CLP (mM)	BUN day 1 (mM)
PBS	0	8.0	23.2
non-specific mouse IgG	0	7.9	15.5
CT-M	10	7.8	13.5
MR-M	30	8.1	24.9
NT-M	70	8.8	8.2

20 It can be seen from Table 4 that the NT-M antibody reduced mortality considerably. After 4 days 70 % of the mice survived when treated with NT-M antibody. When treated with MR-M antibody 30 % of the animals survived and when treated with CT-M antibody 10 % of the animals survived after 4 days. In contrast thereto all mice were dead after 4 days when treated with unspecific mouse IgG. The same result was obtained in the control group where PBS (phosphate buffered saline) was administered to

25 mice. The blood urea nitrogen or BUN test is used to evaluate kidney function, to help diagnose kidney

disease, and to monitor patients with acute or chronic kidney dysfunction or failure. The results of the S-BUN Test revealed that the NT-M antibody was the most effective to protect the kidney.

b) late treatment of sepsis

5

Animal model: 12-15 week-old male C57Bl/6 mice (Charles River Laboratories, Germany) were used for the study. Peritonitis had been surgically induced under light isofluran anesthesia. Incisions were made into the left upper quadrant of the peritoneal cavity (normal location of the cecum). The cecum was exposed and a tight ligature was placed around the cecum with sutures distal to the insertion of the small bowel. One puncture wound was made with a 24-gauge needle into the cecum and small amounts of cecal contents were expressed through the wound. The cecum was replaced into the peritoneal cavity and the laparotomy site was closed. Finally, animals were returned to their cages with free access to food and water. 500µl saline were given s.c. as fluid replacement.

15 Application and dosage of the compound (NT-M FAB2): NT-M FAB2 was tested versus: vehicle and versus control compound treatment. Treatment was performed after full development of sepsis, 6 hours after CLP (late treatment). Each group contained 4 mice and were followed over a period of 4 days.

Group Treatment (10µl/ g bodyweight) dose/ Follow-Up:

- 20 1 NT-M, FAB2 0.2 mg/ml survival over 4 days
- 2 control non-specific mouse IgG, 0.2 mg/ml survival over 4 days
- 3 vehicle: - PBS 10µl/g bodyweight survival over 4 days

Table 5: 4-day mortality

4 day mortality	survival (%)
PBS	0
Non-specific mouse IgG	0
NT-M FAB2	75

25

It can be seen from Table 5 that the NT-M FAB 2 antibody reduced mortality considerably. After 4 days 75 % of the mice survived when treated with NT-M FAB 2 antibody. In contrast thereto all mice were

dead after 4 days when treated with non-specific mouse IgG. The same result was obtained in the control group where PBS (phosphate buffered saline) was administered to mice.

Example 5 - Administration of NT-H in healthy humans

5

The study was conducted in healthy male subjects as a randomized, double-blind, placebo-controlled, study with single escalating doses of NT-H antibody administered as intravenous (i.v.) infusion in 3 sequential groups of 8 healthy male subjects each (1st group 0,5 mg/kg, 2nd group 2mg/kg, 3rd group 8 mg/kg) of healthy male subjects (n=6 active, n = 2 placebo for each group). The main inclusion criteria were written informed consent, age 18 – 35 years, agreement to use a reliable way of contraception and a BMI between 18 and 30 kg/m². Subjects received a single i.v. dose of NT-H antibody (0.5 mg/kg; 2 mg/kg; 8 mg/kg) or placebo by slow infusion over a 1-hour period in a research unit. The baseline ADM-values in the 4 groups did not differ. Median ADM values were 7.1 pg/mL in the placebo group, 6.8 pg/mL in the first treatment group (0.5mg/kg), 5.5 pg/mL in second treatment group (2mg/kg) and 7.1 pg/mL in the third treatment group (8mg/mL). The results show, that ADM-values rapidly increased within the first 1.5 hours after administration of NT-H antibody in healthy human individuals, then reached a plateau and slowly declined (Figure 6).

Example 6 – Methods for the measurement of DPP3 protein and DPP3 activity

20 Generation of antibodies and determination DPP3 binding ability: Several murine antibodies were produced and screened by their ability of binding human DPP3 in a specific binding assay (see Table 6).

Peptides/ conjugates for immunization: DPP3 peptides for immunization were synthesized, see Table 6, (JPT Technologies, Berlin, Germany) with an additional N-terminal cystein (if no cystein is present within the selected DPP3-sequence) residue for conjugation of the peptides to Bovine Serum Albumin (BSA). The peptides were covalently linked to BSA by using Sulfolink-coupling gel (Perbio-science, Bonn, Germany). The coupling procedure was performed according to the manual of Perbio. Recombinant GST-hDPP3 was produced by USBio (United States Biological, Salem, MA, USA).

Immunization of mice, immune cell fusion and screening: Balb/c mice were intraperitoneally (i.p.) injected with 84 µg GST-hDPP3 or 100 µg DPP3-peptide-BSA-conjugates at day 0 (emulsified in TiterMax Gold Adjuvant), 84 µg or 100 µg at day 14 (emulsified in complete Freund's adjuvant) and 42 µg or 50 µg at day 21 and 28 (in incomplete Freund's adjuvant). At day 49 the animal received an intravenous (i.v.) injection of 42 µg GST-hDPP3 or 50 µg DPP3-peptide-BSA-conjugates dissolved in saline. Three days later the mice were sacrificed and the immune cell fusion was performed.

30

Splenocytes from the immunized mice and cells of the myeloma cell line SP2/0 were fused with 1 ml 50% polyethylene glycol for 30 s at 37°C. After washing, the cells were seeded in 96-well cell culture plates. Hybrid clones were selected by growing in HAT medium [RPMI 1640 culture medium supplemented with 20% fetal calf serum and HAT-Supplement]. After one week, the HAT medium was replaced with HT Medium for three passages followed by returning to the normal cell culture medium. The cell culture supernatants were primarily screened for recombinant DPP3 binding IgG antibodies two weeks after fusion. Therefore, recombinant GST-tagged hDPP3 (USBiologicals, Salem, USA) was immobilized in 96-well plates (100 ng/ well) and incubated with 50 µl cell culture supernatant per well for 2 hours at room temperature. After washing of the plate, 50 µl / well POD-rabbit anti mouse IgG was added and incubated for 1 h at RT. After a next washing step, 50 µl of a chromogen solution (3,7 mM o-phenylen-diamine in citrate/ hydrogen phosphate buffer, 0.012% H₂O₂) were added to each well, incubated for 15 minutes at RT and the chromogenic reaction stopped by the addition of 50 µl 4N sulfuric acid. Absorption was detected at 490 nm.

The positive tested microcultures were transferred into 24-well plates for propagation. After retesting the selected cultures were cloned and re-cloned using the limiting-dilution technique and the isotypes were determined.

Mouse monoclonal antibody production

Antibodies raised against GST-tagged human DPP3 or DPP3-peptides were produced via standard antibody production methods (*Marx et al. 1997*) and purified via Protein A. The antibody purities were ≥ 90% based on SDS gel electrophoresis analysis.

Characterization of antibodies – binding to hDPP3 and/ or immunization peptide

To analyze the capability of DPP3/ immunization peptide binding by the different antibodies and antibody clones a binding assay was performed:

Solid phase: Recombinant GST-tagged hDPP3 (SEQ ID NO. 34) or a DPP3 peptide (immunization peptide, SEQ ID NO. 35) was immobilized onto a high binding microtiter plate surface (96-Well polystyrene microplates, Greiner Bio-One international AG, Austria, 1 µg/well in coupling buffer [50 mM Tris, 100 mM NaCl, pH7,8], 1h at RT). After blocking with 5% bovine serum albumin, the microplates were vacuum dried.

Labelling procedure (tracer): 100 µg (100 µl) of the different antiDPP3 antibodies (detection antibody, 1 mg/ ml in PBS, pH 7.4) were mixed with 10 µl acridinium NHS-ester (1 mg/ml in acetonitrile, InVent GmbH, Germany; EP 0 353 971) and incubated for 30 min at room temperature. Labelled antiDPP3 antibody was purified by gel-filtration HPLC on Shodex Protein 5 µm KW-803 (Showa Denko, Japan). The purified labelled antibody was diluted in assay buffer (50 mmol/l potassium phosphate, 100 mmol/l NaCl, 10 mmol/l Na₂-EDTA, 5 g/l bovine serum albumin, 1 g/l murine IgG, 1 g/l bovine IgG, 50 µmol/l amastatin, 100 µmol/l leupeptin, pH 7.4). The final concentration was approx. 5-7*10⁶ relative light

units (RLU) of labelled compound (approx. 20 ng labelled antibody) per 200 μ l. acridinium ester chemiluminescence was measured by using a Centro LB 960 luminometer (Berthold Technologies GmbH & Co. KG).

5 hDPP3 binding assay: the plates were filled with 200 μ l of labelled and diluted detection antibody (tracer) and incubated for 2-4 h at 2-8 °C. Unbound tracer was removed by washing 4 times with 350 μ l washing solution (20 mM PBS, pH 7.4, 0.1 % Triton X-100). Well-bound chemiluminescence was measured by using the Centro LB 960 luminometer (Berthold Technologies GmbH & Co. KG).

Characterization of antibodies – hDPP3-inhibition analysis

To analyze the capability of DPP3 inhibition by the different antibodies and antibody clones a DPP3 activity assay with known procedure (Jones et al., 1982) was performed. Recombinant GST-tagged hDPP3 was diluted in assay buffer (25 ng/ml GST-DPP3 in 50 mM Tris-HCl, pH7,5 and 100 μ M ZnCl₂) and 200 μ l of this solution incubated with 10 μ g of the respective antibody at room temperature. After 1 hour of pre-incubation, fluorogenic substrate Arg-Arg- β NA (20 μ l, 2mM) was added to the solution and the generation of free β NA over time was monitored using the Twinkle LB 970 microplate
10 fluorometer (Berthold Technologies GmbH & Co. KG) at 37 °C. Fluorescence of β NA is detected by exciting at 340 nm and measuring emission at 410 nm. Slopes (in RFU/ min) of increasing fluorescence of the different samples are calculated. The slope of GST-hDPP3 with buffer control is appointed as 100 % activity. The inhibitory ability of a possible capture-binder is defined as the decrease of GST-hDPP3 activity by incubation with said capture-binder in percent.

20 The following table represents a selection of obtained antibodies and their binding rate in Relative Light Units (RLU) as well as their relative inhibitory ability (%; table 6). The monoclonal antibodies raised against the below depicted DPP3 regions, were selected by their ability to bind recombinant DPP3 and/ or immunization peptide, as well as by their inhibitory potential.

All antibodies raised against the GST-tagged, full length form of recombinant hDPP3 show a strong
25 binding to immobilized GST-tagged hDPP3. Antibodies raised against the SEQ ID NO.: 35 peptide bind as well to GST-hDPP3. The SEQ ID NO.: 35 antibodies also strongly bind to the immunization peptide.

Table 6: list of antibodies raised against full-length or sequences of hDPP3 and their ability to bind hDPP3 (SEQ ID NO.: 34) or immunization peptide (SEQ ID NO.: 35) in RLU, as well as the maximum inhibition of recombinant GST-hDPP3.

Sequence number	Antigen/ Immunogen	hDPP3 region	Clone	hDPP3 binding [RLU]	immunization peptide binding [RLU]	Max. inhibition of hDPP3
SEQ ID NO.: 34	GST tagged recombinant FL-hDPP3	1-737	2552	3.053.621	0	65%
			2553	3.777.985	0	35%
			2554	1.733.815	0	30%
			2555	3.805.363	0	25%
SEQ ID NO.: 35	CETVINPETGEQIQSWYRSGE	474-493	1963	141.822	2.163.038	60%
			1964	100.802	2.041.928	60%
			1965	99.493	1.986.794	70%
			1966	118.097	1.990.702	65%
			1967	113.736	1.909.954	70%
			1968	105.696	2.017.731	65%
			1969	82.558	2.224.025	70%

The development of a luminescence immunoassay for the quantification of DPP3 protein concentrations (DPP3-LIA) as well as an enzyme capture activity assay for the quantification of DPP3 activity (DPP3-ECA) have been described recently (*Rehfeld et al. 2019. JALM 3(6): 943-953*), which is incorporated here in its entirety by reference.

Example 7 – DPP3 in shock

DPP3 concentration in plasma of patients with sepsis/ septic shock and cardiogenic shock was determined and related to the short term-mortality of the patients.

10 a) Study Cohort – Sepsis/Septic Shock

In 574 plasma samples from patients of the Adrenomedullin and Outcome in Severe Sepsis and Septic Shock (AdrenOSS-1) study DPP3 was measured. AdrenOSS-1 is a prospective, observational, multinational study including 583 patients admitted to the intensive care unit with sepsis or septic shock (Hollinger et al., 2018 Aug 22;3(6):1424-1433). 292 patients were diagnosed with septic shock.

15 b) Study Cohort – Cardiogenic Shock

Plasma samples from 108 patients that were diagnosed with cardiogenic shock were screened for DPP3. Blood was drawn within 6 h from detection of cardiogenic shock. Mortality was followed for 7 days.

5 hDPP3 immunoassay: An immunoassay (LIA) or an activity assays (ECA) detecting the amount of human DPP3 (LIA) or the activity of human DPP3 (ECA), respectively, was used for determining the DPP3 level in patient plasma. Antibody immobilization, labelling and incubation were performed as described in Rehfeld et al. (*Rehfeld et al. 2019. JALM 3(6): 943-953*).

10 Results: Short-term patients' survival in sepsis patients was related to the DPP3 plasma concentration at admission. Patients with DPP3 plasma concentration above 40.5 ng/mL (3rd Quartile) had an increased mortality risk compared to patients with DPP3 plasma concentrations below this threshold (Figure 7A). Applying this cut-off to the sub-cohort of septic shock patients, revealed an even more pronounced risk for short-term mortality in relation to high DPP3 plasma concentrations (Figure 7B). When the same cut-off is applied to patients with cardiogenic shock, also an increased risk for short-term mortality within 7 days is observed in patients with high DPP3 (Figure 7C).

Example 8 – NT-ADM antibodies in patients with septic shock (AdrenOSS-2)

AdrenOSS-2 is a double-blind, placebo-controlled, randomized, multicenter, proof of concept and dose-finding phase II clinical trial to investigate the safety, tolerability and efficacy of the N-terminal ADM antibody named Adrecizumab in patients with septic shock and elevated adrenomedullin (*Geven et al. BMJ Open 2019;9:e024475*). In total, 301 patients with septic shock and bio-ADM concentration > 70 pg/mL were randomized (2:1:1) to treatment with a single intravenous infusion over approximately 1 hour with either placebo (n=152), adrecizumab 2 ng/kg (n=72) or adrecizumab 4 ng/kg (n=77). All-cause mortality within 28 (90) days after inclusion was 25.8% (34.8%). Mean age was 68.4 years and 61% were male. For the per protocol analysis, n=294 patients remained eligible, and 14-day all-cause mortality rate was 18.5%.

In patients treated with Adrecizumab (both doses combined, per protocol population), a trend to lower short term mortality (14 days post admission) was observed compared to placebo (Hazard ratio (HR) 0.701 [0.408-1.21], p=0.100) (figure 8). Surprisingly, in patients with a DPP3 concentration on admission below 50 ng/mL, the treatment effect was more pronounced (n=244, HR 0.426, p=0.007) (figure 9), while in patients with an elevated DPP3 (above 50 ng/mL, n=44), outcome was comparable between Adrecizumab and placebo (HR 1.69, p=0.209) (figure 10).

Treatment effects for different DPP3 thresholds (14-day mortality) are summarized in table 7.

Table 7 Hazard risks (HR) for 14-day mortality with different pre-dose DPP3 concentrations

	n	HR	p-value (1-sided, log rank)
all	294	0.701	0.100
DPP3 <90	273	0.606	0.049
DPP3 <70	261	0.546	0.027
DPP3 <60	254	0.449	0.008
DPP3 <50	244	0.426	0.007
DPP3 <40	227	0.385	0.005

Example 9:

To show that the anti-ADM treatment works also in patients with bio-ADM levels lower than 70 pg/mL, it was investigated whether in the included patient population (all with bio-ADM >70 pg/mL) patients would exhibit a different treatment effect depending on their pre-dose bio-ADM concentration. No such

interaction was detected which in other words means, that the beneficial treatment effect was detectable for all patients independent of their pre-dose bio-ADM concentration. This finding strongly suggests, that treatment works also in patients with bio-ADM levels lower than 70 pg/mL, with the likely (and acceptable) restriction that it such effect might not be expected in patients with bio-ADM concentrations
5 in the healthy normal range.

P-value for interaction term between treatment and pre-dose bio-ADM:

- Per Protocol population: $p=0.279$
- Per Protocol population /with pre-dose DPP3<70 ng/mL: $p=0.150$

This is further illustrated in Kaplan Meier Plots, where the patient populations were separated by their
10 pre-dose bio-ADM concentration (below/above bio-ADM median) (see Figure 11a and Figure 11b).

SEQUENCES

SEQ ID No.: 1

GYTFSRYW

5

SEQ ID No.: 2

ILPGSGST

SEQ ID No.: 3

10 TEGYEYDGFYD

SEQ ID No.: 4

QSIVYSNGNTY

15 SEQUENCE "RVS" (not part of the Sequencing Listing):

RVS

SEQ ID No.: 5

FQGSHIPYT

20

SEQ ID No.: 6 (AM-VH-C)

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPBGHGLEWIGEILPGSGSTNYNE
KFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFYDWGQGTTTLTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV

25 TVPSSSLGTQTYICNVNHHKPSNTKVDKRVEPK

SEQ ID No.: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSKKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFYDWGQGTTTVTVSSASTKG

PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID No.: 8 (AM-VH2-E40)

5 QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCTEGYEYDGFQDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

10 SEQ ID No.: 9 (AM-VH3-T26-E55)

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCTEGYEYDGFQDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

15

SEQ ID No.: 10 (AM-VH4-T26-E40-E55)

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCTEGYEYDGFQDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
20 VTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID No.: 11 (AM-VL-C)

DVLLSQTPSLPVS LGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
25 LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No.: 12 (AM-VL1)

DVVM TQSPSLPVT LGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDSGVP
30 DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No.: 13 (AM-VL2-E40)

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
5 EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No.: 14 (human ADM 1-21)

YRQSMNNFQGLRSFGCRFGTC

10 SEQ ID No.: 15 (human ADM 21-32)

CTVQKLAHQIYQ

SEQ ID No.: 16 (human ADM C-42-52)

CAPRSKISPQGY-CONH₂

15

SEQ ID No.: 17 (murine ADM 1-19)

YRQSMNQGSRSNGCRFGTC

SEQ ID No.: 18 (murine ADM 19-31)

20 CTFQKLAHQIYQ

SEQ ID No.: 19 (murine ADM C-40-50)

CAPRNKISPQGY-CONH₂

25 SEQ ID No.: 20 (mature human Adrenomedullin (mature ADM); amidated ADM; bio-ADM); amino
acids 1-52 or amino acids 95 – 146 of pro-ADM

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVAPRSKISPQGY-CONH₂

30 SEQ ID No.: 21 (Murine ADM 1-50)

YRQSMNQGSRSNGCRFGTCTFQKLAHQIYQLTDKDKDGMAPRNKISPQGY-CONH₂

SEQ ID No.: 22 (1-21 of human ADM):

YRQSMNNFQGLRSFGCRFGTC

5

SEQ ID No.: 23 (1-42 of human ADM):

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA

SEQ ID No.: 24 (aa 43 – 52 of human ADM)

10 PRSKISPQGY-NH₂

SEQ ID No.: 25 (aa 1-14 of human ADM)

YRQSMNNFQGLRSF

15 SEQ ID No.: 26 (aa 1-10 of human ADM)

YRQSMNNFQG

SEQ ID No.: 27 (aa 1-6 of human ADM)

YRQSMN

20

SEQ ID No.: 28 (aa 1-32 of human ADM)

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQ

SEQ ID No.: 29 (aa 1-40 murine ADM)

25 YRQSMNQGSRSNGCRFGTCTFQKLAHQIYQLTDKDKDGMA

SEQ ID No.: 30 (aa 1-31 murine ADM)

YRQSMNQGSRSNGCRFGTCTFQKLAHQIYQL

SEQ ID No.: 31 (proADM: 164 amino acids (22 – 185 of preproADM))

ARLDVASEF RKKWNKWALS RGKRELRMSS SYPTGLADV K AGPAQTLIRP QDMKGASRSP
EDSSPDAARI RVKRYRQSMN NFQGLRSFGC RFGTCTVQKL AHQIYQFTDK DKDNVAPRSK
ISPQGYGRRR RRSLEAGPG RTLVS SKPQA HGAPAPPSGS APHFL

5

SEQ ID NO: 32 (Adrecizumab heavy chain)

QVQLVQSGAEVKKPGSSVKV SCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
KFQGRVTITADTSTSTAYMELSSLRSED TAVYYCTEGYEYDGF DYWGQGTTVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
10 TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTTPVLD
SDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

15

SEQ ID NO: 33 (Adrecizumab light chain)

DVVLTSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADY
20 EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No. 34 – human DPP3 (amino acid 1-737)

MADTQYILPNDIGVSSLDCREAFRLSPTERLYAYHLSRAAWYGGLAVLLQTSPEAPYIYALL
25 SRLFRAQDPDQLRQHAAEGLTEEEYQAF LVYAAGVYSNMGNYSFGDTKFVPNLPKEKLE
RVILGSEAAQQHP EEVRLWQTCGELMFSLEPRLRHLGLGKEGITT YFSGNCTMEDAKLAQD
FLDSQNLSAYNTRLFKEVDGEGKPYEVR LASVLGSEPSLDSEVTSKLKSYEFRGSPFQVTRG
DYAPILQKVVEQLEKAKAYAANSHQGQMLAQYIESFTQGSIEAHKRGSRFWIQDKGPIVESYI
GFIESYRDPFGSRGEFEGFVAVVNKAMS AKFERLVASAEQLLKELPWPPTFEKDKFLTPDFTS
30 LDVLT FAGSGIPAGINIPNYDDL RQTEGFKNVSLGNVLAVAYATQREKLT FLEEDDKDLYILW
KGPSFDVQVGLHELLGHGSGKLFVQDEKGAFNFDQETVINPETGEQIQSWYRSGETWDSKFS
TIASSYEECRAESVGLYLCLHPQVLEIFGFEGADAEDVIYVNWLN MV RAGLLALEFYTP EAFN
WRQAHMQARFVILRVLLEAGEGLVTITPTTGSDGRPDARVRLDRSKIRSVGKPALERFLRRLQ

VLKSTGDTVAGGRALYEGYATVTDAPPECFLTLRDTVLLRKESRKLIVQPNTRLEGSDVQLLE
YEASAAGLIRSFSEFPEDGPELEEILTQLATADARFWKGPSEAPSGQA

SEQ ID No. 35 – human DPP3 (amino acid 474-493 (N-Cys)) – immunization peptide with additional
5 N-terminal Cystein

CETVINPETGEQIQSWYRSGE

SEQ ID No. 36 - **IGHV1-69*11**

10 QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGTANYAQ
KFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARYYYYYGMDVWGQGTTTVTVSS

SEQ ID No. 37 - **HB3**

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPBGHGLEWIGEILPGSGSTNYNE
15 KFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFQYWGQGTTTLTVSS