

Anti-ADM-antibodies binding to the free N-terminus for accelerated transition of ADM-Gly to bio-ADM in patients with ADM-Gly/ bio-ADM ratio above a threshold and combination with vitamin C

5 Field of the invention

Subject matter of the present invention is an anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or an anti-ADM non-Ig scaffold for use in a patient to accelerate the conversion of ADM-Gly to ADM-NH₂ in said patient, wherein said patient is characterized by having a ratio of pro-Adrenomedullin or a fragment thereof to ADM-NH₂ (SEQ ID No. 20) above a certain threshold in a sample of bodily fluid, wherein said pro-Adrenomedullin or fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34) and wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:
YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23).

Background

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. Pre-proADM is subsequently converted into the 164 amino acid pro-ADM (SEQ ID No. 31) by cleavage of the N-terminal signal-peptide. Pro-ADM is further processed into pro-ADM N-terminal 20 peptide (PAMP; SEQ ID No. 32), midregional pro-ADM (MR-proADM; SEQ ID No. 33), adrenotensin pro-ADM 153–185 (CT-pro ADM; SEQ ID No. 34) and immature ADM, a C-terminally glycine-extended version of ADM (ADM-Gly; SEQ ID No. 21). This is converted into the mature bioactive form of ADM (bio-ADM; ADM-NH₂; SEQ ID No. 20) by enzymatic amidation of its C-terminus. More than half of the known neural and endocrine peptides require the formation of

a C-terminal alpha-amide group to gain full biological activity (*Guembe et al. 1999. J Histochem Cytochem 47(5): 623–36; Vishwanatha et al. 2013. Handbook of Biologically Active Peptides Peptidylglycine Amidating Monooxygenase (PAM). Second Edi. Elsevier Inc.*). This final step of peptide hormone biosynthesis involves the action of a bifunctional enzyme, the peptidylglycine alpha-amidating monooxygenase (PAM), that specifically recognizes C-terminal glycine (CT-Gly) residues in its substrates. PAM cleaves glyoxylate from the peptides CT-Gly residue in a two-step enzymatic reaction leading to the formation of c-terminally alpha-amidated peptide hormones, wherein the resulting alpha-amide group originates from the cleaved CT-Gly (*Prigge et al. 2000. Cellular and Molecular Life Sciences 57(8): 1236–59*). This amidation reaction takes place in the lumen of secretory granules prior to exocytosis of the amidated product (*Martinez et al. 1996. Am J Pathol 149(2): 707-16*).

In humans, the PAM gene is located at chromosome 5q21.1 having a length of 160 kb containing 25 known exons (*Gaier et al. 2014. BMC Endocrine Disorders 14*). At least 6 isoforms are known to be generated by alternative splicing (SEQ ID No. 39-44). The PAM enzyme was found to be expressed at different levels in almost all mammalian cell types, with significant expression in airway epithelium, endothelial cells, ependymal cells in the brain, adult atrium, brain, kidney, pituitary, gastrointestinal tract and reproductive tissues (*Chen et al. 2018. Diabetes Obes Metab 20 Suppl 2: 64-76; Oldham et al. 1992. Biochem Biophys Res Commun 184(1): 323–29; Schafer et al. 1992. J Neurosci 12(1): 222–34*).

The largest known PAM isoform 1 (SEQ ID No. 39) is the precursor protein (1-973 amino acids). The N-terminal signal sequence (amino acids 1-20) assures direction of the nascent PAM polypeptide into the secretory lumen of endoplasmic reticulum and is subsequently cleaved co-translationally. Afterwards the PAM-pro-peptide is processed by the same machinery used for the biosynthesis of integral membrane proteins and secreted proteins including cleavage of the pro-region (amino acids 21-30), assuring proper folding, disulfide bond formation, phosphorylation and glycosylation (*Bousquet-Moore et al. 2010. J Neurosci Res 88(12):2535-45*). The PAM cDNA encodes two distinct enzymatic activities: the first enzymatic activity is named peptidyl-glycine alpha-hydroxylating monooxygenase (PHM; EC 1.14.17.3), is an enzyme, capable of catalyzing the conversion of a C-terminal glycine residue to an alpha-hydroxy-glycine; the second activity is named peptidyl-a-hydroxy-glycine alpha-amidating lyase (PAL; EC 4.3.2.5) and is an enzyme capable of catalyzing the conversion of an alpha-hydroxy-glycine to an alpha-amide with subsequent glyoxylate release. The

sequential action of these separate enzymatic activities results in the overall peptidyl-glycine alpha amidating activity. The first enzymatic activity (PHM) is located directly upstream of the pro-region (within amino acids 31-494 of isoform 1 (SEQ ID No. 45)). The second catalytic activity (PAL) is located after exon 16 in isoform 1 within amino acids 495-817 (SEQ ID No. 46).

Both activities may be encoded together within one polypeptide as a membrane-bound protein (isoforms 1, 2, 5, 6; corresponding to SEQ ID No. 39, 40, 43 and 44) as well within one polypeptide as a soluble protein lacking the transmembrane domain (TMD; isoforms 3 and 4; corresponding to SEQ ID No. 41 and 42). While isoforms 1, 2, 5 and 6 remain in the outer plasma membrane after fusion of secretory vesicles with the plasma membrane with subsequent endocytosis and recycling or degradation, soluble PAM isoforms lacking the TMD (isoforms 3 and 4) (amino acids 864-887) are co-secreted with the peptide hormones (*Wand et al. 1985 Metabolism 34(11): 1044-52*). Furthermore, prohormone convertases may convert membrane bound PAM protein into soluble PAM protein by cleavage within the flexible region (exons 25/26) connecting PAL with the TMD during the secretory pathway (*Bousquet-Moore et al. 2010. J Neurosci Res 88(12): 2535-45*). The PHM subunit may be cleaved from soluble or membrane bound PAM within the secretory pathway by prohormone convertases that address a double-basic cleavage-site in the exon 16 region. Furthermore, during endocytosis the full-length PAM protein may be also converted into a soluble form due to the action of alpha- and gamma secretases (*Bousquet-Moore et al. 2010. J Neurosci Res 88(12): 2535-45*). Membrane bound PAM from late endosome can be further secreted in form of exosomal vesicles.

PHM and PAL activities, as well as the activity of the full-length PAM were determined in several human tissues and body fluids. However, the separated PHM and PAL activities in soluble forms will also lead to formation of c-terminally alpha amidated products from c-terminally glycinated substrates when allowed to perform their separate reactions in the same compartment, body fluid or in an *in vitro* experimental setup. How the transfer of the PHM hydroxylated product to the PAL takes place is not exactly understood to date. There is evidence that the hydroxylated product is released into solution and is not directly transferred from PHM to PAL (*Yin et al. 2011. PLoS One 6(12): e28679*). Also not clear to date is the source of PAM in circulation.

PHM is a copper dependent monooxygenase responsible for stereo-specific hydroxylation of the c-terminal glycine at the alpha-carbon atom. During the hydroxylation reaction ascorbate is believed to be the naturally occurring reducing agent, while the oxygen in the newly formed hydroxyl group was shown to originate from molecular oxygen. The catalytic action of PAL involves proton abstraction from the PHM-formed hydroxy-glycine by a protein-backbone derived base and a nucleophilic attack of hydroxyl-group oxygen to the divalent metal leading to a cleavage of glyoxylate and formation of a c-terminal amide.

Thus the term "amidating activity", "alpha-amidating activity", "peptidyl-glycine alpha-amidating activity" or "PAM activity" refers to the sequential enzymatic activities of PHM and PAL, independent of the present splice variant or mixtures of splice variants or post-translationally modified PAM enzymes or soluble, separated PHM or PAL activities or soluble PHM and membrane bound PAL or combinations of all mentioned forms leading to the formation of alpha-amidated products of peptide or non-peptide character from glycinated substrates of peptide or non-peptide character. In other words, the term "amidating activity", "alpha-amidating activity", "peptidyl-glycine alpha-amidating activity" or "PAM activity" may be described as the sequential action of enzymatic activities located within amino acids 31 to 817 in the propeptide encoded by the human PAM cDNA, independent of present splice-variants or mixtures thereof.

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*). 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. As mentioned above, 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 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 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 mentioned review articles above, *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*). 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 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 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*). Moreover, plasma concentrations of ADM are elevated in patients with heart failure and correlate with disease 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*).

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Kitamura and colleagues showed that the concentration of mature ADM and ADM-Gly was significantly elevated in plasma of hypertensive patients compared to healthy volunteers (*Kitamura et al. 1998. Biochem Biophys Res Comm 244(2): 551-5*). In both groups mature ADM was much lower than ADM-Gly. However, the ratio of mature ADM to ADM-Gly was not significantly different between hypertensive and non-hypertensive subjects.

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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 N-terminal 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 N-terminal 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.

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

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

WO2019/154900 describes a non-neutralizing N-terminal anti-ADM antibody for use in therapy and prevention of dementia. Moreover, WO2019/154900 describes a method for diagnosing and monitoring a (preventive) therapy of dementia by determining a ratio of the level of mature ADM to the level of pro-Adrenomedullin or a fragment thereof.

WO2013/072512 describes a non-neutralizing N-terminal anti-ADM antibody that is an ADM stabilizing antibody enhancing the half-life ($t_{1/2}$ half retention time) of adrenomedullin in serum, blood, plasma.

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*). In addition, antibodies against the mid-

regional part of ADM (MR-ADM antibodies) also significantly improved the survival in mice with CLP-induced sepsis, but to a lower extent when compared to N-terminal anti-ADM antibodies (*Struck et al. 2013. Intensive Care Med Exp 1(1):22*).

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: 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*). 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 endothelial cells (ECs; predominantly barrier stabilization), whereas ADM's detrimental effects on vascular smooth muscle cells (VSMCs; vasodilation) in the interstitium are reduced.

Vitamin C (ascorbic acid) is a water-soluble vitamin with a variety of antioxidant, anti-inflammatory, and microvascular effects. Vitamin C levels are known to be decreased in

critical illness and are associated with severity of illness. Supplemental vitamin C has shown promise in both animal models of sepsis and human trials in the intensive care unit (ICU) setting:

Preclinical research in early sepsis revealed that vitamin C prevented sepsis-induced cytokine
5 surges that activate and sequester neutrophils in lung, thus damaging alveolar capillaries
(*Fisher et al. 2012. Physiol Lung Cell Mol Physiol. 303(1): L20-L32; Fisher et al. 2011. Crit*
*Care Med. 39 (6): 1454-1460). Vitamin C increased alveolar fluid clearance by preventing
activated neutrophil accumulation in alveolar spaces, limiting alveolar epithelial water-
channel damage, and promoting their increased expression (*Fisher et al. 2011. Crit Care*
10 *Med. 39 (6): 1454-1460). In addition, vitamin C prevented neutrophil extracellular trap
formation, a biological event in activated neutrophils that promotes vascular injury
(*Mohammed et al. 2013 Nutrients 5(8): 3131-3151).***

In a recent, phase I trial in 24 medical intensive care unit (ICU) patients with severe sepsis,
15 high dose administration of vitamin C reduced the extent of multiple organ failure and
attenuated circulating injury biomarker levels (*Fowler et al. 2014. J Transl Med 12: 32*). In a
double-blinded randomized clinical trial, 28 adult surgical patients with septic shock had a
significantly decreased vasopressor requirement, a faster weaning of vasopressors and a
significantly lower 28-day mortality in the ascorbic acid, compared to the placebo group
20 (*Zhabet et al. 2016. Res Pharm Pract 5: 94–100*). Moreover, intravenous vitamin C therapy
reduced mortality in septic patients from 46% in the placebo group to almost 30% in the
vitamin C group at day 28 (*Fowler et al. 2019. JAMA 322(13):1261-1270*).

Surprisingly, the present invention shows that the ratio of ADM-Gly and bioactive ADM is
25 highly variable although current literature describes that only 5-20% of ADM is ADM-Gly
(*Kitamura et al. 1998. Biochemical and Biophysical Research Communications. 244(2): 551-*
555). Moreover, our data show for the first time that the conversion of ADM-Gly to bioactive
ADM also occurs within the circulation, notwithstanding that the amidation of peptides,
especially ADM, is described so far as an intracellular rather than an intravascular process
30 (*Kumar, Mains, Eipper 2016. Journal of Molecular Endocrinology. 56(4): T63-T76*). The
present invention also clearly demonstrates that N-terminal and mid-regional anti-ADM
antibodies, in addition to its effects described above, accelerate the conversion of ADM-Gly
to bioactive ADM in critically ill patients with an acute disease or condition. Surprisingly,
antibodies binding to the mid-regional part of ADM show a stronger effect than antibodies

binding to the N-terminal part of ADM and/ or ADM-Gly. In addition, the present invention clearly demonstrates that those N-terminal and mid-regional anti-ADM antibodies in combination with ascorbate significantly increase the enzymatic amidation of glycinated adrenomedullin to mature ADM catalyzed by PAM in plasma.

Description of the invention

Subject-matter of the present application is an anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said patient is characterized by having a ratio of pro-Adrenomedullin or a fragment thereof to ADM-NH₂ (SEQ ID No. 20) above a certain threshold in a sample of bodily fluid, wherein said pro-Adrenomedullin or fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34) and wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23).

In one embodiment, an anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said patient is characterized by having a ratio of pro-Adrenomedullin or a fragment thereof to ADM-NH₂ (SEQ ID No. 20) above a certain threshold in a sample of bodily fluid, wherein said pro-Adrenomedullin or fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34) and wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23).

and wherein the anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold is used in combination with L-ascorbic acid.

- 5 One 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 the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure
10 (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein 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-Gly and/ or ADM-NH₂:
15 YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

- One 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 the treatment of a critically ill patient suffering from an acute disease or condition selected from
20 the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in
25 said patient, wherein 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-Gly and/ or ADM-NH₂: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14). and wherein the anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold is used in combination with L-ascorbic acid.

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Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response

syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said 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.

Another preferred embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 21-42) of ADM-Gly and/or ADM-NH₂: CTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 48).

Another preferred embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 21-42) of ADM-Gly and/or ADM-NH₂: CTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 48).

Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung)

in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 21-32) of ADM-Gly and/ or ADM-NH₂: CTVQKLAHQIYQ (SEQ ID No.: 15).

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Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said
10 anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 21-32) of ADM-Gly and/ or ADM-NH₂: CTVQKLAHQIYQ (SEQ ID No.: 15).

Another embodiment of the present application relates to an anti-adrenomedullin (ADM)
15 antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and
20 symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 27-39) of ADM-Gly and/ or ADM-NH₂: AHQIYQFTDKDKD (SEQ ID No.: 49).

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Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said
30 anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 27-39) of ADM-Gly and/ or ADM-NH₂: AHQIYQFTDKDKD (SEQ ID No.: 49).

One embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody
35 or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a

critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein in a sample of bodily fluid of said patient the level of pro-Adrenomedullin or a fragment thereof consisting of the group of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34) and ADM-NH₂ (SEQ ID No. 20) is determined.

One preferred embodiment of the present application relates to an Anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein in a sample of bodily fluid of said patient a ratio of the level of ADM-Gly (SEQ ID No. 21) and ADM-NH₂ (SEQ ID No. 20) is determined and the patient is treated with said anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold if the ratio is above a certain threshold, wherein the ADM-Gly/ ADM-NH₂ ratio is in a range between 1 and 10, preferably between 1.5 and 7.5, preferably between 2 and 5, most preferred the threshold is 2.5.

Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating

ADM-Gly in said 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.

5 A further embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and
10 symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said sample is selected from the group comprising human citrate plasma, heparin plasma and EDTA plasma.

15 Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure
20 (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein the ratio of pro-Adrenomedullin or a fragment thereof and ADM-NH₂ of the patient is determined. In a most preferred embodiment, the ratio is measured
25 using an immunoassay.

Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from
30 the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating

ADM-Gly in said patient, wherein an immunoassay is used for determining the ratio of pro-Adrenomedullin or a fragment thereof and ADM-NH₂, wherein said pro-Adrenomedullin or fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34).

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Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said immunoassay is a sandwich immunoassay, preferably a fully automated assay.

One embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein the assay sensitivity of said assay for the detection of ADM-NH₂ is able to quantify ADM-NH₂ of healthy subjects and is < 70 pg/ml, preferably < 40 pg/ml and more preferably < 10 pg/ml.

Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and

symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein the assay sensitivity of said assay for ADM-Gly is able to quantify ADM-Gly of healthy subjects and is 20 pg/ml, preferably 15 pg/ml and more preferably 10 pg/ml.

One embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein the assay sensitivity of said assay for the detection of MR-proADM is able to quantify MR-proADM of healthy subjects and is < 0.5 nmol/L, preferably < 0.4 nmol/L and more preferably < 0.2 nmol/L.

Another embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein the assay sensitivity of said assay for PAMP is able to quantify PAMP of healthy subjects and is < 0.5 pmol/L, preferably < 0.25 pmol/L and more preferably < 0.1 pmol/L.

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One embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome

(SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein the assay sensitivity of said assay for the detection of CT-proADM is able to quantify CT-proADM of healthy subjects and is < 100 pmol/L, preferably < 75 pmol/L and more preferably < 50 pmol/L.

A further embodiment of the present application relates to an anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein the level of pro-Adrenomedullin or a fragment thereof and ADM-NH₂ (SEQ ID No. 20) is determined by using one binder to said pro-Adrenomedullin or a fragment thereof and a second binder to ADM-NH₂ (SEQ ID No. 20), wherein said proAdrenomedullin or a fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34) and wherein both binders are selected from the group comprising an antibody, an antibody fragment or a non-Ig-Scaffold binding to said pro-Adrenomedullin or a fragment thereof and ADM-NH₂.

Another embodiment of the present application relates to an anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy of a patient, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23), wherein in a sample of bodily fluid of said patient the level of PAM and/or its isoforms and/or fragments thereof is determined and the patient is treated with said anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold, if the level of PAM is below a threshold.

Another embodiment of the present application relates to an anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy of a patient, wherein said level of PAM and/or its isoforms and/or fragments thereof is the total concentration of PAM and/or its isoforms and/or fragments thereof having at least 12 amino acids or the activity of PAM and/or its isoforms and/or fragments thereof comprising the sequences SEQ ID No. 39, SEQ ID No. 40, SEQ ID No. 41, SEQ ID No. 42, SEQ ID No. 43, SEQ ID No. 44, SEQ ID No. 45, SEQ ID No. 46 and SEQ ID No. 47.

It is to be understood by the skilled artisan, that the PAM isoform sequences (SEQ ID No. 39 to 44) as represented in the sequence list, contain an N-terminal signal sequence (amino acid 1-20), that is cleaved off prior to secretion of the protein. Therefore, in a preferred embodiment the PAM isoform sequences (SEQ ID No. 39 to 44) do not contain the N-terminal signal sequence.

Another embodiment of the present application relates to an anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy of a patient, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal and/or mid-regional part (amino acid 1-42) of ADM-Gly and/or ADM-NH₂: YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23) and is to be used in combination with L-ascorbic acid.

In a preferred embodiment, the anti-Adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold in combination with L-ascorbic acid,

- a. for use in therapy of an acute disease or acute condition of a patient for stabilizing the systemic circulation of said patient wherein said patient is in need of stabilizing the systemic circulation and exhibits a heart rate of > 100 beats/min and/or < 65 mm Hg mean arterial pressure and wherein stabilizing the systemic circulation means increasing the mean arterial pressure over 65 mmHg, or
- b. for use in the prevention of a heart rate increase to > 100 beats/min and/or a mean arterial pressure decrease to < 65 mm Hg in patients having an acute disease or acute condition, or
- c. for use in therapy of an acute disease or acute condition of a patient that suffers from a chronic and/or acute disease or acute condition for prevention or reduction of organ dysfunction or prevention of organ failure in said patient and wherein said

organ is selected from the group comprising heart, kidney, liver, lungs, pancreas, small intestines and spleen, or

d. for use in therapy or prevention of SIRS, meningitis, sepsis, shock, *e.g.*, septic shock in a patient

5 e. for the reduction of the mortality risk in patient with SIRS, meningitis, sepsis, shock, *e.g.*, septic shock.

wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

10 YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23).

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 intervention and therapy
15 of a patient and its use in combination with L-ascorbic acid, wherein said L-ascorbic acid is a single enantiomer, a mixture of enantiomers, a mixture of diastereomers or a pharmaceutically acceptable salt, solvate, hydrate or prodrug thereof.

According to the present invention it has been found that the administration of an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold may be used in a
20 patient to accelerate the conversion of ADM-Gly to ADM-NH₂, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No.: 23).

25 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 can be referred to as “anti-ADM antibodies”, “anti-ADM antibody fragments”, or “anti-ADM non-Ig scaffolds”.

30 In one embodiment, the ascorbic acid compound is L-ascorbic acid or a pharmaceutically acceptable salt thereof; or a pharmaceutically acceptable solvate or hydrate thereof. L-Ascorbic acid is also known as vitamin C, L-xyloascorbic acid, 3-oxo-L-gulofuranolactone (enol form), L-3-ketothreohexuronic acid lactone, antiscorbutic vitamin, cevitamic acid,

adenex, allercorb, ascorin, ascortear, ascorvit, cantan, cantaxin, catavin C, cebicure, cebion, cecon, cegiolan, celaskon, celin, cenetone, cereon, cergona, cescorbat, cetamid, cetabe, cetemican, cevalin, cevatine, cevex, cevimin, ce-vi-sol, cevitan, cevitec, cewin, ciamin, cipca, concemin, C-vin, daviamon C, duoscorb, hybrin, laroscorbine, lemascorb, planavit C, 5 proscorbin, redoxon, ribena, scorbacid, scorbu-C, testascorbic, vicelat, vitacee, vitacimin, vitacin, vitascorbol, and xitix.

In one embodiment, the ascorbic acid compound is L-ascorbic acid. In another embodiment, the ascorbic acid compound is a pharmaceutically acceptable salt of L-ascorbic acid, or a 10 pharmaceutically acceptable solvate or hydrate thereof.

Suitable bases for forming a pharmaceutically acceptable salt of L-ascorbic acid include, but are not limited to, inorganic bases, such as magnesium hydroxide, calcium hydroxide, potassium hydroxide, zinc hydroxide, and sodium hydroxide; and organic bases, such as 15 primary, secondary, tertiary, and quaternary, aliphatic and aromatic amines, including, but not limited to, L-arginine, benethamine, benzathine, choline, deanol, diethanolamine, diethylamine, dimethylamine, dipropylamine, diisopropylamine, 2-(diethylamino)-ethanol, ethanolamine, ethylamine, ethylenediamine, isopropylamine, N-methyl-glucamine, hydrabamine, 1H-imidazole, L-lysine, morpholine, 4-(2-hydroxyethyl)-morpholine, 20 methylamine, piperidine, piperazine, propylamine, pyrrolidine, 1-(2-hydroxyethyl)-pyrrolidine, pyridine, quinuclidine, quinoline, isoquinoline, triethanolamine, trimethylamine, triethylamine, N-methyl-D-glucamine, 2-amino-2-(hydroxymethyl)-1,3-propanediol, and tromethamine.

25 In one embodiment, the ascorbic acid compound is an alkali or alkaline earth metal salt of L-ascorbic acid, or a pharmaceutically acceptable solvate or hydrate thereof. In another embodiment, the ascorbic acid compound is sodium, potassium, calcium, or magnesium L-ascorbate, or a pharmaceutically acceptable solvate or hydrate thereof. In yet another embodiment, the ascorbic acid compound is sodium L-ascorbate, or a pharmaceutically 30 acceptable solvate or hydrate thereof. In yet another embodiment, the ascorbic acid compound is sodium L-ascorbate, which is also known as vitamin C sodium, ascorbin, sodascorbate, natrascorb, cenolate, ascorbicin, or cebitate. In yet another embodiment, the ascorbic acid compound is potassium L-ascorbate, or a pharmaceutically acceptable solvate or hydrate thereof. In yet another embodiment, the ascorbic acid compound is calcium L-ascorbate, or a

pharmaceutically acceptable solvate or hydrate thereof. In yet another embodiment, the ascorbic acid compound is calcium L-ascorbate. In yet another embodiment, the ascorbic acid compound is magnesium L-ascorbate, or a pharmaceutically acceptable solvate or hydrate thereof. In still another embodiment, the ascorbic acid compound is magnesium L-ascorbate.

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In certain embodiments, the ascorbic acid compound is D-ascorbic acid or a pharmaceutically acceptable salt, or a pharmaceutically acceptable solvate or hydrate thereof.

In one embodiment, the ascorbic acid compound is D-ascorbic acid. In another embodiment, the ascorbic acid compound is a pharmaceutically acceptable salt of D-ascorbic acid, or a pharmaceutically acceptable solvate or hydrate thereof.

Suitable bases for forming a pharmaceutically acceptable salt of D-ascorbic acid include, but are not limited to, inorganic bases, such as magnesium hydroxide, calcium hydroxide, potassium hydroxide, zinc hydroxide, and sodium hydroxide; and organic bases, such as primary, secondary, tertiary, and quaternary, aliphatic and aromatic amines, including, but not limited to, L-arginine, benethamine, benzathine, choline, deanol, diethanolamine, diethylamine, dimethylamine, dipropylamine, diisopropylamine, 2-(diethylamino)-ethanol, ethanolamine, ethylamine, ethylenediamine, isopropylamine, N-methyl-glucamine, hydrabamine, 1H-imidazole, L-lysine, morpholine, 4-(2-hydroxyethyl)-morpholine, methylamine, piperidine, piperazine, propylamine, pyrrolidine, 1-(2-hydroxyethyl)-pyrrolidine, pyridine, quinuclidine, quinoline, isoquinoline, triethanolamine, trimethylamine, triethylamine, N-methyl-D-glucamine, 2-amino-2-(hydroxymethyl)-1,3-propanediol, and tromethamine.

25

In one embodiment, the ascorbic acid compound is an alkali or alkaline earth metal salt of D-ascorbic acid, or a pharmaceutically acceptable solvate or hydrate thereof. In another embodiment, the ascorbic acid compound is sodium, potassium, calcium, or magnesium D-ascorbate, or a pharmaceutically acceptable solvate or hydrate thereof. In yet another embodiment, the ascorbic acid compound is sodium D-ascorbate, or a pharmaceutically acceptable solvate or hydrate thereof. In yet another embodiment, the ascorbic acid compound is sodium D-ascorbate, which is also known as vitamin C sodium, ascorbin, sodascorbate, natrascorb, cenolate, ascorbicin, or cebitate. In yet another embodiment, the ascorbic acid compound is potassium D-ascorbate, or a pharmaceutically acceptable solvate or hydrate

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thereof. In yet another embodiment, the ascorbic acid compound is calcium D-ascorbate, or a pharmaceutically acceptable solvate or hydrate thereof. In yet another embodiment, the ascorbic acid compound is calcium D-ascorbate. In yet another embodiment, the ascorbic acid compound is magnesium D-ascorbate, or a pharmaceutically acceptable solvate or hydrate thereof. In still another embodiment, the ascorbic acid compound is magnesium D-ascorbate.

The term "solvate" refers to a complex or aggregate formed by one or more molecules of a solute, e.g., a compound provided herein, and one or more molecules of a solvent, which present in stoichiometric or non-stoichiometric amount. Suitable solvents include, but are not limited to, water, methanol, ethanol, n-propanol, isopropanol, and acetic acid. In certain embodiments, the solvent is pharmaceutically acceptable. In one embodiment, the complex or aggregate is in a crystalline form. In another embodiment, the complex or aggregate is in a non-crystalline form. Where the solvent is water, the solvate is a hydrate. Examples of hydrates include, but are not limited to, a hemihydrate, monohydrate, dihydrate, trihydrate, tetrahydrate, and pentahydrate.

In one embodiment, the ascorbic acid compound in each of the pharmaceutical compositions is independently L-ascorbic acid or a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate or hydrate thereof. In another embodiment, the ascorbic acid compound in each of the pharmaceutical compositions is independently an alkali or alkaline earth metal salt of L-ascorbic acid, or a pharmaceutically acceptable solvate or hydrate thereof; or a mixture thereof. In yet another embodiment, the ascorbic acid compound in each of the pharmaceutical compositions is independently sodium, potassium, calcium, or magnesium salt of L-ascorbic acid, or a pharmaceutically acceptable solvate or hydrate thereof; or a mixture thereof. In yet another embodiment, the ascorbic acid compound in each of the pharmaceutical compositions is independently sodium L-ascorbate. In yet another embodiment, the ascorbic acid compound in each of the pharmaceutical compositions is independently calcium L-ascorbate. In yet another embodiment, the ascorbic acid compound in each of the pharmaceutical compositions is independently magnesium L-ascorbate. In still another embodiment, the ascorbic acid compound in each of the pharmaceutical compositions is independently a mixture of two or three of sodium L-ascorbate, calcium L-ascorbate, and magnesium L-ascorbate.

In a specific embodiment said anti-ADM antibody or anti-ADM antibody fragment binding to ADM or anti-ADM non-Ig scaffold binding to ADM may be administered in a patient to accelerate the conversion of ADM-Gly to ADM-NH₂ with help of a diagnostic method. Said diagnostic method is described below.

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

Said ratio level is determined that maybe the ratio of the level of pro-Adrenomedullin or a
10 fragment thereof and ADM-NH₂ (SEQ ID No. 20), wherein said pro-Adrenomedullin or
fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-
proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34)
in a sample of bodily fluid of said subject to the level of bio-ADM determined in a sample of
bodily fluid of said subject and said marker ratio is compared to a threshold ratio. If said ratio
15 level of pro-Adrenomedullin or a fragment thereof to ADM-NH₂ is above a certain threshold
level, the anti-ADM antibody or anti-ADM antibody fragment binding to ADM or anti-ADM
non-Ig scaffold binding to ADM is administered as therapy or intervention to said patient.

This means that in a specific embodiment of the present invention said anti-ADM antibody or
20 anti-ADM antibody fragment binding to ADM or anti-ADM non-Ig scaffold binding to ADM
is for use in a patient to accelerate the conversion of ADM-Gly to ADM-NH₂, wherein a
sample of bodily fluid taken from said patient exhibits an elevated level ratio of pro-
Adrenomedullin or a fragment thereof to ADM-NH₂ above a certain threshold, wherein said
pro-Adrenomedullin or fragment thereof is selected from the group consisting of PAMP
25 (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-
proADM (SEQ ID No. 34).

Subject matter of the present invention is a diagnostic method, wherein the level of ADM-Gly
and ADM-NH₂ is determined by using a binder to ADM-Gly and ADM-NH₂.

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Subject matter of the present invention is a diagnostic method, wherein the binder is selected
from the group comprising an antibody, an antibody fragment or a non-Ig-Scaffold binding to
pro-Adrenomedullin or a fragment thereof and ADM-NH₂, wherein said pro-Adrenomedullin

or fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34).

The term "sample" as used herein refers to a sample of bodily fluid obtained for the purpose of diagnosis, prognosis, or evaluation of a subject of interest, such as a patient. Preferred test samples include blood, serum and plasma. In addition, one of skill in the art would realize that some test samples would be more readily analyzed following a fractionation or purification procedure, for example, separation of whole blood into serum or plasma components.

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 diagnostic method said sample is selected from the group comprising human citrate plasma, heparin plasma and EDTA plasma.

In a specific embodiment of the present invention, an assay is used for determining the ratio of pro-Adrenomedullin or a fragment thereof and ADM-NH₂, wherein said level of pro-Adrenomedullin or a fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34), wherein the assay sensitivity of said assay for ADM-NH₂ is able to quantify the mature ADM-NH₂ of healthy subjects and is < 70 pg/ml, preferably < 40 pg/ml and more preferably < 10 pg/ml.

In a specific embodiment of the present invention, an assay is used for determining the ratio of pro-Adrenomedullin or a fragment thereof and ADM-NH₂, wherein said level of pro-Adrenomedullin or a fragment thereof is ADM-Gly (SEQ ID No. 21) and wherein the assay sensitivity of said assay for ADM-Gly is able to quantify ADM-Gly of healthy subjects and is 20 pg/ml, preferably 15 pg/ml and more preferably 10 pg/ml.

In a specific embodiment of the present invention, an assay is used for determining the ratio of pro-Adrenomedullin or a fragment thereof and ADM-NH₂, wherein said level of pro-Adrenomedullin or a fragment thereof is MR-proADM (SEQ ID No. 33) and wherein the assay sensitivity of said assay is able to quantify MR-proADM of healthy subjects and is < 0.5 nmol/L, preferably < 0.4 nmol/L and more preferably < 0.2 nmol/L.

In a specific embodiment of the present invention, an assay is used for determining the ratio of pro-Adrenomedullin or a fragment thereof and ADM-NH₂, wherein said level of pro-Adrenomedullin or a fragment thereof is CT-proADM (SEQ ID No. 34) and wherein the assay sensitivity of said assay is able to quantify CT-proADM of healthy subjects and is < 100 pmol/L, preferably < 75 pmol/L and more preferably < 50 pmol/L.

In a specific embodiment of the present invention, an assay is used for determining the ratio of pro-Adrenomedullin or a fragment thereof and ADM-NH₂, wherein said level of pro-Adrenomedullin or a fragment thereof is PAMP (SEQ ID No. 34) and wherein the assay sensitivity of said assay is able to quantify PAMP of healthy subjects and is < 0.5 pmol/L, preferably < 0.25 pmol/L and more preferably < 0.1 pmol/L.

In one embodiment of the subject matter of the present invention the ratio threshold of ADM-Gly and ADM-NH₂ is in a range between 1 and 10, preferably between 1.5 and 7.5, preferably between 2 and 5, most preferred the threshold is 2.5.

For the calculation of the ratio, the concentration of the two markers has to be preferably expressed in the same unit (e.g., pg/ml).

Both marker levels are used to conduct a calculation which maybe either a ratio of both markers (e.g., ratio between ADM-Gly and ADM-NH₂ or ratio between ADM-NH₂ and ADM-Gly), or a mathematical formula in which both markers are introduced or a mathematical algorithm in which both markers are introduced. The outcome of such a ratio or mathematical formula or mathematical algorithm maybe a value that is then compared with a predetermined threshold value and this comparison is then used in a patient to accelerate the conversion of ADM-Gly to ADM-NH₂ with an anti-ADM antibody or anti-ADM antibody fragment binding to ADM or anti-ADM non-Ig scaffold binding to ADM.

Threshold levels can be obtained for instance from a Kaplan-Meier analysis, where the occurrence of a disease is correlated with the quartiles of the marker ratio in the population. According to this analysis, subjects with marker ratios above, e.g., the 75th percentile have a significantly increased risk, e.g., for getting the diseases or suffering from an adverse event (e.g., mortality) according to the invention.

Other preferred threshold values are for instance the 90th, 95th or 99th percentile of a normal population. By using a higher percentile than the 75th percentile, one reduces the number of false positive subjects identified, but one might miss to identify subjects, who are at moderate, albeit still increased risk. Thus, one might adopt the threshold value depending on whether it is considered more appropriate to identify most of the subjects at risk at the expense of also identifying "false positives", or whether it is considered more appropriate to identify mainly the subjects at high risk at the expense of missing several subjects at moderate risk.

The above-mentioned threshold values might be different when using other assays, if these have been calibrated differently from the assay systems used in the present invention.

Therefore, the above-mentioned thresholds 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 assays in question (e.g., bio-ADM assay) with the respective biomarker assay used in the present invention by measuring the respective biomarker (e.g., bio-ADM) 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 (e.g., *Weber et al. 2017. JALM 2(2): 222-233*) 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 13.7 pg/ml (inter quartile range [IQR] 9.6 – 18.7 pg/mL) (*Weber et al. 2017. JALM 2(2): 222-233*).

In a specific embodiment of the diagnostic method, said binder exhibits a binding affinity to pro-Adrenomedullin or a fragment thereof (which is not ADM-NH₂ according to SEQ ID No.: 20) and 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.

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 capture kit; GE Healthcare), (*Lorenz et al. 2011. Antimicrob Agents Chemother. 55 (1): 165–173*).

- 5 In a specific embodiment of the diagnostic method, an assay is used for determining the level of pro-Adrenomedullin or a fragment thereof and ADM-NH₂, wherein said level of pro-Adrenomedullin or a fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34) and wherein such assay is a sandwich assay, preferably a fully automated
10 assay.

In one embodiment of the invention, it may be a so-called POC-test (point-of-care) that is a test 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
15 is the immunochromatographic test technology.

In one embodiment of the diagnostic method 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
20 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 Centauer®, Brahms Kryptor®, Biomerieux Vidas®, Alere Triage®.

25 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.

30

In a specific embodiment of the diagnostic method, at least one of said two binders is labeled in order to be detected.

Subject matter of the present invention is an anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold for use in a patient to accelerate the conversion of ADM-Gly to ADM-NH₂, wherein said patient is a critically ill patient suffering from an acute disease or condition selected from the group comprising:
5 severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia.

10 Heart failure (HF) is a cardiac condition that occurs, when a problem with the structure or function of the heart impairs its ability to supply sufficient blood flow to meet the body's needs. It can cause a large variety of symptoms, particularly shortness of breath (SOB) at rest or during exercise, signs of fluid retention such as pulmonary congestion or ankle swelling and objective evidence of an abnormality of the structure or function of the heart at rest.

15

Heart failure is a clinical syndrome characterized by a constellation of symptoms and signs caused by cardiac dysfunction. It is one of the major causes of morbidity and mortality in the developed countries, with a prevalence of 1-2%. Heart failure can be grouped into chronic HF and acute HF. Patients with chronic HF can be grouped into stable chronic HF, worsening
20 signs and symptoms of chronic HF and acute decompensation of chronic HF. Acute heart failure (AHF) is defined as a rapid onset of signs and symptoms of heart failure resulting in the need for urgent therapy or hospitalization. AHF can present as acute de novo HF (new onset of AHF in a patient without previous cardiac dysfunction) or acute decompensation of chronic HF. AHF is the leading cause of hospitalization in adults older than 65 years of age.

25 Despite marked improvements in the prognosis of chronic heart failure patients primarily related to therapeutic advances over the past few decades, both short- and long-term outcomes remain very poor once patients are hospitalized for decompensated heart failure. Nearly 25% of patients hospitalized for AHF need readmission within 30 days of hospital discharge while <50% survive beyond 5 years after hospitalization.

30

Heart failure comprises a wide range of patients, from those with normal left ventricular ejection fraction (LVEF) typically considered as $\geq 50\%$, also known as HF with preserved EF (HFpEF) to those with reduced LVEF, typically considered as $< 40\%$, also known as HF with reduced EF (HFrEF). Patients with an LVEF in the range of 40–49% represent a 'grey area',

which is defined as HF with mid-range EF (HFmrEF) (*Ponikowski et al. 2016. European Heart Journal 18(8): 891-975*).

Symptoms/signs of congestion (left-sided) are defined as orthopnoea, paroxysmal nocturnal dyspnoea, pulmonary rales (bilateral), peripheral edema (bilateral). Symptoms/signs of congestion (right-sided) are defined as jugular venous dilatation, peripheral edema (bilateral), congested hepatomegaly, hepatojugular reflux, ascites, symptoms of gut congestion (for review see table 12.2 in *Ponikowski et al. 2016. Eur Heart J. ehv128*).

Edema is an accumulation of fluid in the intercellular tissue that results from an abnormal expansion in interstitial fluid volume. The fluid between the interstitial and intravascular spaces is regulated by the capillary hydrostatic pressure gradient and the oncotic pressure gradient across the capillary (*Trayes et al. 2013. Am Fam Physician 88(2):102-110*). The accumulation of fluid occurs when local or systemic conditions disrupt this equilibrium, leading to increased capillary hydrostatic pressure, increased plasma volume, decreased plasma oncotic pressure (hypoalbuminemia), increased capillary permeability, or lymphatic obstruction.

Clinically, edema manifests as swelling: the amount of interstitial fluid is determined by the balance of fluid homeostasis, and the increased secretion of fluid into the interstitium, or the impaired removal of the fluid can cause edema. A rise in hydrostatic pressure occurs in cardiac failure. Causes of edema which are generalized to the whole body can cause edema in multiple organs and peripherally. For example, severe heart failure can cause pulmonary edema, pleural effusions, ascites and peripheral edema.

Pulmonary edema is fluid accumulation in the air spaces and parenchyma of the lungs. It leads to impaired gas exchange and may cause respiratory failure. It is due to either failure of the left ventricle of the heart to adequately remove blood from the pulmonary circulation ("cardiogenic pulmonary edema"), or an injury to the lung parenchyma or vasculature of the lung ("noncardiogenic pulmonary edema") (*Ware and Matthay 2005. N. Engl. J. Med. 353 (26): 2788-96*). Treatment is focused on three aspects: firstly, improving respiratory function, secondly, treating the underlying cause, and thirdly avoiding further damage to the lung. Pulmonary edema, especially acute, can lead to fatal respiratory distress or cardiac arrest due to hypoxia. It is a cardinal feature of congestive heart failure.

The term "acute" is used to mean rapid onset and to describe exacerbated or decompensated heart failure, referring to episodes in which a patient can be characterized as having a change in heart failure signs and symptoms resulting in a need for urgent therapy or hospitalization.

- 5 The term "chronic" refers to long duration. Chronic heart failure is a long-term condition, usually kept stable by the treatment of symptoms (stable chronic HF).

10 Stable chronic HF is characterized by: the presence of structural or functional failure of the heart that impairs its ability to supply sufficient blood flow to meet body's needs, the absence of volume overload (manifested by pulmonary and/ or systemic congestion) and/ or profound depression of cardiac output (manifested by hypotension, renal insufficiency and/ or a shock syndrome), and whereas the patient is not in need of urgent therapy or therapy adjustment and does not require hospitalization.

- 15 Chronic HF with worsening signs and symptoms is characterized by: the presence of structural or functional failure of the heart that impairs its ability to supply sufficient blood flow to meet body's needs, volume overload (manifested by pulmonary and/ or systemic congestion) and/ or profound depression of cardiac output (manifested by hypotension, renal insufficiency and/ or a shock syndrome), and whereas the patient is not in need of urgent
20 therapy and does not require hospitalization, but is in need of therapy adjustment.

Chronic heart failure may also decompensate (termed acute decompensated heart failure or acute decompensated chronic heart failure), which is most commonly the result from an intercurrent illness (such as pneumonia), myocardial infarction, arrhythmias, uncontrolled
25 hypertension or a patient's failure to maintain fluid restriction, diet or medication. After treatment, patients with acute decompensated chronic HF may return to a stable chronic compensated status (stable chronic HF).

- 30 New onset acute HF and acute decompensated chronic HF are characterized by: the presence of structural or functional failure of the heart that impairs its ability to supply sufficient blood flow to meet body's needs, volume overload (manifested by pulmonary and/ or systemic congestion) and/ or profound depression of cardiac output (manifested by hypotension, renal insufficiency and/ or a shock syndrome), and whereas the patient is in need of urgent therapy or therapy adjustment and does require hospitalization.

- Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (see *Singer et al. 2016. JAMA 315(8): 801-810*). Organ dysfunction can be identified as an acute change in total SOFA score ≥ 2 points consequent to the infection. The baseline SOFA score can be assumed to be zero in patients not known to have preexisting organ dysfunction. A SOFA score ≥ 2 reflects an overall mortality risk of approximately 10% in a general hospital population with suspected infection. Even patients presenting with modest dysfunction can deteriorate further, emphasizing the seriousness of this condition and the need for prompt and appropriate intervention, if not already being instituted. Sepsis is a life-threatening condition that arises when the body's response to an infection injures its own tissues and organs. Patients with suspected infection who are likely to have a prolonged ICU stay or to die in the hospital can be promptly identified at the bedside with qSOFA, i.e., alteration in mental status, systolic blood pressure ≤ 100 mm Hg, or respiratory rate ≥ 22 /min.
- Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality. Patients with septic shock can be identified with a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain mean arterial pressure (MAP) ≥ 65 mm Hg and having a serum lactate level >2 mmol/L (18 mg/dL) despite adequate volume resuscitation. With these criteria, hospital mortality is in excess of 40%.

- Dementia is a clinical syndrome characterized by a cluster of symptoms and signs manifested by difficulties in memory, disturbances in language, psychological and psychiatric changes, and impairments in activities of daily living. The different causes (sometimes referred to as subtyping) of dementia syndrome are: Alzheimer's disease (about 50% of cases), vascular dementia (about 25%), mixed Alzheimer's disease and vascular dementia (included in the above, 25%), Lewy body dementia (15%) and others (about 5% combined) including frontotemporal dementia, focal dementias (such as progressive aphasia), subcortical dementias (such as Parkinson's disease dementia), and secondary causes of dementia syndrome (such as intracranial lesions).

Alzheimer's disease (AD) is the most prevalent form of dementia. AD is increasing rapidly in frequency as the world's population ages and more people enter the major risk period for this age-related disorder. From the 5.3 million US citizens affected now, the number of victims

will increase to 13 million or more by 2050; worldwide the total number of affected individuals will increase to a staggering 100 million (*Alzheimer's Association. 2015 Alzheimer's disease facts and figures. Alzheimers Dement 11: 332–84*). Key molecular mechanisms and histopathological hallmarks in the AD brain comprise a dynamic cascade of biochemical events including the pathological amyloidogenic cleavage of the amyloid precursor protein (APP), the generation of various beta-amyloid species including the amyloid-beta peptide ($A\beta_{1-42}$), dimers, trimers, oligomers and subsequent amyloid aggregation and deposition in plaques, abnormal hyperphosphorylation and aggregation of tau protein, progressive intracellular neurofibrillary degeneration, changes within the innate immune system and inflammation.

About 5% of patients develop symptoms before age 65 and are characterized as patients with “early-onset Alzheimer's disease” (EOAD). Most of these patients have the sporadic form of the disease, but 10-15% have a genetic form that is generally inherited as an autosomal dominant fashion. Three genes have been suggested to be involved in the development of EOAD: Presenilin 1 and 2 and the amyloid precursor protein (APP) gene. Other candidate genes are also under investigation. Genetic forms tend to start at age 30 or 40 and have an aggressive course while sporadic EOAD tend to start after age 50 and have, in general, a temporal profile similar to the “late onset Alzheimer's disease” (LOAD) one.

Mental status testing evaluates memory, ability to solve simple problems and other thinking skills. Such tests give an overall sense of whether a person is aware of symptoms, knows the date, time, and where he or she is, can remember a short list of words, follow instructions and do simple calculations. The mini-mental state exam (MMSE) and the mini-cog test are two commonly used tests. The MMSE or Folstein test is a 30-point questionnaire that is used extensively in clinical and research settings to measure cognitive impairment (*Pangman, et al. 2000. Applied Nursing Research 13 (4): 209–213; Folstein et al. 1975. Journal of Psychiatric Research. 12 (3): 189–98*). During the MMSE, a health professional asks a patient a series of questions designed to test a range of everyday mental skills. The maximum MMSE score is 30 points. A score of 20 to 24 suggests mild dementia, 13 to 20 suggests moderate dementia, and less than 12 indicates severe dementia. On average, the MMSE score of a person with Alzheimer's declines about two to four points each year. Advantages to the MMSE include requiring no specialized equipment or training for administration, and has both validity and reliability for the diagnosis and longitudinal assessment of Alzheimer's disease. During the

- mini-cog, a person is asked to complete two tasks, remember and a few minutes later repeat the names of three common objects and draw a face of a clock showing all 12 numbers in the right places and a time specified by the examiner. The results of this brief test can help a physician determine if further evaluation is needed. Other tests are also used, such as the
- 5 Hodkinson abbreviated mental test score (*Hodkinson 1972. Age and ageing. 1 (4): 233–8*) or the General Practitioner Assessment of Cognition, computerized tests such as CoPs and Mental Attributes Profiling System as well as longer formal tests for deeper analysis of specific deficits.
- 10 Mild cognitive impairment (MCI) is a heterogeneous clinical condition with several underlying causes. However, the large proportion of MCI represents a transitional state between healthy aging and very mild AD (*DeCarli 2003. Lancet Neurol. 2: 15–21*). Accordingly, studies suggest that MCI subjects tend to progress to clinically probable AD at a rate of approximately 10%–15% per year (*Markesbery 2010. J Alzheimers Dis. 19: 221–228*).
- 15 Alzheimer's disease is usually diagnosed based on the person's medical history, history from relatives, and behavioral observations. The presence of characteristic neurological and neuropsychological features and the absence of alternative conditions is supportive. Advanced medical imaging with computed tomography (CT) or magnetic resonance imaging (MRI), and
- 20 with single-photon emission computed tomography (SPECT) or positron emission tomography (PET) can be used to help exclude other cerebral pathology or subtypes of dementia. Moreover, it may predict conversion from prodromal stages (mild cognitive impairment) to Alzheimer's disease. Assessment of intellectual functioning including memory testing can further characterize the state of the disease. Medical organizations have created
- 25 diagnostic criteria to ease and standardize the diagnostic process for practicing physicians. The diagnosis can be confirmed with very high accuracy post-mortem when brain material is available and can be examined histologically.
- To date, only symptomatic treatments exist for this disease, all trying to counterbalance the
- 30 neurotransmitter disturbance. Three cholinesterase inhibitors are currently available and have been approved for the treatment of mild to moderate AD. A further therapeutic option available for moderate to severe AD is memantine, an N-methyl-D-aspartate receptor noncompetitive antagonist. Treatments capable of stopping or at least effectively modifying the course of AD, referred to as 'disease-modifying' drugs, are still under extensive research.

New therapies are urgently needed to treat affected patients and to prevent, defer, slow the decline, or improve the symptoms of AD. It has been estimated that the overall frequency of the disease would be decreased by nearly 50% if the onset of the disease could be delayed by 5 years. Symptomatic treatments are drugs aimed at cognitive enhancement or control of neuropsychiatric symptoms and typically work through neurotransmitter mechanisms; disease-modifying therapies or treatments (DMTs) are agents that prevent, delay, or slow progression and target the underlying pathophysiologic mechanisms of AD. Currently there are more than 100 agents in the AD treatment development pipeline (*Cummings et al. 2017. Alzheimer's & Dementia: Translational Research & Clinical Interventions* 3: 367-384).

Dementia with Lewy bodies (DLB) is a type of dementia that worsens over time. Additional symptoms may include fluctuations in alertness, visual hallucinations, slowness of movement, trouble walking, and rigidity. DLB is the most common cause of dementia after Alzheimer's disease and vascular dementia. It typically begins after the age of 50. About 0.1% of those over 65 are affected. Men appear to be more commonly affected than women. The underlying mechanism involves the formation of Lewy bodies in neurons, consisting of alpha-synuclein protein. A diagnosis may be suspected based on symptoms, with blood tests and medical imaging done to rule out other possible causes. At present no cure for DLB exists. Treatments are supportive and attempt to relieve some of the motor and psychological symptoms associated with the disease. Acetylcholinesterase inhibitors, such as donepezil, may provide some benefit. Some motor problems may improve with levodopa. For review see *McKeith et al. 2017. Neurology* 89: 88-100.

Vascular dementia (VaD), also known as multi-infarct dementia (MID) and vascular cognitive impairment (VCI), is dementia caused by problems in the supply of blood to the brain, typically a series of minor strokes, leading to worsening cognitive decline that occurs step by step. The term refers to a syndrome consisting of a complex interaction of cerebrovascular disease and risk factors that lead to changes in the brain structures due to strokes and lesions, and resulting changes in cognition. The temporal relationship between a stroke and cognitive deficits is needed to make the diagnosis. Differentiating the different dementia syndromes can be challenging, due to the frequently overlapping clinical features and related underlying pathology. In particular, Alzheimer's dementia often co-occurs with vascular dementia. People with vascular dementia present with progressive cognitive impairment, acutely or sub-

acutely as in mild cognitive impairment, frequently step-wise, after multiple cerebrovascular events (strokes). For review see *Venkat et al. 2015. Exp Neurol 272: 97–108.*

Frontotemporal dementia (FTD) is the clinical presentation of frontotemporal lobar degeneration, which is characterized by progressive neuronal loss predominantly involving the frontal or temporal lobes, and typical loss of over 70% of spindle neurons, while other neuron types remain intact. FTD accounts for 20% of young-onset dementia cases. Signs and symptoms typically manifest in late adulthood, more commonly between the ages of 55 and 65, approximately equally affecting men and women. Common signs and symptoms include significant changes in social and personal behavior, apathy, blunting of emotions, and deficits in both expressive and receptive language. Currently, there is no cure for FTD, but there are treatments that help alleviate symptoms. For review see *Bott et al. 2014. Neurodegener Dis Manag 4(6): 439–454.*

In one specific embodiment, wherein 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-Gly and/ or ADM-NH₂: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14), the patient is not suffering from the disease or condition dementia or Alzheimer's disease.

In another specific embodiment 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 the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein 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-Gly and/ or ADM-NH₂: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14), wherein said disease or condition is not dementia or Alzheimer's disease.

As used herein, organ dysfunction denotes a condition or a state of health where an organ does not perform its expected function. "Organ failure" denotes an organ dysfunction to such

a degree that normal homeostasis cannot be maintained without external clinical intervention. Said organ failure may pertain an organ selected from the group comprising kidney, liver, heart, lung, nervous system. By contrast, organ function represents the expected function of the respective organ within physiologic ranges. The person skilled in the art is aware of the
5 respective function of an organ during medical examination.

Organ dysfunction may be defined by the sequential organ failure assessment score (SOFA-Score) or the components thereof. The SOFA score, previously known as the sepsis-related organ failure assessment score (*Singer et al. 2016. JAMA 315(8): 801-10*) is used to track a
10 person's status during the stay in an intensive care unit (ICU) to determine the extent of a person's organ function or rate of failure. The score is based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems each scored from 0 to 4 with an increasing score reflecting worsening organ dysfunction. The criteria for assessment of the SOFA score are described for example in Lamden et al. (for
15 review see *Lamden et al. 2019. Critical Care 23: 374*). SOFA score may traditionally be calculated on admission to ICU and at each 24-h period that follows. In particular, said organ dysfunction is selected from the group comprising renal decline, cardiac dysfunction, liver dysfunction or respiratory tract dysfunction.

20 The quick SOFA Score (quickSOFA or qSOFA) was introduced by the Sepsis-3 group in February 2016 as a simplified version of the SOFA Score as an initial way to identify patients at high risk for poor outcome with an infection (*Angus et al. 2016. Critical Care Medicine. 44 (3): e113-e121*). The qSOFA simplifies the SOFA score drastically by only including its 3 clinical criteria and by including "any altered mentation" instead of requiring a GCS <15.
25 qSOFA can easily and quickly be repeated serially on patients. The score ranges from 0 to 3 points. One point is given for: low blood pressure (SBP $\leq$ 100 mm Hg), high respiratory rate ($\geq$ 22 breaths/min) and altered mentation (GCS $\leq$ 15). The presence of 2 or more qSOFA points near the onset of infection was associated with a greater risk of death or prolonged intensive care unit stay. These are outcomes that are more common in infected patients who
30 may be septic than those with uncomplicated infection. Based upon these findings, the Third International Consensus Definitions for Sepsis recommends qSOFA as a simple prompt to identify infected patients outside the ICU who are likely to be septic (*Seymour et al. 2016. JAMA 315(8): 762-774*).

The term “to accelerate the conversion” is defined in the present application as an increased conversion of glycinated adrenomedullin (ADM-Gly) to mature ADM (ADM-NH₂) in presence of an anti-ADM antibody or an anti-adrenomedullin antibody fragment or an anti-ADM non-Ig scaffold within a certain period of time.

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Furthermore, 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 means that said antibody or antibody fragment or non-Ig scaffold binds to one specific region encompassing at least 4 amino acids within the target ADM. Monospecific antibodies or fragments or non-Ig scaffolds according to the invention are antibodies or fragments or 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.

15

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.

20 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.

25

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.

30

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 CDRs. 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 multispecific 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.

5

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),
15 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
20 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).

25

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.

30

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 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
5 directed approach guided by molecular modelling (*Almagro and Fransson 2008, Front Biosci. 13: 1619-33*).

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.

10 In a preferred embodiment, the anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to an epitope of preferably at least 4 or at least 5 amino acids in length of the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

15 YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23).

An epitope, also known as antigenic determinant, is the part of an antigen (e.g., peptide or protein) 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
20 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.

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
25 by the interaction of contiguous amino acid residues. 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.

30 In a specific embodiment, the anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23)

and needs the free N-terminus (amino acid 1) of ADM and/ or ADM-Gly for binding.

In another specific embodiment of the invention the anti-ADM antibody or anti-ADM
5 antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23)

and does not bind the free N-terminus (amino acid 1) of ADM and/ or ADM-Gly.

10

In one specific embodiment of the invention the anti-ADM antibody or anti-ADM antibody
fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at
least 5 amino acids within the N-terminal part (amino acid 1-21) of ADM-Gly and/ or ADM-
NH₂: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No.: 14).

15

In one specific embodiment of the invention the anti-ADM antibody or anti-ADM antibody
fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at
least 5 amino acids within the N-terminal part (amino acid 1-21) of ADM-Gly and/ or ADM-
NH₂: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No.: 14) and needs the free N-terminus
20 (amino acid 1) of ADM and/ or ADM-Gly for binding.

20

In one specific embodiment of the invention the anti-ADM antibody or anti-ADM antibody
fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at
least 5 amino acids within the N-terminal part (amino acid 1-21) of ADM-Gly and/ or ADM-
25 NH₂: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No.: 14) and does not bind the free N-
terminus (amino acid 1) of ADM and/ or ADM-Gly.

25

In another preferred embodiment said anti-ADM-antibody or anti-ADM antibody fragment or
anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5
30 amino acids within the N-terminal part (amino acid 1-14) of ADM-Gly and/ or ADM-NH₂:
YRQSMNNFQGLRSF (SEQ ID No.: 25).

30

In another preferred embodiment said anti-ADM-antibody or anti-ADM antibody fragment or
anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5

amino acids within the N-terminal part (amino acid 1-14) of ADM-Gly and/ or ADM-NH₂: YRQSMNNFQGLRSF (SEQ ID No.: 25) and needs the free N-terminus (amino acid 1) of ADM and/ or ADM-Gly for binding.

- 5 In another preferred embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the N-terminal part (amino acid 1-14) of ADM-Gly and/ or ADM-NH₂: YRQSMNNFQGLRSF (SEQ ID No.: 25) and does not bind the free N-terminus (amino acid 1) of ADM and/ or ADM-Gly.

10

In another preferred embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the N-terminal part (amino acid 1-10) of ADM-Gly and/ or ADM-NH₂: YRQSMNNFQG (SEQ ID No.: 26).

15

In another preferred embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the N-terminal part (amino acid 1-10) of ADM-Gly and/ or ADM-NH₂: YRQSMNNFQG (SEQ ID No.: 26) and needs the free N-terminus (amino acid 1) of ADM and/ or ADM-Gly for binding.

20

In another preferred embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the N-terminal part (amino acid 1-10) of ADM-Gly and/ or ADM-NH₂: YRQSMNNFQG (SEQ ID No.: 26) and does not bind the free N-terminus (amino acid 1) of ADM and/ or ADM-Gly.

25

In a very specific embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the N-terminal part (amino acid 1-6) of ADM-Gly and/ or ADM-NH₂: YRQSMN (SEQ ID No.: 27) and needs the free N-terminus (amino acid 1) of ADM and/ or ADM-Gly for binding.

30

In another very specific embodiment of the invention the anti-ADM antibody or anti-adrenomedullin 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. N-terminal end means that the amino acid 1, that is “Y” of SEQ ID No. 14, 20, 22, 23, 25, 26, 27 is mandatory for antibody binding. The antibody or fragment or scaffold would neither bind N-terminal extended nor N-terminal modified ADM nor N-terminal degraded ADM-Gly and/ or ADM-NH₂. This means that said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold binds only to a region within the sequence of ADM-Gly and/ or ADM-NH₂ if the N-terminal end of ADM is free. The anti-ADM antibody or anti-ADM antibody fragment or non-Ig scaffold would not bind to a region within the sequence of ADM-Gly and/ or ADM-NH₂ 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 ADM-Gly and/ or ADM-NH₂ sequence.

In another specific embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the mid-regional part (amino acid 21-42) of ADM-Gly and/ or ADM-NH₂.

In another specific embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the mid-regional part (amino acid 21-32) of ADM-Gly and/ or ADM-NH₂: CTVQKLAHQIYQ (SEQ ID No.: 15).

In another specific embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is directed to and can bind to preferably at least 4, or at least 5 amino acids within the mid-regional part (amino acid 27-39) of ADM-Gly and/ or ADM-NH₂: AHQIYQFTDKDKD (SEQ ID No.: 49).

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 aa 43 – 52 of ADM (SEQ ID No.: 24).

In one specific embodiment it is preferred to use an anti-ADM antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold according to the present invention, wherein said anti-adrenomedullin antibody or said anti-adrenomedullin antibody fragment or non-Ig scaffold leads to an increase of the ADM-NH₂ level or ADM-NH₂ immunoreactivity in serum, blood, plasma of at least 10 %, preferably at least 50 %, more preferably >50 %, most preferably >100%.

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.

10

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 ("humanization").

Subject matter of the present invention in one aspect is a humanized CDR-grafted antibody or antibody fragment thereof, wherein said antibody recognizes or binds to the N-terminal part of ADM-Gly and/ or ADM-NH₂ and needs the free N-terminus (amino acid 1) of ADM-Gly and/ or ADM-NH₂ for binding for use in a patient to accelerate the conversion of ADM-Gly to ADM-NH₂, wherein the humanized CDR-grafted antibody or antibody fragment thereof comprises an antibody heavy chain (H chain) comprising:

SEQ ID No.:1

25 GYTFSRYW

SEQ ID No.: 2

ILPGSGST

30 and/or

SEQ ID No.: 3

TEGYEYDGFY

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

SEQ ID No.: 4

QSIVYSNGNTY

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

RVS

and/or

10 SEQ ID No.: 5

FQGSHIPYT.

One specific embodiment of the invention is a humanized and/ or human monoclonal antibody or an antibody fragment thereof, wherein said antibody recognizes or binds to the N-terminal part (amino acid 1-21) of ADM-Gly and/ or ADM-NH₂:
15 YRQSMNNFQGLRSFGCRFGTC (SEQ ID No.: 14) and needs the free N-terminus (amino acid 1) of ADM-Gly and/ or ADM-NH₂ for binding for use in a patient to accelerate the conversion of ADM-Gly to ADM-NH₂ wherein the heavy chain comprises at least one CDR selected from the group comprising:

20

SEQ ID No.: 1

GYTFSRYW

SEQ ID No.: 2

25 ILPGSGST

SEQ ID No.: 3

TEGYEYDGFYD

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

SEQ ID No.: 4

QSIVYSNGNTY

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

RVS

SEQ ID No.: 5

FQGSHIPYT.

5

In a more specific embodiment of the invention subject matter of the invention is a humanized and/ or human monoclonal antibody or antibody fragment thereof, wherein said antibody recognizes or binds to the N-terminal part (amino acid 1-21) of ADM-Gly and/ or ADM-NH₂: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No.: 14) and needs the free N-terminus (amino acid 1) of ADM-Gly and/ or ADM-NH₂ for binding for use in a patient to accelerate the conversion of ADM-Gly to ADM-NH₂ wherein the heavy chain comprises the sequences:

SEQ ID No.: 1

GYTFSTRYW

15

SEQ ID No.: 2

ILPGSGST

SEQ ID No.: 3

20 TEGYEYDGFYD

and wherein the light chain comprises the sequences:

SEQ ID No.: 4

25 QSIVYSNGNTY

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

RVS

30 SEQ ID No.: 5

FQGSHIPYT.

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, 35 and 36.

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-Gly and/ or ADM-NH₂ in such that affinity constant is greater than 10⁻⁷ M, preferred 10⁻⁸ M, preferred 5 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 affinity constants may be determined according to the method as described in Example 1.

10 Subject matter of the present invention is a human or humanized monoclonal antibody or fragment that binds to ADM-Gly and/ or ADM-NH₂, wherein said antibody or fragment binds to the N-terminal (amino acid 1-21) of ADM-Gly and/ or ADM-NH₂: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No.: 14) and needs the free N-terminus (amino acid 1) of ADM-Gly and/ or ADM-NH₂ for binding, for use in a patient to accelerate the 15 conversion of ADM-Gly to ADM-NH₂, wherein said antibody or fragment comprises a sequence selected from the group comprising:

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

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGS
20 TNYNEKFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFQDYWGQGTT
LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVTVPSDDLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID No.: 7 (AM-VH1)

25 QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPGGGLEWMGRILPGSG
STNYAQKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCTEGYEYDGFQDYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVTVPSDDLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGGGLEWMGRILPGSG
STNYAQKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCTEGYEYDGFQDYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVTVPSDDLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKV SCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSG
STNYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCTEGYEYDGF DYWGQGT
5 TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKV SCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSG
10 STNYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCTEGYEYDGF DYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

15 DVLLSQTPLSLPVS LGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

20 SEQ ID No.: 12 (AM-VL1)

DVVMTQSPSLPVT LGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSN
RDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

25

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

DVVMTQSPSLPVT LGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSN
RDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
30 KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No.: 35 (heavy chain HAM8101)

QVQLVQSGAEVKKPGSSVKV SCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGS
TNYNQKFQGRVTITADTSTSTAYMELSSLRSED TAVYYCTEGYEYDGF DYWGQGT

VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
 PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
 5 QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFS_cSVMHEALHNHYTQKSLSLSPGK

SEQ ID No.: 36 (light chain HAM 8101)

DVVLTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGQSPRLLIYRVSNR
 10 FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAA
 PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
 DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Subject matter of the present invention is further a human and/ or humanized monoclonal
 15 antibody or fragment that binds to ADM-Gly and/ or ADM-NH₂, wherein said antibody or
 fragment binds to the N-terminal part (amino acid 1-21) of ADM-Gly and/ or ADM-NH₂:
 YRQSMNNFQGLRSFGCRFGTC (SEQ ID No.: 14) and needs the free N-terminus (amino
 acid 1) of ADM-Gly and/ or ADM-NH₂ for binding for use in a patient to accelerate the
 conversion of ADM-Gly to ADM-NH₂, wherein said antibody or fragment comprises the
 20 following sequence as a heavy chain:

SEQ ID No.: 35

QVQLVQSGAEVKKPGSSVKV_cCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGS
 TNYNQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCTEGYEYDGF₂DYWGQGTT
 25 VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
 PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
 QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 30 SDGSFFLYSKLTVDKSRWQQGNVFS_cSVMHEALHNHYTQKSLSLSPGK

and comprises the following sequence as a light chain:

SEQ ID No.: 36

DVVLTSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

5

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

SEQ ID No.: 35

10 QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGS
TNYNQKFQGRVTITADTSTSTAYMELSSLRSEDNAVYYCTEGYEYDGFQDYWGQGTT
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
15 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

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

SEQ ID No.: 36

DVVLTSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNR
25 FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

30

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.

Subject matter of the present invention is a pharmaceutical formulation for use in intervention and therapy of congestion 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 a patient to accelerate the conversion of ADM-Gly to ADM-NH₂, comprising an antibody or fragment or scaffold according to the present invention, wherein said patient is a critically ill patient suffering from an acute disease or acute condition. Said acute disease or condition is selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory
- 10 response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms, e.g., congestion or edema), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia.
- 15 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 a solution, preferably a ready-to-use solution.

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

- 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.

30

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.

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 to be administered systemically.

5 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 the treatment of a critically ill patient suffering from an
10 acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in
15 order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, wherein said patient is characterized by having a ratio of pro-Adrenomedullin or a fragment thereof to ADM-NH₂ (SEQ ID No. 20) above a certain threshold in a sample of bodily fluid, wherein said pro-Adrenomedullin or fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID
20 No. 34) and wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:
YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No.
25 23).

2. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an
acute disease or condition selected from the group comprising: severe infections (e.g.,
30 meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly

in said patient according to embodiment 1, wherein 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-Gly and/ or ADM-NH₂: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

5

3. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g.,
10 septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, according to embodiments 1 and 2, wherein said anti-ADM antibody or
15 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.

4. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g.,
20 meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in
25 order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, according to embodiment 1, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 21-42) of ADM-Gly and/ or ADM-NH₂: CTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 48).

30

5. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g.,
35 meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated

heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, according to embodiment 4, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 21-32) of ADM-Gly and/ or ADM-NH₂: CTVQKLAHQIYQ (SEQ ID No.: 15).

6. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient, according to claim 1, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 21-42) of ADM-Gly and/ or ADM-NH₂: CTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 48).

7. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to claim 6, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 21-32) of ADM-Gly and/ or ADM-NH₂: CTVQKLAHQIYQ (SEQ ID No.: 15).

8. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to claim 6, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the mid-regional part (amino acid 27-39) of ADM-Gly and/ or ADM-NH₂: AHQIYQFTDKDKD (SEQ ID No.: 49).

9. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an

acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to embodiments 1 - 8, wherein in a sample of bodily fluid of said patient the level of pro-Adrenomedullin or a fragment thereof consisting of the group of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34) and ADM-NH₂ (SEQ ID No. 20) is determined.

10. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to embodiments 1 - 9, wherein in a sample of bodily fluid of said patient a ratio of the level of ADM-Gly (SEQ ID No. 21) and ADM-NH₂ (SEQ ID No. 20) is determined and the patient is treated with said anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold if the ratio is above a certain threshold, wherein the ADM-Gly/ ADM-NH₂ ratio is in a range between 1 and 10, preferably between 1.5 and 7.5, preferably between 2 and 5, most preferred the threshold is 2.5.

11. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial

infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to embodiments 1 - 9, wherein the sample of bodily fluid of said patient is selected from the group of blood, serum, plasma, urine, cerebrospinal fluid (CSF), and saliva.

5

12. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to embodiment 11, wherein said sample is selected from the group comprising human citrate plasma, heparin plasma and EDTA plasma.

10

15

13. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to embodiments 1 - 12, wherein an immunoassay is used for determining the ratio of pro-Adrenomedullin or a fragment thereof and ADM-NH₂, wherein said pro-Adrenomedullin or fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34).

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14. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g.,

meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to embodiment 13, wherein said immunoassay is a sandwich immunoassay, preferably a fully automated assay.

15. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to embodiments 1 - 14, wherein the assay sensitivity of said assay for the detection of ADM-NH₂ is able to quantify ADM-NH₂ of healthy subjects and is < 70 pg/ml, preferably < 40 pg/ml and more preferably < 10 pg/ml.

20

16. Anti-adrenomedullin (ADM) antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g., meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to embodiments 1 - 14, wherein the assay sensitivity of said assay for ADM-Gly is able to quantify ADM-Gly of healthy subjects and is 20 pg/ml, preferably 15 pg/ml and more preferably 10 pg/ml.

30

17. Anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in the treatment of a critically ill patient suffering from an acute disease or condition selected from the group comprising: severe infections (e.g.,

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meningitis, systemic inflammatory response syndrome (SIRS), sepsis), shock (e.g., septic shock, cardiogenic shock), acute heart failure (including acute decompensated heart failure, chronic heart failure with worsening signs and symptoms), myocardial infarction, stroke, organ dysfunction (e.g., kidney, liver, heart, lung) or dementia in order to accelerate the conversion of ADM-Gly to ADM-NH₂ of circulating ADM-Gly in said patient according to embodiments 1 -16, wherein the level of pro-Adrenomedullin or a fragment thereof and ADM-NH₂ (SEQ ID No. 20) is determined by using one binder to said pro-Adrenomedullin or a fragment thereof and a second binder to ADM-NH₂ (SEQ ID No. 20), wherein said proAdrenomedullin or a fragment thereof is selected from the group consisting of PAMP (SEQ ID No. 32), MR-proADM (SEQ ID No. 33), ADM-Gly (SEQ ID No. 21) and CT-proADM (SEQ ID No. 34), and wherein both binders are selected from the group comprising an antibody, an antibody fragment or a non-Ig-Scaffold binding to said pro-Adrenomedullin or a fragment thereof and ADM-NH₂.

18. Anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy of a patient, wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23), wherein in a sample of bodily fluid of said patient the level of peptidylglycine alpha-amidating monooxygenase (PAM) and/or its isoforms and/or fragments thereof is determined and the patient is treated with said anti-ADM antibody or an anti-ADM antibody fragment or anti-ADM non-Ig scaffold, if the level of peptidylglycine alpha-amidating monooxygenase (PAM) is below a threshold.

19. Anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy of a patient according to embodiment 19, wherein said level of PAM and/or its isoforms and/or fragments thereof is the total concentration of PAM and/or its isoforms and/or fragments thereof having at least 12 amino acids or the activity of PAM and/or its isoforms and/or fragments thereof comprising the sequences SEQ ID No. 39, SEQ ID No. 40, SEQ ID No. 41, SEQ ID No. 42, SEQ ID No. 43, SEQ ID No. 44, SEQ ID No. 45, SEQ ID No. 46 and SEQ ID No. 47.

20. Anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold for use in therapy of a patient according to any of the embodiments 1-19, wherein the anti-adrenomedullin (ADM) antibody or an anti-adrenomedullin antibody fragment or anti-ADM non-Ig scaffold is used in combination with L-ascorbic acid.

5

21. Anti-Adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold in combination with L-ascorbic acid,

10

a. for use in therapy of an acute disease or acute condition of a patient for stabilizing the systemic circulation of said patient wherein said patient is in need of stabilizing the systemic circulation and exhibits a heart rate of > 100 beats /min and/ or < 65 mm Hg mean arterial pressure and wherein stabilizing the systemic circulation means increasing the mean arterial pressure over 65 mmHg, or

15

b. for use in the prevention of a heart rate increase to > 100 beats/min and/or a mean arterial pressure decrease to < 65 mm Hg in patients having an acute disease or acute condition, or

20

c. for use in therapy of an acute disease or acute condition of a patient that suffers from a chronic and/or acute disease or acute condition for prevention or reduction of organ dysfunction or prevention of organ failure in said patient and wherein said organ is selected from the group comprising heart, kidney, liver, lungs, pancreas, small intestines and spleen, or

d. for use in therapy or prevention of SIRS, meningitis, sepsis, shock, *e.g.*, septic shock in a patient

e. for the reduction of the mortality risk in a patient with SIRS, meningitis, sepsis, shock, *e.g.*, septic shock,

25

wherein said anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold binds to the N-terminal and/ or mid-regional part (amino acid 1-42) of ADM-Gly and/ or ADM-NH₂:

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA (SEQ ID No. 23).

30

22. Anti-Adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold in combination with L-ascorbic acid according to embodiment 21, wherein said L-ascorbic acid is a single enantiomer, a mixture of enantiomers, a mixture of diastereomers or a pharmaceutically acceptable salt, solvate, hydrate or prodrug thereof.

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: Illustration of antibody formats – bivalent antibodies and bispecific antibodies

Fig. 2:

- 10 a: Dose response curve of human ADM. Maximal cAMP stimulation was adjusted to 100% activation
- b: Dose/ inhibition curve of human ADM 22-52 (ADM-receptor antagonist) in the presence of 5.63 nM hADM.
- c: Dose/ inhibition curve of CT-H in the presence of 5.63 nM hADM
- 15 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
- 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
- 20 h: Dose/ inhibition curve of CT-M in the presence of 0.67 nM mADM
- 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
- 25 l: shows the inhibition of ADM by F(ab)₂ NT-M and by Fab NT-M

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.

30 **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: Alignment of the Fab with homologous human framework sequences.

Fig. 6: This figure shows a typical ADM-Gly dose/ signal curve.

Fig. 7: Influence of HAM8101 on the formation of bio-ADM by human recombinant PAM. The change of the bio-ADM signal per minute (RLU/min) is expressed in %. The relative bio-ADM signal for the antibody concentration of 0 µg/mL was set as 100%.

Fig. 8: Influence of HAM8101 on the formation of bio-ADM by human native PAM (with and without exogenous ADM-Gly as substrate).

Fig. 9: Influence of NT- and MR-anti-ADM antibodies on native human adrenomedullin maturation activity (AMA).

Fig. 10: ADM-concentration in healthy human subjects after NT-H application at different doses up to 60 days.

Fig. 11: ADM maturation activities (AMA) for subjects before and after administration of HAM8101 (average of activities from n=3 samples).

Fig. 12: Ratio ADM-Gly/bioADM in Phase1A clinical trial samples at different time-points (pre application and 1h, 4h, 24h after application of HAM 8101).

Fig. 13: Correlation between bio-ADM and ADM-Gly/ bio-ADM ratio in sepsis and septic shock patients (AdrenOSS-1) ($r=0.21$, $p=0.003$).

Fig. 14: Kaplan-Meier-Plot of ADM-Gly/ bio-ADM ratio for sepsis and septic shock patients (AdrenOSS-1) for 28-day survival outcome.

Fig. 15: ADM maturation activities (AMA) in plasma with HAM8101 and different concentrations of ascorbate.

EXAMPLES

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.

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

Several anti-human and anti-murine ADM antibodies were produced and their affinity constants were determined (see tables 1 and 2).

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.

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*).

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 antibody production by means of phage display:

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 spacers to the adrenomedullin peptide sequence. A mix of
10 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*;
15 *Schütte 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. Biotechn. 2011*), captured from the culture supernatant via immobilized metal ion affinity chromatography and
20 purified by a size exclusion chromatography.

Affinity Constants:

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
25 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 capture kit; GE Healthcare). (*Lorenz et al. 2011. Antimicrob Agents Chemother. 55(1): 165–173*).

30

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:

Table 1:

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}
SEQ ID: 49	AHQIYQFTDKDKDC	27-39-C	MR-CT-H	-

The following is a list of further obtained monoclonal antibodies:

5 **Table 2:**

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

NT-M	Mouse	ADM/381	2.4×10^{-8}	
NT-M	Mouse	ADM/382	1.6×10^{-8}	
NT-M	Mouse	ADM/383	1.8×10^{-8}	
NT-M	Mouse	ADM/384	1.7×10^{-8}	
NT-M	Mouse	ADM/385	1.7×10^{-8}	
NT-M	Mouse	ADM/403	1.2×10^{-8}	
NT-M	Mouse	ADM/395	1.2×10^{-8}	
NT-M	Mouse	ADM/396	3.0×10^{-8}	
NT-M	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	$< 1 \times 10^{-8}$	
MR-CT-H	Mouse	ADM/2901		
MR-CT-H	Mouse	ADM/2902		
MR-CT-H	Mouse	ADM/ 2903		
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	$< 1 \times 10^{-8}$	
hADM	Phage display	ADM/B7	$< 1 \times 10^{-8}$	
hADM	Phage display	ADM/C7	$< 1 \times 10^{-8}$	
hADM	Phage display	ADM/G3	$< 1 \times 10^{-8}$	
hADM	Phage display	ADM/B6	$< 1 \times 10^{-8}$	
hADM	Phage display	ADM/B11	$< 1 \times 10^{-8}$	
hADM	Phage display	ADM/D8	$< 1 \times 10^{-8}$	

hADM	Phage display	ADM/D11	$<1 \times 10^{-8}$	
hADM	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 16h, respectively.

10 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 x 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 x g for 2 minutes. 0.5ml of the prepared IgG sample were 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 x 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 x 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 × 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: 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). The following steps were done to achieve the humanized sequence: Total RNA was extracted from NT-H hybridomas using the Qiagen kit. For first-round RT-PCR the 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.

The reaction set up was as follows: 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
 5 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.

10 The reaction setup was as follows: 2x PCR mix 10 µl; Primer set 2 µl; First-round PCR product 8 µl; Total volume 20 µl; 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.

15 After PCR is finished, run PCR reaction samples 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
 20 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 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–*
 25 *498; Harris and Bajorath 1995. Protein Sci. 4: 306–310*).

Annotation for the antibody fragment sequences (SEQ ID No.: 7-13, 35 and 36): bold and underline are the CDR 1, 2, 3 chronologically arranged.

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

QVQLQQSGAELMKPGASVKISCKAT**GYTFSRYW**IEWVKQRPBGHGLEWIGE**ILPGSG**
STNYNEKFKGKATITADTSSNTAYMQLSSLTSEDSAVYYC**TEGYEYDGF**FDYWGQGT
 TLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
 FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID No.: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPGQGLEWMGRILPGS
GSTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFFDYWGQ
 5 GTTQTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
 HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGS
 10 GSTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFFDYWGQ
 GTTQTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
 HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

15 QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGS
GSTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFFDYWGQ
 GTTQTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
 HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGS
GSTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFFDYWGQ
 GTTQTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
 HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

25

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

DVLLSQTPSLPVS LGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSN
 RFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFOGSHIPYTFGGGTKLEIKRTVA
 APSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDS
 30 KDSTYSLSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

SEQ ID No.: 12 (AM-VL1)

DVVMQTQSPLSPVTLGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLLIYRVSN
 RD SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFOGSHIPYTFGQGTKLEIKRTVA

APSVFIFPPSDEQLKSGTASVVCLLNNFYPPREKVKQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSLTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

5 DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSN
RDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFOGSHIPYTFGGQTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPPREKVKQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSLTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

10 SEQ ID No.: 35

QVQLVQSGAEVKKPGSSVKVCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSG
STNYNQKFQGRVTITADTSTAYMELSSLRSEDTAVYYCTEGYEYDGFYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
15 PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

20 SEQ ID No.: 36

DVVLTSQPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPGQSPRLLIYRVSN
RFGSVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFOGSHIPYTFGGGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPPREKVKQWKVDNALQSGNSQESVTEQDS
25 KDSTYSLSSLTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

The effect of selected ADM-antibodies on ADM-bioactivity was tested in a human
30 recombinant Adrenomedullin receptor cAMP functional assay (Adrenomedullin Bioassay).
The following materials were used: Cell line CHO-K1, Adrenomedullin receptor (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).

5

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
10 (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 (SEQ ID No. 20), at the following final antibody concentrations: 100 µg/ml, 20 µg/ml, 4 µg/ml, 0.8 µg/ml, 0.16 µg/ml. The anti-m-ADM
20 antibodies (NT-M, MR-M, CT-M) were tested for antagonist activity in human recombinant adrenomedullin receptor (FAST-027C) cAMP functional assay in the presence of 0.67 nM Mouse ADM 1-50 (SEQ ID No. 22), 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 figs. 2 a to 2 l). The maximal inhibition by the individual
25 antibody is given in table 3.

Table 3: Maximal inhibition of ADM-antibodies

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

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).

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). Tube-bound chemiluminescence was measured by using the LB 953 (Berthold, Germany). 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.

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. 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 - Immunoassay for the detection of ADM-Gly

ADM-Gly was quantified as based on Weber et al. (*Weber et al. 2017. JALM 2(2): 222-233*) for bioactive ADM with the following modifications: the tracer-antibody used for ADM-Gly detection, labelled with MACN-acridinium-NHS, was directed to the C-terminal glycine of ADM-Gly. The assay was calibrated with synthetic ADM-Gly. The limit of detection (LOD) was 10 pg/mL of ADM-Gly. Cross-reactivity of antibody directed to the C-terminal glycine of ADM with bio-ADM was in the range between 6 and 50 % in a concentration dependent manner. All determined ADM-Gly concentrations were corrected for cross-reactivity as follows: For each ADM-Gly quantification additional quantification of bio-ADM in corresponding samples was performed using the sphingotest® bio-ADM immunoassay. The corresponding bio-ADM values were used to determine the signal (RLU) generated with the antibody directed to C-terminal glycine of ADM on a bio-ADM calibration curve. The determined signal (RLU) was used to calculate the false-positive ADM-Gly concentration (pg/mL) using the ADM-Gly calibration curve. This concentration was subtracted from the initially determined ADM-Gly concentration. A typical standard curve is shown in Figure 6.

Example 5 – (Invitro) effect of NT- and MR-ADM-antibodies on ADM-Gly to bio-ADM conversion

a) Recombinant human PAM

The formation of bio-ADM from c-terminally glycinated 1-53 Adrenomedullin (ADM-Gly) by recombinant human PAM and the influence of the N-terminal anti-Adrenomedullin antibodies on the bio-ADM formation from ADM-Gly by PAM was investigated.

In a first step, 200µl per well ADM-Gly (50 ng/mL) dissolved in buffer (300 mM potassium-phosphate, 100 mM NaCl, 10 mM Na-EDTA, 5 g/L BSA, pH 7.0) was added to microtiter plate wells that were precoated with an anti-MR-ADM antibody, and were

incubated for 1h at 22°C under agitation (600 rpm). Unbound material was removed by washing. In a second step, varying concentrations (0 – 100 µg/mL) of either N-terminal- (HAM8101), C-terminal anti-Adrenomedullin antibody specifically blocking the glycinated C-terminus of ADM-Gly or an unspecific antibody (control antibody) was added to the wells (200 µl per well) for 1h at 22°C under agitation (600 rpm). The respective antibodies were diluted in buffer as described above. Unbound antibodies were removed by washing. Amidation reaction was initiated by addition of PAM reaction buffer (100 mM Tris-HCl, 5 µM CuSO₄, 2 mM L-Ascorbate, 50 µM amastatin and 200 µM leupeptin) containing 50 µg/mL of recombinant human PAM-containing protein solution (InVivo Biotech Services GmbH, Hennigsdorf). The amidation reaction was performed at 37°C and was stopped by addition of EDTA (10 mM final concentration) at 0 minutes (t=0) and after 40 minutes (t=40). The plates were washed again and labelled anti-ADM antibody specific to the amidated C-Terminus of ADM) was added as tracer and incubated for 1 hour at 22°C and 600 rpm. After a final washing step, the remaining chemiluminescence was measured for 1 s per well with a Centro LB 960 microtiter plate luminescence reader (Berthold Technologies). To evaluate the velocity of the enzyme PAM, the signal for bio-ADM measured at t=0 minutes was subtracted from the signal for bio-ADM at t=40 minutes for each antibody concentration. The signal (t40-t0) for each antibody-concentration was normalized to the signal (t40-t0) without antibody addition, which was set at 100%.

As shown in Figure 7, the control antibody, having no specificity to Adrenomedullin, showed no influence on the activity of the PAM enzyme. The C-terminal anti-Adrenomedullin antibody specifically recognizing ADM-Gly inhibits the reaction of the PAM enzyme in a concentration-dependent manner, as it blocks the C-terminal glycine residue that represents the substrate for the PAM enzyme. Surprisingly, the N-terminal anti-ADM-antibody (HAM8101) has a significant accelerating effect on the conversion of ADM-Gly to bio-ADM catalyzed by PAM in a concentration dependent manner. The PAM-accelerating effect of HAM8101 was detected for concentrations up to 10 µg/mL. Concentrations of more than 10µg/mL did not further increase the PAM activity. The PAM activity at a concentration of 10 µg/mL of HAM8101 was 233% when compared to the reaction without antibody addition.

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b) Native human PAM

In a further experiment we investigated the formation of bio-ADM from ADM-Gly by native human plasma PAM and the influence of N-terminal- and mid-regional anti-adrenomedullin antibodies.

For the testing of N-terminal anti-ADM antibody HAM8101 the experiment was set up as follows:

Human Li-Heparin plasma (pool of 3 specimen) was used as source of human native PAM. The amidation reaction was performed in a total volume of 120 μ l at 37°C. 96 μ l of plasma were spiked with either HAM8101 (375 μ g/mL final concentration) or ADM-Gly (5 ng/mL final concentration) or with both. As control, equal volumes of 100 mM Tris-HCl, pH 7.5 were added to untreated plasma. The prepared samples were allowed to chill for 15 minutes at room temperature. The amidation reaction was started by addition of 24 μ l of PAM-reaction buffer resulting in final concentrations of 2 mM L-Ascorbate and 5 μ M CuSO₄, respectively.

10 The final concentrations of HAM8101 and ADM-Gly were 300 μ g/mL and 4 ng/mL, respectively. The reaction was allowed to proceed for 90 minutes at 37°C. After 0 min, 30 min, 60 min and 90 min of incubation, the reaction was stopped by addition of 20 mM EDTA (final concentration). The concentration of bio-ADM in the reaction sample was quantified using the sphingotest® bio-ADM immunoassay as described recently (*Weber et al. 2017, JALM 2(2): 222-233*).

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For testing of mid-regional anti-ADM antibody in comparison to HAM8101 and four additional N-terminal anti-ADM antibodies the experiment was set-up as follows:

Human serum was used as source of human native PAM. Each sample (20 μ l) was diluted two-fold in 100 mM Tris-HCl in duplicate. The amidation reaction was initiated by addition of 160 μ l of NT-ADM antibodies (HAM8101, AK1373, AK1388, AK1398 or AK1434) or MR-ADM antibodies (ADM43, ADM38, ADM41, ADM2901, ADM2902 and ADM2903) containing PAM-reaction buffer (100 mM Tris-HCl, pH 7.5, 6.25 μ M CuSO₄, 2.5 mM L-Ascorbate, 125 μ g/mL Catalase, 62.5 μ M Amastatin, 250 μ M Leupeptin, and 36 ng/mL synthetic 1-53 Adrenomedullin-Gly as substrate). The final antibody concentrations were 100 μ g/mL, respectively. Afterwards, 100 μ l of each individual reaction of duplicated samples were combined and transferred into 20 μ l of 200 mM EDTA to terminate the amidation reaction and thus to generate a t=0 minutes reaction time-point followed by incubation at 37°C for 40 minutes. Afterwards the non-terminated reactions were stopped with 10 μ l of

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200 mM EDTA. To determine the PAM activity in NT-ADM antibody containing samples, bio-ADM was quantified in each reaction using the sphingotest® bio-ADM immunoassay (Weber et al. 2017, supra). The MR-ADM antibody containing reaction was transferred to an alternative bio-ADM assay (description below). A control reaction without antibodies was measured in both bio-ADM assays. For each sample the difference between t=40 min and

t=0 min bio-ADM concentration was calculated. PAM activity is described as ng bio-ADM formed per hour and L of sample and was normalized to the reaction without antibody addition, which was set as 100%.

Alternative bio-ADM assay: All components and conditions were as described by Weber et al., 2017 with an N-terminal anti-ADM antibody as solid-phase capture antibody instead of a mid-regional anti-ADM antibody.

No change in bio-ADM concentration was detected in samples without addition of exogenous ADM-Gly, neither in absence or in presence of HAM8101. When ADM-Gly was added to the sample, a linear formation of bio-ADM was detected within 90 minutes. When HAM8101 was present in the reaction in addition to ADM-Gly, a linear formation of bio-ADM was detected within 90 minutes that was increased by the factor of 4 after 90 minutes when compared to the reaction without HAM8101 (Figure 8). All tested antibodies binding to the N-terminus of ADM increased the amidating activity, while HAM8101 showed the strongest influence (Figure 9). All tested antibodies binding to the mid-regional part of ADM (either antibodies ADM38, ADM 41 and ADM43 directed to SEQ ID No. 15 with amino acids 21-32 of ADM or antibodies ADM2901, ADM2902 and ADM2903 directed to SEQ ID No. 49 with amino acids 27-39 of ADM) increased the amidating activity, while ADM43 showed the strongest influence. Moreover, the tested antibody ADM43 binding to MR-ADM increased the formation of bio-ADM significantly more than the NT-ADM antibodies. This clearly demonstrates, that the conversion of ADM-Gly to bio-ADM by human native PAM is significantly increased by N-terminal as well as mid-regional anti-ADM antibodies.

Example 6 - Administration of NT-H in healthy humans and its in vivo effect on ADM-Gly to bio-ADM conversion

The study was described in Geven et al. (*Geven et al. 2017. Intensive Care Med Exp 5 (Suppl 2): 0427*). Briefly, the study was conducted in healthy male subjects as a randomized, double-blind, placebo-controlled, study with single escalating doses of NT-H antibody (HAM8101) 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 (HAM8101)

(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
5 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 (HAM8101) in healthy human individuals, then reached a plateau and slowly declined (Figure 10). Administration of NT-H antibody (HAM8101) was
10 safe as the antibody did not influence heart rate, mean arterial pressure, peripheral oxygen saturation or temperature. Moreover, there were no significant differences between groups in routine haematological and biochemical safety laboratory measurements.

The formation of bio-ADM from ADM-Gly by recombinant human PAM enzyme and the
15 *in vivo* influence of the N-terminal anti-Adrenomedullin antibody HAM8101 using samples from healthy subjects that received HAM8101 was investigated by measuring the bio-ADM maturation activity (AMA).

Samples from n=3 subjects from each administration group before and 1 hour after
20 administration of HAM8101 were used. Activity was determined as described for Serum samples in example 5b). The assay was calibrated using recombinant human PAM of known activity (InVivo Biotech Services GmbH, Hennigsdorf). Calibrators, controls and samples were treated in the same manner. The produced bio-ADM in the reaction-samples was quantified using the sphingotest® bio-ADM assay (*Weber et al., 2017*). For each sample the
25 difference of the t=40 min and t=0 min signal (RLUs, relative light units) was calculated and the signal (RLU(t40 - t0min)) of the calibrator was used to determine the AMA in tested serum samples. AMA is expressed as ng bio-ADM formed per hour and Liter of sample (ng/[h*L]).

30 Figures 11 A and B show bio-ADM maturation activities (AMA) for each group before and 1, 4 and 24 hours after administration of HAM8101 as an average of activities from n=3 specimen. In all three groups AMA with ADM-Gly as substrate was increased after administration of the N-terminal anti-ADM antibody HAM8101 when compared to the activity before HAM8101 administration (Fig. 11 A). While the difference in group 1 (0.5

mg/kg) was not significant in a one-way ANOVA, the differences in activity in group 2 (2 mg/kg) and group 3 (8 mg/kg) were significant ($p < 0.05$ in group 2 and 3, respectively). In group 1 the AMA was increased by approximately 60%, 40% and 52% after 1 hour, 4 hours and 24 hours, respectively. In group 2 the AMA was increased by approximately 27%, 25% and 40% after 1 hour, 4 hours and 24 hours, respectively. In group 3 the AMA was increased by approximately 65%, 48% and 60% after 1 hour, 4 hours and 24 hours, respectively (Fig. 11 B). These results clearly demonstrate, that the conversion of ADM-Gly to bio-ADM by human native PAM is significantly increased by the presence of circulating N-terminal anti-ADM antibody HAM8101. To further demonstrate the enhanced conversion of ADM-Gly to bio-ADM by human native PAM we determined the bioADM and ADM-Gly levels in the samples described above. While the ratio of ADM-Gly/bio-ADM was above 1 in each group before administration of HAM8101, it surprisingly decreased to approximately 0.75 after 1h in group 1, to 0.63 in group 2 and to 0.43 in group 3 (Figure 12). Four hours after administration of HAM8101 the ratio further decreased to approximately 0.38, 0.25 and 0.38 in groups 1, 2 and 3 respectively. No further change of the ratio was detected in groups 1 and 2 after 24 hours, while a slight increase to approximately 0.5 was detected in group 3. These results clearly demonstrate, that the increased conversion of ADM-Gly to bio-ADM by human native PAM due to administration of HAM8101 shifts the ADM-Gly/bio-ADM ratio towards bio-ADM.

Example 7 – Adrenomedullin and Outcome in Sepsis and Septic Shock 1 (AdrenOSS-1) study

AdrenOSS-1 was a European prospective observational study. Twenty-four centers in five countries (France, Belgium, The Netherlands, Italy, and Germany) contributed to the trial achievement of 583 enrolled patients (recruited from June 2015 to May 2016). The study protocol was approved by the local ethics committees and was conducted in accordance with the Declaration of Helsinki. The study enrolled patients aged 18 years and older who were (1) admitted to the ICU for sepsis or septic shock or (2) transferred from another ICU in the state of sepsis and septic shock within less than 24 h after admission. Included patients were stratified by severe sepsis and septic shock based on definitions for sepsis and organ failure from 2001 (*Levy et al. 2003. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med. 31(4):1250–6*). The term “sepsis” refers to the updated definition of Sepsis-3 (*Singer et al. 2016 The Third International Consensus*

Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 315(8):801–10). Patients were treated according to local practice, and treatments as well as procedures were registered. The primary endpoint was 28-day mortality. Secondary endpoints concerned organ failure (as defined by the Sequential Organ Failure Assessment [SOFA] score) and organ support, vasopressor/inotrope use, fluid balance, and use of renal replacement therapy (RRT).

Upon admission, demographics (age, sex), body mass index, presence of septic shock, type of ICU admission, organ dysfunction scores (SOFA, Acute Physiologic Assessment and Chronic Health Evaluation II [APACHE II]), origin of sepsis, pre-existing comorbidities (i.e., treated within the last year), past medical history, laboratory values, and organ support were recorded, and blood was drawn for measurement of bio-ADM and other markers. After patient enrollment, the following data were collected daily during the first week: SOFA score, antimicrobial therapies, fluid balance, ventilation status, Glasgow Coma Scale score, central venous pressure, need for RRT, invasive procedures for sepsis control, and vasopressor/inotrope treatment. Moreover, discharge status and mortality were recorded on day 28 after ICU admission. Blood for the central laboratory was sampled within 24 h after ICU admission and on day 2 (mean 47 h, SD 9 h) after the first sample. Samples were subsequently processed and stored at -80°C .

Bio-ADM was measured using a recently developed immunoassay as described in Weber et al. (*Weber et al. 2017. JALM 2(2): 222-233*). ADM-Gly was measured as described in example 4 in a randomly chosen subset of available samples ($n=170$). Bio-ADM and the ADM-Gly/ bio-ADM ratio correlated significantly ($r=0.25$, $p=0.0011$) (Fig. 13).

In the patient population described above (patients from AdrenOSS-I with sepsis, severe sepsis or septic shock) the ratio of plasma ADM-Gly and bioADM on day 1 after admission was determined as described above. Using the ratio's median as a simple cut-off value of 3.01, the population was segmented in two groups (above and below 3.01) and the corresponding 28-day survival rates were depicted in a Kaplan-Meier-Plot (Figure 14). Patients with an ADM-Gly/ bio-ADM ratio below 3.01 on the day of admission had a high survival rate of 84.7% (95% CI: 77.4-92.7), whereas, when the ratio increased over 3.01, the survival rate was lowered to 71.8% (95% CI: 62.8-82). The two ADM-Gly/ bio-ADM ratio groups could stratify the survival probability, and the HR was significantly higher when ADM-Gly/ bio-ADM ratio above median was compared with the ratio group below median (HR= 2.04).

Example 8 – Comparison of ADM-Gly/ bio-ADM ratio in healthy, healthy-treated and critically ill patients (survivors and non-survivors)

Critically ill subjects were from the AdrenOSS-I cohort described in example 7. Healthy and healthy HAM8101 treated subjects were from the study cohort described in example 6 (*Geven et al. 2017. Intensive Care Med Exp 5 (Suppl 2): 0427*). Bio-ADM was determined as described by Weber et al., 2017. ADM-Gly was determined as described in example 4.

Table 4: comparison of ADM-Gly/ bio-ADM ratios

	ADM-Gly/bioADM Ratio	Standard deviation	n Subjects
Critically ill (non-survivors)	5.2	4.97	37
Critically ill (survivors)	3.9	3.67	133
Healthy	1.29	0.13	9
Healthy HAM8101 treated (1h)	0.61	0.33	9
Healthy HAM8101 treated (4h)	0.33	0.13	9

10

Description of results:

Critically ill patients who died within of 28 days after admission to ICU showed an ADM-Gly/bio-ADM ratio of 5.2. The ratio of critically ill subjects surviving 28 days after ICU admission had a surprisingly significantly lower ADM-Gly/ bio-ADM ratio of 3.9 (p=0.0116). In comparison to critically ill subjects, healthy subject had a further significantly reduced ADM-Gly/ bio-ADM ratio of 1.29 (p<0.05). Surprisingly, healthy subjects who received HAM8101 had a further significantly reduced ADM-Gly/ bio-ADM ratio 1h after HAM8101 administration (p=0.0002). Four hours after administration of HAM8101 the ratio further decreased to 0.33 (p=0.0354) showing a direct influence of HAM8101 on ADM-Gly to bio-ADM conversion rate here expressed as the ratio of ADM-Gly/ bio-ADM.

20

Example 9 – Comparison of MR-proADM/ bio-ADM ratio in healthy, healthy-treated and critically ill patients (survivors and non-survivors)

The same patients as described in example 8 were also analysed for their MR-proADM/ ADM-NH₂ ratio.

25

Table 5: comparison of MR-proADM/ bio-ADM ratios

	MR-proADM/bio-ADM ratio	Standard deviation	n Subjects
Critically ill (non-survivors)	293.2	178.8	36
Critically ill (survivors)	283.9	131.7	130
Healthy	239.4	50.1	24
Healthy HAM8101 treated (1h)	44.6	32.1	18
Healthy HAM8101 treated (4h)	42.4	32.0	18

Description of results:

- 5 Critically ill patients who died within of 28 days after admission to ICU showed a MR-proADM/bio-ADM ratio of 293.2, whereas the ratio of critically ill subjects surviving 28 days after ICU admission had a lower ratio of 283.9. In comparison to critically ill subjects, healthy subject had a reduced MR-proADM/ bio-ADM ratio of 239.4. Surprisingly, healthy subjects who received HAM8101 had a further significantly reduced MR-proADM/ bio-ADM ratio 1h after HAM8101 administration ($p < 0.0001$). Four hours after administration of HAM8101 the ratio did not decrease any further.

Example 10 - Influence of N-terminal anti-Adrenomedullin antibodies and L-ascorbate on the velocity of bio-ADM formation from exogenous ADM-Gly by PAM

15

- A serial antibody-dilution with concentrations of 0, 5, 10, 50, 100, 500 and 3000 $\mu\text{g/mL}$ per well, respectively (in 20 μl) were pipetted into 96-well micro-titer plates. Then 160 μl PAM reaction buffer (100 mM Tris-HCl, 6.25 μM CuSO_4 , 62.5 μM amastatin, 250 μM leupeptin, 36 ng/mL ADM-Gly) with varying L-ascorbate concentrations was added to the wells and incubated for 15 min. Afterwards the reaction was started by addition of 20 μl of Li-Heparin plasma, containing native PAM. Immediately after addition of plasma, 100 μl from each well were removed and inactivated by addition of EDTA (20 mM final concentration) to generate a $t=0$ minutes reaction point. Inactivated and non-inactivated samples were incubated for 40 minutes at 37°C and the reaction in non-inactivated samples was stopped as described above. For quantification of produced ADM-NH₂ the sphingotest® bio-ADM Kit (*Weber et al. 2017*) was utilized according to manufacturer's manual. The ADM maturation activity (AMA) was calculated according to equation 1 (DF = dilution factor of sample matrix).

$$AMA = \frac{[bio - ADM; t = 40min] \left(\frac{ng}{L}\right) - [bio - ADM; t = 0min] \left(\frac{ng}{L}\right) * 60min * DF}{40min * 1h}$$

Increasing concentrations of ascorbate in the range of 0.4 – 2 mM led to increasing ADM maturation activities (Figure 15). The overall ascorbate dependency of the amidating reaction was not changed by addition of HAM8101, i.e., the activity optimum remained at 0.8 – 2 mM of ascorbate. However, the presence of HAM8101 in the amidation assay showed a concentration dependent positive effect on the amidation for each ascorbate concentration assayed. The presence of 100 µg/mL of HAM8101 in the amidation assay increased the bio-ADM formation of PAM by 40%. These results clearly demonstrate that a combination of N-terminal anti-ADM antibodies with ascorbate significantly enhance the activity of the PAM enzyme and the formation of bio-ADM in plasma.

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)

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGS

TNYNEKFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFYDYGQGT

LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF

25 PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID No.: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVCKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSG

STNYAQKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCTEGYEYDGFYDYGQGT

30 TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT

FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSG
STNYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCTEGYEYDGFQDYWGQGT
5 TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSG
10 STNYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCTEGYEYDGFQDYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

15 QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSG
STNYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCTEGYEYDGFQDYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

DVLLSQTPSLPVS LGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

25

SEQ ID No.: 12 (AM-VL1)

DVVMQTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSN
RD SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
30 KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

DVVMQTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSN
RD SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVA

APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

5 YRQSMNNFQGLRSFGCRFGTC

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

CTVQKLAHQIYQ

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

CAPRSKISPQGY-CONH₂

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

YRQSMNQGSRSNGCRFGTC

15

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

CTFQKLAHQIYQ

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

20 CAPRNKISPQGY-CONH₂

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-

25 CONH₂

SEQ ID No.: 21 (Adrenomedullin 1-52-Gly (ADM 1-52-Gly): amino acids 95 – 147 of
preproADM)

YRQSMN NFQGLRSFGC RFGTCTVQKL AHQIYQFTDK DKDNVAPRSK ISPQGYG

30

SEQ ID No.: 22 (Murine ADM 1-50)

YRQSMNQGSRSNGCRFGTCTFQKLAHQIYQLTDKDKDGMAPRNKISPQGY-CONH₂

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

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA

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

PRSKISPQGY-NH₂

5

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

YRQSMNNFQGLRSF

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

10 YRQSMNNFQG

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

YRQSMN

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

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQ

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

YRQSMNQGSRSNGCRFGTCTFQKLAHQIYQLTDKDKDGMA

20

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

YRQSMNQGSRSNGCRFGTCTFQKLAHQIYQL

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

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

30 SEQ ID No.: 32 (Proadrenomedullin N-20 terminal peptide, PAMP: amino acids 22 – 41 of preproADM)

ARLDVASEF RKKWKNWALS R

SEQ ID No.: 33 (Midregional proAdrenomedullin, MR-proADM: amino acids 45 – 92 of preproADM)

ELRMSS SYPTGLADVK AGPAQTLIRP QDMKGASRSP EDSSPDAARI RV

- 5 SEQ ID No.: 34 (C-terminal proAdrenomedullin, CT-proADM: amino acids 148 – 185 of preproADM)

RRR RRLPEAGPG RTL VSSKPQA HGAPAPPSGS APHFL

SEQ ID No.: 35 (heavy chain, HAM8101)

- 10 QVQLVQSGAEVKKPGSSVKV SCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGS
TNYNQKFQGRVTITADTSTSTAYMELSSLRSED AVYYCTEGYEYDGFQDYWGQGTT
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTF
PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
15 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD
SDGSFFLYSKLTVDKSRWQQGNVFS ScSVMHEALHNHYTQKSLSLSPGK

SEQ ID No.: 36 (light chain, HAM 8101)

- 20 DVVLTQSPLSLPVTGLQPASISCRSSQSIVYSNGNTYLEWYLQRPQGSPRLLIYRVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

- 25 SEQ ID No.: 37 – IGHV1-69*11

QVQLVQSGAEVKKPGSSVKV SCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGT
ANYAQKFQGRVTITADESTSTAYMELSSLRSED AVYYCARYYYYYYGMDVWGQGT
TVTVSS

- 30 SEQ ID No. 38: - HB3

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGS
TNYNEKFKGKATITADTSSNTAYMQLSSLTSEDS AVYYCTEGYEYDGFQDYWGQGTT
LTVSS

SEQ ID No: 39 - Prepro-PAM isoform 1 AS 1-973

	10	20	30	40	50
	MAGRVPSLLV	LLVFPSSCLA	FRSPLSVFKR	FKETTRPFSN	ECLGTTRPVV
	60	70	80	90	100
5	PIDSSDFALD	IRMPGVTPKQ	SDTYFCMSMR	IPVDEEAFVI	DFKPRASMDT
	110	120	130	140	150
	VHHMLLFGCN	MPSSTGSYWF	CDEGTCTDKA	NILYAWARNA	PPTRLPKGVG
	160	170	180	190	200
	FRVGGETGSK	YFVLQVHYGD	ISAFRDNNKD	CSGVSLHLTR	LPQPLIAGMY
10	210	220	230	240	250
	LMMSVDTVIP	AGEKVVNSDI	SCHYKNYPMH	VFAYRVHTHH	LGKVVSGYRV
	260	270	280	290	300
	RNGQWTLIGR	QSPQLPQAFY	PVGHPVDVSF	GDLLAARCVF	TGEGRTEATH
	310	320	330	340	350
15	IGGTSSDEMC	NLYIMYYMEA	KHAVSFMTCT	QNVAPDMFRT	IPPEANIPIP
	360	370	380	390	400
	VKSDMVMME	HHKETEKDK	IPLLQQPKRE	EEEVLDAQDF	YSLLSKLLGE
	410	420	430	440	450
	REDVVHVHKY	NPTEKAESES	DLVAEIANVV	QKKDLGRSDA	REGAEHERGN
20	460	470	480	490	500
	AILVRDRIHK	FHRLVSTLRP	PESRVFSLQQ	PPPGEGTWEP	EHTGDFHMEE
	510	520	530	540	550
	ALDWPGVYLL	PGQVSGVALD	PKNNLVIFHR	GDHVWDGNSF	DSKFVYQQIG
	560	570	580	590	600
25	LGPIEEDTIL	VIDPNNAAVL	QSSGKNLFYL	PHGLSIDKDG	NYWVTDVALH
	610	620	630	640	650
	QVFKLDPNNK	EGPVLILGRS	MQPGSDQNHF	CQPTDVAVDP	GTGAIYVSDG
	660	670	680	690	700
	YCNSRIVQFS	PSGKFITQWG	EESGSSPLP	GQFTVPHSLA	LVPLLGLQCV
30	710	720	730	740	750
	ADRENGRIQC	FKTDTKEFVR	EIKHSSFGRN	VFAISYIPGL	LFAVNGKPHF
	760	770	780	790	800
	GDQEPVQGFV	MNFSNGEIID	IFKPVRKHFD	MPHDIVASED	GTVYIGDAHT
	810	820	830	840	850
35	NTVWKFTLTE	KLEHRSVKKA	GIEVQEIKEA	EAVVETKMEN	KPTSSELQKM
	860	870	880	890	900
	QEKQKLIKEP	GSGVPVVLIT	TLLVIPVVVL	LAIAIFIRWK	KSRAFGDSEH
	910	920	930	940	950
	KLETSSGRVL	GRFRGKGGG	LNLGNFFASR	KGYSRKGFD	LSTEGSDQEK
40	960	970			
	EDDGSESEEE	YSAPLPALAP	SSS		

SEQ ID NO: 40 - Prepro-PAM isoform 2 AS 1-868

	10	20	30	40	50
	MAGRVPSLLV	LLVFPSSCLA	FRSPLSVFKR	FKETTRPFSN	ECLGTTRPVV
	60	70	80	90	100
5	PIDSSDFALD	IRMPGVTPKQ	SDTYFCMSMR	IPVDEEAFVI	DFKPRASMDT
	110	120	130	140	150
	VHHMLLFGCN	MPSSTGSYWF	CDEGTCTDKA	NILYAWARNA	PPTRLPKGVG
	160	170	180	190	200
	FRVGGETGSK	YFVLQVHYGD	ISAFRDNNKD	CSGVSLHLTR	LPQPLIAGMY
10	210	220	230	240	250
	LMMSVDTVIP	AGEKVVNSDI	SCHYKNYPMH	VFAYRVHTHH	LGKVVSGYRV
	260	270	280	290	300
	RNGQWTLIGR	QSPQLPQAFY	PVGHPVDVSF	GDLLAARCVF	TGEGRTEATH
	310	320	330	340	350
15	IGGTSSDEMC	NLYIMYYMEA	KHAVSFMTCT	QNVAPDMFRT	IPPEANIPIP
	360	370	380	390	400
	VKSDMVMME	HHKETEKDK	IPLLQQPKRE	EEEVLDDQDFH	MEEALDWPGV
	410	420	430	440	450
	YLLPGQVSGV	ALDPKNNLVI	FHRGDHVWDG	NSFDSKFVYQ	QIGLGPPEED
20	460	470	480	490	500
	TILVIDPNNA	AVLQSSGKNL	FYLPHGSLID	KDGNVWVTDV	ALHQVFKLDP
	510	520	530	540	550
	NNKEGPVLIL	GRSMQPGSDQ	NHFCQPTDVA	VDPGTGAIYV	SDGYCNSRIV
	560	570	580	590	600
25	QFSPSGKFIT	QWGEESSGSS	PLPGQFTVPH	SLALVPLLQ	LCVADRENGR
	610	620	630	640	650
	IQCFKTDKE	FVREIKHSSF	GRNVFAISYI	PGLLFAVNGK	PHFGDQEPVQ
	660	670	680	690	700
	GFVMNFSNGE	IIDIFKPVRK	HFDMPHDIVA	SEDGTVYIGD	AHTNTVWKFT
30	710	720	730	740	750
	LTEKLEHRVS	KKAGIEVQEI	KEAEAVVETK	MENKPTSSEL	QKMQEKQKLI
	760	770	780	790	800
	KEPGSGVPVV	LITLLVIPV	VVLLAIAIFI	RWKKSRAGD	SEHKLETSSG
	810	820	830	840	850
35	RVLGRFRGKG	SGGLNLGNFF	ASRKGYSRKG	FDRLSTEGSD	QEKEDDGSES
	860				
	EEEYSAPLPA	LAPSSS			

SEQ ID No.: 41 - Prepro-PAM isoform 3 AS (amino acids 829-896 of SEQ ID No. 1 missing)

	10	20	30	40	50
	MAGRVP	SLLV	LLVFPSSCLA	FRSPLSVFKR	FKETTRPFSN
	60	70	80	90	100
5	PIDSSDFALD	IRMPGVTPKQ	SDTYFCMSMR	IPVDEEAFVI	DFKPRASMDT
	110	120	130	140	150
	VHHMLLFGCN	MPSSTGSYWF	CDEGTCTDKA	NILYAWARNA	PPTRLPKGVG
	160	170	180	190	200
	FRVGGETGSK	YFVLQVHYGD	ISAFRDNNKD	CSGVSLHLTR	LPQPLIAGMY
10	210	220	230	240	250
	LMMSVDTVIP	AGEKVVNSDI	SCHYKNYPMH	VFAYRVHTHH	LGKVVSGYRV
	260	270	280	290	300
	RNGQWTLIGR	QSPQLPQAFY	PVGHPVDVSF	GDLLAARCVF	TGEGRTEATH
	310	320	330	340	350
15	IGGTSSDEMC	NLYIMYYMEA	KHAVSFMTCT	QNVAPDMFRT	IPPEANIPIP
	360	370	380	390	400
	VKSDMVMMEH	HHKETEKYKDK	IPLLQQPKRE	EEEVLDQGDF	YSLLSKLLGE
	410	420	430	440	450
	REDVVHVHKY	NPTEKAESES	DLVAEIANVV	QKKDLGRSDA	REGAEHERGN
20	460	470	480	490	500
	AILVRDRIHK	FHRLVSTLRP	PESRVFSLQQ	PPPGEGTWEP	EHTGDFHMEE
	510	520	530	540	550
	ALDWPGVYLL	PGQVSGVALD	PKNNLVIFHR	GDHVWDGNSF	DSKFVYQQIG
	560	570	580	590	600
25	LGPIEEDTIL	VIDPNNAAVL	QSSGKNLFYL	PHGLSIDKDG	NYWVTDVALH
	610	620	630	640	650
	QVFKLDPNNK	EGPVLILGRS	MQPGSDQNHF	CQPTDVAVDP	GTGAIYVSDG
	660	670	680	690	700
	YCNSRIVQFS	PSGKFITQWG	EESGSSPLP	GQFTVPHSLA	LVPLLGLQCV
30	710	720	730	740	750
	ADRENGRIQC	FKTDTKEFVR	EIKHSSFGRN	VFAISYIPGL	LFAVNGKPHF
	760	770	780	790	800
	GDQEPVQGFV	MNFSNGEIID	IFKPVRKHFD	MPHDIVASED	GTVYIGDAHT
	810	820	830	840	850
35	NTVWKFTLTE	KLEHRSVKKA	GIEVQEIKDS	EHKLETSSGR	VLGRFRGKGS
	860	870	880	890	900
	GGLNLGNFFA	SRKGYSRKGF	DRLSTEGSDQ	EKEDDGSESE	EEYSAPLPAL
	905				
	APSSS				

SEQ ID No. 42 - Prepro-PAM isoform 4 (amino acids 829-914 of SEQ ID No. 1 missing)

	10	20	30	40	50
	MAGRVP	SLLV	LLVFPSSCLA	FRSPLSVFKR	FKETTRPFSN
	ECLGTT	RPVV			
	60	70	80	90	100
5	PIDSSD	FALD	IRMPGV	TPKQ	SDTYFCMSMR
	110	120	130	140	150
	VHHMLL	FGCN	MPSSTGSYWF	CDEGTCTDKA	NILYAWARNA
	160	170	180	190	200
	FRVGGE	TGSK	YFVLQVHYGD	ISAFRDNNKD	CSGVSLHLTR
10	210	220	230	240	250
	LMMSVD	TVIP	AGEKVVNSDI	SCHYKNYPMH	VFAYRVHTHH
	260	270	280	290	300
	RNGQWT	LIGR	QSPQLPQAFY	PVGHPVDVSF	GDLLAARCVF
	310	320	330	340	350
15	IGGTSS	DEMC	NLYIMYYMEA	KHAVSFMTCT	QNVAPDMFRT
	360	370	380	390	400
	VKSDMV	MMHE	HHKETEKDK	IPLLQQPKRE	EEEVLDQGDF
	410	420	430	440	450
	REDVVH	VHKY	NPTEKAESES	DLVAEIANVV	QKKDLGRSDA
20	460	470	480	490	500
	AILVRD	RIHK	FHRLVSTLRP	PESRVFSLQQ	PPPGEGTWEP
	510	520	530	540	550
	ALDWPG	VYLL	PGQVSGVALD	PKNNLVIFHR	GDHVWDGNSF
	560	570	580	590	600
25	LGPIEE	DTIL	VIDPNNAAVL	QSSGKNLFYL	PHGLSIDKDG
	610	620	630	640	650
	QVFKLD	PNNK	EGPVLILGRS	MQPGSDQNHF	CQPTDVAVDP
	660	670	680	690	700
	YCNSRI	VQFS	PSGKFITQWG	EESGSSPLP	GQFTVPHSLA
30	710	720	730	740	750
	ADRENG	RIQC	FKTDTKEFVR	EIKHSSFGRN	VFAISYIPGL
	760	770	780	790	800
	GDQEPV	QGFV	MNFSNGEIID	IFKPVRKHFD	MPHDIVASED
	810	820	830	840	850
35	NTVWKFT	LTLE	KLEHRSVKKA	GIEVQEIKGK	GSGGLNLGNF
	860	870	880		
	GFDRLST	EGS	DQEKEDDGSE	SEEEYSAPLP	ALAPSSS

SEQ ID No. 43 - Prepro-PAM Isoform 5 (Isoform 1 with an additional aa in position 896)

	10	20	30	40	50
	MAGRVPSLLV	LLVFPSSCLA	FRSPLSVFKR	FKETTRPFSN	ECLGTTRPVV
	60	70	80	90	100
5	PIDSSDFALD	IRMPGVTPKQ	SDTYFCMSMR	IPVDEEAFVI	DFKPRASMDT
	110	120	130	140	150
	VHHMLLFGCN	MPSSTGSYWF	CDEGTCTDKA	NILYAWARNA	PPTRLPKGVG
	160	170	180	190	200
	FRVGGETGSK	YFVLQVHYGD	ISAFRDNNKD	CSGVSLHLTR	LPQPLIAGMY
10	210	220	230	240	250
	LMMSVDTVIP	AGEKVVNSDI	SCHYKNYPMH	VFAYRVHTHH	LGKVVSGYRV
	260	270	280	290	300
	RNGQWTLIGR	QSPQLPQAFY	PVGHPVDVSF	GDLLAARCVF	TGEGRTEATH
	310	320	330	340	350
15	IGGTSSDEMC	NLYIMYYMEA	KHAVSFMTCT	QNVAPDMFRT	IPPEANIPIP
	360	370	380	390	400
	VKSDMVMME	HHKETEKDK	IPLLQQPKRE	EEEVLDAQDF	YSLLSKLLGE
	410	420	430	440	450
	REDVVHVHKY	NPTEKAESES	DLVAEIANVV	QKKDLGRSDA	REGAEHERGN
20	460	470	480	490	500
	AILVRDRIHK	FHRLVSTLRP	PESRVFSLQQ	PPPGEGTWEP	EHTGDFHMEE
	510	520	530	540	550
	ALDWPGVYLL	PGQVSGVALD	PKNNLVIFHR	GDHVWDGNSF	DSKFVYQQIG
	560	570	580	590	600
25	LGPIEEDTIL	VIDPNNAAVL	QSSGKNLFYL	PHGLSIDKDG	NYWVTDVALH
	610	620	630	640	650
	QVFKLDPNNK	EGPVLILGRS	MQPGSDQNHF	CQPTDVAVDP	GTGAIYVSDG
	660	670	680	690	700
	YCNSRIVQFS	PSGKFITQWG	EESGSSSPLP	GQFTVPHSLA	LVPLLGLQCV
30	710	720	730	740	750
	ADRENGRIQC	FKTDTKEFVR	EIKHSSFGRN	VFAISYIPGL	LFAVNGKPHF
	760	770	780	790	800
	GDQEPVQGFV	MNFSNGEIID	IFKPVRKHFD	MPHDIVASED	GTVYIGDAHT
	810	820	830	840	850
35	NTVWKFTLTE	KLEHRSVKKA	GIEVQEIKEA	EAVVETKMEN	KPTSSELQKM
	860	870	880	890	900
	QEKQKLIKEP	GSGVPVVLIT	TLLVIPVVVL	LAIAIFIRWK	KSRAFGADSE
	910	920	930	940	950
	HKLETSSGRV	LGRFRGKGS	GLNLGNFFAS	RKGYSRKGFD	RLSTEGSDQE
40	960	970			
	KEDDGSESEE	EYSAPLPALA	PSSS		

SEQ ID No. 44 - Prepro-PAM Isoform 6 (amino acids 897-914 of SEQ ID No. 1 missing)

	10	20	30	40	50
	MAGRVPSLLV	LLVFPSSCLA	FRSPLSVFKR	FKETTRPFSN	ECLGTTRPVV
	60	70	80	90	100
5	PIDSSDFALD	IRMPGVTPKQ	SDTYFCMSMR	IPVDEEAFVI	DFKPRASMDT
	110	120	130	140	150
	VHHMLLFGCN	MPSSTGSYWF	CDEGTCTDKA	NILYAWARNA	PPTRLPKGVG
	160	170	180	190	200
	FRVGGETGSK	YFVLQVHYGD	ISAFRDNNKD	CSGVSLHLTR	LPQPLIAGMY
10	210	220	230	240	250
	LMMSVDTVIP	AGEKVVNSDI	SCHYKNYPMH	VFAYRVHTHH	LGKVVSGYRV
	260	270	280	290	300
	RNGQWTLIGR	QSPQLPQAFY	PVGHPVDVSF	GDLLAARCVF	TGEGRTEATH
	310	320	330	340	350
15	IGGTSSDEMC	NLYIMYYMEA	KHAVSFMTCT	QNVAPDMFRT	IPPEANIPIP
	360	370	380	390	400
	VKSDMVMME	HHKETEKDK	IPLLQQPKRE	EEEVLDDQGF	YSLLSKLLGE
	410	420	430	440	450
	REDVVHVHKY	NPTEKAESES	DLVAEIANVV	QKKDLGRSDA	REGAEHERGN
20	460	470	480	490	500
	AILVRDRIHK	FHRLVSTLRP	PESRVFSLQQ	PPPGEGTWEP	EHTGDFHMEE
	510	520	530	540	550
	ALDWPGVYLL	PGQVSGVALD	PKNNLVIFHR	GDHVWDGNSF	DSKFVYQQIG
	560	570	580	590	600
25	LGPIEEDTIL	VIDPNNAAVL	QSSGKNLFYL	PHGLSIDKDG	NYWVTDVALH
	610	620	630	640	650
	QVFKLDPNNK	EGPVLILGRS	MQPGSDQNHF	CQPTDVAVDP	GTGAIYVSDG
	660	670	680	690	700
	YCNSRIVQFS	PSGKFITQWG	EESGSSPLP	GQFTVPHSLA	LVPLLGLQCV
30	710	720	730	740	750
	ADRENGRIQC	FKTDTKEFVR	EIKHSSFGRN	VFAISYIPGL	LFAVNGKPHF
	760	770	780	790	800
	GDQEPVQGFV	MNFSNGEIID	IFKPVRKHFD	MPHDIVASED	GTVYIGDAHT
	810	820	830	840	850
35	NTVWKFTLTE	KLEHRSVKKA	GIEVQEIKEA	EAVVETKMEN	KPTSSELQKM
	860	870	880	890	900
	QEKQKLIKEP	GSGVPVVLIT	TLLVIPVVVL	LAIAIFIRWK	KSRAFGGKGS
	910	920	930	940	950
	GGLNLGNFFA	SRKGYSRKGF	DRLSTEGSDQ	EKEDDGSESE	EEYSAPLPAL
40	APSSS				

SEQ ID No. 45 - PHM subunit of PAM

	10	20	30	40	50
	FKETTRPFSN	ECLGTTTPVV	PIDSSDFALD		IRMPGVTPKQ
	SDTYFCMSMR				
5	60	70	80	90	100
	IPVDEEAFVI	DFKPRASMDT	VHHMLLFGCN	MPSSTGSYWF	CDEGTCTDKA
	110	120	130	140	150
	NILYAWARNA	PPTRLPGKVG	FRVGGETGSK	YFVLQVHYGD	ISAFRDNNKD
	160	170	180	190	200
10	CSGVSLHLTR	LPQPLIAGMY	LMMSVDTVIP	AGEKVVNSDI	SCHYKNYPMH
	210	220	230	240	250
	VFAYRVHTHH	LGKVVSGYRV	RNGQWTLIGR	QSPQLPQAFY	PVGHPVDVSF
	260	270	280	290	300
	GDLLAARCVF	TGEGRTEATH	IGGTSSDEMC	NLYIMYYMEA	KHAVSFMTCT
15	310	320	330	340	350
	QNVAPDMFRT	IPPEANIPIP	VKSDMVMME	HHKETEKYKDK	IPLLQQPKRE
	360	370	380	390	400
	EEEVLDQGDF	YSLLSKLLGE	REDVVHVHKY	NPTEKAESES	DLVAEIANVV
	410	420	430	440	450
20	QKKDLGRSDA	REGAEHERGN	AILVRDRIHK	FHRLVSTLRP	PESRVFSLQQ
	460				
	PPPGEGTWEP	EHTG			

25 SEQ ID No. 46 - PAL subunit of PAM

	10	20	30	40	50
	DFHMEEALDW	PGVYLLPGQV	SGVALDPKNN	LVI FHRGDHV	WDGNSFDSKF
	60	70	80	90	100
	VYQQIGLGPI	EEDTILVIDP	NNAAVLQSSG	KNLFYLPHGL	SIDKDGNYWV
30	110	120	130	140	150
	TDVALHQVFK	LDPNNKEGPV	LILGRSMQPG	SDQNHFCQPT	DVAVDPGTGA
	160	170	180	190	200
	IYVSDGYCNS	RIVQFSPSGK	FITQWGEES	GSSPLPGQFT	VPHSLALVPL
	210	220	230	240	250
35	LGQLCVADRE	NGRIQCCKTD	TKEFVREIKH	SSFGRNVFAI	SYIPGLLFAV
	260	270	280	290	300
	NGKPHFGDQE	PVQGFVMNFS	NGEIIDIFKP	VRKHFDMPHD	IVASEDGTIV
	310	320			
	IGDAHTNTVW	KFTLTEKLEH	RSV		

SEQ ID No. 47 - Sequence of recombinant human PAM

	10	20	30	40	50
	SPLSVFKRFK	ETTRPFSNEC	LGTTTPVVPI	DSSDFALDIR	MPGVTPKQSD
	60	70	80	90	100
5	TYFCMSMRIP	VDEEAFVIDF	KPRASMDTVH	HMLLFGCNMP	SSTGSYWFC
	110	120	130	140	150
	EGTCTDKANI	LYAWARNAPP	TRLPGKVGFR	VGGETGSKYF	VLQVHYGDIS
	160	170	180	190	200
	AFRDNNKDCS	GVSLHLTRLP	QPLIAGMYLM	MSVDTVIPAG	EKVVNSDISC
10	210	220	230	240	250
	HYKNYPMHVF	AYRVHTHHLG	KVVSGYRVRN	GQWTLIGRQS	PQLPQAFYPV
	260	270	280	290	300
	GHPVDVSFGD	LLAARCVFTG	EGRTEATHIG	GTSSDEMCNL	YIMYYMEAKH
	310	320	330	340	350
15	AVSFMTCQTN	VAPDMFRTIP	PEANIPIPVK	SDMVMMEHH	KETEKDKIP
	360	370	380	390	400
	LLQQPKREEE	EVLDQGDFYS	LLSKLLGERE	DVVHVHKYNP	TEKAESSEDL
	410	420	430	440	450
	VAEIANVVQK	KDLGRSDARE	GAEHERGNAI	LVRDRIHKFH	RLVSTLRPPE
20	460	470	480	490	500
	SRVFSLQQPP	PGEGTWEPEH	TGDFHMEEAL	DWPGVYLLPG	QVSGVALDPK
	510	520	530	540	550
	NNLVIFHRGD	HVWDGNSFDS	KFVYQQIGLG	PIEEDTILVI	DPNNAAVLQS
	560	570	580	590	600
25	SGKNLFYLP	GLSIDKDGNY	WVTDVALHQV	FKLDPNNKEG	PVLILGRSMQ
	610	620	630	640	650
	PGSDQNHFCQ	PTDVAVDPGT	GAIYVSDGYC	NSRIVQFSPS	GKFITQWGEE
	660	670	680	690	700
	SSGSSPLPGQ	FTVPHSLALV	PLLGQLCVAD	RENGRIQCFK	TDTKEFVREI
30	710	720	730	740	750
	KHSSFGRNVF	AIYIPGLLF	AVNGKPHFGD	QEPVQGFVMN	FSNGEIIDIF
	760	770	780	790	800
	KPVRKHFDMP	HDIVASEDGT	VYIGDAHTNT	VWKFTLTEKL	EHRSVKKAGI
	810				
35	EVQEIKEAEA	VVGS			

SEQ ID No. 48 - MR-ADM 21-42

CTVQKLAHQIYQFTDKDKDNVA

40 SEQ ID No. 49 - MR-ADM 27-39

AHQIYQFTDKDKD