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(54) Title: DPP3 FOR THERAPY GUIDANCE, MONITORING AND STRATIFICATION OF NT-ADM ANTIBODIES IN PATIENTS WITH SHOCK

(57) Abstract: The present application is directed to to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/or in a patient running into shock. In particular, the method comprises providing a sample from said patient, determining a level of DPP3 in said sample, and wherein the level of DPP3 in said sample is indicative of whether a treatment with an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is required. In a preferred embodiment of the invention, the method comprises additionally determining in a sample from said patient a level of ADM-NH2.

DPP3 for therapy guidance, monitoring and stratification of NT-ADM antibodies in patients with shock

Field of the invention

The invention relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/or in a patient running into shock. In particular, the method comprises providing a sample from said patient, determining a level of Dipeptidyl peptidase 3 (DPP3) in said sample, and wherein the level of DPP3 in said sample is indicative of whether a treatment with an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is required. In a preferred embodiment of the invention, the method comprises additionally determining in a sample from said patient a level of ADM-NH_2. Furthermore, the invention also relates to a kit for carrying out the method of the present invention.

Background

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

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

Structural analysis of DPP3 revealed the catalytic motifs HELLGH (hDPP3 450-455) and EECRAE (hDPP3 507-512), as well as following amino acids, that are important for substrate binding and hydrolysis: Glu316, Tyr, 318, Asp366, Asn391, Asn394, His568, Arg572, Arg577, Lys666 and Arg669

(*Prajapati & Chauhan 2011; Kumar et al. 2016*; numbering refers to the sequence of human DPP3, see SEQ ID NO. 1). Considering all known amino acids or sequence regions that are involved in substrate binding and hydrolysis, the active site of human DPP3 can be defined as the area between amino acids 316 and 669.

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

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

Circulating DPP3 levels were shown to be increased in cardiogenic shock patients and were associated with an increased risk of short-term mortality and severe organ dysfunction (*Deaniau et al. 2020. Eur J Heart Fail. 22(2):290-299*). Moreover, DPP3 measured at inclusion discriminated cardiogenic shock patients who did develop refractory shock vs. non-refractory shock and a DPP3 concentration ≥ 59.1 ng/mL was associated with a greater risk of death (*Takagi et al. 2020. Eur J Heart Fail. 22(2):279-286*).

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

exactly investigated, in particular the physiologically active peptides ADM and "PAMP", a peptide comprising 20 amino acids (22-41), which follows the 21 amino acids of the signal peptide in pre-proADM. The discovery and characterization of ADM in 1993 triggered intensive research activity, the results of which have been summarized in various review articles, in the context of the present description, reference being made in particular to the articles to be found in an issue of "Peptides" devoted to ADM in particular (*Takahashi 2001. Peptides 22: 1691; Eto 2001. Peptides 22: 1693-1711*). A further review is Hinson et al. 2000 (*Hinson et al. 2000. Endocrine Reviews 21(2):138-167*). In the scientific investigations to date, it has been found, inter alia, that ADM may be regarded as a polyfunctional regulatory peptide. It is released into the circulation in an inactive form extended by glycine (*Kitamura et al. 1998. Biochem Biophys Res Commun 244(2): 551-555*). There is also a binding protein (*Pio et al. 2001. The Journal of Biological Chemistry 276(15): 12292-12300*), which is specific 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 above-mentioned review articles *Eto et al. 2001* and *Hinson et al. 2000* see also *Kuwasaki et al. 1997. FEBS Lett 414(1): 105-110; Kuwasaki et al. 1999. Ann. Clin. Biochem. 36: 622-628; Tsuruda et al. 2001 Life Sci. 69(2): 239-245 and EP-A2 0 622 458*). 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 unusually high concentrations of ADM are observed in sepsis, and the highest concentrations in septic shock (*Eto 2001. Peptides 22: 1693-1711; Hirata et al. Journal of Clinical Endocrinology and Metabolism 81(4): 1449-1453; Ehlenz et al. 1997. Exp Clin Endocrinol Diabetes 105: 156-162; Tomoda et al. 2001. Peptides 22: 1783-1794; Ueda et al. 1999. Am. J. Respir. Crit. Care Med.160: 132-136* and *Wang et al. 2001. Peptides 22: 1835-1840*).

Plasma concentrations of ADM are elevated in patients with heart failure and correlate with disease severity (*Hirayama et al. 1999. J Endocrinol 160: 297–303; Yu et al. 2001. Heart 86: 155–160*). High plasma ADM is an independent negative prognostic indicator in these subjects (*Poyner et al. 2002. Pharmacol Rev 54: 233–246*).

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

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

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

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

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

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

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

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

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

It is the surprising finding of the present invention, that in patients with shock and patients running into shock, the level of DPP3 in a bodily fluid sample is to be used for the therapy guidance and/ or therapy mornitoring and/ or therapy stratification with an anti-ADM antibody and/ or anti-ADM antibody fragment and/ or anti-ADM non-Ig scaffold. Moreover, the results of the present invention clearly show, that patients with shock will have most benefit of a therapy with an anti-ADM antibody if the level of DPP3 in a bodily fluid sample is below a threshold.

Description of the invention

Subject matter of the present invention is a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, the method comprising:

- determining the level of dipeptidyl peptidase 3 (DPP3) in a sample of bodily fluid of said patient,
- comparing said level of determined DPP3 to a pre-determined threshold, and
- and wherein the level of DPP3 in said sample is indicative of whether a treatment with an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is required, and

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: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

Subject matter of the present invention is a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, the method comprising:

- determining the level of dipeptidyl peptidase 3 (DPP3) in a sample of bodily fluid of said patient,
- comparing said level of determined DPP3 to a pre-determined threshold, and
- administering an anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold to said patient, wherein said patient is treated with said anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold if said determined level of DPP3 is below a pre-determined threshold, and

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: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

Subject-matter of the present application is a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, the method comprising:

- determining the level of dipeptidyl peptidase 3 (DPP3) in a sample of bodily fluid of said patient,

- comparing said level of determined DPP3 to a pre-determined threshold, and
- administering an anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold to said patient,

wherein said patient is treated if said determined level of DPP3 is below a pre-determined threshold, and

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: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

One embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said shock is selected from the group comprising shock due to hypovolemia, cardiogenic shock, obstructive shock and distributive shock, in particular cardiogenic shock or septic shock.

One preferred embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein

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

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said pre-determined threshold of DPP3 in a sample of bodily fluid of said subject is between 20 and 120 ng/mL, more preferred between 30 and 80 ng/mL, even more preferred between 40 and 60 ng/mL, most preferred said threshold is 50 ng/mL.

Another specific embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein either the level of DPP3 protein and/or the level of active DPP3 is determined and compared to a pre-determined threshold.

Another preferred embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the level of DPP3 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to DPP3.

One embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said determination comprises the use of a capture-binder that binds specifically to full-length DPP3, wherein said capture-binder may be selected from the group of antibody, antibody fragment or non-IgG scaffold.

A further embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the amount of DPP3 protein and/or DPP3 activity is determined in a bodily fluid sample of said subject and wherein said determination comprises the use of a capture-binder that binds specifically to full-length DPP3, wherein said capture-binder is an antibody.

One embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the amount of DPP3 protein and/or DPP3 activity is determined in a bodily fluid sample of said subject and wherein said determination comprises the use of a capture-binder that binds specifically to full-length DPP3, wherein said capture-binder is immobilized on a surface.

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the amount of DPP3 protein and/or DPP3 activity is determined in a bodily fluid sample of said subject and wherein said separation step is a washing step that removes ingredients of the sample that are not bound to said capture-binder from the captured DPP3.

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock,

wherein the method for determining DPP3 activity in a bodily fluid sample of said subject comprises the steps:

- contacting said sample with a capture-binder that binds specifically to full-length DPP3,
- separating DPP3 bound to said capture binder,
- adding substrate of DPP3 to said separated DPP3,
- quantifying of said DPP3 activity by measuring and quantifying the conversion of a substrate of DPP3.

Another specific embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the DPP3 activity is determined in a bodily fluid sample of said subject and wherein DPP3 substrate conversion is detected by a method selected from the group comprising: fluorescence of fluorogenic substrates (e.g. Arg-Arg-βNA, Arg-Arg-AMC), color change of chromogenic substrates, luminescence of substrates coupled to aminoluciferin (Promega Protease-Glo™ Assay), mass spectrometry, HPLC/ FPLC (reversed phase chromatography, size exclusion chromatography), thin layer chromatography, capillary zone electrophoresis, gel electrophoresis followed by activity staining (immobilized, active DPP3) or western blot (cleavage products).

Another preferred embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the DPP3 activity is determined in a bodily fluid sample of said subject and wherein said substrate may be selected from the group comprising: angiotensin II, III and IV, Leu-enkephalin, Met-enkephalin, endomorphin 1 and 2, valorphin, β-casomorphin, dynorphin, proctolin, ACTH and MSH, or di-peptides coupled to a fluorophore, a chromophore or aminoluciferin wherein the di-peptide is Arg-Arg.

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the DPP3 activity is determined in a bodily fluid sample of said subject and wherein said substrate may be selected from the group comprising: A di-peptide coupled to a fluorophore, a chromophore or aminoluciferin wherein the di-peptide is Arg-Arg.

One embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said patient is additionally characterized by having a level of ADM-NH_2 above a threshold.

One specific embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said threshold of ADM-NH_2 in a sample of bodily fluid of said patient is between 40 and 100 pg/mL, more preferred between 50 and 90 pg/mL, even more preferred between 60 and 80 pg/mL, most preferred said threshold is 70 pg/mL.

One preferred embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the level of ADM-NH_2 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to ADM-NH_2.

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the sample of bodily fluid of said patient is selected from the group of blood, serum, plasma, urine, cerebrospinal fluid (CSF), and saliva.

Another specific embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the level of DPP3 and the level of ADM-NH_2 is determined in combination.

Another preferred embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the level of DPP3 and the level of ADM-NH_2 is determined simultaneously.

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein the level of DPP3 and the level of ADM-NH_2 is determined using a point-of-care device.

Another preferred embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said point-of-care device is a microfluidic device.

As used herein, a microfluidic device has a plurality of chambers arranged at different positions which are connected in parallel and into which a fixed amount of fluid may be efficiently distributed without using a separate driving source, wherein said device includes a platform having a center of rotation and including at least one microfluidic structure. Microfluidic devices are used to perform biological or chemical reactions by manipulating small amounts of fluid.

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, 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.

A further embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said antibody, antibody fragment or non-Ig scaffold does not bind to the C-terminal portion of ADM, having the sequence amino acid 43-52 of ADM: PRSKISPQGY-NH_2 (SEQ ID NO: 24).

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said antibody or fragment is a monoclonal antibody or fragment that binds to ADM or an antibody fragment thereof, wherein the heavy chain comprises the sequences:

CDR1: SEQ ID NO: 1

GYTFSRYW

CDR2: SEQ ID NO: 2

ILPGSGST

CDR3: SEQ ID NO: 3

TEGYEYDGFDY

and wherein the light chain comprises the sequences:

CDR1: SEQ ID NO: 4

QSIVYSNGNTY

CDR2:

RVS

CDR3: SEQ ID NO: 5

FQGSHIPYT.

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said antibody or fragment comprises a sequence selected from the group comprising as a VH region:

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

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGSTNYNE
KFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFDYWGQGTTLTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

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

DVLLSQTPLSLPVSLGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 12 (AM-VL1)

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

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

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC.

Another embodiment of the present application relates to a method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, wherein said antibody or fragment comprises the following sequence as a heavy chain:

SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
KFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK

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

SEQ ID NO: 33

DVVLTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPGQSPRLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The LIA is a one-step chemiluminescence sandwich immunoassay that uses white high-binding polystyrene microtiter plates as solid phase. These plates are coated with monoclonal anti-DPP3 antibody AK2555 (capture antibody). The tracer anti-DPP3 antibody AK2553 is labeled with MA70-acridinium-NHS-ester and used at a concentration of 20 ng per well. Twenty microliters of samples (e.g. serum, heparin-plasma, citrate-plasma or EDTA-plasma derived from patients' blood) and calibrators are pipetted into coated white microtiter plates. After adding the tracer antibody AK2553, the microtiter plates are incubated for 3 h at room temperature and 600 rpm. Unbound tracer is then removed by 4 washing steps (350 µL per well). Remaining chemiluminescence is measured for 1s per well by using a microtiter plate luminometer. The concentration of DPP3 is determined with a 6-point calibration curve. Calibrators and samples are preferably run in duplicate.

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

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

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

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

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

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

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

Mature ADM, bio-ADM and ADM-NH_2 is used synonymously throughout this application and is a molecule according to SEQ ID No.: 20.

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

In a specific embodiment an assay is used for determining the level ADM-NH_2, wherein the assay sensitivity of said assay is able to quantify the mature ADM-NH_2 of healthy subjects and is < 70 pg/ml, preferably < 40 pg/ml and more preferably < 10 pg/ml.

In a specific embodiment of the invention the threshold for ADM-NH_2 is between 40 and 100 pg/mL, more preferred between 50 and 90 pg/mL, even more preferred between 60 and 80, most preferred a threshold of 70 pg/ml is applied.

In a specific embodiment of the invention a threshold for plasma ADM-NH_2 is the 5fold median concentration, preferably the 4fold median concentration, more preferred the 3fold median concentration, most preferred the 2fold median concentration of a normal healthy population.

In a specific embodiment, said binder exhibits a binding affinity to ADM-NH_2 of at least 10^7 M^{-1}, preferred 10^8 M^{-1}, preferred affinity is greater than 10^9 M^{-1}, most preferred greater than 10^{10} M^{-1}. A person skilled in the art knows that it may be considered to compensate lower affinity by applying a higher dose of compounds and this measure would not lead out-of-the-scope of the invention.

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*).

In a specific embodiment, said binder is selected from the group comprising an antibody or an antibody fragment or a non-Ig scaffold binding to ADM-NH_2.

In a specific embodiment an assay is used for determining the level of ADM-NH_2, wherein such assay is a sandwich assay, preferably a fully automated assay.

In one embodiment such assay for determining the level of the biomarkers (DPP3 and/ or ADM-NH_2) is a sandwich immunoassay using any kind of detection technology including but not restricted to enzyme label, chemiluminescence label, electrochemiluminescence label, preferably a fully automated assay. In one embodiment of the 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®, BiomerieuxVidas®, Alere Triage®.

A variety of immunoassays are known and may be used for the assays and methods of the present invention, these include: mass spectrometry (MS), luminescence immunoassay (LIA), radioimmunoassays ("RIA"), homogeneous enzyme-multiplied immunoassays ("EMIT"), enzyme linked immunoadsorbent assays ("ELISA"), apoenzyme reactivation immunoassay ("ARIS"), luminescence-based bead arrays, magnetic beads based arrays, protein microarray assays, rapid test formats such as for instance dipstick immunoassays, immuno-chromatographic strip tests, rare cryptate assay and automated systems/ analysers.

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

In one embodiment of the invention, it may be a so-called POC-test (point-of-care) that is a test technology, which allows performing the test within less than 1 hour near the patient without the

requirement of a fully automated assay system. One example for this technology is the immunochromatographic test technology, e.g. a microfluidic device.

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

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

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

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

In the context of the present invention, fluorescence based assays comprise the use of dyes, which may for instance be selected from the group comprising FAM (5-or 6-carboxyfluorescein), VIC, NED, Fluorescein, Fluoresceinisothiocyanate (FITC), IRD-700/800, Cyanine dyes, auch as CY3, CY5, CY3.5, CY5.5, Cy7, Xanthen, 6-Carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), TET, 6-Carboxy-4',5'-dichloro-2',7'-dimethodyfluorescein (JOE), N,N,N',N'-Tetramethyl-6-carboxyrhodamine (TAMRA), 6-Carboxy-X-rhodamine (ROX), 5-Carboxyrhodamine-6G (R6G5), 6-carboxyrhodamine-6G (RG6), Rhodamine, Rhodamine Green,

Rhodamine Red, Rhodamine 110, BODIPY dyes, such as BODIPY TMR, Oregon Green, Coumarines such as Umbelliferone, Benzimides, such as Hoechst 33258; Phenanthridines, such as Texas Red, Yakima Yellow, Alexa Fluor, PET, Ethidiumbromide, Acridinium dyes, Carbazol dyes, Phenoxazine dyes, Porphyrine dyes, Polymethin dyes, and the like.

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

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

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

The ADM-NH_2 levels of the present invention have been determined with the described ADM-NH_2 assay (*Weber et al. 2017. JALM 2(2):1-4*). The DPP3 levels of the present invention have been determined with the described DPP3-assays as outlined in the examples (*Rehfeld et al. 2019. JALM 3(6): 943-953*). The mentioned threshold values above might be different in other assays, if these have been calibrated differently from the assay systems used in the present invention. Therefore, the mentioned cut-off values above shall apply for such differently calibrated assays accordingly, taking into account the differences in calibration. One possibility of quantifying the difference in calibration is a method comparison analysis (correlation) of the assay in question with the respective biomarker assay used in the present invention by measuring the respective biomarker (e.g. bio-ADM, DPP3) in samples using both methods. Another possibility is to determine with the assay in question, given this test has sufficient analytical sensitivity, the median biomarker level of a representative normal population, compare results with the median biomarker levels as described in the literature and recalculate the calibration based on the difference obtained by this comparison. With the calibration used in the present invention, samples from normal (healthy) subjects have been measured: median plasma bio-ADM (mature ADM-NH_2) was 24.7 pg/ml, the lowest value 11 pg/ml and the 99th percentile 43 pg/ml *(Marino et al. 2014. Critical Care 18:R34)*. With the calibration used in the present invention, samples from 5,400 normal (healthy) subjects (swedish single-center prospective population-based Study (MPP-

RES)) have been measured: median (interquartile range) plasma DPP3 was 14.5 ng/ml (11.3 ng/ml – 19 ng/ml).

As used herein, the term "therapy guidance" refers to application of certain therapies or medical interventions based on the value of one or more biomarkers and/or clinical parameter and/or clinical scores.

The term "therapy monitoring" in the context of the present invention refers to the monitoring and/or adjustment of a therapeutic treatment of said patient, for example by obtaining feedback on the efficacy of the therapy.

The term "therapy stratification" in particular relates to grouping or classifying patients into different groups, such as therapy groups that receive or do not receive therapeutic measures depending on their classification.

A particular advantage of the method of the present invention is that patients with shock or patients running into shock can be stratified with respect to the required therapy, wherein said therapy is the administration of an anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold that binds to the N-terminal part (amino acid 1-21) of ADM: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14). The stratified patient groups may include patients that require an initiation of treatment and patients that do not require initiation of treatment.

Another particular advantage of the present invention is that the method can discriminate patients who are more likely to benefit from said therapy from patients who are not likely to benefit from said therapy.

In a preferred embodiment, the treatment is initiated or changed immediately upon provision of the result of the sample analysis indicating the level of DPP3 and/ or ADM-NH_2 in the sample. In further embodiments, the treatment may be initiated within 12 hours, preferably 6, 4, 2, 1, 0.5, 0.25 hours or immediately after receiving the result of the sample analysis.

In some embodiments, the method comprises or consists of a single and/ or multiple measurement of DPP3 and/ or ADM-NH_2 in a sample from a patient in a single sample and/or multiple samples obtained at essentially the same time point, in order to guide and/ or monitor and/ or stratify a therapy, wherein

said therapy is the administration of an anti-ADM antibody or anti-ADM fragment or anti-ADM non-Ig scaffold that binds to the N-terminal part (amino acid 1-21) of ADM: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

It is particularly preferable to determine the level of DPP3 in the same sample as the level of ADM-NH_2 in the context of the method of the present invention. In this embodiment, both biomarkers DPP3 and ADM-NH_2 can be determined in the same sample at the same time in a multiplex assay format or at different time points in a multiplex assay format or single assay format. Multiplex assays can be duplex assays for determining both markers, wherein the assay may be a point of care assay that can be performed immediately after sample isolation in the same place where the patient is encountered.

The present invention further relates to a kit for carrying out the method of the invention, comprising detection reagents for determining the level of DPP3 and additionally for determining the level of ADM-NH_2 in a sample from a patient.

It may also preferably be determined as a point of care assay that can be carried out directly at the place where the patient encounters the medical personnel, such as, for example, an emergency department or primary care unit. Furthermore, the assay for detection of DPP3 and additionally ADM-NH_2 may be an assay, preferably a duplex assay and/or a point of care assay that is automated or semi-automated.

In a preferred embodiment the present invention is related to methods and kits for determining the level of DPP3 and additionally for determining the level of ADM-NH_2 and optionally further biomarkers in a sample from a patient.

Said further biomarkers may be selected from the group comprising procalcitonin (PCT), C-reactive protein (CRP), lactate.

The present invention further relates to a kit for carrying out the method of the invention, comprising detection reagents for determining DPP3 and additionally for determining the level of ADM-NH_2, in a sample from a patient, and reference data, such as a reference and/ or threshold level, corresponding to a level of DPP3 in said sample between 20 and 120 ng/mL, more preferred between 30 and 80 ng/mL, even more preferred between 40 and 60 ng/mL, most preferred 50 ng/mL, wherein said reference data is preferably stored on a computer readable medium and/or employed in the form of computer

executable code configured for comparing the determined DPP3 and additionally for determining the level of ADM-NH_2 to said reference data.

In one embodiment of the method described herein, the method additionally comprises comparing the determined level of DPP3 and additonally ADM-NH_2 in patients with shock or patients running into shock to a reference and/ or threshold level, wherein said comparing is carried out in a computer processor using computer executable code.

The methods of the present invention may in part be computer-implemented. For example, the step of comparing the detected level of a marker, e.g. DPP3 and/ or ADM-NH_2, with a reference and/ or threshold level can be performed in a computer system. For example, the determined values may be entered (either manually by a health professional or automatically from the device(s) in which the respective marker level(s) has/have been determined) into the computer-system. The computer-system can be directly at the point-of-care (e.g. primary care unit or ED) or it can be at a remote location connected via a computer network (e.g. via the internet, or specialized medical cloud-systems, optionally combinable with other IT-systems or platforms such as hospital information systems (HIS)). Alternatively, or in addition, the associated therapy guidance and/ or therapy stratification will be displayed and/or printed for the user (typically a health professional such as a physician).

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

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

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

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

losses/insensible losses (e.g. burns, heat stroke) or third-space loss in the setting of pancreatitis, cirrhosis, intestinal obstruction, trauma, or

- in case of obstructive shock said patient may have suffered a cardiac tamponade, tension pneumothorax, pulmonary embolism or aortic stenosis, or
- in case of distributive shock said patient has septic shock, neurogenic shock, anaphylactic shock or shock due to adrenal crisis.

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

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

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

in Europe. Despite advances in treatment mainly by early revascularization with subsequent mortality reduction, CS remains the leading cause of death in AMI with mortality rates still approaching 40–50% according to recent registries and randomized trials (*Goldberg et al. 2009. Circulation 119: 1211–1219*).

Obstructive shock is due to a physical obstruction of the great vessels or the heart itself. Several conditions can result in this form of shock (e.g. cardiac tamponade, tension pneumothorax, pulmonary embolism, aortic stenosis). For review see *Koya and Paul 2018. Shock. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019-2018 Oct 27*.

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

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

Septic shock is a potentially fatal medical condition that occurs when sepsis, which is organ injury or damage in response to infection, leads to dangerously low blood pressure and abnormalities in cellular metabolism. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) defines septic shock as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone. Patients with septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia. This combination is associated with hospital mortality rates greater than 40% (*Singer et al. 2016. JAMA. 315 (8): 801–10)*. The primary infection is most commonly caused by bacteria, but also may be by fungi, viruses or parasites. It may be located in any part of the body, but most commonly in the lungs, brain, urinary tract, skin or abdominal organs. It can cause multiple organ dysfunction syndrome (formerly known as multiple organ failure) and death. Frequently, people with septic shock are cared for in intensive care units. It most commonly affects children, immunocompromised individuals, and the elderly, as their immune systems cannot deal with infection as effectively as those of healthy adults. The mortality rate from septic shock is approximately 25–50%.

In one embodiment of the invention said patient is a critically ill patient having shock or running into shock at the time the sample of bodily fluid of said patient is taken.

"A patient running into shock" is defined as a critically ill patient that does not have shock at the time the bodily fluid is taken from said patient, but has an increased risk of developing shock.

In a specific embodiment said shock is septic shock or cardiogenic shock.

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

Due to these positive results, a humanized version of an N-terminal anti-ADM antibody, named Adrecizumab, has been developed for further clinical development. Beneficial effects of Adrecizumab on vascular barrier function and survival were recently demonstrated in preclinical models of systemic inflammation and sepsis (*Geven et al. 2018. Shock 50(6):648-654*). In this study, pre-treatment with

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

A mechanistic explanation for this increase could be that the excess of antibody in the circulation may drain ADM from the interstitium to the circulation, since ADM is small enough to cross the endothelial barrier, whereas the antibody is not (*Geven et al. 2018. Shock. 50(2):132-140*). 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 ADMs detrimental effects on vascular smooth muscle cells (VSMCs; vasodilation) in the interstitium are reduced.

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".

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

higher, most preferably at least 1000-fold higher than towards other molecules comprised in a sample containing the molecule of interest. It is well known in the art how to make antibodies and to select antibodies with a given specificity.

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

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

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

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

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

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

- a molecule or molecules binding to ADM, which upon addition to a culture of an eukaryotic cell line, which expresses functional human recombinant ADM receptor composed of CRLR (calcitonin receptor like receptor) and RAMP3 (receptor-activity modifying protein 3), reduces the amount of cAMP produced by the cell line through the action of parallel added human synthetic ADM peptide, wherein said added human synthetic ADM is added in an amount that in the absence of the non-neutralizing antibody to be analyzed, leads to half-maximal stimulation of cAMP synthesis, wherein the reduction of cAMP by said molecule(s) binding to ADM takes place to an extent, which is not more than 80%, even when the non-neutralizing molecule(s) binding to ADM to be analyzed is added in an amount, which is 10-fold more than the amount, which is needed to obtain the maximal reduction of cAMP synthesis obtainable with the non-neutralizing antibody to be analyzed.

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

An antibody or fragment according to the present invention is a protein including one or more polypeptides substantially encoded by immunoglobulin genes that specifically binds an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha (IgA), gamma (IgG_1, IgG_2, IgG_3, IgG_4), delta (IgD), epsilon (IgE) and mu (IgM) constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin light chains are generally about 25 Kd or 214 amino acids in length.

Full-length immunoglobulin heavy chains are generally about 50 Kd or 446 amino acid in length. Light chains are encoded by a variable region gene at the NH_2-terminus (about 110 amino acids in length) and a kappa or lambda constant region gene at the COOH-terminus. Heavy chains are similarly encoded by a variable region gene (about 116 amino acids in length) and one of the other constant region genes.

The basic structural unit of an antibody is generally a tetramer that consists of two identical pairs of immunoglobulin chains, each pair having one light and one heavy chain. In each pair, the light and heavy chain variable regions bind to an antigen, and the constant regions mediate effector functions. Immunoglobulins also exist in a variety of other forms including, for example, Fv, Fab, and $(Fab')_2$, as well as bifunctional hybrid antibodies and single chains (*e.g.*, Lanzavecchia et al. 1987. Eur. J. Immunol.

17:105; Huston et al. 1988. Proc. Natl. Acad. Sci. U.S.A., 85:5879-5883; Bird et al. 1988. Science 242:423-426; Hood et al. 1984, Immunology, Benjamin, N.Y., 2nd ed.; Hunkapiller and Hood 1986. Nature 323:15-16). An immunoglobulin light or heavy chain variable region includes a framework region interrupted by three hypervariable regions, also called complementarity determining regions (CDR's) (see, Sequences of Proteins of Immunological Interest, E. Kabat et al. 1983, U.S. Department of Health and Human Services). As noted above, the CDRs are primarily responsible for binding to an epitope of an antigen. An immune complex is an antibody, such as a monoclonal antibody, chimeric antibody, humanized antibody or human antibody, or functional antibody fragment, specifically bound to the antigen.

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

accomplished, for example, by EBV infection or by fusing a human B cell with a myeloma or hybridoma cell to produce a trioma cell. Human antibodies can also be produced by phage display methods (see, *e.g.* WO91/17271; WO92/001047; WO92/20791), or selected from a human combinatorial monoclonal antibody library (see the Morphosys website). Human antibodies can also be prepared by using transgenic animals carrying a human immunoglobulin gene (for example, see WO93/12227; WO 91/10741).

Thus, the anti-ADM antibody may have the formats known in the art. Examples are human antibodies, monoclonal antibodies, humanized antibodies, chimeric antibodies, CDR-grafted antibodies. In a preferred embodiment antibodies according to the present invention are recombinantly produced antibodies as *e.g.* IgG, a typical full-length immunoglobulin, or antibody fragments containing at least the F-variable domain of heavy and/or light chain as *e.g.* chemically coupled antibodies (fragment antigen binding) including but not limited to Fab-fragments including Fab minibodies, single chain Fab antibody, monovalent Fab antibody with epitope tags, *e.g.* Fab-V5Sx2; bivalent Fab (mini-antibody) dimerized with the CH3 domain; bivalent Fab or multivalent Fab, *e.g.* formed via multimerization with the aid of a heterologous domain, *e.g.* via dimerization of dHLX domains, *e.g.* Fab-dHLX-FSx2; F(ab')$_2$-fragments, scFv-fragments, multimerized multivalent or/and multi-specific scFv-fragments, bivalent and/or bispecific diabodies, BITE® (bispecific T-cell engager), trifunctional antibodies, polyvalent antibodies, *e.g.* from a different class than G; single-domain antibodies, *e.g.* nanobodies derived from camelid or fish immunoglobulines and numerous others.

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

In a preferred embodiment the anti-ADM antibody format is selected from the group comprising Fv fragment, scFv fragment, Fab fragment, scFab fragment, F(ab)$_2$ fragment and scFv-Fc Fusion protein. In another preferred embodiment the antibody format is selected from the group comprising scFab fragment, Fab fragment, scFv fragment and bioavailability optimized conjugates thereof, such as PEGylated fragments. One of the most preferred formats is the scFab format.

Non-Ig scaffolds may be protein scaffolds and may be used as antibody mimics as they are capable to bind to ligands or antigens. Non-Ig scaffolds may be selected from the group comprising tetranectin-based non-Ig scaffolds (*e.g.* described in US 2010/0028995), fibronectin scaffolds (*e.g.* described in EP

1 266 025; lipocalin-based scaffolds (*e.g.* described in *WO 2011/154420*); ubiquitin scaffolds (*e.g.* described in *WO 2011/073214*), transferrin scaffolds (*e.g.* described in *US 2004/0023334*), protein A scaffolds (*e.g.* described in *EP 2 231 860*), ankyrin repeat based scaffolds (*e.g.* described in *WO 2010/060748*), microproteins preferably microproteins forming a cysteine knot) scaffolds (*e.g.* described in *EP 2314308*), Fyn SH3 domain based scaffolds (*e.g.* described in *WO 2011/023685*) EGFR-A-domain based scaffolds (*e.g.* described in *WO 2005/040229*) and Kunitz domain based scaffolds (*e.g.* described in *EP 1 941 867*).

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

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

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

In a preferred embodiment the ADM antibody format is selected from the group comprising Fv fragment, scFv fragment, Fab fragment, scFab fragment, $F(ab)_2$ fragment and scFv-Fc Fusion protein. In another preferred embodiment the antibody format is selected from the group comprising scFab fragment, Fab fragment, scFv fragment and bioavailability optimized conjugates thereof, such as PEGylated fragments. One of the most preferred formats is scFab format.

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

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

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

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

In one specific embodiment of the invention the anti-Adrenomedullin (ADM) antibody or anti-ADM antibody fragment binding to adrenomedullin or anti-ADM non-Ig scaffold binding to adrenomedullin is provided for use in therapy or prevention of shock in a patient, wherein said antibody or fragment or scaffold binds to a region of preferably at least 4, or at least 5 amino acids within the sequence of amino acid 1-21 of mature human ADM: YRQSMNNFQGLRSFGCRFGTC SEQ ID No.: 14.

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

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

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

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

In another preferred embodiment said anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold recognizes and binds to the N-terminal end (amino acid 1) of adrenomedullin. N-terminal end means that the amino acid 1, that is "Y" of SEQ ID No. 20, 14 or 23, respectively and is mandatory for binding. The antibody or fragment or scaffold would neither bind N-terminal extended nor N-terminal modified Adrenomedullin nor N-terminal degraded adrenomedullin. This means in another preferred embodiment said anti-ADM-antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold binds only to a region within the sequence of mature ADM if the N-terminal end of ADM is free. In said embodiment the anti-ADM antibody or anti-ADM antibody fragment or non-Ig scaffold would not bind to a region within the sequence of mature ADM if said sequence is *e.g.* comprised within pro-ADM.

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

In another specific embodiment pursuant to the invention the herein provided anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold does not bind to the C-terminal portion of ADM, *i.e.* the amino acid 43 – 52 of ADM: PRSKISPQGY-NH_2 (SEQ ID No.: 24).

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

Conformational and linear epitopes interact with the paratope based on the 3-D conformation adopted by the epitope, which is determined by the surface features of the involved epitope residues and the shape or tertiary structure of other segments of the antigen. A conformational epitope is formed by the 3-D conformation adopted by the interaction of discontiguous amino acid residues. A linear or a

sequential epitope is an epitope that is recognized by antibodies by its linear sequence of amino acids, or primary structure and is formed by the 3-D conformation adopted by the interaction of contiguous amino acid residues.

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

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

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

The following steps may be conducted:

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

- The half-life of ADM is calculated for ADM incubated in absence and presence of an ADM stabilizing antibody or an adrenomedullin stabilizing antibody fragment or an adrenomedullin stabilizing non-Ig scaffold, respectively.
- The enhancement of half-life is calculated for the stabilized ADM in comparison to ADM that has been incubated in absence of an ADM stabilizing antibody or an adrenomedullin stabilizing antibody fragment or an adrenomedullin stabilizing non-Ig scaffold.

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

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

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

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

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

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

In a specific embodiment according to the present invention a non-neutralizing anti-ADM antibody or anti-ADM antibody fragment or ADM non-Ig scaffold is used, wherein said anti-ADM antibody or an anti-ADM antibody fragment blocks the bioactivity of ADM to less than 80 %, preferably less than 50% (of baseline values). It has to be understood that said limited blocking of the bioactivity (meaning reduction of the bioactivity) of ADM occurs even at excess concentration of the antibody, fragment or scaffold, meaning an excess of the antibody, fragment or scaffold in relation to ADM. Said limited blocking is an intrinsic property of the ADM binder itself in said specific embodiment. This means that said antibody, fragment or scaffold has a maximal inhibition of 80% or 50% respectively. In a preferred embodiment said anti-ADM antibody, anti-ADM antibody fragment or anti-ADM non-Ig scaffold would block the bioactivity / reduce the bioactivity of anti-ADM to at least 5 %. The stated above means that approximately 20% or 50% or even 95% residual ADM bioactivity remains present, respectively.

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

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

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

A maximal inhibition in said ADM bioassay of 50% means that said anti-ADM antibody or said anti-ADM antibody fragment or said anti-ADM non-Ig scaffold, respectively, blocks the bioactivity of ADM

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

However, by the present specification and in the above context the expression "blocks the bioactivity of ADM" in relation to the herein disclosed anti-ADM antibodies, anti-ADM antibody fragments, and anti-ADM non-Ig scaffolds should be understood as mere decreasing the bioactivity of ADM from 100% to 20% remaining ADM bioactivity at maximum, preferably decreasing the ADM bioactivity from 100% to 50% remaining ADM bioactivity; but in any case there is ADM bioactivity remaining that can be determined as detailed above.

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

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

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

Such a modulating anti-ADM antibody or modulating anti-ADM antibody fragment or a modulating anti-ADM non-Ig scaffold offers the advantage that the dosing of the administration is facilitated. The combination of partially blocking or partially reducing ADM bioactivity and increase of the in vivo half-life (increasing the ADM bioactivity) leads to beneficial simplicity of anti-ADM antibody or an anti-

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

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.

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

GYTFSRYW (SEQ ID No.:1),

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

TEGYEYDGFDY (SEQ ID No.: 3)

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

QSIVYSNGNTY (SEQ ID No.: 4),

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

FQGSHIPYT (SEQ ID No.: 5).

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

GYTFSRYW (SEQ ID No.:1),

ILPGSGST (SEQ ID No.: 2),

TEGYEYDGFDY (SEQ ID No.: 3)

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

QSIVYSNGNTY (SEQ ID No.: 4),

RVS (not part of the Sequencing Listing),

FQGSHIPYT (SEQ ID No.: 5).

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

GYTFSRYW (SEQ ID No.: 1),

ILPGSGST (SEQ ID No.: 2),

TEGYEYDGFDY (SEQ ID No.: 3)

and wherein the light chain comprises the sequences:

QSIVYSNGNTY (SEQ ID No.: 4),

RVS (not part of the Sequencing Listing),

FQGSHIPYT (SEQ ID No.: 5).

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

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

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

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

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGSTNYNE
KFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFDYWGQGTTLTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

DVLLSQTPLSLPVSLGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ

LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 12 (AM-VL1)

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

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

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC.

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

SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
KFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFScSVMHEALHNHY
TQKSLSLSPGK

and comprises the following sequence as a light chain:

SEQ ID NO: 33

DVVLTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPGQSPRLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC.

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

SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
KFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK

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

SEQ ID NO: 33

DVVLTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPGQSPRLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

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

wherein the heavy chain comprises the sequences:

CDR1: SEQ ID NO: 1

GYTFSRYW

CDR2: SEQ ID NO: 2

ILPGSGST

CDR3: SEQ ID NO: 3

TEGYEYDGFDY

and wherein the light chain comprises the sequences:

CDR1: SEQ ID NO: 4

QSIVYSNGNTY

CDR2:

RVS

CDR3: SEQ ID NO: 5

FQGSHIPYT.

This means, in one embodiment of the invention the CDR's do not exhibit any variations of the sequence. Any variation of the above sequence is outside of the CDR's in said embodiment.

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.

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

The term "pharmaceutical formulation" means a pharmaceutical ingredient in combination with at least one pharmaceutically acceptable excipient, which is in such form as to permit the biological activity of a pharmaceutical ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. The term "pharmaceutical ingredient" means a therapeutic composition which can be optionally combined with pharmaceutically acceptable excipients to provide a pharmaceutical formulation or dosage form.

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

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

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

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

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

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.

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

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

1. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, the method comprising:
    - determining the level of dipeptidyl peptidase 3 (DPP3) in a sample of bodily fluid of said patient,
    - comparing said level of determined DPP3 to a pre-determined threshold, and
    - administering an anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold to said patient,

    wherein said patient is treated if said determined level of DPP3 is below a pre-determined threshold, and

    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: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

2. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiment 1, wherein said shock is selected from the group comprising shock due to hypovolemia, cardiogenic shock, obstructive shock and distributive shock, in particular cardiogenic shock or septic shock.

3. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiment 1 and 2, wherein
    - in case of cardiogenic shock said patient may have suffered an acute coronary syndrome (e.g. acute myocardial infarction) or wherein said patient has heart failure (e.g. acute decompensated heart failure), myocarditis, arrhythmia, cardiomyopathy, valvular heart disease, aortic dissection with acute aortic stenosis, traumatic chordal rupture or massive pulmonary embolism, or
    - in case of hypovolemic shock said patient may have suffered a hemorrhagic disease including gastrointestinal bleed, trauma, vascular etiologies (e.g. ruptured abdominal aortic aneurysm, tumor eroding into a major blood vessel) and spontaneous bleeding in the setting of anticoagulant use or a non-hemorrhagic disease including vomiting, diarrhea, renal loss, skin losses/insensible losses (e.g. burns, heat stroke) or third-space loss in the setting of pancreatitis, cirrhosis, intestinal obstruction, or
    - in case of obstructive shock said patient may have suffered a cardiac tamponade, tension pneumothorax, pulmonary embolism or aortic stenosis, or

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

4. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 3, wherein said pre-determined threshold of DPP3 in a sample of bodily fluid of said subject is between 20 and 120 ng/mL, more preferred between 30 and 80 ng/mL, even more preferred between 40 and 60 ng/mL, most preferred said threshold is 50 ng/mL.

5. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 4, wherein either the level of DPP3 protein and/or the level of active DPP3 is determined and compared to a pre-determined threshold.

6. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 5, wherein the level of DPP3 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to DPP3.

7. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiment 1 to 6, wherein said determination comprises the use of a capture-binder that binds specifically to full-length DPP3 wherein said capture-binder may be selected from the group of antibody, antibody fragment or non-IgG scaffold.

8. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 7, wherein the amount of DPP3 protein and/or DPP3 activity is determined in a bodily fluid sample of said subject and wherein said determination comprises the use of a capture-binder that binds specifically to full-length DPP3 wherein said capture-binder is an antibody.

9. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 8, wherein the amount of DPP3 protein and/or DPP3 activity is determined in a bodily fluid sample of said subject and wherein said determination comprises the use of a capture-binder that binds specifically to full-length DPP3 wherein said capture-binder is immobilized on a surface.

10. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 9, wherein the amount of DPP3 protein and/or DPP3 activity is determined in a bodily fluid sample of said subject and wherein said separation step is a washing step that removes ingredients of the sample that are not bound to said capture-binder from the captured DPP3.

11. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 10, wherein the method for determining DPP3 activity in a bodily fluid sample of said subject comprises the steps:

- contacting said sample with a capture-binder that binds specifically to full-length DPP3,
- separating DPP3 bound to said capture binder,
- adding substrate of DPP3 to said separated DPP3,
- quantifying of said DPP3 activity by measuring and quantifying the conversion of a substrate of DPP3.

12. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 11, wherein the DPP3 activity is determined in a bodily fluid sample of said subject and wherein DPP3 substrate conversion is detected by a method selected from the group comprising: fluorescence of fluorogenic substrates (e.g. Arg-Arg-βNA, Arg-Arg-AMC), color change of chromogenic substrates, luminescence of substrates coupled to aminoluciferin (Promega Protease-Glo™ Assay), mass spectrometry, HPLC/ FPLC (reversed phase chromatography, size exclusion chromatography), thin layer chromatography, capillary zone electrophoresis, gel electrophoresis followed by activity staining (immobilized, active DPP3) or western blot (cleavage products).

13. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 12, wherein the DPP3 activity is determined in a bodily fluid sample of said subject and wherein said substrate may be selected from the group comprising: angiotensin II, III and IV, Leu-enkephalin, Met-enkephalin, endomorphin 1 and 2, valorphin, β-casomorphin, dynorphin, proctolin, ACTH and MSH, or di-peptides coupled to a fluorophore, a chromophore or aminoluciferin wherein the di-peptide is Arg-Arg.

14. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 13, wherein the DPP3 activity is determined in a bodily fluid sample of said subject and wherein said substrate may

be selected from the group comprising: A di-peptide coupled to a fluorophore, a chromophore or aminoluciferin wherein the di-peptide is Arg-Arg.

15. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 14, wherein said patient is additionally characterized by having a level of ADM-NH_2 above a threshold.

16. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiment 15, wherein said threshold of ADM-NH_2 in a sample of bodily fluid of said patient is between 40 and 100 pg/mL, more preferred between 50 and 90 pg/mL, even more preferred between 60 and 80 pg/mL, most preferred said threshold is 70 pg/mL.

17. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 15 and 16, wherein the level of ADM-NH_2 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to ADM-NH_2.

18. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 17, wherein the sample of bodily fluid of said patient is selected from the group of blood, serum, plasma, urine, cerebrospinal fluid (CSF), and saliva.

19. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 18, wherein the level of DPP3 and the level of ADM-NH_2 is determined in combination.

20. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 19, wherein the level of DPP3 and the level of ADM-NH_2 is determined simultaneously.

21. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 19 and 20, wherein the level of DPP3 and the level of ADM-NH_2 is determined using a point-of-care device.

22. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiment 21, wherein said point-of-care device is a microfluidic device.

23. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 22, 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.

24. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 23, wherein said antibody, antibody fragment or non-Ig scaffold does not bind to the C-terminal portion of ADM, having the sequence amino acid 43-52 of ADM: PRSKISPQGY-NH_2 (SEQ ID NO: 24).

25. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 24, wherein said antibody or fragment is a monoclonal antibody or fragment that binds to ADM or an antibody fragment thereof, wherein the heavy chain comprises the sequences:

CDR1: SEQ ID NO: 1

GYTFSRYW

CDR2: SEQ ID NO: 2

ILPGSGST

CDR3: SEQ ID NO: 3

TEGYEYDGFDY

and wherein the light chain comprises the sequences:

CDR1: SEQ ID NO: 4

QSIVYSNGNTY

CDR2:

RVS

CDR3: SEQ ID NO: 5

FQGSHIPYT.

26. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 25, wherein said antibody or fragment comprises a sequence selected from the group comprising as a VH region:

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

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGSTNYN
EKFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFDYWGQGTTLTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSGSTNY
AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGSTNY
AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGSTNY
AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTNY
AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

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

DVLLSQTPLSLPVSLGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFSGV
PDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 12 (AM-VL1)

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDSG
VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSGV
PDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

27. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to embodiments 1 to 25, wherein said antibody or fragment comprises the following sequence as a heavy chain:

SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYN
QKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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

SEQ ID NO: 33

DVVLTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPGQSPRLLIYRVSNRFSGV
PDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

FIGURE DESCRIPTION

Fig. 1a:

Illustration of antibody formats – Fv and scFv-Variants.

Fig. 1b:

Illustration of antibody formats – heterologous fusions and bifunctional antibodies.

Fig. 1c:

Illustration of antibody formats – bivalental antibodies and bispecific antibodies.

Fig. 2:

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 5.63nM hADM.

c: Dose/ inhibition curve of CT-H in the presence of 5.63 nM hADM.

d: Dose/ inhibition curve of MR-H in the presence of 5.63 nM hADM.

e: Dose/ inhibition curve of NT-H in the presence of 5.63 nM hADM.

f: Dose response curve of mouse ADM. Maximal cAMP stimulation was adjusted to 100% activation.

g: Dose/ inhibition curve of human ADM 22-52 (ADM-receptor antagonist) in the presence of 0,67 nM mADM.

h: Dose/ inhibition curve of CT-M in the presence of 0,67 nM mADM.

i: Dose/ inhibition curve of MR-M in the presence of 0,67 nM mADM.

j: Dose/ inhibition curve of NT-M in the presence of 0,67 nM mADM.

k: Shows the inhibition of ADM by F(ab)2 NT-M and by Fab NT-M.

l: shows the inhibition of ADM by F(ab)2 NT-M and by Fab NT-M.

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.

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

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

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

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

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

EXAMPLES

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

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

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.

Human Antibodies:

Human Antibodies were produced by means of phage display according to the following procedure: The human naive antibody gene libraries HAL7/8 were used for the isolation of recombinant single chain F-Variable domains (scFv) against adrenomedullin peptide. The antibody gene libraries were screened with a panning strategy comprising the use of peptides containing a biotin tag linked via two different spacers to the adrenomedullin peptide sequence. A mix of panning rounds using non-specifically bound antigen and streptavidin bound antigen were used to minimize background of non-specific binders. The eluted phages from the third round of panning have been used for the generation of monoclonal scFv expressing E. coli strains. Supernatant from the cultivation of these clonal strains has been directly used for an antigen ELISA testing (see also *Hust et al. 2011. Journal of Biotechnology 152, 159–170; Schütte 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 purified by a size exclusion chromatography.

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

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

Table 1: immunization peptides

Sequence Number	**Antigen/Immunogen**	**ADM Region**	**Designation**	**Affinity constants Kd (M)**
SEQ ID: 14	YRQSMNNFQGLRSFGCRFGTC	1-21	NT-H	5.9×10^{-9}
SEQ ID: 15	CTVQKLAHQIYQ	21-32	MR-H	2×10^{-9}
SEQ ID: 16	CAPRSKISPQGY-NH_2	C-42-52	CT-H	1.1×10^{-9}
SEQ ID: 17	YRQSMNQGSRSNGCRFGTC	1-19	NT-M	3.9×10^{-9}
SEQ ID: 18	CTFQKLAHQIYQ	19-31	MR-M	4.5×10^{-10}
SEQ ID: 19	CAPRNKISPQGY-NH_2	C-40-50	CT-M	9×10^{-9}

The following is a list of further obtained monoclonal antibodies:

Table 2:

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

	Mouse	ADM/379	1.6×10^{-8}	
	Mouse	ADM/380	1.8×10^{-8}	
	Mouse	ADM/381	2.4×10^{-8}	
	Mouse	ADM/382	1.6×10^{-8}	
	Mouse	ADM/383	1.8×10^{-8}	
	Mouse	ADM/384	1.7×10^{-8}	
	Mouse	ADM/385	1.7×10^{-8}	
	Mouse	ADM/403	1.2×10^{-8}	
	Mouse	ADM/395	1.2×10^{-8}	
	Mouse	ADM/396	3.0×10^{-8}	
	Mouse	ADM/397	1.5×10^{-8}	
MR-M	Mouse	ADM/38	4.5×10^{-10}	68
MR-M	Mouse	ADM/39	5.9×10^{-9}	72
CT-M	Mouse	ADM/65	9.0×10^{-9}	100
CT-M	Mouse	ADM/66	1.6×10^{-8}	100
NT-H	Mouse	ADM/33	5.9×10^{-8}	38
NT-H	Mouse	ADM/34	1.6×10^{-8}	22
MR-H	Mouse	ADM/41	1.2×10^{-8}	67
MR-H	Mouse	ADM/42	$<1 \times 10^{-8}$	
MR-H	Mouse	ADM/43	2.0×10^{-9}	73
MR-H	Mouse	ADM/44	$<1 \times 10^{-8}$	
CT-H	Mouse	ADM/15	$<1 \times 10^{-8}$	
CT-H	Mouse	ADM/16	1.1×10^{-9}	100
CT-H	Mouse	ADM/17	3.7×10^{-9}	100
CT-H	Mouse	ADM/18	$<1 \times 10^{-8}$	
hADM	Phage display	ADM/A7	$<1 \times 10^{-8}$	
	Phage display	ADM/B7	$<1 \times 10^{-8}$	
	Phage display	ADM/C7	$<1 \times 10^{-8}$	

	Phage display	ADM/G3	$<1x10^{-8}$	
	Phage display	ADM/B6	$<1x10^{-8}$	
	Phage display	ADM/B11	$<1x10^{-8}$	
	Phage display	ADM/D8	$<1x10^{-8}$	
	Phage display	ADM/D11	$<1x10^{-8}$	
	Phage display	ADM/G12	$<1x10^{-8}$	

Generation of antibody fragments by enzymatic digestion: The generation of Fab and $F(ab)_2$ fragments was done by enzymatic digestion of the murine full-length antibody NT-M. Antibody NT-M was digested using a) the pepsin-based $F(ab)_2$ Preparation Kit (Pierce 44988) and b) the papain-based Fab Preparation Kit (Pierce 44985). The fragmentation procedures were performed according to the instructions provided by the supplier. Digestion was carried out in case of $F(ab)_2$-fragmentation for 8h at 37°C. The Fab-fragmentation digestion was carried out for 16 h, respectively.

Procedure for Fab Generation and Purification: The immobilized papain was equilibrated by washing the resin with 0.5 ml of Digestion Buffer and centrifuging the column at 5000 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 were 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 0.5 mL 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 2 mL of PBS, centrifuge again for 1 minute and the flow-through discarded. The sample was applied to the column and resuspended by inversion. Incubation was done at room temperature with end-over-end mixing for 10 minutes. The column was centrifuged for 1 minute, saving the flow-through with the Fab fragments. (References: *Mariani et al. 1991. Mol. Immunol. 28: 69-77; Beale 1987. Exp Comp Immunol 11:287-96; Ellerson et al. 1972. FEBS Letters 24(3):318-22; Kerbel and Elliot 1983. Meth Enzymol 93:113-147; Kulkarni et al. 1985. Cancer Immunol Immunotherapy 19:211-4; Lamoyi 1986. Meth Enzymol 121:652-663; Parham et al. 1982. J Immunol Meth 53:133-73; Raychaudhuri et al. 1985. Mol Immunol 22(9):1009-19; Rousseaux et al. 1980. Mol Immunol 17:469-82; Rousseaux et al. 1983. J Immunol Meth 64:141-6; Wilson et al. 1991. J Immunol Meth 138:111-9*).

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

The following steps were done to achieve the humanized sequence:

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

Reaction Setup: 5x QIAGEN® OneStep RT-PCR Buffer 5.0 µl, dNTP Mix (containing 10 mM of each dNTP) 0.8 µl, Primer set 0.5 µl, QIAGEN® OneStep RT-PCR Enzyme Mix 0.8 µl,

Template RNA 2.0 µl, RNase-free water to 20.0 µl, Total volume 20.0 µl PCR condition: Reverse transcription: 50°C, 30 min; Initial PCR activation: 95°C, 15 min Cycling: 20 cycles of 94°C, 25 sec; 54°C, 30 sec; 72°C, 30 sec; Final extension: 72°C, 10 min Second-round semi-nested PCR: The RT-PCR products from the first-round reactions were further amplified in the second-round PCR. 12 individual heavy chain and 11 light chain RT-PCR reactions were set up using semi-nested primer sets specific for antibody variable regions.

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

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

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

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

QVQLQQSGAELMKPGASVKISCKAT**GYTFSRYW**IEWVKQRPGHGLEWIGE**ILPGSGST**NYN EKFKGKATITADTSS*NTAYMQLSSLTSEDSAVYYC**TEGYEYDGFDY**WGQGTTLTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKRV*EPK

SEQ ID No.: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKAS**GYTFSRYW**ISWVRQAPGQGLEWMGR**ILPGSGST**NY
AQKFQGRVTITADE*STSTAYMELSSLRSEDTAVYYC**TEGYEYDGFDY**WGQGTTVTVSSASTKGPSV*
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
*TQTYICNVNHKPSNTKVDKRV***EPK**

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

QVQLVQSGAEVKKPGSSVKVSCKAS**GYTFSRYW**IEWVRQAPGQGLEWMGR**ILPGSGST**NY
AQKFQGRVTITADE*STSTAYMELSSLRSEDTAVYYC**TEGYEYDGFDY**WGQGTTVTVSSASTKGPSV*
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
*TQTYICNVNHKPSNTKVDKRV***EPK**

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

QVQLVQSGAEVKKPGSSVKVSCKAT**GYTFSRYW**ISWVRQAPGQGLEWMGE**ILPGSGST**NY
AQKFQGRVTITADE*STSTAYMELSSLRSEDTAVYYC**TEGYEYDGFDY**WGQGTTVTVSSASTKGPSV*
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
*TQTYICNVNHKPSNTKVDKRV***EPK**

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

QVQLVQSGAEVKKPGSSVKVSCKAT**GYTFSRYW**IEWVRQAPGQGLEWMGE**ILPGSGST**NY
AQKFQGRVTITADE*STSTAYMELSSLRSEDTAVYYC**TEGYEYDGFDY**WGQGTTVTVSSASTKGPSV*
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
*TQTYICNVNHKPSNTKVDKRV***EPK**

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

DVLLSQTPLSLPVSLGDQATISCRSS**QSIVYSNGNTY**LEWYLQKPGQSPKLLIY**RVS**NRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYC**FQGSHIPYT**FGGGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No.: 12 (AM-VL1)

DVVMTQSPLSLPVTLGQPASISCRSS**QSIVYSNGNTY**LNWFQQRPGQSPRRLIY**RVS**NRDSGV
PDRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGSHIPYT**FGQGTKLEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

DVVMTQSPLSLPVTLGQPASISCRSS**QSIVYSNGNTY**LEWFQQRPGQSPRRLIY**RVS**NRDSGV
PDRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGSHIPYT**FGQGTKLEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 32 (Adrecizumab heavy chain)

QVQLVQSGAEVKKPGSSVKVSCKAS**GYTFSRYW**IEWVRQAPGQGLEWIGE**ILPGSGST**NYN
QKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYC**TEGYEYDGFDY**WGQGTTVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 33 (Adrecizumab light chain)

DVVLTQSPLSLPVTLGQPASISCRSS**QSIVYSNGNTY**LEWYLQRPGQSPRLLIY**RVS**NRFSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGSHIPYT**FGGGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC

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 recombinant Adrenomedullin receptor cAMP functional assay (Adrenomedullin Bioassay).

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

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

CHO-K1 cells expressing human recombinant adrenomedullin receptor (FAST-027C) grown prior to the test in media without antibiotic were detached by gentle flushing with PBS-EDTA (5 mM EDTA), recovered by centrifugation and resuspended in assay buffer (KRH: 5 mM KCl, 1.25 mM $MgSO_4$, 124 mM NaCl, 25 mM HEPES, 13.3 mM Glucose, 1.25 mM KH_2PO_4, 1.45 mM $CaCl_2$, 0.5 g/l BSA).

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

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

Antibodies testing cAMP-HTRF assay

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

The anti-m-ADM antibodies (NT-M, MR-M, CT-M) were tested for antagonist activity in human recombinant ADM receptor (FAST-027C) cAMP functional assay in the presence of 0.67 nM Mouse ADM 1-50, at the following final antibody concentrations: 100 µg/ml, 20 µg/ml, 4 µg/ml, 0.8 µg/ml, 0.16 µg/ml. Data were plotted relative inhibition vs. antagonist concentration (see figures 2 a to 2 l). The maximal inhibition by the individual antibody is given in table 3.

Table 3: maximal inhibition of bio-ADM activity

Antibody	**Maximal inhibition of ADM bioactivity (ADM-Bioassay) (%)**
NT-H	38
MR-H	73
CT-H	100
NT-M FAB	26
NT-M FAB2	28
NT-M	45
MR-M	66
CT-M	100
Non specific mouse IgG	0

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

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

Immunoassay for the quantification of human Adrenomedullin

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

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: 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 - Sepsis Mortality

a) Early treatment of sepsis

Animal model: 12-15 week-old male C57Bl/6 mice (Charles River Laboratories, Germany) were used for the study. Peritonitis had been surgically induced under light isofluran anesthesia. Incisions were

made into the left upper quadrant of the peritoneal cavity (normal location of the cecum). The cecum was exposed and a tight ligature was placed around the cecum with sutures distal to the insertion of the small bowel. One puncture wound was made with a 24-gauge needle into the cecum and small amounts of cecal contents were expressed through the wound. The cecum was replaced into the peritoneal cavity and the laparotomy site was closed. Finally, animals were returned to their cages with free access to food and water. 500µl saline were given s.c. as fluid replacement.

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

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

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

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

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

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

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

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

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

Table 4: 4-day mortality and BUN concentrations

4-day mortality	**survival (%)**	**BUN pre CLP (mM)**	**BUN day 1 (mM)**
PBS	0	8.0	23.2
non-specific mouse IgG	0	7.9	15.5
CT-M	10	7.8	13.5

MR-M	30	8.1	24.9
NT-M	70	8.8	8.2

It can be seen from Table 4 that the NT-M antibody reduced mortality considerably. After 4 days 70 % of the mice survived when treated with NT-M antibody. When treated with MR-M antibody 30 % of the animals survived and when treated with CT-M antibody 10 % of the animals survived after 4 days. In contrast thereto all mice were dead after 4 days when treated with unspecific mouse IgG. The same result was obtained in the control group where PBS (phosphate buffered saline) was administered to mice. The blood urea nitrogen or BUN test is used to evaluate kidney function, to help diagnose kidney disease, and to monitor patients with acute or chronic kidney dysfunction or failure. The results of the S-BUN Test revealed that the NT-M antibody was the most effective to protect the kidney.

b) late treatment of sepsis

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

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

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

1 NT-M, FAB2 0.2 mg/ml survival over 4 days

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

3 vehicle: - PBS 10µl/g bodyweight survival over 4 days

Table 5: 4-day mortality

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

It can be seen from Table 5 that the NT-M FAB 2 antibody reduced mortality considerably. After 4 days 75 % of the mice survived when treated with NT-M FAB 2 antibody. In contrast thereto all mice were dead after 4 days when treated with non-specific mouse IgG. The same result was obtained in the control group where PBS (phosphate buffered saline) was administered to mice.

Example 5 - Administration of NT-H in healthy humans

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

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

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

Peptides/ conjugates for immunization: DPP3 peptides for immunization were synthesized, see Table 6, (JPT Technologies, Berlin, Germany) with an additional N-terminal cystein (if no cystein is present

within the selected DPP3-sequence) residue for conjugation of the peptides to Bovine Serum Albumin (BSA). The peptides were covalently linked to BSA by using Sulfolink-coupling gel (Perbio-science, Bonn, Germany). The coupling procedure was performed according to the manual of Perbio. Recombinant GST-hDPP3 was produced by USBio (United States Biological, Salem, MA, USA).

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

Splenocytes from the immunized mice and cells of the myeloma cell line SP2/0 were fused with 1 ml 50% polyethylene glycol for 30 s at 37°C. After washing, the cells were seeded in 96-well cell culture plates. Hybrid clones were selected by growing in HAT medium [RPMI 1640 culture medium supplemented with 20% fetal calf serum and HAT-Supplement]. After one week, the HAT medium was replaced with HT Medium for three passages followed by returning to the normal cell culture medium. The cell culture supernatants were primarily screened for recombinant DPP3 binding IgG antibodies two weeks after fusion. Therefore, recombinant GST-tagged hDPP3 (USBiologicals, Salem, USA) was immobilized in 96-well plates (100 ng/ well) and incubated with 50 µl cell culture supernatant per well for 2 hours at room temperature. After washing of the plate, 50 µl / well POD-rabbit anti mouse IgG was added and incubated for 1 h at RT. After a next washing step, 50 µl of a chromogen solution (3,7 mM o-phenylen-diamine in citrate/ hydrogen phosphate buffer, 0.012% H_2O_2) were added to each well, incubated for 15 minutes at RT and the chromogenic reaction stopped by the addition of 50 µl 4N sulfuric acid. Absorption was detected at 490 mm.

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

Mouse monoclonal antibody production

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

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

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

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

Labelling procedure (tracer): 100 µg (100 µl) of the different antiDPP3 antibodies (detection antibody, 1 mg/ ml in PBS, pH 7.4) were mixed with 10 µl acridinium NHS-ester (1 mg/ml in acetonitrile, InVent GmbH, Germany; EP 0 353 971) and incubated for 30 min at room temperature. Labelled antiDPP3 antibody was purified by gel-filtration HPLC on Shodex Protein 5 µm KW-803 (Showa Denko, Japan). The purified labelled antibody was diluted in assay buffer (50 mmol/l potassium phosphate, 100 mmol/l NaCl, 10 mmol/l Na_2-EDTA, 5 g/l bovine serum albumin, 1 g/l murine IgG, 1 g/l bovine IgG, 50 µmol/l amastatin, 100 µmol/l leupeptin, pH 7.4). The final concentration was approx. 5-7*10^6 relative light units (RLU) of labelled compound (approx. 20 ng labelled antibody) per 200 µl. acridinium ester chemiluminescence was measured by using a Centro LB 960 luminometer (Berthold Technologies GmbH & Co. KG).

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

Characterization of antibodies – hDPP3-inhibition analysis

To analyze the capability of DPP3 inhibition by the different antibodies and antibody clones a DPP3 activity assay with known procedure (Jones et al., 1982) was performed. Recombinant GST-tagged hDPP3 was diluted in assay buffer (25 ng/ ml GST-DPP3 in 50 mM Tris-HCl, pH7,5 and 100 µM $ZnCl_2$) and 200 µl of this solution incubated with 10 µg of the respective antibody at room temperature. After 1 hour of pre-incubation, fluorogenic substrate Arg-Arg-βNA (20 µl, 2mM) was added to the solution and the generation of free βNA over time was monitored using the Twinkle LB 970 microplate fluorometer (Berthold Technologies GmbH & Co. KG) at 37 °C. Fluorescence of βNA is detected by

exciting at 340 nm and measuring emission at 410 nm. Slopes (in RFU/ min) of increasing fluorescence of the different samples are calculated. The slope of GST-hDPP3 with buffer control is appointed as 100 % activity. The inhibitory ability of a possible capture-binder is defined as the decrease of GST-hDPP3 activity by incubation with said capture-binder in percent.

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

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

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

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

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

Example 7 – DPP3 in shock

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

a) Study Cohort – Sepsis/Septic Shock

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

b) Study Cohort – Cardiogenic Shock

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

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

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

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

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

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

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

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

	n	HR	p-value (1-sided, log rank)
all	294	0.701	0.100
DPP3 <70	261	0.546	0.027
DPP3 >70	27	1.24	0.384
DPP3 <60	254	0.449	0.008
DPP3 >60	34	1.62	0.231
DPP3 <50	244	0.426	0.007
DPP3 >50	44	1.69	0.209
DPP3 <40	227	0.385	0.005
DPP3 >40	61	1.35	0.286

SEQUENCES

SEQ ID No.: 1

GYTFSRYW

SEQ ID No.: 2

ILPGSGST

SEQ ID No.: 3

TEGYEYDGFDY

SEQ ID No.: 4

QSIVYSNGNTY

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

RVS

SEQ ID No.: 5

FQGSHIPYT

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

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGSTNYNE
KFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFDYWGQGTTLTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID No.: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG

PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTNYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

DVLLSQTPLSLPVSLGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No.: 12 (AM-VL1)

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

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

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

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

YRQSMNNFQGLRSFGCRFGTC

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

CTVQKLAHQIYQ

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

CAPRSKISPQGY-$CONH_2$

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

YRQSMNQGSRSNGCRFGTC

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

CTFQKLAHQIYQ

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

CAPRNKISPQGY-$CONH_2$

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-$CONH_2$

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

YRQSMNQGSRSNGCRFGTCTFQKLAHQIYQLTDKDKDGMAPRNKISPQGY-$CONH_2$

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

YRQSMNNFQGLRSFGCRFGTC

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

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDKDKDNVA

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

PRSKISPQGY-NH2

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

YRQSMNNFQGLRSF

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

YRQSMNNFQG

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

YRQSMN

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

YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQ

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

YRQSMNQGSRSNGCRFGTCTFQKLAHQIYQLTDKDKDGMA

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

YRQSMNQGSRSNGCRFGTCTFQKLAHQIYQL

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

ARLDVASEF RKKWNKWALS RGKRELRMSS SYPTGLADVK AGPAQTLIRP QDMKGASRSP
EDSSPDAARI RVKRYRQSMN NFQGLRSFGC RFGTCTVQKL AHQIYQFTDK DKDNVAPRSK
ISPQGYGRRR RRSLPEAGPG RTLVSSKPQA HGAPAPPSGS APHFL

SEQ ID NO: 32 (Adrecizumab heavy chain)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNYNQ
KFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 33 (Adrecizumab light chain)

DVVLTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPGQSPRLLIYRVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

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

MADTQYILPNDIGVSSLDCREAFRLLSPTERLYAYHLSRAAWYGGLAVLLQTSPEAPYIYALL
SRLFRAQDPDQLRQHALAEGLTEEEYQAFLVYAAGVYSNMGNYKSFGDTKFVPNLPKEKLE
RVILGSEAAQQHPEEVRGLWQTCGELMFSLEPRLRHLGLGKEGITTYFSGNCTMEDAKLAQD
FLDSQNLSAYNTRLFKEVDGEGKPYYEVRLASVLGSEPSLDSEVTSKLKSYEFRGSPFQVTRG
DYAPILQKVVEQLEKAKAYAANSHQGQMLAQYIESFTQGSIEAHKRGSRFWIQDKGPIVESYI
GFIESYRDPFGSRGEFEGFVAVVNKAMSAKFERLVASAEQLLKELPWPPTFEKDKFLTPDFTS
LDVLTFAGSGIPAGINIPNYDDLRQTEGFKNVSLGNVLAVAYATQREKLTFLEEDDKDLYILW
KGPSFDVQVGLHELLGHGSGKLFVQDEKGAFNFDQETVINPETGEQIQSWYRSGETWDSKFS
TIASSYEECRAESVGLYLCLHPQVLEIFGFEGADAEDVIYVNWLNMVRAGLLALEFYTPEAFN
WRQAHMQARFVILRVLLEAGEGLVTITPTTGSDGRPDARVRLDRSKIRSVGKPALERFLRRLQ
VLKSTGDVAGGRALYEGYATVTDAPPECFLTLRDTVLLRKESRKLIVQPNTRLEGSDVQLLE
YEASAAGLIRSFSERFPEDGPELEEILTQLATADARFWKGPSEAPSGQA

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

CETVINPETGEQIQSWYRSGE

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

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGTANYAQ KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARYYYYYGMDVWGQGTTVTVSS

SEQ ID No. 37 - **HB3**

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGSTNYNE KFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFDYWGQGTTLTVSS

CLAIMS

1. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock, the method comprising:
   - determining the level of dipeptidyl peptidase 3 (DPP3) in a sample of bodily fluid of said patient,
   - comparing said level of determined DPP3 to a pre-determined threshold, and
   - and wherein the level of DPP3 in said sample is indicative of whether a treatment with an anti-ADM antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold is required, and

   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: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

2. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claim 1, the method comprising:
   - determining the level of dipeptidyl peptidase 3 (DPP3) in a sample of bodily fluid of said patient,
   - comparing said level of determined DPP3 to a pre-determined threshold, and
   - administering an anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold to said patient, wherein said patient is treated with said anti-adrenomedullin (ADM) antibody or anti-ADM antibody fragment or anti-ADM non-Ig scaffold if said determined level of DPP3 is below a pre-determined threshold, and

   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: YRQSMNNFQGLRSFGCRFGTC (SEQ ID No. 14).

3. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claim 1 or 2, wherein said shock is selected from the group comprising shock due to hypovolemia, cardiogenic shock, obstructive shock and distributive shock, in particular cardiogenic shock or septic shock.

4. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to according to claims 1 to 3, wherein

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

5. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 4, wherein said pre-determined threshold of DPP3 level in a sample of bodily fluid of said subject is between 20 and 120 ng/mL, more preferred between 30 and 80 ng/mL, even more preferred between 40 and 60 ng/mL, most preferred said threshold is 50 ng/mL.

6. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 5, wherein either the level of DPP3 protein and/or the level of active DPP3 is determined and compared to a pre-determined threshold.

7. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 6, wherein the level of DPP3 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to DPP3.

8. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claim 1 to 7, wherein said

determination comprises the use of a capture-binder that binds specifically to full-length DPP3 wherein said capture-binder may be selected from the group of antibody, antibody fragment or non-IgG scaffold.

9. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 8, wherein the amount of DPP3 protein and/or DPP3 activity is determined in a bodily fluid sample of said subject and wherein said determination comprises the use of a capture-binder that binds specifically to full-length DPP3 wherein said capture-binder is an antibody.

10. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 9, wherein the amount of DPP3 protein and/or DPP3 activity is determined in a bodily fluid sample of said subject and wherein said determination comprises the use of a capture-binder that binds specifically to full-length DPP3 wherein said capture-binder is immobilized on a surface.

11. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 10, wherein the amount of DPP3 protein and/or DPP3 activity is determined in a bodily fluid sample of said subject and wherein said separation step is a washing step that removes ingredients of the sample that are not bound to said capture-binder from the captured DPP3.

12. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 11, wherein the method for determining DPP3 activity in a bodily fluid sample of said subject comprises the steps:

- contacting said sample with a capture-binder that binds specifically to full-length DPP3,
- separating DPP3 bound to said capture binder,
- adding substrate of DPP3 to said separated DPP3,
- quantifying of said DPP3 activity by measuring and quantifying the conversion of a substrate of DPP3.

13. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 12, wherein the DPP3 activity is determined in a bodily fluid sample of said subject and wherein DPP3 substrate conversion is detected by a method selected from the group comprising: fluorescence of fluorogenic substrates (e.g. Arg-Arg-βNA, Arg-Arg-AMC), color change of chromogenic

substrates, luminescence of substrates coupled to aminoluciferin (Promega Protease-Glo™ Assay), mass spectrometry, HPLC/ FPLC (reversed phase chromatography, size exclusion chromatography), thin layer chromatography, capillary zone electrophoresis, gel electrophoresis followed by activity staining (immobilized, active DPP3) or western blot (cleavage products).

14. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 13, wherein the DPP3 activity is determined in a bodily fluid sample of said subject and wherein said substrate may be selected from the group comprising: angiotensin II, III and IV, Leu-enkephalin, Met-enkephalin, endomorphin 1 and 2, valorphin, β-casomorphin, dynorphin, proctolin, ACTH and MSH, or di-peptides coupled to a fluorophore, a chromophore or aminoluciferin wherein the di-peptide is Arg-Arg.

15. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 14, wherein the DPP3 activity is determined in a bodily fluid sample of said subject and wherein said substrate may be selected from the group comprising: A di-peptide coupled to a fluorophore, a chromophore or aminoluciferin wherein the di-peptide is Arg-Arg.

16. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 15, wherein said patient is additionally characterized by having a level of ADM-NH_2 above a threshold.

17. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claim 16 wherein said threshold of ADM-NH_2 in a sample of bodily fluid of said patient is between 40 and 100 pg/mL, more preferred between 50 and 90 pg/mL, even more preferred between 60 and 80 pg/mL, most preferred said threshold is 70 pg/mL.

18. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 16 and 17, wherein the level of ADM-NH_2 is determined by contacting said sample of bodily fluid with a capture binder that binds specifically to ADM-NH_2.

19. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 18, wherein

the sample of bodily fluid of said patient is selected from the group of blood, serum, plasma, urine, cerebrospinal fluid (CSF), and saliva.

20. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 19, wherein the level of DPP3 and the level of ADM-NH_2 is determined in combination.

21. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claim 20, wherein the level of DPP3 and the level of ADM-NH_2 is determined simultaneously.

22. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 20 and 21, wherein the level of DPP3 and the level of ADM-NH_2 is determined using a point-of-care device.

23. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claim 22, wherein said point-of-care device is a microfluidic device.

24. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 23, 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.

25. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 24, wherein said antibody, antibody fragment or non-Ig scaffold does not bind to the C-terminal portion of ADM, having the sequence amino acid 43-52 of ADM: PRSKISPQGY-NH_2 (SEQ ID NO: 24).

26. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 25, wherein said antibody or fragment is a monoclonal antibody or fragment that binds to ADM or an antibody fragment thereof, wherein the heavy chain comprises the sequences:

CDR1: SEQ ID NO: 1

GYTFSRYW

CDR2: SEQ ID NO: 2

ILPGSGST

CDR3: SEQ ID NO: 3

TEGYEYDGFDY

and wherein the light chain comprises the sequences:

CDR1: SEQ ID NO: 4

QSIVYSNGNTY

CDR2:

RVS

CDR3: SEQ ID NO: 5

FQGSHIPYT.

27. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 26, wherein said antibody or fragment comprises a sequence selected from the group comprising as a VH region:

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

QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGSTNY
NEKFKGKATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFDYWGQGTTLTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID NO: 7 (AM-VH1)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWISWVRQAPGQGLEWMGRILPGSGSTN
YAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWMGRILPGSGSTN
YAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWISWVRQAPGQGLEWMGEILPGSGSTN
YAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

QVQLVQSGAEVKKPGSSVKVSCKATGYTFSRYWIEWVRQAPGQGLEWMGEILPGSGSTN
YAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

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

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

DVLLSQTPLSLPVSLGDQATISCRSSQSIVYSNGNTYLEWYLQKPGQSPKLLIYRVSNRFSG
VPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 12 (AM-VL1)

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLNWFQQRPGQSPRRLIYRVSNRDSG
VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

DVVMTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWFQQRPGQSPRRLIYRVSNRDSG
VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGQGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

28. A method for therapy guidance and/ or therapy monitoring and/ or therapy stratification in a patient with shock and/ or in a patient running into shock according to claims 1 to 26, wherein said antibody or fragment comprises the following sequence as a heavy chain:

SEQ ID NO: 32

QVQLVQSGAEVKKPGSSVKVSCKASGYTFSRYWIEWVRQAPGQGLEWIGEILPGSGSTNY
NQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCTEGYEYDGFDYWGQGTTVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

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

SEQ ID NO: 33

DVVLTQSPLSLPVTLGQPASISCRSSQSIVYSNGNTYLEWYLQRPGQSPRLLIYRVSNRFSG
VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHIPYTFGGGTKLEIKRTVAAPSVFIFPP

SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

or a sequence that is > 95% identical to it

wherein the heavy chain comprises the sequences:

CDR1: SEQ ID NO: 1

GYTFSRYW

CDR2: SEQ ID NO: 2

ILPGSGST

CDR3: SEQ ID NO: 3

TEGYEYDGFDY

and wherein the light chain comprises the sequences:

CDR1: SEQ ID NO: 4

QSIVYSNGNTY

CDR2:

RVS

CDR3: SEQ ID NO: 5

FQGSHIPYT.

FIGURES

Fig. 1a:

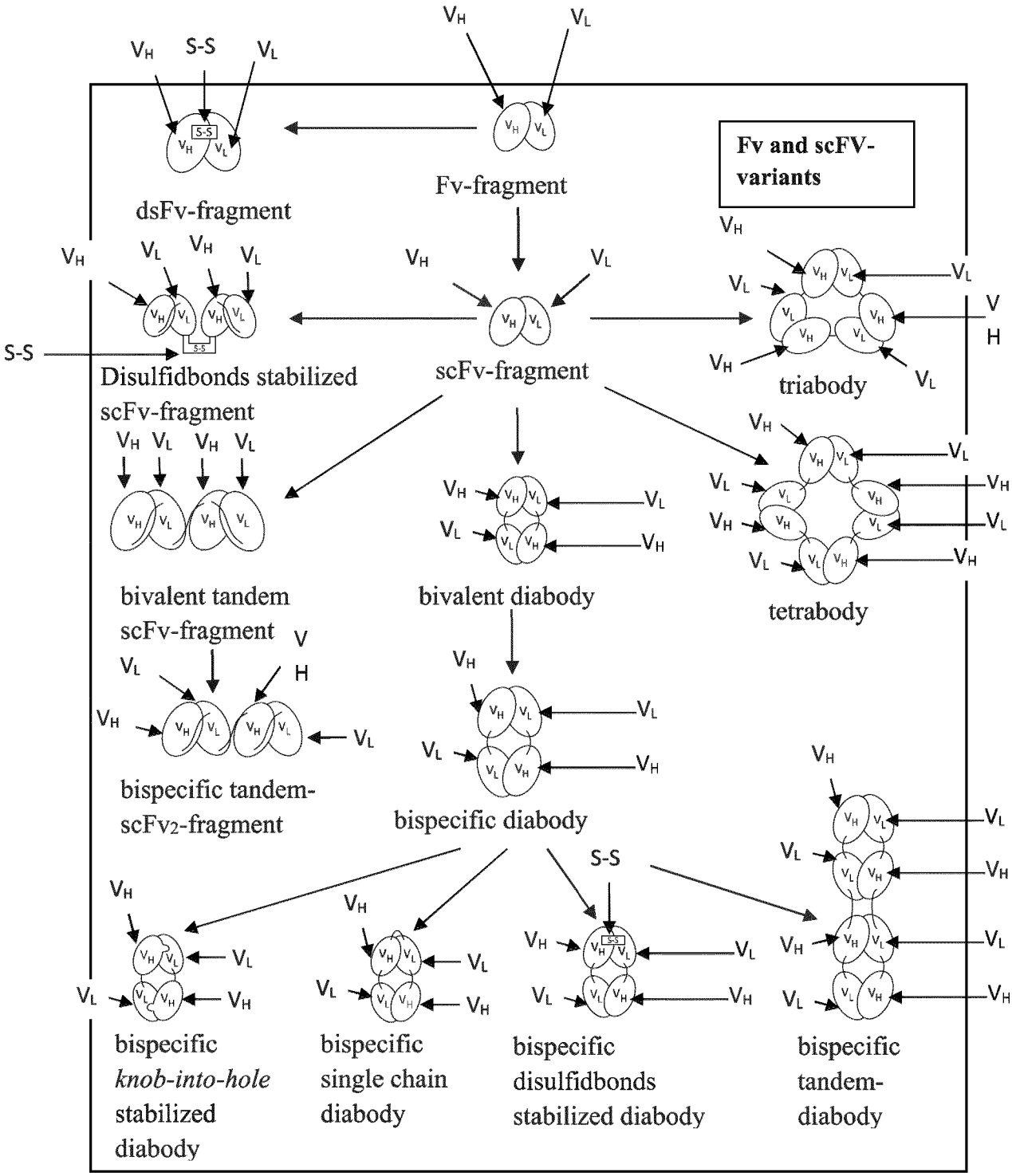


Fig. 1b:

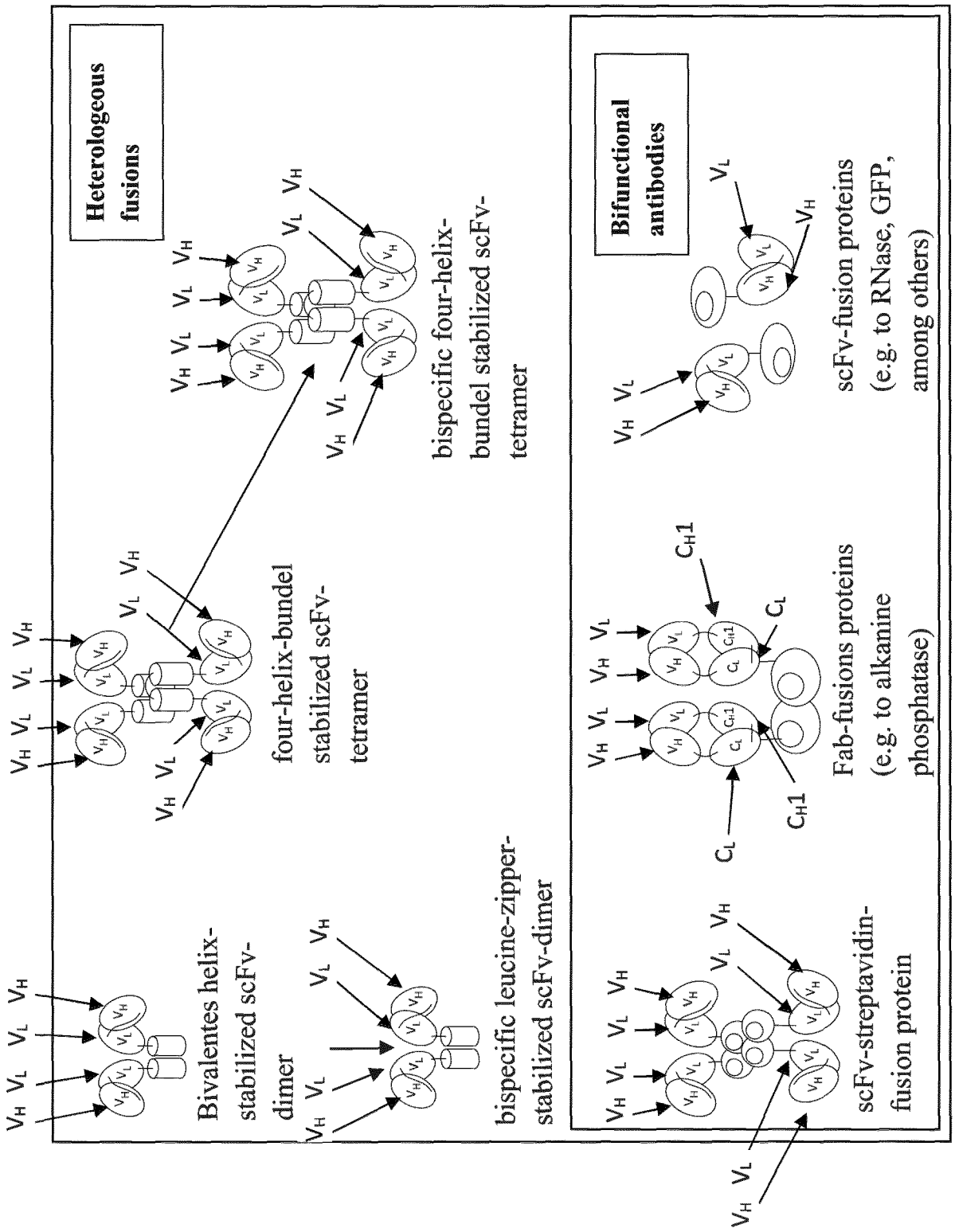

Heterologeous fusions
Bivalentes helix-stabilized scFv-dimer
four-helix-bundel stabilized scFv-tetramer
bispecific four-helix-bundel stabilized scFv-tetramer
bispecific leucine-zipper-stabilized scFv-dimer
Bifunctional antibodies
scFv-streptavidin-fusion protein
Fab-fusions proteins (e.g. to alkanine phosphatase)
scFv-fusion proteins (e.g. to RNase, GFP, among others)

Fig. 1c:

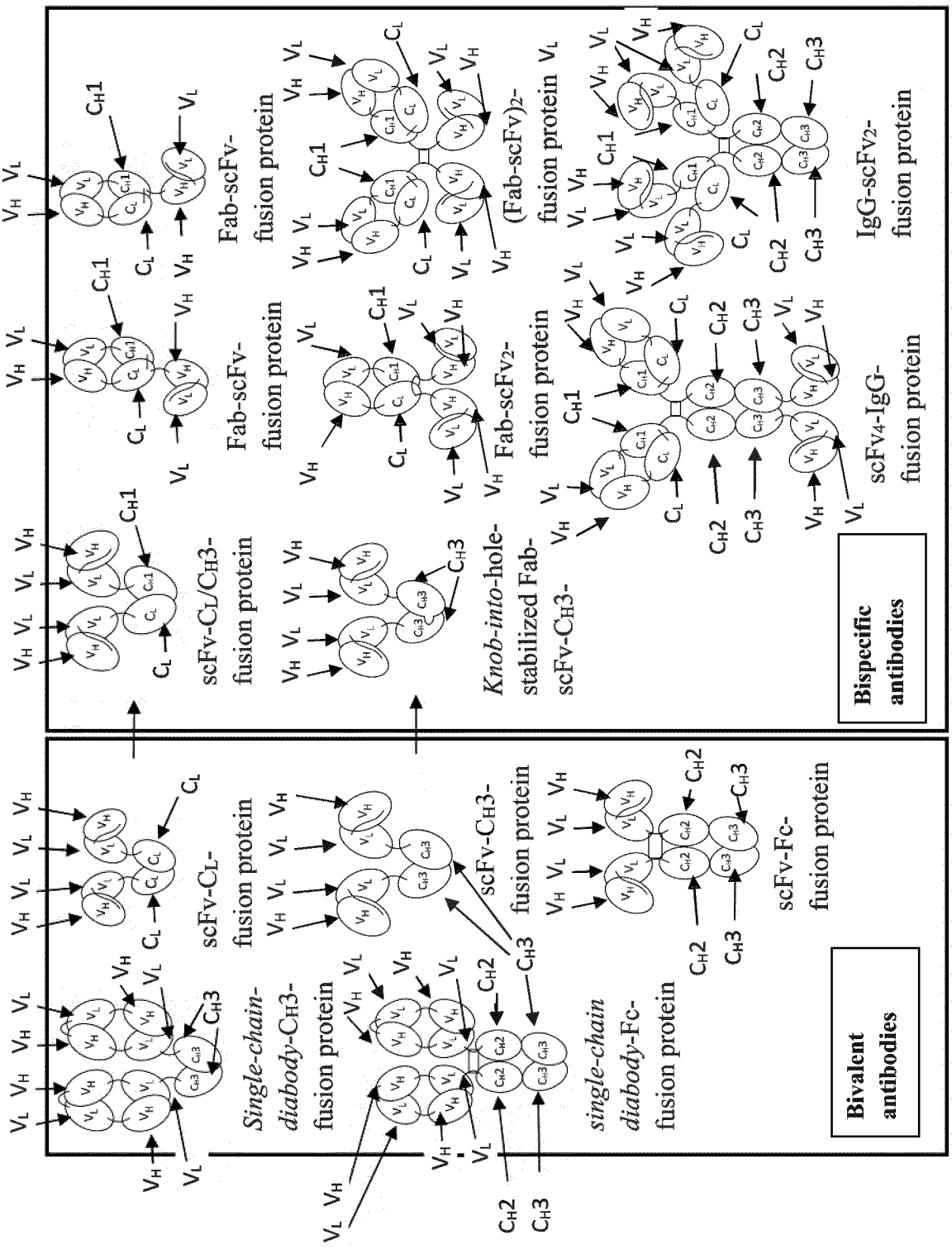

Bivalent antibodies
Single-chain-diabody-CH3-fusion protein
scFv-CL-fusion protein
scFv-CH3-fusion protein
single-chain diabody-Fc-fusion protein
scFv-Fc-fusion protein
Bispecific antibodies
scFv-CL/CH3-fusion protein
Fab-scFv-fusion protein
Knob-into-hole-stabilized Fab-scFv-CH3-fusion protein
Fab-scFv2-fusion protein
(Fab-scFv)2-fusion protein
scFv4-IgG-fusion protein
IgG-scFv2-fusion protein

Fig. 2 a:

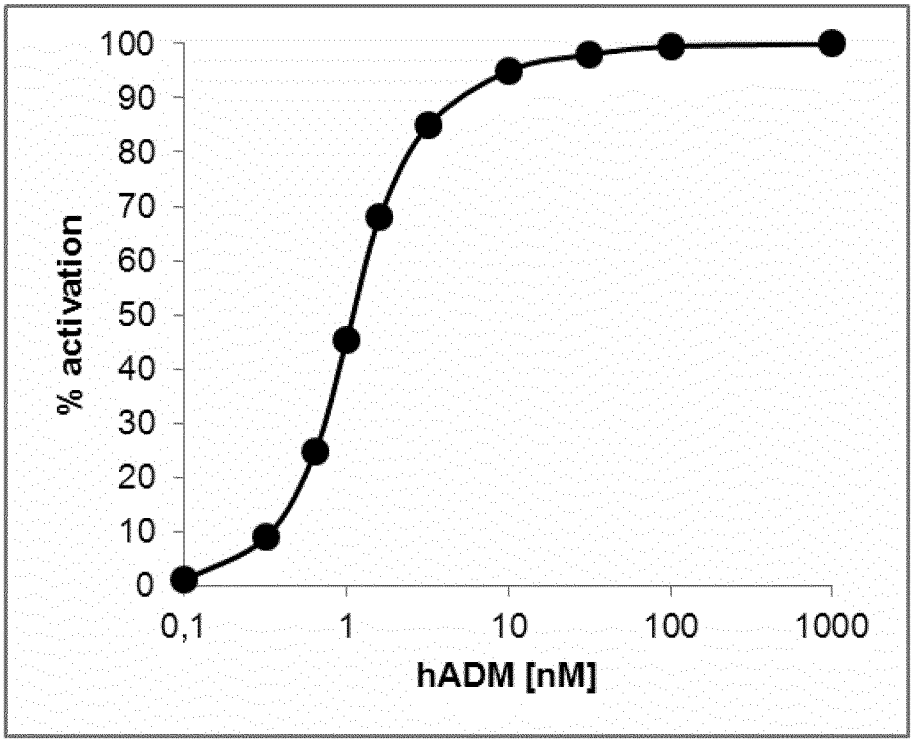

100
90
80
70
60
50
40
30
20
10
0
% activation
0,1
1
10
100
1000
hADM [nM]

Fig. 2 b:

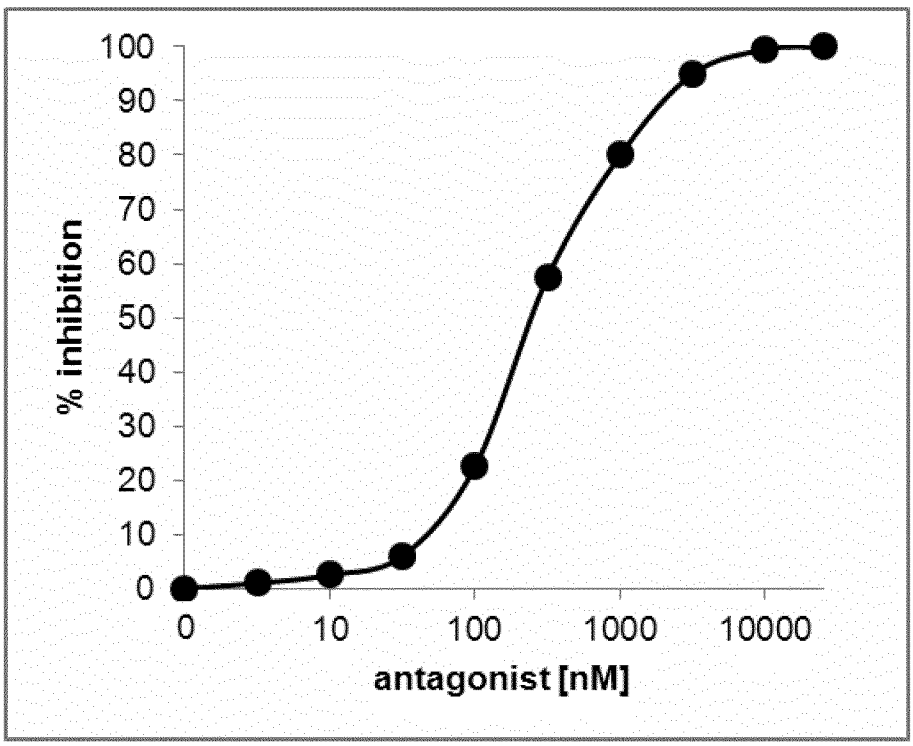

100
90
80
70
60
50
40
30
20
10
0
% inhibition
0
10
100
1000
10000
antagonist [nM]

Fig. 2 c:

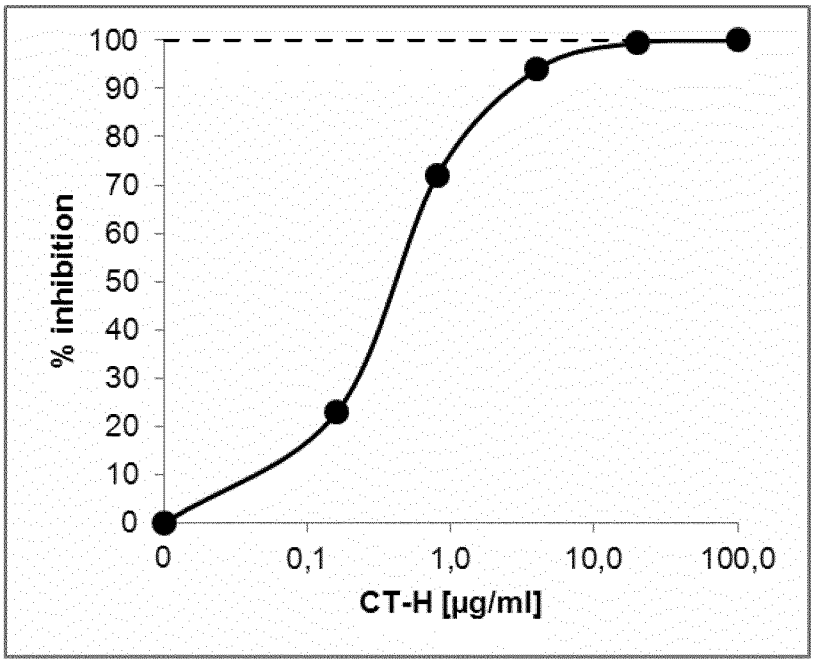

100
90
80
70
60
50
40
30
20
10
0
% inhibition
0
0,1
1,0
10,0
100,0
CT-H [µg/ml]

Fig. 2 d:

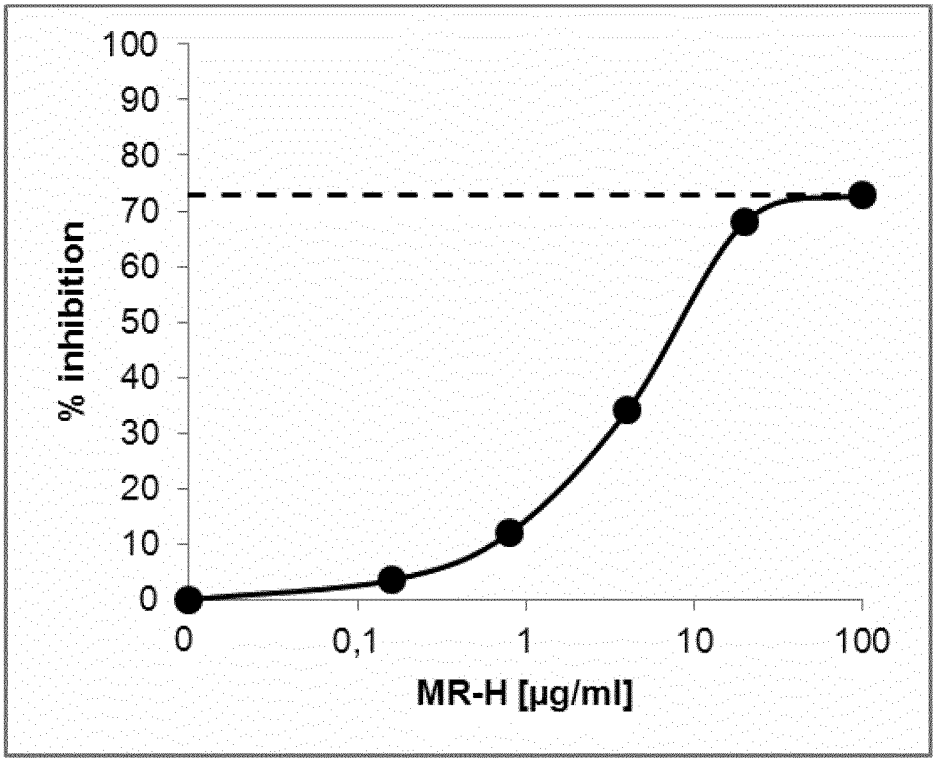
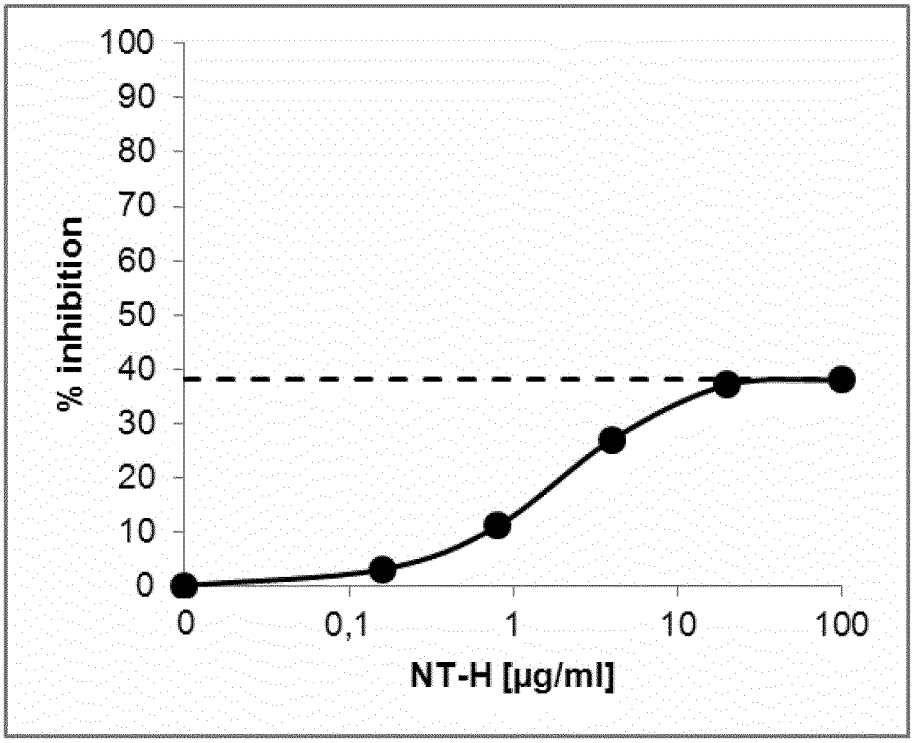

100
90
80
70
60
50
40
30
20
10
0
% inhibition
0
0,1
1
10
100
MR-H [µg/ml]

Fig. 2 e: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90
80
70
60
50
40
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20
10
0
% inhibition
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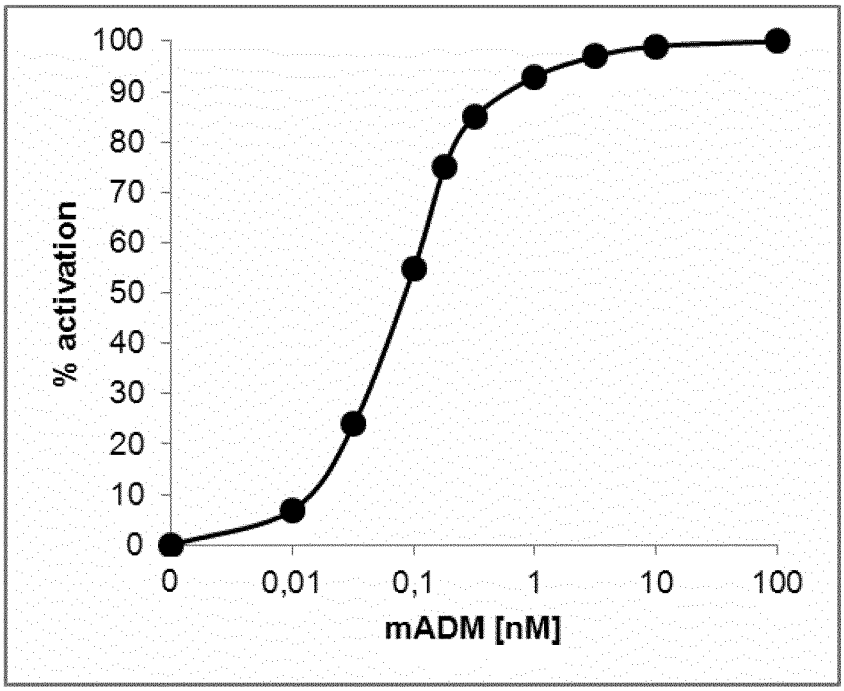
0,1
1
10
100
NT-H [µg/ml]

Fig. 2 f: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80
70
60
50
40
30
20
10
0
% activation
0
0,01
0,1
1
10
100
mADM [nM]

Fig. 2 g:

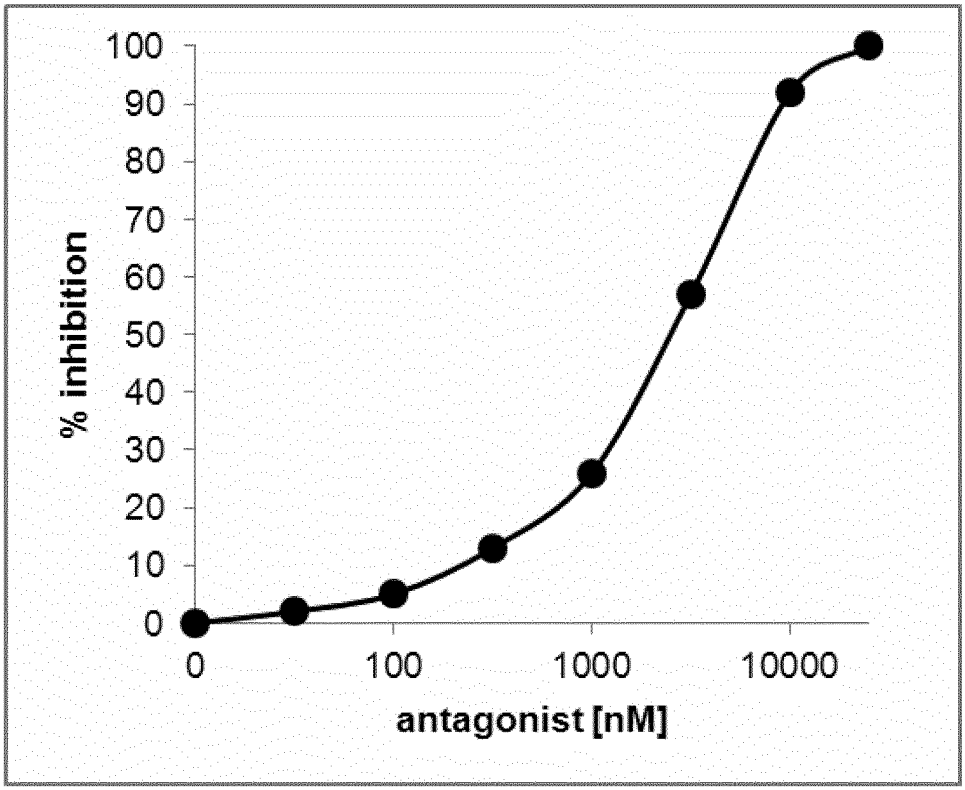

100
90
80
70
60
50
40
30
20
10
0
% inhibition
0
100
1000
10000
antagonist [nM]

Fig. 2 h:

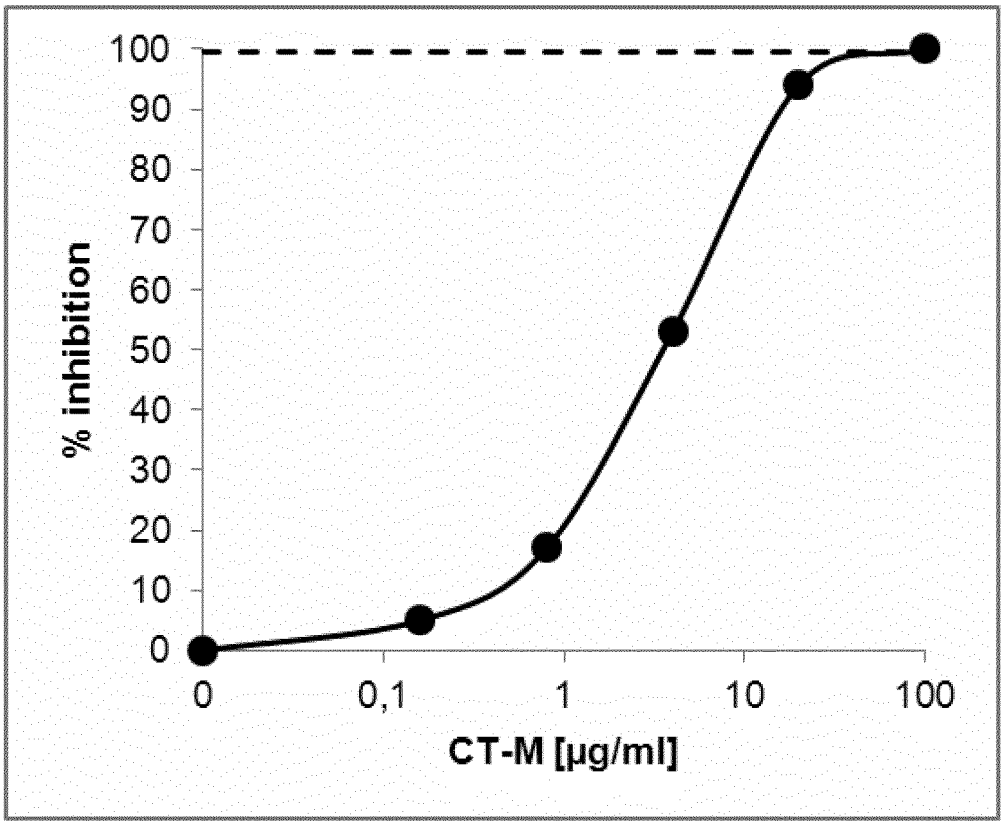
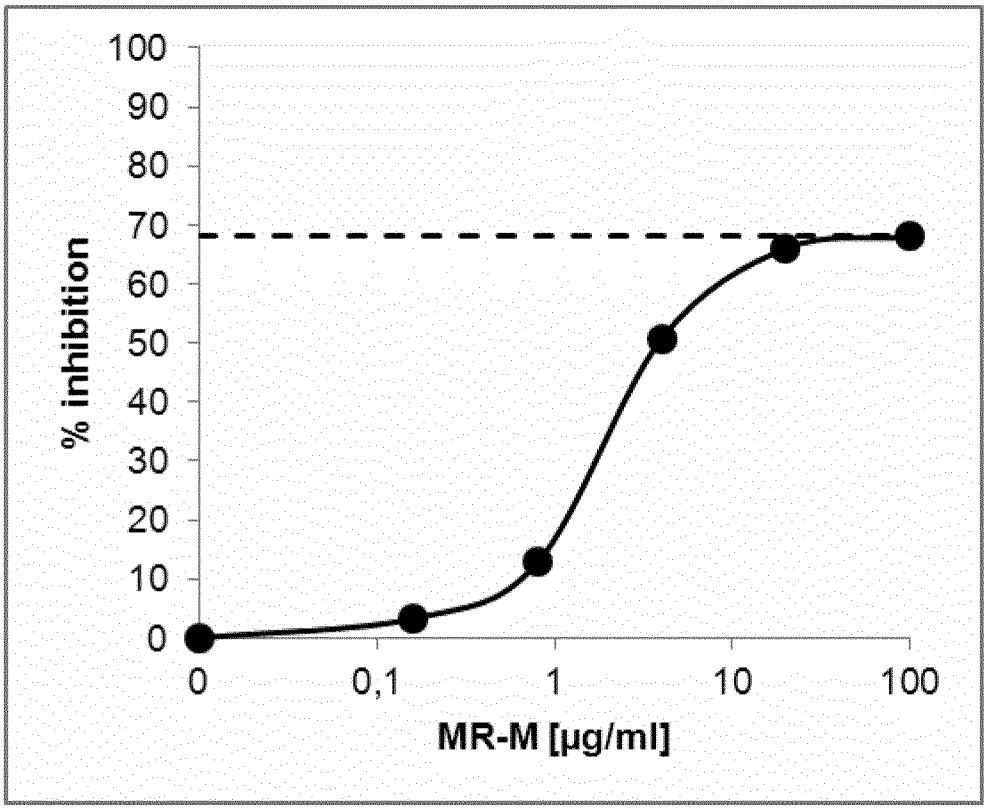

100
90
80
70
60
50
40
30
20
10
0
% inhibition
0
0,1
1
10
100
CT-M [µg/ml]

Fig. 2 i:


100
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80
70
60
50
40
30
20
10
0
% inhibition
0
0,1
1
10
100
MR-M [µg/ml]

Fig. 2 j:

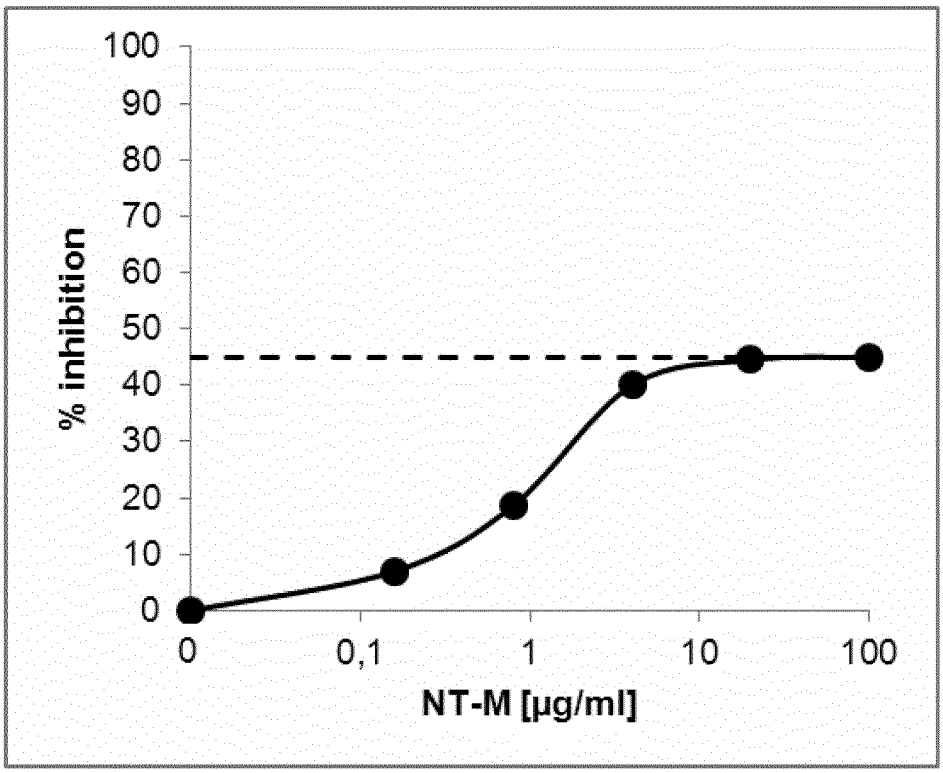
100
90
80
70
60
50
40
30
20
10
0
% inhibition
0
0,1
1
10
100
NT-M [µg/ml]

Fig. 2 k:

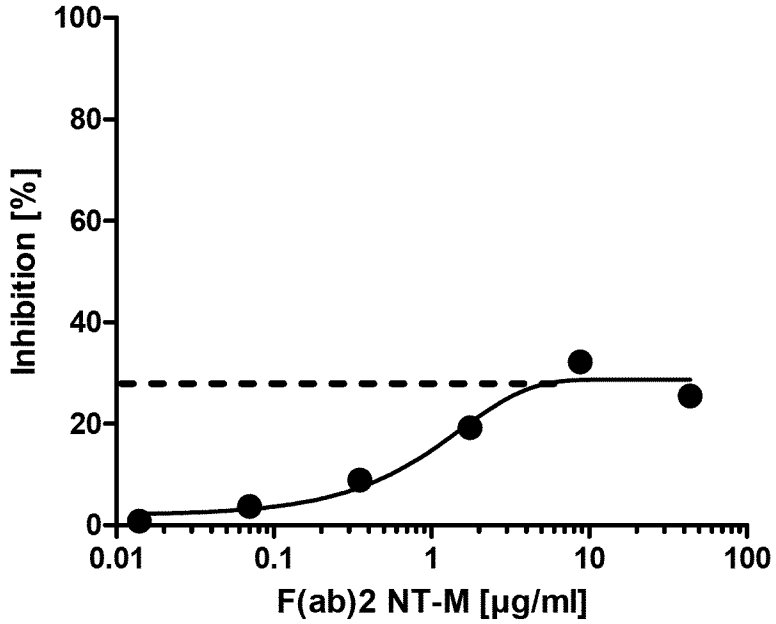
100
80
60
40
20
0
Inhibition [%]
0.01
0.1
1
10
100
F(ab)2 NT-M [µg/ml]

Fig. 2 l:

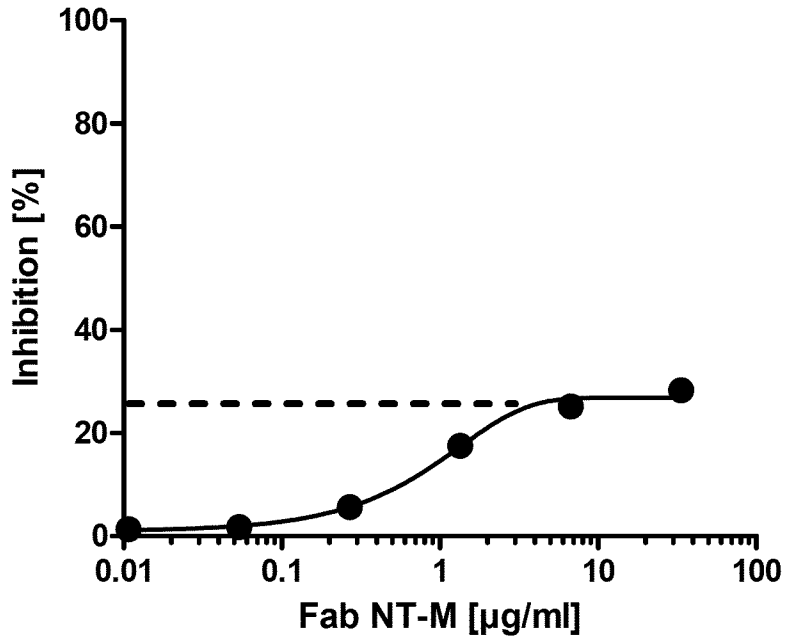
100
80
60
40
20
0
Inhibition [%]
0.01
0.1
1
10
100
Fab NT-M [µg/ml]

Fig. 3:

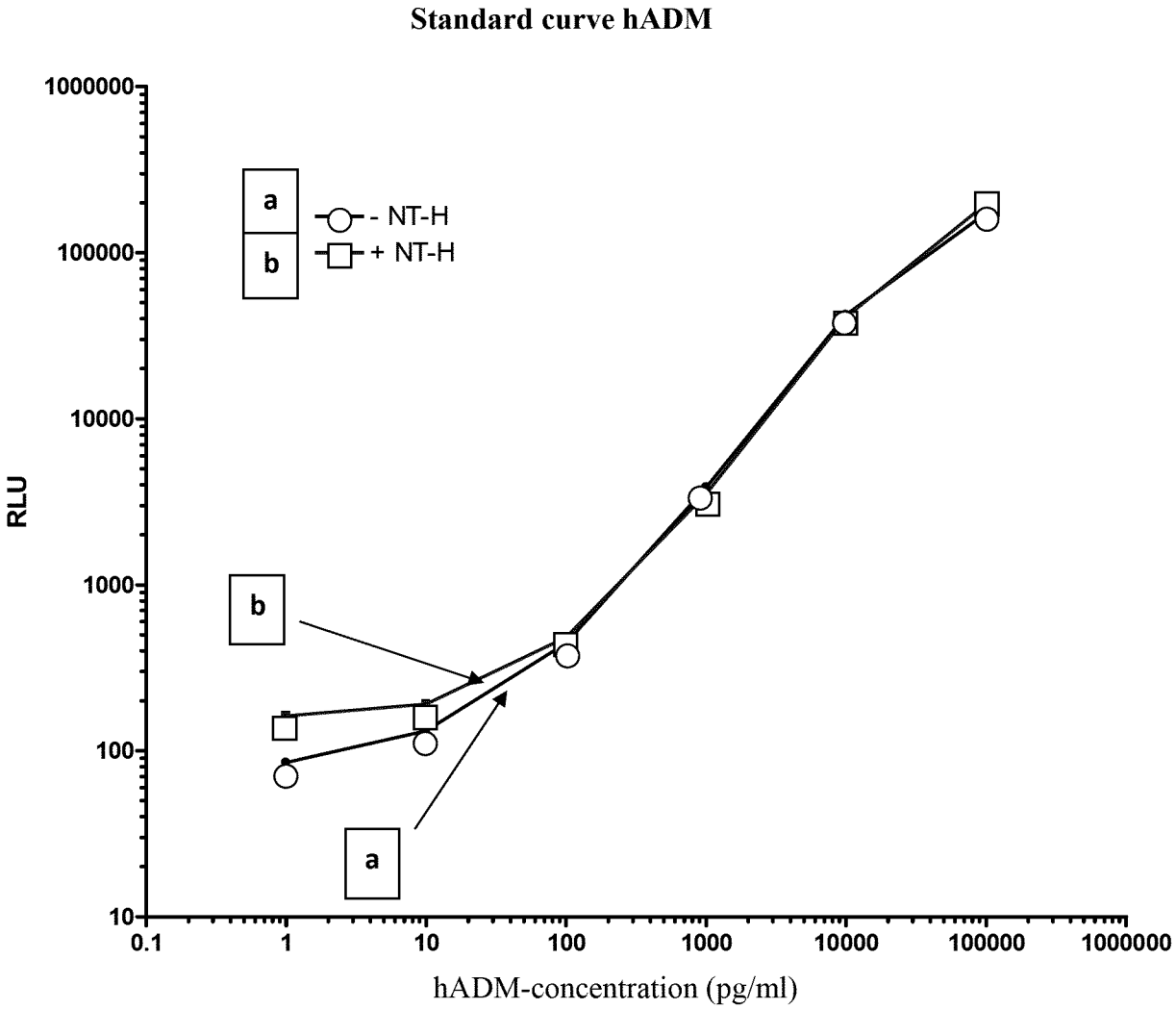
Standard curve hADM
1000000
100000
10000
1000
100
10
RLU
a
b
- NT-H
+ NT-H
b
a
0.1
1
10
100
1000
10000
100000
1000000
hADM-concentration (pg/ml)

Fig. 4:

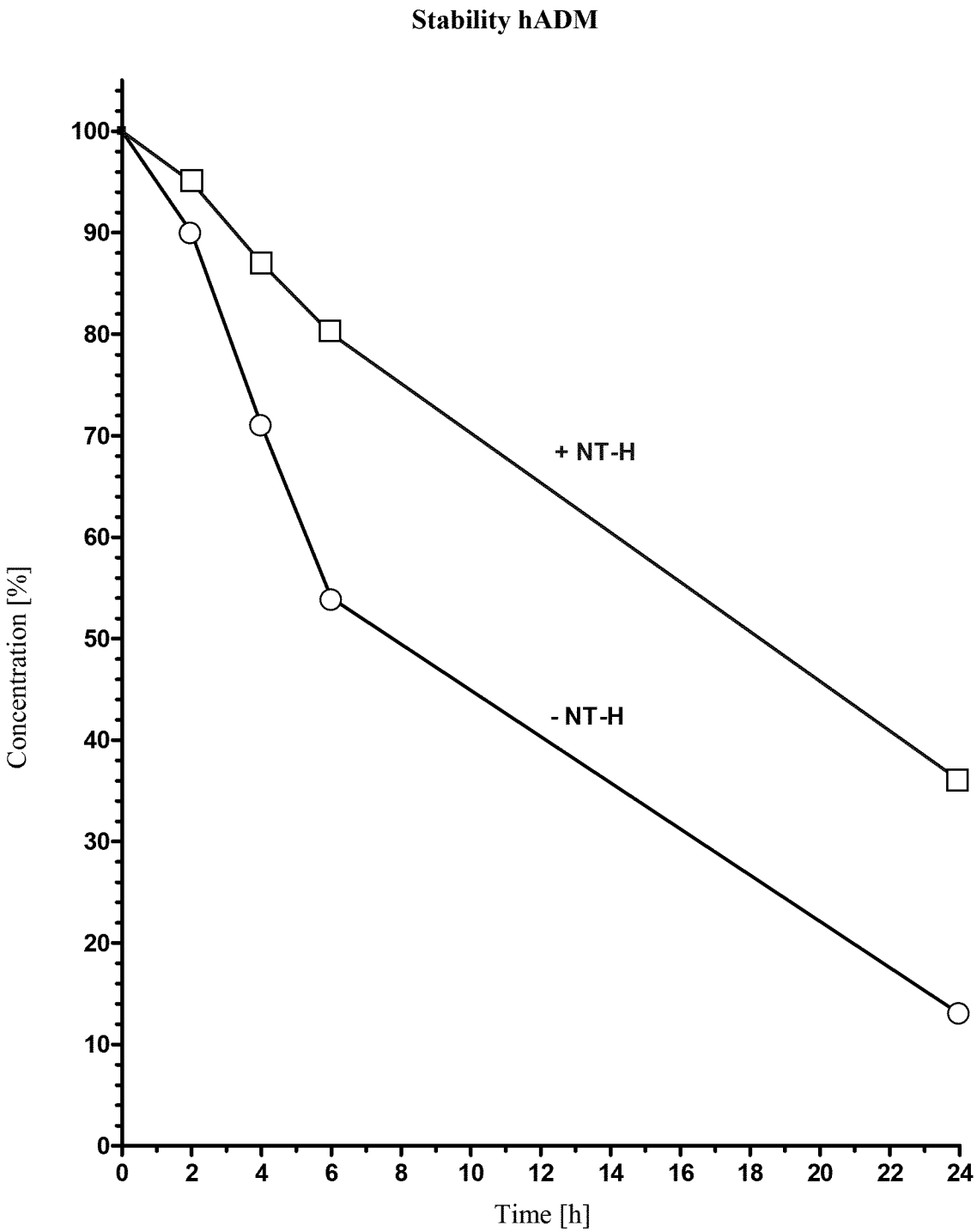
Stability hADM
Concentration [%]
Time [h]
+ NT-H
- NT-H
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
16
18
20
22
24

Fig 5

Alignment (ClustalW2): Identical amino acids are illustrated by stars; points indicate conservative changes.

```
IGHV1:QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGTANYAQKFQGR
      VTITADESTSTAYMELSSLRSEDTAVYYCARYYYYYGMDVWGQGTTVTVSS
HB3:  QVQLQQSGAELMKPGASVKISCKATGYTFSRYWIEWVKQRPGHGLEWIGEILPGSGSTNYNEKFKGK
      ATITADTSSNTAYMQLSSLTSEDSAVYYCTEGYEYDGFDYWGQGTTLTVSS
      **** *****: ***:***:****:* *** * *.**:* **:****:*.*:*  *::** :**:*:
      .***** *:.****:**** ***:*****:. * * *:* ******:***
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Fig. 6

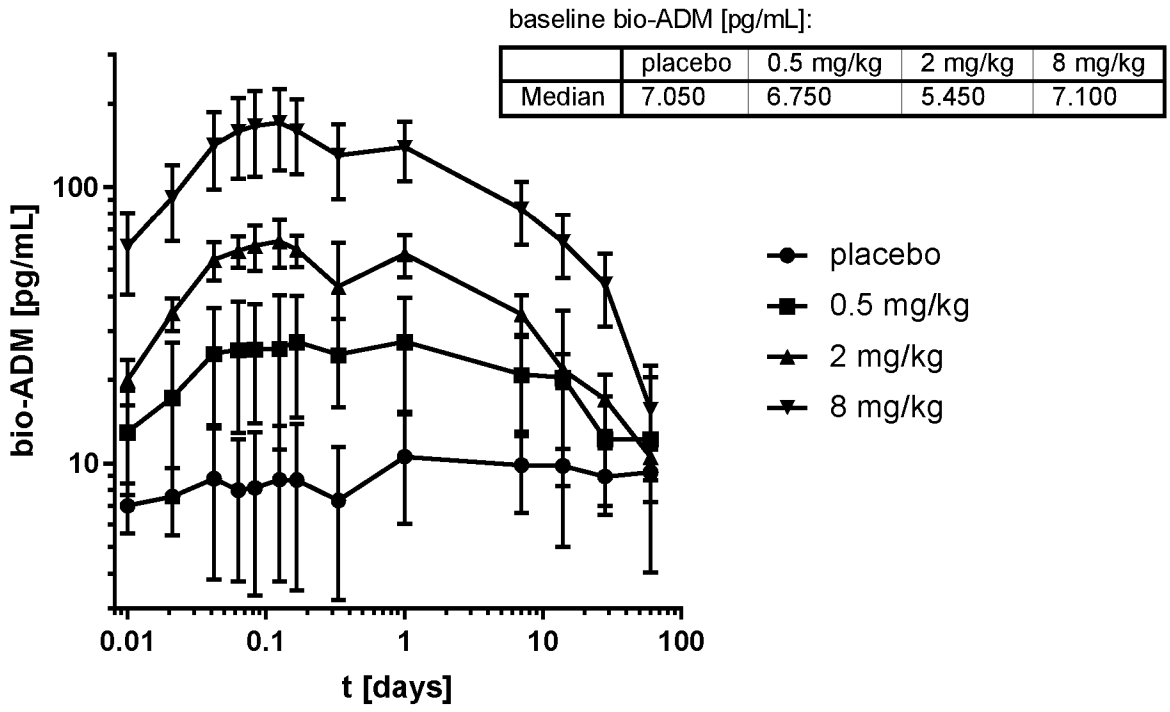


Fig. 7

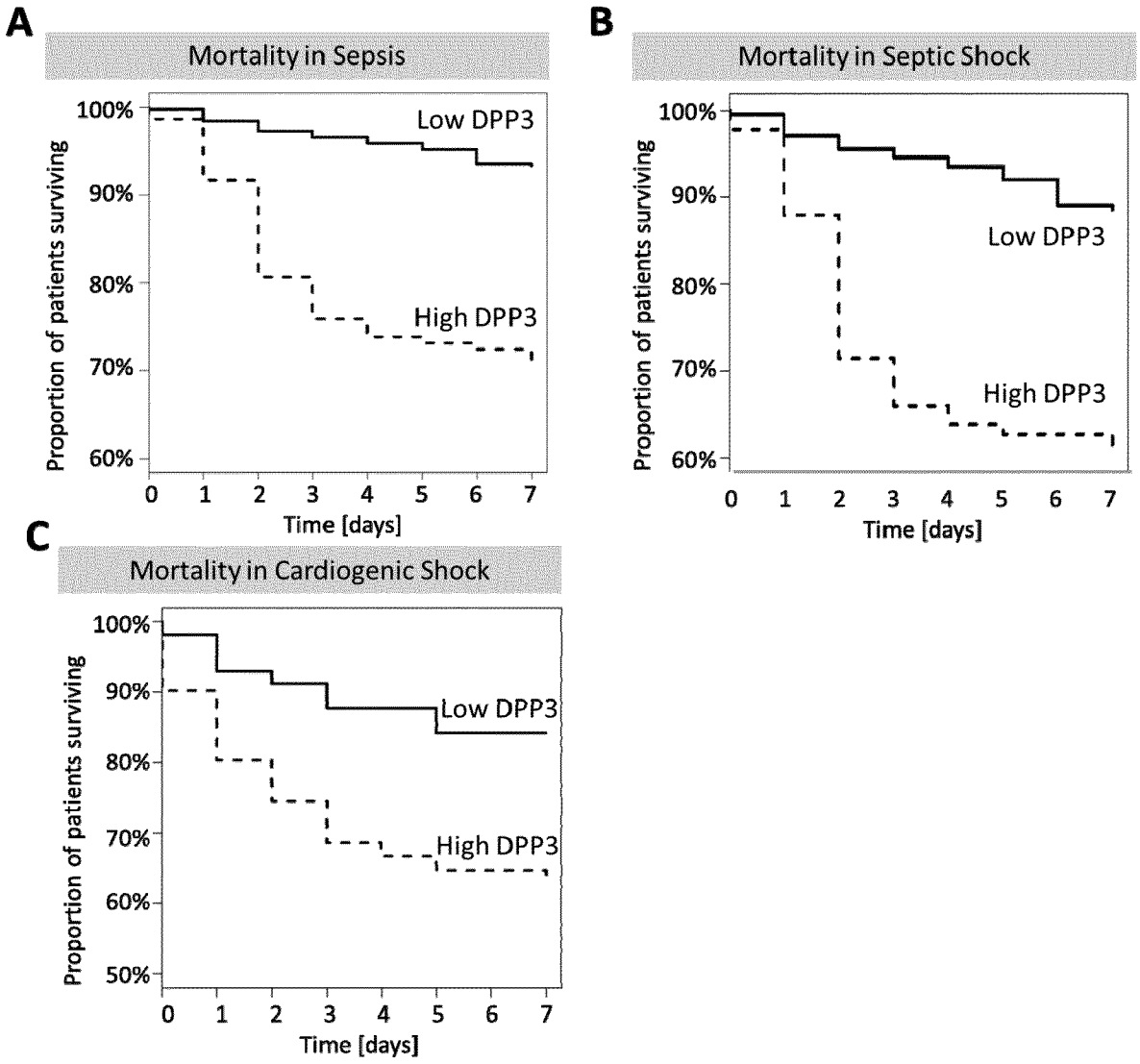

A
Mortality in Sepsis
Proportion of patients surviving
100%
90%
80%
70%
60%
Low DPP3
High DPP3
0 1 2 3 4 5 6 7
Time [days]
B
Mortality in Septic Shock
Proportion of patients surviving
100%
90%
80%
70%
60%
Low DPP3
High DPP3
0 1 2 3 4 5 6 7
Time [days]
C
Mortality in Cardiogenic Shock
Proportion of patients surviving
100%
90%
80%
70%
60%
50%
Low DPP3
High DPP3
0 1 2 3 4 5 6 7
Time [days]

Fig. 8

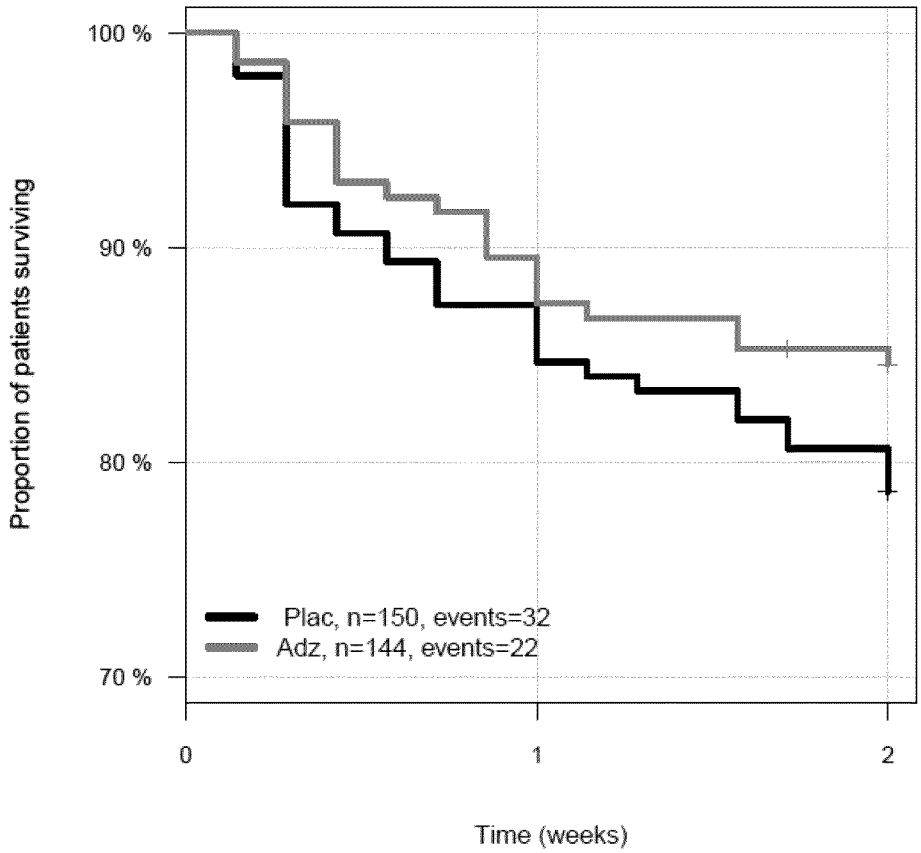

100 %
90 %
80 %
70 %
Proportion of patients surviving
Plac, n=150, events=32
Adz, n=144, events=22
0
1
2
Time (weeks)

Fig. 9

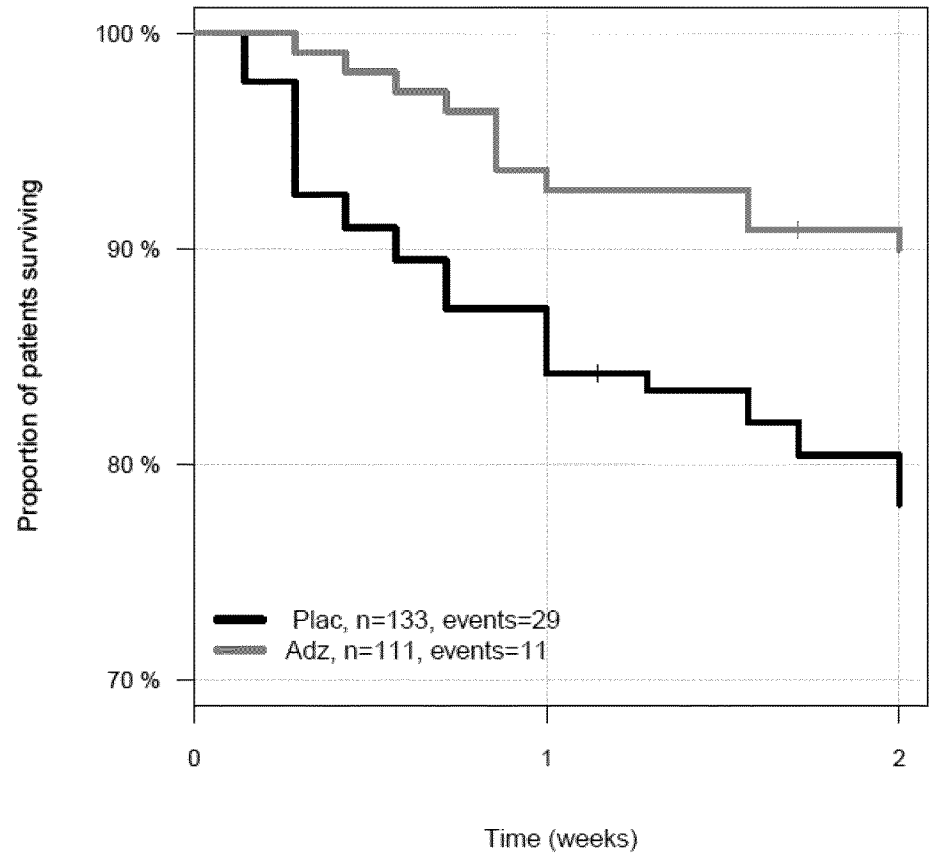
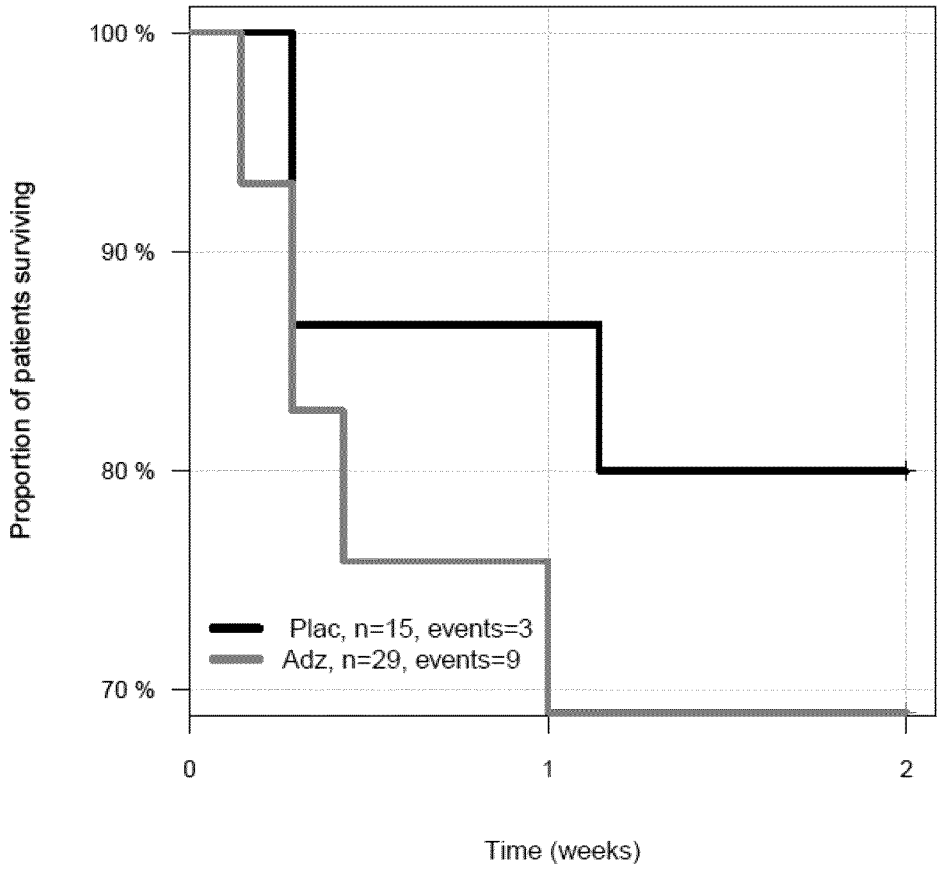

100 %
90 %
80 %
70 %
Proportion of patients surviving
Plac, n=133, events=29
Adz, n=111, events=11
0
1
2
Time (weeks)

Fig. 10


100 %
90 %
80 %
70 %
Proportion of patients surviving
Plac, n=15, events=3
Adz, n=29, events=9
0
1
2
Time (weeks)

SEQUENCE LISTING

<110> 4TEEN4 Pharmaceuticals GmbH

<120> DPP3 for therapy guidance, monitoring and stratification of NT-ADM antibodies in patients with shock

<130> T75115WO

<150> 20159848.9
<151> 2020-02-27

<160> 37

<170> PatentIn version 3.5

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Gly Tyr Thr Phe Ser Arg Tyr Trp
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Ile Leu Pro Gly Ser Gly Ser Thr
1 5

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Thr Glu Gly Tyr Glu Tyr Asp Gly Phe Asp Tyr
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<210> 4
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Phe Gln Gly Ser His Ile Pro Tyr Thr

1 5

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20 25 30

Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Thr Glu Gly Tyr Glu Tyr Asp Gly Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
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Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
210 215

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<212> PRT

<213> Homo sapiens

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```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Arg Tyr
            20                  25                  30


Trp Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Arg Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Thr Glu Gly Tyr Glu Tyr Asp Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125


Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140


Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160


Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175


Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190


Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205


Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
    210                 215
```

<210> 8
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<212> PRT
<213> Homo sapiens

<400> 8

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15
```

```
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Arg Tyr
            20                  25                  30

Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Thr Glu Gly Tyr Glu Tyr Asp Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205

Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
    210                 215
```

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<212> PRT
<213> Homo sapiens

<400> 9

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1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser Arg Tyr
            20                  25                  30
```

```
Trp Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Thr Glu Gly Tyr Glu Tyr Asp Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205

Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
    210                 215
```

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<213> Homo sapiens

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```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser Arg Tyr
            20                  25                  30

Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60
```

```
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Thr Glu Gly Tyr Glu Tyr Asp Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125


Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140


Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160


Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175


Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190


Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205


Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
    210                 215
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<212> PRT
<213> Homo sapiens

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Asp Val Leu Leu Ser Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                   10                  15


Asp Gln Ala Thr Ile Ser Cys Arg Ser Ser Gln Ser Ile Val Tyr Ser
            20                  25                  30


Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45


Pro Lys Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60


Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
```

```
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95

Ser His Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
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<212> PRT
<213> Homo sapiens

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Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1               5                   10                  15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val Tyr Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Asn Trp Phe Gln Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Arg Arg Leu Ile Tyr Arg Val Ser Asn Arg Asp Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95

Ser His Ile Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
```

```
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125


Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140


Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160


Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175


Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190


Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205


Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210>  13
<211>  219
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<213>  Homo sapiens

<400>  13

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1               5                   10                  15


Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val Tyr Ser
            20                  25                  30


Asn Gly Asn Thr Tyr Leu Glu Trp Phe Gln Gln Arg Pro Gly Gln Ser
        35                  40                  45


Pro Arg Arg Leu Ile Tyr Arg Val Ser Asn Arg Asp Ser Gly Val Pro
    50                  55                  60


Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80


Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95


Ser His Ile Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125
```

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130   135   140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145   150   155   160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165   170   175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180   185   190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195   200   205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210   215

<210> 14
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<213> Homo sapiens

<400> 14

Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys
1   5   10   15

Arg Phe Gly Thr Cys
            20

<210> 15
<211> 12
<212> PRT
<213> Homo sapiens

<400> 15

Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr Gln
1   5   10

<210> 16
<211> 12
<212> PRT
<213> Homo sapiens

<400> 16

Cys Ala Pro Arg Ser Lys Ile Ser Pro Gln Gly Tyr
1   5   10

<210> 17
<211> 19
<212> PRT
<213> Mus musculus

<400> 17

Tyr Arg Gln Ser Met Asn Gln Gly Ser Arg Ser Asn Gly Cys Arg Phe

1 5 10 15

Gly Thr Cys

<210> 18
<211> 12
<212> PRT
<213> Mus musculus

<400> 18

Cys Thr Phe Gln Lys Leu Ala His Gln Ile Tyr Gln
1 5 10

<210> 19
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<400> 19

Cys Ala Pro Arg Asn Lys Ile Ser Pro Gln Gly Tyr
1 5 10

<210> 20
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<213> Homo sapiens

<400> 20

Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys
1 5 10 15

Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr Gln
20 25 30

Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile Ser
35 40 45

Pro Gln Gly Tyr
50

<210> 21
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<212> PRT
<213> Mus musculus

<400> 21

Tyr Arg Gln Ser Met Asn Gln Gly Ser Arg Ser Asn Gly Cys Arg Phe
1 5 10 15

Gly Thr Cys Thr Phe Gln Lys Leu Ala His Gln Ile Tyr Gln Leu Thr
20 25 30

Asp Lys Asp Lys Asp Gly Met Ala Pro Arg Asn Lys Ile Ser Pro Gln
35 40 45

Gly Tyr
    50

<210> 22
<211> 21
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<213> Homo sapiens

<400> 22

Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys
1   5   10   15

Arg Phe Gly Thr Cys
            20

<210> 23
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<212> PRT
<213> Homo sapiens

<400> 23

Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys
1   5   10   15

Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr Gln
            20   25   30

Phe Thr Asp Lys Asp Lys Asp Asn Val Ala
        35   40

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<400> 24

Pro Arg Ser Lys Ile Ser Pro Gln Gly Tyr Asn His
1   5   10

<210> 25
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<400> 25

Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe
1   5   10

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<400> 26

Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly
1   5   10

<210> 27
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<400> 27

Tyr Arg Gln Ser Met Asn
1   5

<210> 28
<211> 32
<212> PRT
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<400> 28

Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys
1   5   10   15

Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr Gln
            20   25   30

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<213> Mus musculus

<400> 29

Tyr Arg Gln Ser Met Asn Gln Gly Ser Arg Ser Asn Gly Cys Arg Phe
1   5   10   15

Gly Thr Cys Thr Phe Gln Lys Leu Ala His Gln Ile Tyr Gln Leu Thr
            20   25   30

Asp Lys Asp Lys Asp Gly Met Ala
        35   40

<210> 30
<211> 31
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<213> Mus musculus

<400> 30

Tyr Arg Gln Ser Met Asn Gln Gly Ser Arg Ser Asn Gly Cys Arg Phe
1   5   10   15

Gly Thr Cys Thr Phe Gln Lys Leu Ala His Gln Ile Tyr Gln Leu
            20   25   30

<210> 31
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<213> Homo sapiens

<400> 31

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1 5 10 15

Ala Leu Ser Arg Gly Lys Arg Glu Leu Arg Met Ser Ser Ser Tyr Pro
20 25 30

Thr Gly Leu Ala Asp Val Lys Ala Gly Pro Ala Gln Thr Leu Ile Arg
35 40 45

Pro Gln Asp Met Lys Gly Ala Ser Arg Ser Pro Glu Asp Ser Ser Pro
50 55 60

Asp Ala Ala Arg Ile Arg Val Lys Arg Tyr Arg Gln Ser Met Asn Asn
65 70 75 80

Phe Gln Gly Leu Arg Ser Phe Gly Cys Arg Phe Gly Thr Cys Thr Val
85 90 95

Gln Lys Leu Ala His Gln Ile Tyr Gln Phe Thr Asp Lys Asp Lys Asp
100 105 110

Asn Val Ala Pro Arg Ser Lys Ile Ser Pro Gln Gly Tyr Gly Arg Arg
115 120 125

Arg Arg Arg Ser Leu Pro Glu Ala Gly Pro Gly Arg Thr Leu Val Ser
130 135 140

Ser Lys Pro Gln Ala His Gly Ala Pro Ala Pro Pro Ser Gly Ser Ala
145 150 155 160

Pro His Phe Leu

<210> 32
<211> 448
<212> PRT
<213> Homo sapiens

<400> 32

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Arg Tyr
20 25 30

Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Asn Gln Lys Phe
50 55 60

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Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Thr Glu Gly Tyr Glu Tyr Asp Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125


Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140


Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160


Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175


Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190


Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205


Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
    210                 215                 220


His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
225                 230                 235                 240


Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255


Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            260                 265                 270


Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285


Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    290                 295                 300


Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320


Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                325                 330                 335


Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
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            340                 345                 350

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360                 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                405                 410                 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420                 425                 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445


<210>  33
<211>  219
<212>  PRT
<213>  Homo sapiens

<400>  33

Asp Val Val Leu Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1               5                   10                  15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val Tyr Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Arg Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95

Ser His Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140
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```
Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160


Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175


Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190


Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205


Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210>  34
<211>  737
<212>  PRT
<213>  Homo sapiens

<400>  34

Met Ala Asp Thr Gln Tyr Ile Leu Pro Asn Asp Ile Gly Val Ser Ser
1               5                   10                  15


Leu Asp Cys Arg Glu Ala Phe Arg Leu Leu Ser Pro Thr Glu Arg Leu
            20                  25                  30


Tyr Ala Tyr His Leu Ser Arg Ala Ala Trp Tyr Gly Gly Leu Ala Val
        35                  40                  45


Leu Leu Gln Thr Ser Pro Glu Ala Pro Tyr Ile Tyr Ala Leu Leu Ser
    50                  55                  60


Arg Leu Phe Arg Ala Gln Asp Pro Asp Gln Leu Arg Gln His Ala Leu
65                  70                  75                  80


Ala Glu Gly Leu Thr Glu Glu Glu Tyr Gln Ala Phe Leu Val Tyr Ala
                85                  90                  95


Ala Gly Val Tyr Ser Asn Met Gly Asn Tyr Lys Ser Phe Gly Asp Thr
            100                 105                 110


Lys Phe Val Pro Asn Leu Pro Lys Glu Lys Leu Glu Arg Val Ile Leu
        115                 120                 125


Gly Ser Glu Ala Ala Gln Gln His Pro Glu Glu Val Arg Gly Leu Trp
    130                 135                 140


Gln Thr Cys Gly Glu Leu Met Phe Ser Leu Glu Pro Arg Leu Arg His
145                 150                 155                 160


Leu Gly Leu Gly Lys Glu Gly Ile Thr Thr Tyr Phe Ser Gly Asn Cys
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                165                 170                 175

Thr Met Glu Asp Ala Lys Leu Ala Gln Asp Phe Leu Asp Ser Gln Asn
            180                 185                 190

Leu Ser Ala Tyr Asn Thr Arg Leu Phe Lys Glu Val Asp Gly Glu Gly
        195                 200                 205

Lys Pro Tyr Tyr Glu Val Arg Leu Ala Ser Val Leu Gly Ser Glu Pro
    210                 215                 220

Ser Leu Asp Ser Glu Val Thr Ser Lys Leu Lys Ser Tyr Glu Phe Arg
225                 230                 235                 240

Gly Ser Pro Phe Gln Val Thr Arg Gly Asp Tyr Ala Pro Ile Leu Gln
                245                 250                 255

Lys Val Val Glu Gln Leu Glu Lys Ala Lys Ala Tyr Ala Ala Asn Ser
            260                 265                 270

His Gln Gly Gln Met Leu Ala Gln Tyr Ile Glu Ser Phe Thr Gln Gly
        275                 280                 285

Ser Ile Glu Ala His Lys Arg Gly Ser Arg Phe Trp Ile Gln Asp Lys
    290                 295                 300

Gly Pro Ile Val Glu Ser Tyr Ile Gly Phe Ile Glu Ser Tyr Arg Asp
305                 310                 315                 320

Pro Phe Gly Ser Arg Gly Glu Phe Glu Gly Phe Val Ala Val Val Asn
                325                 330                 335

Lys Ala Met Ser Ala Lys Phe Glu Arg Leu Val Ala Ser Ala Glu Gln
            340                 345                 350

Leu Leu Lys Glu Leu Pro Trp Pro Pro Thr Phe Glu Lys Asp Lys Phe
        355                 360                 365

Leu Thr Pro Asp Phe Thr Ser Leu Asp Val Leu Thr Phe Ala Gly Ser
    370                 375                 380

Gly Ile Pro Ala Gly Ile Asn Ile Pro Asn Tyr Asp Asp Leu Arg Gln
385                 390                 395                 400

Thr Glu Gly Phe Lys Asn Val Ser Leu Gly Asn Val Leu Ala Val Ala
                405                 410                 415

Tyr Ala Thr Gln Arg Glu Lys Leu Thr Phe Leu Glu Glu Asp Asp Lys
            420                 425                 430

Asp Leu Tyr Ile Leu Trp Lys Gly Pro Ser Phe Asp Val Gln Val Gly
        435                 440                 445
```

```
Leu His Glu Leu Leu Gly His Gly Ser Gly Lys Leu Phe Val Gln Asp
    450                 455                 460

Glu Lys Gly Ala Phe Asn Phe Asp Gln Glu Thr Val Ile Asn Pro Glu
465                 470                 475                 480

Thr Gly Glu Gln Ile Gln Ser Trp Tyr Arg Ser Gly Glu Thr Trp Asp
                485                 490                 495

Ser Lys Phe Ser Thr Ile Ala Ser Ser Tyr Glu Glu Cys Arg Ala Glu
            500                 505                 510

Ser Val Gly Leu Tyr Leu Cys Leu His Pro Gln Val Leu Glu Ile Phe
        515                 520                 525

Gly Phe Glu Gly Ala Asp Ala Glu Asp Val Ile Tyr Val Asn Trp Leu
    530                 535                 540

Asn Met Val Arg Ala Gly Leu Leu Ala Leu Glu Phe Tyr Thr Pro Glu
545                 550                 555                 560

Ala Phe Asn Trp Arg Gln Ala His Met Gln Ala Arg Phe Val Ile Leu
                565                 570                 575

Arg Val Leu Leu Glu Ala Gly Glu Gly Leu Val Thr Ile Thr Pro Thr
            580                 585                 590

Thr Gly Ser Asp Gly Arg Pro Asp Ala Arg Val Arg Leu Asp Arg Ser
        595                 600                 605

Lys Ile Arg Ser Val Gly Lys Pro Ala Leu Glu Arg Phe Leu Arg Arg
    610                 615                 620

Leu Gln Val Leu Lys Ser Thr Gly Asp Val Ala Gly Gly Arg Ala Leu
625                 630                 635                 640

Tyr Glu Gly Tyr Ala Thr Val Thr Asp Ala Pro Pro Glu Cys Phe Leu
                645                 650                 655

Thr Leu Arg Asp Thr Val Leu Leu Arg Lys Glu Ser Arg Lys Leu Ile
            660                 665                 670

Val Gln Pro Asn Thr Arg Leu Glu Gly Ser Asp Val Gln Leu Leu Glu
        675                 680                 685

Tyr Glu Ala Ser Ala Ala Gly Leu Ile Arg Ser Phe Ser Glu Arg Phe
    690                 695                 700

Pro Glu Asp Gly Pro Glu Leu Glu Glu Ile Leu Thr Gln Leu Ala Thr
705                 710                 715                 720
```

Ala Asp Ala Arg Phe Trp Lys Gly Pro Ser Glu Ala Pro Ser Gly Gln
725 730 735

Ala

<210> 35
<211> 21
<212> PRT
<213> Homo sapiens

<400> 35

Cys Glu Thr Val Ile Asn Pro Glu Thr Gly Glu Gln Ile Gln Ser Trp
1 5 10 15

Tyr Arg Ser Gly Glu
20

<210> 36
<211> 118
<212> PRT
<213> Homo sapiens

<400> 36

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> 37
<211> 118
<212> PRT
<213> Homo sapiens

<400> 37

```
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser Arg Tyr
            20                  25                  30


Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
        35                  40                  45


Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60


Lys Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65                  70                  75                  80


Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95


Thr Glu Gly Tyr Glu Tyr Asp Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Thr Leu Thr Val Ser Ser
        115
```