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Brain natriuretic peptide engrafted antibodies

Abstract

The present invention relates to an antibody or a fragment thereof comprising at least one heterologous amino acid sequence incorporated within at least one CDR region of said antibody or fragment thereof, wherein said at least one heterologous amino acid sequence comprises an N-terminal linker sequence (Ntls), a Brain Natriuretic Peptide (BNP) and a C-terminal linker sequence (Ctls). Optionally, at least a portion of said at least one CDR region is replaced by said at least one heterologous amino acid sequence incorporated therein. The present invention further relates to such antibody or fragment thereof for use in a method for treatment, a composition comprising such antibody or fragment thereof, a nucleic acid or a mixture of nucleic acids encoding such antibody or fragment thereof, a host cell comprising such nucleic acid or such mixture of nucleic acids and to a process for producing such antibody or fragment thereof.

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Background/Summary

CROSS REFERENCE TO RELATED APPLICATIONS

(1) This application is a national stage application under 35 U.S.C. § 371 of International Application No. PCT/EP2019/059101, filed internationally on Apr. 10, 2019, which claims the benefit of priority to European Application No. 18167106.6, filed Apr. 12, 2018.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

(2) The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 777052044500SUBSEQLIST.TXT, date recorded: May 26, 2022, size: 215,279 bytes).

FIELD OF THE INVENTION

(3) The present invention relates to an antibody or a fragment thereof comprising at least one heterologous amino acid sequence incorporated within at least one CDR region of said antibody or fragment thereof, wherein said at least one heterologous amino acid sequence comprises an N-terminal linker sequence (Ntls), a Brain Natriuretic Peptide (BNP) and a C-terminal linker sequence (Ctls). Optionally, at least a portion of said at least one CDR region is replaced by said at least one heterologous amino acid sequence incorporated therein. At least 12 amino acid residues are present between amino acid residue HC (heavy chain) res25 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRH1; amino acid residue HC res51 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRH2; amino acid residue HC res92 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRH3; amino acid residue LC (light chain) res26 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRL1; amino acid residue LC res49 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRL2; and/or amino acid residue LC res88 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRL3. Additionally, at least 9 amino acid residues are present between the last amino acid residue of the BNP and amino acid residue HC res35a according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH1; amino acid residue HC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH2; amino acid residue HC res106 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH3; amino acid residue LC res 32 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL1; amino acid residue LC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL2; and/or amino acid residue LC res98 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL3. The present invention further relates to such antibody or fragment thereof for use in a method for treatment, a composition comprising such antibody or fragment thereof, a nucleic acid or a mixture of nucleic acids encoding such antibody or fragment thereof, a host cell comprising such nucleic acid or such mixture of nucleic acids and to a process for producing such antibody or fragment thereof.

BACKGROUND OF THE INVENTION

(4) Natriuretic peptides are a family of three structurally related peptides with neurohumoral actions. Atrial Natriuretic Peptide (ANP) is a peptide of 28 amino acids comprising a central ring

structure formed by a disulfide bridge between cysteine residues 7 and 23. Human ANP is expressed as a 153 amino acid long pre-pro-hormone in atrial myocyte cells. Signal peptide cleavage yields the prohormone form, which is subsequently further cleaved into the mature ANP and the N-terminal remnant, known as NT-proANP. Similar to ANP, also Brain Natriuretic Peptide (BNP) and C-Type Natriuretic Peptide (CNP) are produced from precursor proteins and comprise a central ring structure. ANP is mainly produced and released by cardiomyocytes of the left and right heart atria, whereas BNP is mainly produced by cardiomyocytes of the ventricles. CNP is synthesized by endothelial cells of blood vessels. Apart from these locations natriuretic peptides are also produced in smaller amounts in other parts of the body, e.g., in brain, kidney and adrenal gland. Natriuretic peptides are encoded by three separate genes, NPPA, NPPB, and NPPC. The amino acid sequences of the three peptides are highly conserved in mammals (Potter et al., Handb Exp Pharmacol. 2009; (191):341-66). Yet, significant sequence modifications of natriuretic peptides such as truncations, amino acid exchanges as well chimeric fusions (e.g. CD-NP (McKie et al., Curr Heart Fail Rep. 2010 September; 7 (3): 93-9)) have been described to result in potent natriuretic peptides that activate or bind to cellular receptors and can elicit relevant physiological effects.

(5) Natriuretic peptides bind to three different, membrane-bound receptor types—NPR-A, NPR-B, and NPR-C—thereby mediating their biological effects. ANP and BNP bind with greatest affinity to NPR-A; in contrast, CNP has the highest affinity for the NPR-B receptor. NPR-A and NPR-B comprise a (particulate) guanylate cyclase domain (pGC) whose enzymatic activity causes an increase in (intracellular) cyclic guanosine monophosphate (cGMP). As a second messenger, cGMP regulates diverse cellular processes. The NPR-C receptor exhibits no guanylate cyclase activity and is also termed “clearance” receptor, as it can bind natriuretic peptides, which leads to their degradation by endocytosis. An additional signaling function of the NPR-C receptor via modulation of cAMP has been described (Anand-Srivastava, Peptides. 2005 June; 26 (6): 1044-59).

(6) The cardiac hormones ANP and BNP are excreted upon stretching of the ventricles and atria, e.g. due to excessive plasma volume. They exert vasodilating effects via relaxation of vascular smooth muscle and lead to a reduction in blood pressure. In the kidney ANP causes i.a. an increase in urinary excretion (diuresis), as well as an increase in the concentration of sodium ions in the urine (natriuresis). ANP is considered to constitute a compensatory antagonist of the renin-angiotensin-aldosterone system (RAAS), which is over-activated in a number of cardiovascular diseases. In addition, ANP exerts other neuro-humoral effects, including an inhibitory effect on the sympathetic nervous system, as well as a complex regulatory effect on the baroreflex (Woods et al., Clin Exp Pharmacol Physiol. 2004 November; 31 (11): 791-4). For ANP, as well as BNP and CNP, anti-inflammatory, anti-hypertrophic and anti-fibrotic effects have been demonstrated in animal models for different diseases (e.g. Knowles et al., 2001, J. Clin. Invest. 107: 975-984; Dahrouj et al., J Pharmacol Exp Ther. 2013 January; 344 (1): 96-102; Baliga et al., Br J Pharmacol. 2014 July; 171 (14): 3463-75; Mitaka et al. Intensive Care Med Exp. 2014 December; 2 (1): 28; Werner et al., Basic Res Cardiol. 2016 March; 111 (2): 22; Kimura et al., Respir Res. 2016 Feb. 19; 17: 19). Activation of NPR-B by CNP is plays a significant role in bone growth (Yasoda et al., Clin. Calcium. 2009 July; 19 (7): 1003-8) and vascular endothelium integrity (Moyes et al., J Clin Invest. 2014 September; 124 (9): 4039-51).

(7) The broad spectrum of physiological effects of natriuretic peptides and their receptors make them attractive targets in drug discovery (Lumsden et al., Curr Pharm Des. 2010; 16 (37): 4080-8; Buglioni et al., Annu Rev Med. 2016; 67: 229-43). For example, the natriuretic cGMP system may be suppressed under various pathophysiological conditions, which may result in hypertension, increased cell proliferation, fibrosis, inflammation, endothelial dysfunction, diabetes, metabolic syndrome, atherosclerosis, cardiac insufficiency, myocardial infarction, pulmonary hypertension, ocular and renal diseases, bone disorders, stroke and/or sexual dysfunction.

(8) A major hurdle for the therapeutic use of natriuretic peptides is their very short plasma half-life

of only a few minutes in the organism (Hunt et al., J Clin Endocrinol Metab. 1994 June; 78 (6): 1428-35; Kimura et al., Eur J Clin Pharmacol. 2007 July; 63 (7): 699-702). In addition to endocytosis by the NPR-C receptor, the natriuretic peptides are efficiently proteolytically degraded by the enzymes neprilysin (NEP) and insulin degrading enzyme (IDE). The associated short-term biological effects of administered natriuretic peptides have restricted their therapeutic use primarily to acute indications. For example, infusions of recombinant carperitide (ANP) and nesiritide (BNP) are approved for the treatment of acute decompensated heart failure in different countries.

(9) The treatment of chronic diseases would be greatly facilitated by the provision of NPR-A and NPR-B agonists with increased plasma half-lives, higher proteolytic stability and prolonged duration of action.

(10) In recent years, several natriuretic peptide derivatives and variants have been described, e.g., CD-NP (McKie et al., Curr Heart Fail Rep. 2010 September; 7 (3): 93-9), ZD100/MANP (McKie et al., Hypertension. 2010 December; 56 (6): 1152-9), PL-3994 (Edelson et al., Pulm Pharmacol Ther. 2013 April; 26 (2): 229-38), Ularitide (Anker et al., Eur Heart J. 2015 Mar. 21; 36 (12): 715-2), ANX-042 (Pan et al., Proc Natl Acad Sci USA. 2009 Jul. 7; 106 (27): 11282-7) and BMN-111 (Wendt et al., J. Pharmacol Exp Ther. 2015 April; 353 (1): 132-49). The half-life of CD-NP is about 18.5 min (Lee et al., BMC Pharmacology 2007, 7 (Suppl I): P38). Further ANP and CNP derivatives are disclosed in U.S. Pat. No. 9,193,777 and EP 2 432 489 A, respectively.

(11) In addition, natriuretic peptide fusions including Fc fusions, albumin fusion and PEGylated natriuretic peptides have been described. Natriuretic peptide-Fc fusions are for example disclosed in US 2010/0310561, WO 2008/154226, WO 2010/117760, WO 2006/107124, WO 2008/136611 and WO 2008/079995. Natriuretic peptide-albumin fusions are disclosed in U.S. Pat. No. 7,521,424 and US 2014/0148390 and PEGylated natriuretic peptides are disclosed in US 2014/0148390.

(12) WO 2005/060642 describes the generation of ANP and BNP peptide engrafted antibody libraries obtained by inserting ANP or BNP with two randomized flanging amino acids on both ends into the CDRH3 region of a human tetanus toxoid specific antibody. Similarly, WO 2005/082004 discloses the generation of an ANP mimetic engrafted antibody library obtained by replacing the entire original CDRH3 region of a 2G12 antibody with an ANP mimetic peptide flanked by two random amino acid residues on either side. Neither one of WO 2005/060642 and WO 2005/082004 discloses any specific natriuretic peptide engrafted antibodies, let alone functionally characterizes such antibodies.

OBJECTS OF THE INVENTION

(13) In view of the prior art it is an object of the present invention to provide novel natriuretic peptide receptor agonists with increased stability in serum as compared to naturally occurring wild type natriuretic peptides.

SUMMARY OF THE INVENTION

(14) The above stated object is achieved by the teaching of the subject independent claims. The present inventors have surprisingly found that biologically active natriuretic peptide variants with significantly increased stability in serum as compared to naturally occurring wild type natriuretic peptides can be obtained by incorporating a natriuretic peptide amino acid sequence into one of the CDR regions of an immunoglobulin molecule or a fragment thereof, despite the short length and high sequence conservation of immunoglobulin CDR regions, which impose considerable conformational restrains to the incorporation of biologically active peptides. However, the activity of natriuretic peptides incorporated within an immunoglobulin CDR region was shown to vary considerably. The present inventors have found that the decisive factor for a successful incorporation yielding a biologically active natriuretic peptide variant is the number of amino acid residues between the incorporated natriuretic peptide and the nearest neighboring CDR-framework junctions N-terminal and C-terminal from the incorporated natriuretic peptide. Below a certain number of N-terminal and C-terminal flanking amino acid residues between natriuretic peptide and neighboring CDR-framework junctions only natriuretic peptide immunoglobulin fusion constructs

with no or drastically reduced biological activity were obtained. Specific linker sequences flanking the incorporated natriuretic peptide were found to be especially advantageous for achieving high peptide activity, good expression levels and/or low protein fragmentation levels.

(15) Thus, in a first aspect, the present invention relates to an antibody or a fragment thereof comprising at least one heterologous amino acid sequence incorporated within at least one CDR region of said antibody or fragment thereof, wherein said at least one heterologous amino acid sequence comprises an N-terminal linker sequence (Ntls), a natriuretic peptide and a C-terminal linker sequence (Ctls), wherein optionally at least a portion of said at least one CDR region is replaced by said at least one heterologous amino acid sequence incorporated therein, and wherein a) at least 12 amino acid residues are present between i) amino acid residue HC res25 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res S25) and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRH1; ii) amino acid residue HC res51 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res 151) and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRH2; iii) amino acid residue HC res92 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res C96) and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRH3; iv) amino acid residue LC res26 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res S25) and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRL1; v) amino acid residue LC res49 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res Y51) and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRL2; and/or vi) amino acid residue LC res88 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res C90) and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRL3; and wherein b) at least 9 amino acid residues are present between the last amino acid residue of the natriuretic peptide and i) amino acid residue HC res35a according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res M34) in case of an incorporation of said heterologous amino acid sequence within CDRH1; ii) amino acid residue HC res57 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res T58) in case of an incorporation of said heterologous amino acid sequence within CDRH2; iii) amino acid residue HC res106 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res G111) in case of an incorporation of said heterologous amino acid sequence within CDRH3; iv) amino acid residue LC res 32 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res D34) in case of an incorporation of said heterologous amino acid sequence within CDRL1; v) amino acid residue LC res57 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res G59) in case of an incorporation of said heterologous amino acid sequence within CDRL2; and/or vi) amino acid residue LC res98 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res F102) in case of an incorporation of said heterologous amino acid sequence within CDRL3.

(16) In further aspects, the present invention relates to such antibody or fragment thereof for use in a method for treatment, a composition comprising such antibody or fragment thereof, a nucleic acid or a mixture of nucleic acids encoding such antibody or fragment thereof, a host cell comprising such nucleic acid or such mixture of nucleic acids and to a process for producing such antibody or fragment thereof.

DETAILED DESCRIPTION OF THE INVENTION

(17) The present invention may be understood more readily by reference to the following detailed description of the invention and the examples included therein.
(18) In a first aspect, the present invention relates to an antibody or a fragment thereof comprising at least one heterologous amino acid sequence incorporated within at least one CDR region of said antibody or fragment thereof, wherein said at least one heterologous amino acid sequence comprises an N-terminal linker sequence (Ntls), a natriuretic peptide and a C-terminal linker sequence (Ctls), wherein optionally at least a portion of said at least one CDR region is replaced by said at least one heterologous amino acid sequence incorporated therein.
(19) The present inventors have found that biologically active natriuretic peptide variants with significantly increased stability in serum as compared to naturally occurring wild type natriuretic peptides can be obtained by incorporating a natriuretic peptide amino acid sequence into one of the CDR regions of an immunoglobulin molecule or a fragment thereof. This finding was entirely unexpected. As is well known in the art, the short length and high sequence conservation of immunoglobulin CDR regions, which are especially pronounced in CDRL1, CDRL2, CDRL3, CDRH1 and CDRH2, impose considerable conformational restrains to the incorporation of biologically active peptides, and the surrounding immunoglobulin sequences may negatively affect expression, folding and/or biological activity of the incorporated peptide. Indeed, the present inventors have found that the activity of natriuretic peptides incorporated within an immunoglobulin CDR region varied considerably depending on the exact way the natriuretic peptide engrafted antibody was constructed. The decisive factor for a successful incorporation yielding a functional, i.e. biologically active natriuretic peptide variant was shown to be the number of amino acid residues between the incorporated natriuretic peptide and the nearest neighboring CDR-framework junctions N-terminal and C-terminal from the incorporated natriuretic peptide. Below a certain number of N-terminal and C-terminal flanking amino acid residues between natriuretic peptide and the neighboring CDR-framework junctions no biologically active natriuretic peptide immunoglobulin fusion constructs were obtained.
(20) The terms “incorporated”, “inserted”, “integrated”, “engrafted” and “embedded” as well as “incorporation”, “insertion”, “integration”, “engrafting” and “embedding” are used interchangeably herein. Within the context of the present invention, these terms refer to the generation of hybrid polynucleic acids or hybrid polypeptides by the introduction of a heterologous sequence into the original sequence of an antibody or an antibody fragment. Such an incorporation may be done by any means. Typically, the antibody or fragment thereof comprising a natriuretic peptide flanked by an N-terminal and a C-terminal linker sequence is generated by recombinant DNA technology and expression as described herein.
(21) Incorporation of the natriuretic peptide flanked by an N-terminal and a C-terminal linker sequence into a CDR region of the original antibody or antibody fragment sequence may result in the deletion of at least a portion of said CDR region. For instance, cloning of a nucleic acid sequence encoding said heterologous amino acid sequence comprising an N-terminal linker sequence, a natriuretic peptide and a C-terminal linker sequence may be performed such that part of the CDR encoding sequence is replaced by the incorporated heterologous nucleic acid sequence. In particular other embodiments, the incorporation of the heterologous amino acid sequence comprising the natriuretic peptide does not result in the deletion of amino acid residues of the CDR region into which the heterologous amino acid sequence is inserted.
(22) Within the context of the present invention, the term “heterologous amino acid sequence” refers to an amino acid sequence that does not originate from the initial “empty” antibody or fragment thereof, into which it is incorporated. Engrafting of the heterologous amino acid sequence into an antibody or fragment thereof thus yields an engineered, recombinant antibody molecule composed of amino acid sequences of different origin.
(23) The term “natriuretic peptide” refers to peptides that can induce natriuresis, the excretion of sodium by the kidneys. Natriuretic peptides include Atrial Natriuretic Peptide (ANP), Brain

Natriuretic Peptide (BNP), C-Type Natriuretic Peptide (CNP), Dendroaspis natriuretic peptide (DNP) and Urodilatin. Natriuretic peptides within the meaning of the present invention may be of any origin. Natriuretic peptides include natural natriuretic peptides such as wild type natriuretic peptides and mutant versions thereof as well as homolog natriuretic peptides of a different species. The term however also encompasses engineered natriuretic peptides such as engineered chimeric variants of distinct natriuretic peptides. It is known that the usage of codons is different between species. Thus, when expressing a heterologous protein in a target cell, it may be necessary, or at least helpful, to adapt the nucleic acid sequence to the codon usage of the target cell. Methods for designing and constructing derivatives of a given protein are well known to anyone of ordinary skill in the art.

(24) In particular embodiments, the natriuretic peptide is selected from a wild type natriuretic peptide of any species and a functional variant of any such wild type natriuretic peptide. Within the context of the present invention, the term “functional variant of a natriuretic peptide” or “functional natriuretic peptide variant” refers to a natriuretic peptide of any origin, including natural and engineered peptides, that differs in the amino acid sequence and/or the nucleic acid sequence encoding the amino acid sequence of a given natriuretic peptide, such as a wild type natriuretic peptide of a given species, but is still functionally active. Within the context of the present invention, the term “functionally active” refers to the ability of a natriuretic peptide variant to perform the biological functions of a naturally occurring natriuretic peptide, in particular a wild type natriuretic peptide. In particular, “functionally active” means that the natriuretic peptide variant is able to bind to its respective receptor. In case of NPR-A and NPR-B ligands, “functionally active” particularly means the ability to mediate an increase in (intracellular) cyclic guanosine monophosphate (cGMP) by binding to one or both of these receptors.

(25) In particular embodiments, the functional natriuretic peptide variant is able to perform one or more biological functions of a given natriuretic peptide, such as a wild type natriuretic peptide of any given species to at least about 50%, particularly to at least about 60%, to at least about 70%, to at least about 80%, and most particularly to at least about 90%, wherein the one or more biological functions include, but are not limited to, binding of the natriuretic peptide to its respective receptor and/or induction of an increase in intracellular cGMP.

(26) The functional activity of natriuretic peptides can be measured by any methods including in vitro methods that make it possible either to measure the increase of (intracellular) cyclic guanosine monophosphate (cGMP), or to measure changes in cellular processes regulated by cGMP, including the methods described in Examples 3 and 5. In particular embodiments, a (non-engrafted) natriuretic peptide variant is considered functionally active, if its EC.sub.50 value as determined by the fluorescence assay described in Example 3 is below 500 nM, more particularly below 250 nM, more particularly below 150 nM, more particularly below 100 nM, more particularly below 50 nM, most particularly below 25 nM.

(27) Incorporation of such a functional natriuretic peptide variant into one of the CDR regions of an immunoglobulin molecule or a fragment thereof as described herein yields a natriuretic peptide engrafted immunoglobulin with natriuretic peptide functional activity and significantly increased stability in serum as compared to the non-engrafted functional natriuretic peptide variant as shown in the Examples. An natriuretic peptide engrafted immunoglobulin is considered biologically active (i.e. functional), if it gives a significant positive signal in any method that measures the increase of (intracellular) cyclic guanosine monophosphate (cGMP) either directly or indirectly by assessing changes in cellular processes regulated by cGMP. In particular, the functional activity of a natriuretic peptide engrafted immunoglobulin may be assessed by the methods described in Examples 3 and 5. In case of natriuretic peptide engrafted immunoglobulins, significance is typically assessed based on i) comparison to a negative sample such as an empty immunoglobulin scaffold, e.g. construct #209, an antibody comprising SEQ ID NO 65 and SEQ ID NO 66, TPP-5657, ii) comparison to a positive sample, e.g. construct #117, an antibody comprising SEQ ID NO

67 and SEQ ID NO 66, TPP-5661, and iii) dose dependency.
(28) Even though the functional natriuretic peptide variant according to the present invention may contain any number of mutations comprising additions, deletions and/or substitutions of one or more amino acids in comparison to the reference natriuretic peptide, a functional natriuretic peptide variant will typically maintain key features of the corresponding natriuretic peptide, such as key residues within the central ring domain. Conserved residues of natriuretic peptides are for instance described in Lincoln R. Potter et al. (Handb Exp Pharmacol. 2009; (191): 341-366). Thus, in particular embodiments, the functional natriuretic peptide variant shares at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% sequence identity with the sequence shown below:
(29) TABLE-US-00001 (SEQ ID NO: 91)
X.sub.1CFGX.sub.2X.sub.3X.sub.4DRIX.sub.5X.sub.6X.sub.7SX.sub.8LGC
wherein X.sub.1 and X.sub.5 are G or S; X.sub.3 is R or K; X.sub.6 is A or S; and X.sub.2, X.sub.4, X.sub.7 and X.sub.8 may be any amino acid.
(30) In principle, natriuretic peptides of any type may be incorporated within a CDR region of an immunoglobulin or fragment thereof as described herein. In particular, the present inventors have found that the findings for one type of natriuretic peptide regarding both minimal requirements for satisfactory biological activity of the engrafted natriuretic peptide and especially suitable N-terminal and C-terminal amino acid sequences may be conferred to other types of natriuretic peptides. Without wishing to be bound by theory it is hypothesized that these similar requirements for successful embedding of a natriuretic peptide within an immunoglobulin molecule among different natriuretic peptide types may be due to structural similarities and/or mechanisms of action within the natriuretic peptide family.
(31) In particular embodiments, the natriuretic peptide is selected from the group consisting of human ANP having the sequence of SEQ ID NO 23, human BNP having the sequence of SEQ ID NO 24, human CNP having the sequence of SEQ ID NO 25 and a peptide having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or at least 98% sequence identity with any one of SEQ ID NOs 23 to 25. Again, the natriuretic peptide having a sequence deviating from wild type human natriuretic peptides ANP, BNP and CNP may be of any natural origin, e.g. a mutant version of a wild type human natriuretic peptide, or a homolog of a different species, or an engineered natriuretic peptide. Methods for designing and constructing peptide variants are well known to anyone of ordinary skill in the art.
(32) In particular such embodiments, the natriuretic peptide having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or at least 98% sequence identity with any one of SEQ ID NOs 23 to 25 is a functional natriuretic peptide variant.
(33) “Percent (%) sequence identity” with respect to a reference polynucleotide or polypeptide sequence, respectively, is defined as the percentage of nucleic acid or amino acid residues, respectively, in a candidate sequence that are identical to the nucleic acid or amino acid residues, respectively, in the reference polynucleotide or polypeptide sequence, respectively, after aligning the sequences and optionally introducing gaps, if necessary, to achieve the maximum percent sequence identity. Conservative substitutions are not considered as part of the sequence identity. In particular embodiments, any gaps introduced in the candidate sequence and/or the reference sequence may in total not amount to more than 50%, more than 40%, more than 30%, more than 25%, more than 20%, more than 15% or more than 10% of the total amount of residues of the reference sequence. In particular embodiments, the percentage sequence identity is determined without introducing any gaps into the candidate or the reference sequence (i.e. using an ungapped alignment). Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are well within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences,

including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

(34) The natriuretic peptide that shares a given percentage of sequence identity with a given reference natriuretic peptide, e.g., human BNP having the amino acid sequence of SEQ ID NO 24, may contain one or more mutations comprising an addition, a deletion and/or a substitution of one or more amino acids in comparison to the reference natriuretic peptide. According to the teaching of the present invention, said deleted, added and/or substituted amino acids may be consecutive amino acids or may be interspersed over the length of the amino acid sequence of the natriuretic peptide that shares a given percentage of sequence identity with a reference natriuretic peptide, e.g., human BNP having the amino acid sequence of SEQ ID NO 24. On the DNA level, the nucleic acid sequences encoding the natriuretic peptide that shares a given percentage of sequence identity with a given reference natriuretic peptide may differ to a larger extent due to the degeneracy of the genetic code.

(35) According to the teaching of the present invention, any number of amino acids may be added, deleted, and/or substituted, as long as the stipulated amino acid sequence identity with the reference natriuretic peptide is adhered to. In particular embodiments, the stipulated amino acid sequence identity is adhered to and the natriuretic peptide variant is biologically active, i.e. is a functional natriuretic peptide variant. Preferably, the biologic activity of the natriuretic peptide that shares a given percentage of sequence identity with a given reference natriuretic peptide, e.g., human BNP having the amino acid sequence as found in SEQ ID NO 24, is reduced by less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 25% or less than 10% compared to said reference natriuretic peptide as measured in the above described assay.

(36) The term “antibody”, as used herein, is intended to refer to immunoglobulin molecules, particularly dimeric immunoglobulin molecules comprised of four polypeptide chains—two heavy (H) chains and two light (L) chains which are typically inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region can comprise e.g. three domains CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain (CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is typically composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus e.g. in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

(37) As used herein, the term “Complementarity Determining Regions” (CDRs; e.g., CDR1, CDR2, and CDR3) refers to the amino acid residues of an antibody variable domain the presence of which are necessary for antigen binding. Each variable domain typically has three CDR regions identified as CDR1, CDR2 and CDR3. Each complementarity determining region may comprise amino acid residues from a “complementarity determining region” as defined by Kabat (e.g. about residues 24-34 (CDRL1), 50-56 (CDRL2) and 89-97 (CDRL3) in the light chain variable domain and 31-35 (CDRH1), 50-65 (CDRH2) and 95-102 (CDRH3) in the heavy chain variable domain; (Kabat et al., Sequences of Proteins of Immulological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a “hypervariable loop” (e.g. about residues 26-32 (CDRL1), 50-52 (CDRL2) and 91-96 (CDRL3) in the light chain variable domain and 26-32 (CDRH1), 53-55 (CDRH2) and 96-101 (CDRH3) in the heavy chain variable domain (Chothia and Lesk; J Mol Biol 196: 901-917 (1987)). In some instances, a complementarity determining region can include amino acids from both a CDR region defined according to Kabat and a hypervariable loop.

(38) Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different “classes”. There are five major classes of intact antibodies:

IgA, IgD, IgE, IgG, and IgM, and several of these maybe further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. Within the context of the present invention, the term “antibody” includes immunoglobulin molecules of any primary class—including IgG, IgE, IgM, IgD, IgA and IgY—and any subclass—including, IgG1, IgG2, IgG3, IgG4, IgA1 and Ig A2—isolated from nature or prepared by recombinant means and includes all conventionally known antibodies. A preferred class of immunoglobulins for use in the present invention is IgG. The term “antibody” also extends to other protein scaffolds that are able to orient antibody CDR inserts into the same active binding conformation as that found in natural antibodies such that binding of the target antigen observed with these chimeric proteins is maintained relative to the binding activity of the natural antibody from which the CDRs were derived.

(39) Within the context of the present invention, the term “fragment” of an antibody/immunoglobulin refers to any part of an antibody/immunoglobulin that comprises at least one CDR region. Particularly, the antibody fragment according to the present invention retains the ability to increase the serum half-life of a biologically active peptide, preferably a natriuretic peptide, incorporated therein. Antibody fragments according to the present invention include Fab, Fab′, Fab′-SH, F(ab′)2, and Fv fragments; diabodies; single domain antibodies (Dabs); linear antibodies; single-chain antibody molecules (scFv); and disulfide-stabilized Fv antibody fragments (dsFv); as well as multispecific antibodies formed from antibody fragments and fragments comprising a VL or VH domain, which are prepared from intact immunoglobulins or prepared by recombinant means.

(40) The F(ab′).sub.2 or Fab may be engineered to minimize or completely remove the intermolecular disulfide interactions that occur between the CH1 and CL domains. Antibody fragments according to the present invention may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, CH3 and CL domains. Also included are antibody fragments comprising any combination of variable region(s) with a hinge region, CH1, CH2, CH3 and CL domain.

(41) The antibody or fragment thereof constitutes a scaffold that confers stability to the natriuretic peptide incorporated therein. For example, the serum half-life of a natriuretic peptide incorporated within the CDR region of an antibody as described herein may be increased as compared to that of a naturally occurring natriuretic peptide.

(42) Principally, the heterologous amino acid sequence comprising the natriuretic peptide may be incorporated within any immunoglobulin molecule or fragment thereof. In particular, immunoglobulins of any species (including but not limited to human, bovine, murine, rat, pig, dog, shark, *lama* and camel) and any primary class and subclass may be used according to the present invention. For therapeutic use a human or humanized antibody may however be preferable. Within the context of the present invention, the term “human antibody” refers to antibodies having the amino acid sequence of a human immunoglobulin and includes antibodies isolated from human immunoglobulin libraries, from human B cells, or from animals transgenic for one or more human immunoglobulin as well as synthetic human antibodies. In particular embodiments the amino acid light chain and heavy chain sequences of the variable domain derive from human germline sequences LV 1-40 and HV 3-23, respectively (for more information see Example 1).

(43) Within the context of the present invention, the term “humanized antibody” or “humanized antibody fragment” refers to an antibody or fragment thereof that is (i) derived from a non-human source (e.g., a transgenic mouse which bears a heterologous immune system), which antibody is based on a human germline sequence; or (ii) chimeric, wherein the variable domain is derived from a non-human origin and the constant domain is derived from a human origin or (iii) CDR-grafted, wherein the CDRs of the variable domain are from a non-human origin, while one or more frameworks of the variable domain are of human origin and the constant domain (if any) is of human origin.

(44) The antibody or fragment thereof according to the present invention may be monospecific,

bispecific, trispecific or of greater multispecificity.
(45) In the context of the present invention, the term “comprises” or “comprising” means “including, but not limited to”. The term is intended to be open-ended, to specify the presence of any stated features, elements, integers, steps or components, but not to preclude the presence or addition of one or more other features, elements, integers, steps, components or groups thereof. The term “comprising” thus includes the more restrictive terms “consisting of” and “essentially consisting of”. In one embodiment, the term “comprising” as used throughout the application and in particular within the claims may be replaced by the term “consisting of”.
(46) In the context of the present invention, the term “about” or “approximately” means within 80% to 120%, alternatively within 90% to 110%, including within 95% to 105% of a given value.
(47) In the antibody or fragment thereof according to the invention, a) at least 12 amino acid residues are present between i) amino acid residue HC res25 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res S25) and the first amino acid residue of the natriuretic peptide in case of an incorporation of the heterologous amino acid sequence within CDRH1; ii) amino acid residue HC res51 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res 151) and the first amino acid residue of the natriuretic peptide in case of an incorporation of the heterologous amino acid sequence within CDRH2; iii) amino acid residue HC res92 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res C96) and the first amino acid residue of the natriuretic peptide in case of an incorporation of the heterologous amino acid sequence within CDRH3; iv) amino acid residue LC res26 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res S25) and the first amino acid residue of the natriuretic peptide in case of an incorporation of the heterologous amino acid sequence within CDRL1; v) amino acid residue LC res49 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res Y51) and the first amino acid residue of the natriuretic peptide in case of an incorporation of the heterologous amino acid sequence within CDRL2; and/or vi) amino acid residue LC res88 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res C90) and the first amino acid residue of the natriuretic peptide in case of an incorporation of the heterologous amino acid sequence within CDRL3;
and b) at least 9 amino acid residues are present between the last amino acid residue of the natriuretic peptide and i) amino acid residue HC res35a according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res M34) in case of an incorporation of the heterologous amino acid sequence within CDRH1; ii) amino acid residue HC res57 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res T58) in case of an incorporation of the heterologous amino acid sequence within CDRH2; iii) amino acid residue HC res106 according to Kabat (in the heavy chain having the amino acid sequence of SEQ ID NO 65 this corresponds to res G111) in case of an incorporation of the heterologous amino acid sequence within CDRH3; iv) amino acid residue LC res 32 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res D34) in case of an incorporation of the heterologous amino acid sequence within CDRL1; v) amino acid residue LC res57 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res G59) in case of an incorporation of the heterologous amino acid sequence within CDRL2; and/or vi) amino acid residue LC res98 according to Kabat (in the light chain having the amino acid sequence of SEQ ID NO 66 this corresponds to res F102) in case of an incorporation of the heterologous amino acid sequence within CDRL3.
(48) The denomination of the above listed amino acid residues refers to the amino acid position in the original immunoglobulin molecule before incorporation of the heterologous amino acid sequence. Within the context of the present invention, the above listed amino acid residues are

referred to as “reference amino acids” or “reference aa”. These reference amino acid residues lie at or near CDR framework junctions but do not necessarily correspond to standard CDR border definitions (standard CDR border definitions are amino acid residues S25 and W36 for CDRH1; S49 and R67 for CDRH2; K98 and W108 for CDRH3; C22 and W37 for CDRL1; Y51 and G59 for CDRL2; C90 and F102 for CDRL3. Jarasch and Skerra, Proteins 2017 January; 85 (1): 65-71).
(49) The nearest neighboring reference aa N-terminal from the inserted natriuretic peptide plus the amino acid stretch present between said reference aa and the first amino acid residue of the inserted natriuretic peptide are herein referred to as “N-terminal sequence”. The N-terminal sequence comprises the Ntls. In particular embodiments, the N-terminal sequence consists of the Ntls plus the neighboring N-terminal reference aa.
(50) The amino acid stretch present between the last amino acid residue of the inserted natriuretic peptide and the nearest neighboring reference aa C-terminal from the inserted natriuretic peptide plus and said reference aa are herein referred to as “C-terminal sequence”. The C-terminal sequence comprises the Ctls. In particular embodiments, the C-terminal sequence consists of the Ctls plus the neighboring C-terminal reference aa.
(51) In particular embodiments, the Ntls comprises a GS linker sequence; a PN linker sequence; an amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the sequence of any one of SEQ ID NOs 1, 2 or 4, or a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 2 or 4; the sequence of any one of SEQ ID NOs 6, 7, 9, 11, 13, 15, 16, 17, 19 or 21; or a sequence that shares at least 60%, at least 70%, at least 80%, at least 90% or at least 95% sequence identity with any one of SEQ ID NO 6, 7, 9, 11, 13, 15, 16, 17, 19 or 21. The Ntls may also comprise any combination of the above listed amino acid sequences.
(52) In particular such embodiments, the Ntls comprises a GS linker sequence; a PN linker sequence; an amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the sequence of any one of SEQ ID NOs 1, 2 or 4, or a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 2 or 4; the sequence of any one of SEQ ID NOs 6, 7, 9, 11, 13, 15 or 21; a sequence that shares at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% sequence identity with any one of SEQ ID NO 6, 7, 9, 11, 13, 15, or 21; or any combination thereof.
(53) In particular embodiments, the Ctls comprises a GS linker sequence; a PN linker sequence; an amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the sequence of any one of SEQ ID NOs 1, 3 or 5, or a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 3 or 5; the sequence of any one of SEQ ID NOs 6, 8, 10, 12, 14, 15, 17, 18, 19, 20 or 22; or a sequence that shares at least 60%, at least 70%, at least 80%, at least 90% or at least 95% sequence identity with any one of SEQ ID NOs 6, 8, 10, 12, 14, 15, 17, 18, 19, 20 or 22. The Ctls may also comprise any combination of the above listed amino acid sequences.
(54) In particular embodiments, the Ctls comprises a GS linker sequence; a PN linker sequence; an amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the sequence of any one of SEQ ID NOs 1, 3 or 5, or a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 3 or 5; the sequence of any

one of SEQ ID NOs 6, 8, 10, 12, 14, 15, 20 or 22; a sequence that shares at least 60%, at least 70%, at least 80%, at least 90% or at least 95% sequence identity with any one of SEQ ID NOs 6, 8, 10, 12, 14, 15, 20 or 22; or any combination thereof.

(55) In particular embodiments the sequence identity between the sequence comprised in the Ntls and/or the Ctls and any one of SEQ ID NOs 1 to 22 is at least 60%, particularly at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100%.

(56) Within the context of the present invention the term “GS linker sequence” refers to a peptide linker comprising mainly glycine and serine residues. Particularly, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% of the amino acid residues of the GS linker sequence according to the present invention are selected from glycine and serine residues. The GS linker sequence according to the present invention may for example comprise from 1 to 30 amino acid residues in total. Particularly, the GS linker sequence according to the present invention does not comprise more than 3, 2 or 1 amino acid residue(s) other than glycine or serine.

(57) Within the context of the present invention the term “PN linker sequence” refers to a peptide linker comprising mainly proline and asparagine residues. Particularly, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% of the amino acid residues of the PN linker sequence according to the present invention are selected from proline and asparagine residues. The PN linker sequence according to the present invention may for example comprise from 1 to 30 amino acid residues in total. Particularly, the PN linker sequence according to the present invention does not comprise more than 3, 2 or 1 amino acid residue(s) other than proline or asparagine. Other amino acid residues that may be present in a PN linker sequence according to the present invention are for instance lysine or glutamic acid residues.

(58) In particular embodiments, the linker sequence comprised in the Ntls and/or the Ctls and selected from a GS linker sequence; a PN linker sequence; a human IgG antibody scaffold linker sequence; a human IgG fab domain scaffold sequence; a sequence that shares at least 80% sequence identity with the human IgG antibody scaffold linker sequence, the human IgG fab domain scaffold sequence or the sequence of any one of SEQ ID NOs 1 to 5; and a sequence that shares at least 60% sequence identity with any one of SEQ ID NOs 6 to 22, comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid residues. The linker sequence comprised in the Ntls and/or the Ctls may for instance comprise up to 30, 28, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 amino acid residues.

(59) In the case of linkers comprising a sequence of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, it may be particularly advantageous to use a sequence of a scaffold region which is adjacent to the CDR into which the heterologous amino acid sequence is incorporated. For example, the Ntls and/or the Ctls may comprise a linker comprising an amino acid sequence that is part of framework region FRH2 or FRH3 in case the heterologous amino acid sequence is incorporated within the CDRH2 domain. Similarly, the Ntls and/or the Ctls may comprise a linker comprising an amino acid sequence that is part of framework region FRL2 or FRL3 in case the heterologous amino acid sequence is incorporated within the CDRL2 region.

(60) In particular embodiments, the Ntls consists of a GS linker sequence; a PN linker sequence; an amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the sequence of any one of SEQ ID NOs 1, 2 or 4 or a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 2 or 4; the sequence of any one of SEQ ID NOs 6, 7, 9, 11, 13, 15, 16, 17, 19 or 21; a sequence that shares at least 60%, at least 70%, at least 80%, at least 90% or at least 95% sequence identity with any one of SEQ ID NOs 6, 7, 9, 11, 13, 15, 16, 17, 19 or 21; or any combination thereof.

(61) In particular embodiments, the Ctls consists of a GS linker sequence; a PN linker sequence; an

amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the sequence of any one of SEQ ID NOs 1, 3 or 5 a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 3 or 5; the sequence of any one of SEQ ID NOs 6, 8, 10, 12, 14, 15, 17, 18, 19, 20 or 22; a sequence that shares at least 60%, at least 70%, at least 80%, at least 90% or at least 95% sequence identity with any one of SEQ ID NOs 6, 8, 10, 12, 14, 15, 17, 18, 19, 20 or 22; or any combination thereof.

(62) In particular embodiments, both the Ntls and the Ctls comprise at least one of the above listed linker sequences or any combination thereof. In principle, any of the above listed Ntls linker sequences may be combined with any of the above listed Ctls linker sequences. In particular, any linker sequence may be combined with a GS linker. As a non-limiting example, a GS Ctls linker may be combined with an Ntls linker comprising the sequence of any one of SEQ ID NOs 6, 9 or 15 or a sequence that shares at least 60% sequence identity with any one of SEQ ID NOs 6, 9 or 15. A further non-limiting example is a GS Ntls linker combined with a Ctls linker comprising the sequence of SEQ ID NO 15 or a sequence that shares at least 60% sequence identity therewith.

(63) The above listed linker sequences have proven particularly advantageous for achieving good natriuretic peptide activities, given a sufficient total length of the N-terminal and C-terminal flanking sequences. Without wishing to be bound by theory, it is believed that the above listed linker peptide stretches result in a conformation/folding that contributes to a favorable state of the system in presentation of a biologically active natriuretic peptide to its respective receptor with minimal sterical hindrance.

(64) In particular embodiments, i) the Ntls comprises a GS linker sequence; a PN linker sequence; the sequence of SEQ ID NOs 2, 4, 9, 11, 13 or 15; a sequence that shares at least 60% sequence identity with SEQ ID NOs 2, 4, 9, 11, 13 or 15; or any combination thereof and ii) the Ctls comprises a GS linker sequence; a PN linker sequence; the sequence of SEQ ID NOs 3, 5, 12, 14, 15 or 20; a sequence that shares at least 60% sequence identity with SEQ ID NOs 3, 5, 12, 14, 15 or 20; or any combination thereof. These linker sequences have proven particularly useful as they not only achieve high natriuretic peptide activities but also good expression levels in recombinant expression and are not prone to protein fragmentation upon expression (see Table 9).

(65) In particular embodiments, the Ntls and the Ctls each comprise a GS linker sequence; the Ntls and the Ctls each comprise a PN linker sequence; the Ntls and the Ctls each comprise an amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the Ntls comprises the sequence of any one of SEQ ID NOs 1, 2 or 4 or a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 2 or 4 and the Ctls comprises the sequence of any one of SEQ ID NOs 1, 3 or 5 or a sequence that shares at least 80% sequence identity therewith; the Ntls and the Ctls each comprise the sequence of SEQ ID NO 6 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 7 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 8 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 9 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 10 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 11 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 12 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 13 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 14 or a sequence that shares at least 60% sequence identity therewith; the Ntls and the Ctls each comprise the

sequence of SEQ ID NO 15 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 16 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 17 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 17 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 18 or a sequence that shares at least 60% sequence identity therewith; the Ntls and the Ctls each comprise the sequence of SEQ ID NO 19 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 9 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 20 or a sequence that shares at least 60% sequence identity therewith; or the Ntls comprises the sequence of SEQ ID NO 21 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 22 or a sequence that shares at least 60% sequence identity therewith.

(66) In particular such embodiments, the Ntls and the Ctls each comprise a GS linker sequence; the Ntls and the Ctls each comprise a PN linker sequence; the Ntls and the Ctls each comprise an amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the Ntls comprises the sequence of any one of SEQ ID NOs 1, 2 or 4 or a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 2 or 4 and the Ctls comprises the sequence of any one of SEQ ID NOs 1, 3 or 5 or a sequence that shares at least 80% sequence identity therewith; the Ntls and the Ctls each comprise the sequence of SEQ ID NO 6 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 7 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 8 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 9 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 10 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 11 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 12 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 13 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 14 or a sequence that shares at least 60% sequence identity therewith; the Ntls and the Ctls each comprise the sequence of SEQ ID NO 15 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 9 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 20 or a sequence that shares at least 60% sequence identity therewith; or the Ntls comprises the sequence of SEQ ID NO 21 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 22 or a sequence that shares at least 60% sequence identity therewith.

(67) In particular such embodiments, the Ntls and the Ctls each comprise a GS linker sequence; the Ntls and the Ctls each comprise a PN linker sequence; the Ntls comprises the sequence of SEQ ID NO 2 or a sequence that shares at least 80% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 3 or a sequence that shares at least 80% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 4 or a sequence that shares at least 80% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 5 or a sequence that shares at least 80% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 11 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 12 or a sequence that shares at least 60% sequence identity therewith; the Ntls comprises the sequence of SEQ ID NO 13 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 14 or a sequence that shares at least

60% sequence identity therewith; the Ntls and the Ctls each comprise the sequence of SEQ ID NO 15 or a sequence that shares at least 60% sequence identity therewith; or the Ntls comprises the sequence of SEQ ID NO 9 or a sequence that shares at least 60% sequence identity therewith and the Ctls comprises the sequence of SEQ ID NO 20 or a sequence that shares at least 60% sequence identity therewith. These linker combinations have proven particularly useful for achieving high natriuretic peptide activities, good expression levels in recombinant expression and minimal or no protein fragmentation, as shown in Table 9.

(68) In particular embodiments, the Ntls further comprises an anchoring element A1 at its C terminal end and/or the Ctls further comprises an anchoring element A2 at its N terminal end, wherein A1 and A2 predominantly comprise glycine and serine residues. In particular embodiments, A1 and/or A2 comprise at least 1, 2, 3, 4, or 5 amino acid residues. A1 and/or A2 may comprise up to 10, 9, 8, 7, 6 or 5 amino acid residues in total. In particular embodiments, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the amino acid residues of A1 and/or A2 are selected from glycine and serine residues. Particularly A1 and/or A2 do/does not comprise more than 3, 2 or 1 amino acid residue other than glycine or serine.

(69) In particular embodiments the Ntls consists of i) an anchoring element A1 at its C terminal end and ii) a GS linker sequence; a PN linker sequence; an amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the sequence of any one of SEQ ID NOs 1, 2 or 4 or a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 2 or 4; the sequence of any one of SEQ ID NOs 6, 7, 9, 11, 13, 15, 16, 17, 19 or 21; a sequence that shares at least 60% sequence identity with any one of SEQ ID NO 6, 7, 9, 11, 13, 15, 16, 17, 19 or 21; or any combination thereof.

(70) In particular embodiments, the Ctls consists of i) an anchoring element A2 at its N terminal end and ii) a GS linker sequence; a PN linker sequence; an amino acid sequence which is part of a human IgG antibody scaffold or a sequence that shares at least 80% sequence identity therewith, particularly an amino acid sequence which is part of the fab domain scaffold of a human IgG antibody or a sequence that shares at least 80% sequence identity therewith, more particularly the sequence of any one of SEQ ID NOs 1, 3 or 5 a sequence that shares at least 80% sequence identity with any one of SEQ ID NOs 1, 3 or 5; the sequence of any one of SEQ ID NOs 6, 8, 10, 12, 14, 15, 17, 18, 19, 20 or 22; a sequence that shares at least 60% sequence identity with any one of SEQ ID NOs 6, 8, 10, 12, 14, 15, 17, 18, 19, 20 or 22; or any combination thereof.

(71) In particular embodiments, the Ntls and/or the Ctls comprise(s) at least 3, 4, 5, 6, 7, 8, 9 or 10 amino acid residues in total. The Ntls and/or the Ctls may for instance comprise up to 30, 28, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 amino acid residues in total.

(72) In particular embodiments, the amino acid stretch present between i) amino acid residue HC res25 according to Kabat and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRH1; ii) amino acid residue HC res51 according to Kabat and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRH2; iii) amino acid residue HC res92 according to Kabat and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRH3; iv) amino acid residue LC res26 according to Kabat and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRL1; v) amino acid residue LC res49 according to Kabat and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRL2; and/or vi) amino acid residue LC res88 according to Kabat and the first amino acid residue of the natriuretic peptide in case of an incorporation of said heterologous amino acid sequence within CDRL3 comprises the sequence of any one of SEQ ID NOs 26 to 38 or a sequence having at least 80%,

85%, 90%, 95% or at least 98% sequence identity with any one of SEQ ID NOs 26 to 38.
(73) In particular embodiments, the amino acid stretch present between the last amino acid residue of the natriuretic peptide and i) amino acid residue HC res35a according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH1; ii) amino acid residue HC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH2; iii) amino acid residue HC res106 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH3; iv) amino acid residue LC res 32 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL1; v) amino acid residue LC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL2; and/or vi) amino acid residue LC res98 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL3
comprises the sequence of any one of SEQ ID NOs 39 to 51 or a sequence having at least 80%, 85%, 90%, 95% or at least 98% sequence identity with any one of SEQ ID NOs 39 to 51.
(74) In particular embodiments, the heterologous amino acid sequence consists of the Ntls, the natriuretic peptide and the Ctls.
(75) In particular embodiments, the amino acid stretch present between i) amino acid residue HC res25 according to Kabat and amino acid residue HC res35a according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH1; ii) amino acid residue HC res51 according to Kabat and amino acid residue HC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH2; iii) amino acid residue HC res92 according to Kabat and amino acid residue HC res106 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH3; iv) amino acid residue LC res26 according to Kabat and amino acid residue LC res 32 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL1; v) amino acid residue LC res49 according to Kabat and amino acid residue LC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL2; and/or vi) amino acid residue LC res88 according to Kabat and amino acid residue LC res98 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL3 comprises the sequence of any one of SEQ ID NOs 52 to 64 or a sequence having at least 80%, 85%, 90%, 95% or at least 98% sequence identity with any one of SEQ ID NOs 52 to 64.
(76) In particular embodiments, the antibody or fragment thereof comprises at least two natriuretic peptides. In particular embodiments, both natriuretic peptides are comprised in a heterologous amino acid sequence further comprising an Ntls and a Ctls and incorporated within a CDR region of said antibody or fragment thereof, as described herein. The at least two natriuretic peptides may be incorporated within the two corresponding CDR regions of two light chains or two heavy chains or the at least two natriuretic peptides may be incorporated within two separate CDR regions. The at least two natriuretic peptides may be the same or different. In particular such embodiments, the antibody or fragment thereof comprises at least two different natriuretic peptides that are incorporated within at least two CDR regions.
(77) Due to the dimeric structure of antibody molecules, the insertion of one nucleic acid sequence encoding the heterologous amino acid sequence (Ntls-natriuretic peptide-Ctls) into the nucleic acid encoding either the light or the heavy chain of an immunoglobulin molecule typically yields an antibody protein carrying two natriuretic peptides located in the corresponding CDR regions of the two identical light or the two identical heavy chains. However, it is also envisaged to insert two natriuretic peptide encoding nucleic acids into two different CDR encoding regions of the nucleic acid sequences encoding the light and/or the heavy chain, thereby yielding an antibody molecule with four natriuretic peptides located in two corresponding CDR pairs of the dimeric antibody. Also encompassed are dimeric immunoglobulin molecules whose light chains and/or heavy chains are not identical, for instance including dimeric antibodies carrying a single natriuretic peptide as well

as dimeric antibodies carrying two different natriuretic peptides in two corresponding CDR regions of the two light or the two heavy chains.

(78) In particular embodiments, natriuretic peptides are inserted in the CDRH1 and CDRH2, CDRH1 and CDRH3, CDRH2 and CDRH3, CDRH1 and CDRL1, CDRH1 and CDRL2, CDRH1 and CDRL3, CDRH2 and CDRL1, or CDRH2 and CDRL2. In particular embodiments, the antibody or fragment thereof comprises one ANP and one BNP molecule; one ANP and one CNP molecule; or one BNP and one CNP molecule.

(79) In particular embodiments, the natriuretic peptide comprised in the at least one heterologous amino acid sequence incorporated within at least one CDR region of said antibody or fragment thereof is an BNP and the antibody or fragment thereof comprises at least one further natriuretic peptide. In particular embodiments, the at least one further natriuretic peptide is also comprised in a heterologous amino acid sequence further comprising an Ntls and a Ctls and incorporated within a CDR region of said antibody or fragment thereof. In particular embodiments, the BNP and the at least one further natriuretic peptide are incorporated within two corresponding CDR regions of either the two light or the two heavy chains of the antibody or fragment thereof. In particular other embodiments, the BNP and the at least one further natriuretic peptide are incorporated within at least two separate CDR regions. Particularly, said at least one further natriuretic peptide is selected from ANP, BNP and CNP, more particularly from ANP and CNP.

(80) The “empty” antibody molecule not harboring a heterologous amino acid sequence comprising a natriuretic peptide which is composed of two heavy chains having the sequence of SEQ ID NO 65 and two light chains having the sequence of SEQ ID NO 66 is termed TPP-5657. In particular embodiments, an antibody molecule composed of two heavy chains having the sequence of SEQ ID NO 65 or a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity therewith and two light chains having the sequence of SEQ ID NO 66 or a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity therewith serves as the initial or parental antibody, into which the heterologous amino acid sequence comprising the natriuretic peptide is incorporated. In particular such embodiments, the heterologous amino acid sequence comprising the natriuretic peptide is incorporated within a CDR region of one or both heavy chains of such antibody molecule.

(81) In particular such embodiments, the heavy chain(s) comprising the at least one heterologous amino acid sequence incorporated within at least one of its CDR regions has the sequence of any one of SEQ ID NOs 67 to 79 or a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity with any one of SEQ ID NOs 67 to 79. In particular embodiments, the antibody according to the present invention is composed of two identical heavy chains having the sequence of any one of SEQ ID NOs 67 to 79 or a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity with any one of SEQ ID NOs 67 to 79 and two identical light chains having the sequence of SEQ ID NO 66 or a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity therewith.

(82) In particular other embodiments, the heterologous amino acid sequence comprising the natriuretic peptide is incorporated within a CDR region of one or both light chains of such antibody molecule. In particular such embodiments, the light chain(s) comprising the at least one heterologous amino acid sequence incorporated within at least one of its CDR regions has(have) the sequence of any one of SEQ ID NOs 80 or 81 a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity with any one of SEQ ID NOs 80 or 81. In particular embodiments, the antibody according to the present invention is composed of two identical light chains having the sequence of any one of SEQ ID NOs 80 or 81 or a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity with any one of SEQ ID NOs 80 or 81 and two identical heavy chains having the sequence of SEQ ID NO 65 or a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity therewith.

(83) The table below depicts the heavy and light chain composition of exemplary antibodies

according to the present invention.
(84) TABLE-US-00002 TABLE 1 Exemplary natriuretic peptide engrafted antibody constructs TPP-Number Heavy Chain Light Chain TPP-5661 SEQ ID NO 67 SEQ ID NO 66 TPP-10274 SEQ ID NO 68 SEQ ID NO 66 TPP-10282 SEQ ID NO 69 SEQ ID NO 66 TPP-10283 SEQ ID NO 70 SEQ ID NO 66 TPP-10290 SEQ ID NO 71 SEQ ID NO 66 TPP-10294 SEQ ID NO 72 SEQ ID NO 66 TPP-10765 SEQ ID NO 73 SEQ ID NO 66 TPP-10845 SEQ ID NO 74 SEQ ID NO 66 TPP-10847 SEQ ID NO 75 SEQ ID NO 66 TPP-10992 SEQ ID NO 76 SEQ ID NO 66 TPP-13054 SEQ ID NO 77 SEQ ID NO 66 TPP-13061 SEQ ID NO 78 SEQ ID NO 66 TPP-13230 SEQ ID NO 79 SEQ ID NO 66 TPP-10355 SEQ ID NO 65 SEQ ID NO 80 TPP-10361 SEQ ID NO 65 SEQ ID NO 81 TPP-9902 SEQ ID NO 442 SEQ ID NO 66 TPP-11156 SEQ ID NO 443 SEQ ID NO 66 TPP-18034 SEQ ID NO 444 SEQ ID NO 66 TPP-12897 SEQ ID NO 445 SEQ ID NO 447 TPP-12377 SEQ ID NO 445 SEQ ID NO 66 TPP-9465 SEQ ID NO 446 SEQ ID NO 66
(85) Antibodies of the present invention or fragments thereof include naturally occurring purified products, products of chemical synthetic procedures, and products produced by recombinant techniques. Depending on its origin, the antibody or fragment thereof according to the present invention may be glycosylated or non-glycosylated.
(86) For example, standard recombinant DNA methodologies may be used to prepare and/or obtain nucleic acids encoding the heavy and light chains, incorporate these nucleic acids into expression vectors and introduce the vectors into host cells for recombinant expression (see, for example, Sambrook, Fritsch and Maniatis (eds.), Molecular Cloning; A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989); Ausubel, F. M. et al. (eds.) Current Protocols in Molecular Biology, Greene Publishing Associates, (1989); Goeddel, Gene Expression Technology, Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990); and U.S. Pat. No. 4,816,397 by Boss et al.).
(87) Thus, in a second aspect, the present invention relates to a nucleic acid or a mixture of nucleic acids encoding the antibody or fragment thereof according to the present invention. These nucleic acid sequences may be optimized in certain cases for mammalian expression. DNA molecules of the invention are not limited to the sequences disclosed herein, but also include variants thereof.
(88) The present invention further provides recombinant nucleic acid constructs comprising one or more of the nucleic acid sequences according to the present invention. The recombinant nucleic acid construct according to the present invention may for instance comprise a nucleic acid vector, such as a plasmid, into which a nucleic acid molecule encoding an antibody or fragment thereof according to the present invention has been inserted. It is understood that the design of the expression vector, including the selection of regulatory sequences is affected by factors such as the choice of the host cell, the desired protein expression level and whether constitutive or inducible expression is desired.
(89) Useful expression vectors for bacterial use may be constructed by inserting one or more nucleic acid sequences according to the present invention together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. Bacterial expression vectors typically comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and, if desirable, to provide amplification within the bacterial host. Bacterial expression vectors may comprise elements derived from commercially available plasmids such as the well-known cloning vector pBR322 (ATCC 37017). A number of bacterial expression vectors may be advantageously selected depending upon the use intended of the expressed antibody or fragment thereof. For example, if a large quantity of such antibody is desired, vectors mediating high level expression of antibody fusion proteins that are readily purified may be desirable.
(90) Recombinant nucleic acid constructs intended for antibody expression in a eukaryotic host cell may comprise regulatory sequences that are able to control the expression of an open reading frame in a eukaryotic cell, preferably a promoter and a polyadenylation signal. Promoters and

polyadenylation signals are preferably selected to be functional within the specific cell type intended for antibody expression. Examples of suitable promoters include but are not limited to promoters from Cytomegalovirus (CMV), such as the strong CMV immediate early promoter, Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV), Human Immunodeficiency Virus (HIV), such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, Epstein Barr Virus (EBV), adenovirus (e.g., the adenovirus major late promoter (AdMLP)), polyoma and from Rous Sarcoma Virus (RSV), the synthetic CAG promoter composed of the CMV early enhancer element, the promoter, the first exon and the first intron of chicken beta-actin gene and the splice acceptor of the rabbit beta globin gene, as well as promoters from mammalian genes such as actin, myosin, hemoglobin, muscle creatine, and metallothionein. In a particular embodiment, the eukaryotic expression cassette contains the CMV promoter. In the context of the present invention, the term “CMV promoter” refers to the strong immediate-early cytomegalovirus promoter.

(91) Examples of suitable polyadenylation signals include but are not limited to the bovine growth hormone (BGH) polyadenylation site, SV40 polyadenylation signals and LTR polyadenylation signals.

(92) In addition, the recombinant nucleic acid sequence may comprise one or more enhancer sequences. The enhancer can be, for example, an enhancer of mammalian actin, myosin, hemoglobin, muscle creatine or a viral enhancer, e.g. an enhancer from CMV, RSV, SV40 or EBV. For further description of viral regulatory elements, and sequences thereof, see e.g., U.S. Pat. No. 5,168,062 by Stinski, U.S. Pat. No. 4,510,245 by Bell et al. and U.S. Pat. No. 4,968,615 by Schaffner et al.

(93) Regulatory sequences and codons are generally species dependent, so in order to maximize protein production, the regulatory sequences and codons are preferably selected to be effective in the species/cell type intended for antibody expression. The person skilled in the art can produce recombinant DNA molecules that are functional in a given subject species.

(94) The mammalian recombinant expression vectors can also include origins of replication and selectable markers (see e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017). Suitable selectable markers include genes that confer resistance to drugs such as G418, puromycin, hygromycin, blasticidin, zeocin/bleomycin or methotrexate or selectable marker that exploit auxotrophies such as Glutamine Synthetase on a host cell into which the vector has been introduced. For example, the dihydrofolate reductase (DHFR) gene confers resistance to methotrexate, the neo gene confers resistance to G418, the bsd gene from *Aspergillus terreus* confers resistance to blasticidin, puromycin N-acetyl-transferase confers resistance to puromycin, the Sh ble gene product confers resistance to zeocin, and resistance to hygromycin is conferred by the *E. coli* hygromycin resistance gene (hyg or hph). Selectable markers like DHFR or Glutamine Synthetase are also useful for amplification techniques in conjunction with MTX and MSX.

(95) In some embodiments, the nucleic acid sequences encoding the heavy and light chains are inserted into separate vectors. In other embodiments, the nucleic acid sequences encoding the heavy and light chains are inserted into the same vector. In addition, the nucleic acid sequences encoding variable regions of the heavy and/or light chains can be converted, for example, to nucleic acid sequences encoding full-length antibody chains, Fab fragments, or to scFv. The VL- or VH-encoding DNA fragment can be operatively linked, (such that the amino acid sequences encoded by the two DNA fragments are in-frame) to another DNA fragment encoding, for example, an antibody constant region or a flexible linker. As an example, to create a polynucleotide sequence that encodes a scFv, the VH- and VL-encoding nucleic acids can be operatively linked to another fragment encoding a flexible linker such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see e.g., Bird et al. (1988) Science 242:423-426; Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883; McCafferty et al., Nature (1990) 348:552-554). The sequences of human heavy chain and light chain constant regions are known in the art (see e.g., Kabat, E. A., el al. (1991) Sequences of

Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification.

(96) In particular embodiments, the nucleic acid sequences encoding the heavy chain into which the heterologous amino acid sequence comprising the natriuretic peptide is incorporated comprises the sequence of any one of SEQ ID NOs 82 or 83 or a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity with any one of SEQ ID NOs 82 or 83. In particular such embodiments, the nucleic acid sequence encoding the light chain comprises the sequence of SEQ ID NO 84 or a sequence having at least 80%, at least 85%, at least 90% or at least 95% sequence identity therewith.

(97) In a third aspect, the present invention relates to a host cell comprising the nucleic acid or the mixture of nucleic acids according to the present invention. Within the context of the present invention, the terms “host cell”, “host cell line”, and “host cell culture” are used interchangeably and refer to cells into which an exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants”, “transformed cells”, “transfectants”, “transfected cells”, and “transduced cells”, which include the primary transformed/transfected/transduced cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, and the comprised exogenous nucleic acid may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

(98) Transfection of the expression vector into a host cell can be carried out using standard techniques such as electroporation, nucleofection, calcium-phosphate precipitation, lipofection, polycation-based transfection such as polyethlylenimine (PEI)-based transfection and DEAE-dextran transfection.

(99) Suitable host cells include prokaryotic and eukaryotic cells. Examples for prokaryotic host cells are e.g. bacteria and include but are not limited to *Escherichia coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera *Pseudomonas, Streptomyces,* and *Staphylococcus.*

(100) Non limiting examples of eukaryotic hosts cells include yeasts, insects and insect cells, plants and plant cells, transgenic animals and mammalian cells. Suitable mammalian host cells for antibody expression include Chinese Hamster Ovary (CHO cells) such as CHO-K1, CHO-S, CHO-K1SV (including dhfr-CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220 and Urlaub et al., Cell. 1983 June; 33(2):405-12, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) Mol. Biol. 159:601-621; and other knockout cells exemplified in Fan et al., Biotechnol Bioeng. 2012 April; 109(4):1007-15), NSO myeloma cells, COS cells, HEK293 cells, HKB11 cells, BHK21 cells, CAP cells, EB66 cells, and SP2 cells.

(101) Expression may also be transient or semi-stable in expression systems such as HEK293, HEK293T, HEK293-EBNA, HEK293E, HEK293-6E, HEK293-Freestyle, HKB11, Expi293F, 293EBNALT75, CHO Freestyle, CHO—S, CHO-K1, CHO-K1SV, CHOEBNALT85, CHOS-XE, CHO-3E7 or CAP-T cells (for instance Durocher et al., Nucleic Acids Res. 2002 Jan. 15; 30(2):E9).

(102) In a fourth aspect, the present invention relates to a process for producing an antibody or fragment thereof, comprising culturing the host cell according to the present invention. Particularly, the host cell according to the present invention is cultured under conditions suitable for expression of the antibody or fragment thereof.

(103) Antibody expression may be constitutive or regulated (e.g., inducible). For inducible antibody expression the host cell according to the present invention is typically grown to an appropriate cell density followed by de-repression/induction of the selected promoter by appropriate means (e.g., temperature shift or chemical induction such as addition or removal of

small molecule inductors such as tetracycline in conjunction with Tet system) and culturing of the host cell for an additional period.
(104) In particular embodiments, the process for producing an antibody or fragment thereof according to the present invention further comprises the step of recovering the antibody or fragment thereof from the host cell culture. Cells may for instance be harvested by centrifugation, disrupted by physical or chemical means, and the antibody or fragment thereof may be further purified from the resulting crude extract. In some embodiments, the expression vector is designed such that the expressed antibody or fragment thereof is secreted into the culture medium in which the host cells are grown. In that case, the antibody or fragment thereof can be directly recovered from the culture medium using standard protein purification methods.
(105) In particular embodiments, the process according to the present invention further comprises the step of purifying the recovered antibody or fragment thereof. Particularly, the antibody is purified (1) to greater than 90% as determined e.g. by analytical chromatography or by SDS-Capillary Gel electrophoresis (for example on a Caliper LabChip GXII, GX 90 or Biorad Bioanalyzer device), and, more particularly, purification yields an antibody homogeneity of at least about 92.5%, 95%, 98% or 99%; alternatively, the antibody is purified (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence, or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, preferably, silver stain.
(106) Antibodies or fragments thereof according to the present invention can be recovered and purified from recombinant cell cultures by well-known methods including, but not limited to ammonium sulfate or ethanol precipitation, acid extraction, Protein A chromatography, Protein G chromatography, size exclusion chromatography, anion or cation exchange chromatography, phospho-cellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography (“HPLC”) can also be employed for purification (see, e.g., Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y., (1997-2001), e.g., Chapters 1, 4, 6, 8, 9, 10).
(107) In a fifth aspect, the present invention relates to a composition comprising the antibody or fragment thereof according the present invention. Particularly, the composition according to the present invention is a pharmaceutical composition suitable for use in a method for treatment, wherein the antibody or fragment thereof according to the present invention is contained in an amount effective to achieve the intended purpose, i.e. prevention or treatment of a particular disease state.
(108) The composition optionally further comprises at least one pharmaceutically acceptable excipient. In the context of the present invention, the term “excipient” refers to a natural or synthetic substance formulated alongside the active ingredient of a medication. Suitable excipients include antiadherents, binders, coatings, disintegrants, flavors, colors, lubricants, glidants, sorbents, preservatives and sweeteners. Specific examples of pharmaceutically acceptable excipients include but are not limited to saline, buffered saline, dextrose, and water. In the context of the present invention, the term “pharmaceutically acceptable” refers to molecular entities and other ingredients of pharmaceutical compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (e.g., human). The term “pharmaceutically acceptable” may also mean approved by a regulatory agency of a Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and, more particularly, in humans.
(109) The composition according to the present invention may further comprise one or more further therapeutically active agents.
(110) The pharmaceutical composition may be in the form of a solution, a suspension, an enteric coated capsule, a lyophilized powder or any other form suitable for the intended use.

(111) Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable excipients well known in the art in dosages suitable for oral administration. Such excipients enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for ingestion by the patient.
(112) Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl-cellulose, hydroxypropylmethylcellulose, or sodium carboxymethyl cellulose; and gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.
(113) Dragee cores can be provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e. dosage.
(114) Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.
(115) Pharmaceutical formulations for parenteral administration include aqueous solutions of a therapeutically active agent. For injection, the pharmaceutical compositions of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active agent may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the therapeutically active agent to allow for the preparation of highly concentrated solutions.
(116) For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.
(117) The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.
(118) The pharmaceutical composition may be provided as a salt and can be formed with acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder in 1 mM-50 mM histidine, 0.1%-2% sucrose, 2%-7% mannitol at a pH range of 4.5 to 7.5 that is combined with buffer prior to use.
(119) Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Ed. Maack Publishing Co, Easton, Pa.).
(120) After preparation of a pharmaceutical composition comprising the antibody or fragment

thereof according to the present invention, it may be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of the antibody or fragment according to the present invention, such labeling would include amount, frequency and method of administration.

(121) The present invention further provides pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions according to the present invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, reflecting approval by the agency of the manufacture, use or sale of the product for human administration.

(122) In a sixth aspect, the present invention relates to the antibody or fragment thereof according to the present invention or the composition according to the present invention for use in a method for treatment.

(123) Particularly, such method for treatment involves administering to a subject in need thereof a therapeutically effective amount of the antibody or fragment thereof according to the present invention. The subject may be a human or non-human animal (e.g., rabbit, rat, mouse, dog, monkey or other lower-order primate).

(124) Within the context of the present invention, the term “therapeutically effective amount” is defined as the amount of an antibody or fragment thereof according to the present invention that is sufficient to prevent or alleviate disease symptoms of any of the disorders and diseases mentioned herein—either as a single dose or according to a multiple dose regimen, alone or in combination with other agents. In particular embodiments, said “therapeutically effective amount” is toxicologically tolerable. The determination of an effective dose is well within the capability of those skilled in the art. The therapeutically effective amount of a therapeutic agent usually largely depends on particular patient characteristics such as age, weight, gender and disease state, time, frequency and route of administration, drug combination(s), and the nature of the disorder being treated. Common dosage amounts for antibodies vary from 0.1 to 100,000 micrograms, up to a total dose of about 10 g, depending upon the route of administration.

(125) General guidance for its determination can be found, for example, in the publications of the International Conference on Harmonization and in REMINGTON'S PHARMACEUTICAL SCIENCES, chapters 27 and 28, pp. 484-528 (18th ed., Alfonso R. Gennaro, Ed., Easton, Pa.: Mack Pub. Co., 1990). More specifically, determining a therapeutically effective amount will depend on such factors as toxicity and efficacy of the medicament that may be determined using methods well known in the art and found in the foregoing references. In brief, therapeutic efficacy and toxicity of therapeutic agents may be determined in cell culture assays or in animal models, e.g., as ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population), respectively. The dose ratio between ED50 and LD50 is the therapeutic index.

(126) The antibody or fragment thereof according to the present invention is suitable for treatment and/or prophylaxis of cardiovascular, renal, pulmonary, skeletal, ocular, thromboembolic and fibrotic diseases and disorders, dwarfism, achondroplasia as well as other cGMP-related and/or natriuretic peptide responsive disorders. Thus, in particular embodiments, the antibody or fragment thereof is for use in the treatment and/or prophylaxis of any one of these disorders and diseases or any combination thereof.

(127) The antibody or fragment thereof according to the present invention can therefore be used in medicaments for treatment and/or prophylaxis of cardiovascular disorders, for example arterial and pulmonary hypertension, resistant and refractory hypertension, acute and chronic heart failure, coronary heart disease, Bronchiolitis obliterans Syndrome (BOS), tumor related and oncological diseases, graft versus host disease, sickle cell disease, stable and unstable angina pectoris, peripheral and cardiac vascular disorders, arrhythmias, atrial and ventricular arrhythmias and

impaired conduction, for example atrioventricular blocks degrees I-III (AB block I-III), supraventricular tachyarrhythmia, atrial fibrillation, atrial flutter, ventricular fibrillation, ventricular flutter, ventricular tachyarrhythmia, Torsade de pointes tachycardia, atrial and ventricular extrasystoles, AV-junctional extrasystoles, sick sinus syndrome, syncopes, AV-nodal re-entry tachycardia, Wolff-Parkinson-White syndrome, of acute coronary syndrome (ACS), autoimmune cardiac disorders (pericarditis, endocarditis, valvolitis, aortitis, cardiomyopathies), shock such as cardiogenic shock, septic shock and anaphylactic shock, aneurysms, boxer cardiomyopathy (premature ventricular contraction (PVC)), for treatment and/or prophylaxis of thromboembolic disorders and ischaemias such as myocardial ischaemia, myocardial infarction, stroke, cardiac hypertrophy, transient and ischaemic attacks, preeclampsia, inflammatory cardiovascular disorders, spasms of the coronary arteries and peripheral arteries, oedema formation, for example pulmonary oedema, cerebral oedema, renal oedema or oedema caused by heart failure, peripheral circulatory disturbances, reperfusion damage, arterial and venous thromboses, microalbuminuria, myocardial insufficiency, endothelial dysfunction, to prevent restenosis, for example after thrombolysis therapies, percutaneous transluminal angioplasties (PTA), transluminal coronary angioplasties (PTCA), heart transplants and bypass operations, and also micro- and macrovascular damage (vasculitis), increased levels of fibrinogen and of low-density lipoprotein (LDL) and increased concentrations of plasminogen activator inhibitor 1 (PAI-1), and also for treatment and/or prophylaxis of erectile dysfunction and female sexual dysfunction.

(128) In the context of the present invention, the term “heart failure” encompasses both acute and chronic forms of heart failure, and also more specific or related types of disease, such as acute decompensated heart failure, right heart failure, left heart failure, global failure, ischaemic cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, idiopathic cardiomyopathy, congenital heart defects, heart failure associated with heart valve defects, mitral valve stenosis, mitral valve insufficiency, aortic valve stenosis, aortic valve insufficiency, tricuspid valve stenosis, tricuspid valve insufficiency, pulmonary valve stenosis, pulmonary valve insufficiency, combined heart valve defects, myocardial inflammation (myocarditis), chronic myocarditis, acute myocarditis, viral myocarditis, diabetic heart failure, alcoholic cardiomyopathy, cardiac storage disorders, diastolic heart failure and systolic heart failure, heart failure with preserved ejection fraction (HFpEF), heart failure with reduced ejection fraction (HFrEF) and acute phases of worsening of existing chronic heart failure (worsening heart failure).

(129) In addition, the antibody or fragment thereof according to the present invention can also be used for treatment and/or prophylaxis of arteriosclerosis, peripheral artery disease (PAD), impaired lipid metabolism, hypolipoproteinaemias, dyslipidaemias, hypertriglyceridaemias, hyperlipidaemias, hypercholesterolaemias, abetalipoproteinaemia, sitosterolaemia, xanthomatosis, Tangier disease, adiposity, obesity and of combined hyperlipidaemias and metabolic syndrome.

(130) The antibody or fragment thereof according to the present invention can additionally be used for treatment and/or prophylaxis of primary and secondary Raynaud's phenomenon, of microcirculation impairments, claudication, peripheral and autonomic neuropathies, diabetic microangiopathies, diabetic retinopathy, diabetic ulcers on the extremities, gangrene, CREST syndrome, erythematosis, onychomycosis, rheumatic disorders and for promoting wound healing.

(131) The antibody or fragment thereof according to the present invention is also suitable for treating urological disorders, for example benign prostate syndrome (BPS), benign prostate hyperplasia (BPH), benign prostate enlargement (BPE), bladder outlet obstruction (BOO), lower urinary tract syndromes (LUTS, including Feline Urological Syndrome (FUS)), disorders of the urogenital system including neurogenic overactive bladder (OAB) and (IC), incontinence (UI), for example mixed urinary incontinence, urge urinary incontinence, stress urinary incontinence or overflow urinary incontinence (MUI, UUI, SUI, OUI), pelvic pain, benign and malignant disorders of the organs of the male and female urogenital system.

(132) The antibody or fragment thereof according to the present invention is also suitable for

treatment and/or prophylaxis of kidney disorders, in particular of acute and chronic renal insufficiency and acute and chronic renal failure. In the context of the present invention, the term "renal insufficiency" encompasses both acute and chronic manifestations of renal insufficiency, and also underlying or related renal disorders such as renal hypoperfusion, intradialytic hypotension, obstructive uropathy, glomerulopathies, glomerulonephritis, acute glomerulonephritis, glomerulosclerosis, tubulointerstitial diseases, nephropathic disorders such as primary and congenital kidney disease, nephritis, immunological kidney disorders such as kidney transplant rejection and immunocomplex-induced kidney disorders, nephropathy induced by toxic substances, nephropathy induced by contrast agents, diabetic and non-diabetic nephropathy, pyelonephritis, renal cysts, nephrosclerosis, hypertensive nephrosclerosis and nephrotic syndrome which can be characterized diagnostically, for example by abnormally reduced creatinine and/or water excretion, abnormally elevated blood concentrations of urea, nitrogen, potassium and/or creatinine, altered activity of renal enzymes, for example glutamyl synthetase, altered urine osmolarity or urine volume, elevated microalbuminuria, macroalbuminuria, lesions on glomerular and arterioles, tubular dilatation, hyperphosphatemia and/or need for dialysis. The present invention also encompasses the use of the antibody or fragment thereof according to the present invention for treatment and/or prophylaxis of sequelae of renal insufficiency, for example pulmonary oedema, heart failure, uraemia, anaemia, electrolyte disturbances (for example hyperkalaemia, hyponatraemia) and disturbances in bone and carbohydrate metabolism.

(133) In addition, the antibody or fragment thereof according to the present invention are also suitable for treatment and/or prophylaxis of asthmatic disorders, pulmonary arterial hypertension (PAH) and other forms of pulmonary hypertension (PH) including left-heart disease, HIV, sickle cell anaemia, thromboembolisms (CTEPH), sarcoidosis, COPD or pulmonary fibrosis-associated pulmonary hypertension, chronic-obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), acute lung injury (ALI), alpha-1-antitrypsin deficiency (AATD), pulmonary fibrosis, pulmonary emphysema (for example pulmonary emphysema induced by cigarette smoke). Bronchiolitis obliterans Syndrom (BOS), and cystic fibrosis (CF).

(134) The antibody or fragment thereof according to the present invention is also suitable for control of central nervous system disorders characterized by disturbances of the NO/cGMP system. It is suitable in particular for improving perception, concentration, learning or memory after cognitive impairments like those occurring in particular in association with situations/diseases/syndromes such as mild cognitive impairment, age-associated learning and memory impairments, age-associated memory losses, vascular dementia, craniocerebral trauma, stroke, dementia occurring after strokes (post stroke dementia), post-traumatic craniocerebral trauma, general concentration impairments, concentration impairments in children with learning and memory problems, Alzheimer's disease, Lewy body dementia, dementia with degeneration of the frontal lobes including Pick's syndrome, Parkinson's disease, progressive nuclear palsy, dementia with corticobasal degeneration, amyolateral sclerosis (ALS), Huntington's disease, demyelination, multiple sclerosis, thalamic degeneration, Creutzfeld-Jacob dementia, HIV dementia, schizophrenia with dementia or Korsakoff's psychosis. It is also suitable for treatment and/or prophylaxis of central nervous system disorders such as states of anxiety, tension and depression, CNS-related sexual dysfunctions and sleep disturbances, and for controlling pathological disturbances of the intake of food, stimulants and addictive substances.

(135) The antibody or fragment thereof according to the present invention is additionally also suitable for controlling cerebral blood flow and thus represent effective agents for controlling migraine. It is also suitable for the prophylaxis and control of sequelae of cerebral infarct (Apoplexia cerebri) such as stroke, cerebral ischaemias and skull-brain trauma. It can likewise be used for controlling states of pain and tinnitus.

(136) In addition, the antibody or fragment according to the present invention has anti-inflammatory action and can therefore be used as anti-inflammatory agents for treatment and/or

prophylaxis of sepsis (SIRS), multiple organ failure (MODS, MOF), inflammatory disorders of the kidney, chronic intestinal inflammations (IBD, Crohn's disease, UC), pancreatitis, peritonitis, rheumatoid disorders, inflammatory skin diseases and inflammatory eye diseases.

(137) Furthermore, the antibody or fragment thereof according to the present invention can also be used for treatment and/or prophylaxis of autoimmune diseases.

(138) The antibody or fragment thereof is also suitable for treatment and/or prophylaxis of fibrotic disorders of the internal organs, for example the lung, the heart, the kidney, the reproductive system, the bone marrow and in particular the liver, and also dermatological fibroses and fibrotic eye disorders. In the context of the present invention, the term fibrotic disorders includes in particular the following terms: hepatic fibrosis, cirrhosis of the liver, pulmonary fibrosis, endomyocardial fibrosis, nephropathy, glomerulonephritis, interstitial renal fibrosis, fibrotic damage resulting from diabetes, uterine fibroids, endometriosis, bone marrow fibrosis and similar fibrotic disorders, scleroderma, morphea, keloids, hypertrophic scarring (also following surgical procedures), naevi, diabetic retinopathy, proliferative vitroretinopathy and disorders of the connective tissue (for example sarcoidosis).

(139) The antibody or fragment thereof according to the present invention is also suitable for controlling postoperative scarring, for example as a result of glaucoma operations.

(140) The antibody or fragment thereof according to the present invention can likewise be used cosmetically for ageing and keratinized skin.

(141) Moreover, the antibody or fragment thereof according to the present invention is suitable for treatment and/or prophylaxis of hepatitis, neoplasms, osteoporosis, glaucoma and gastroparesis.

(142) The antibody or fragment thereof according to the present invention is moreover suitable for treatment and/or prophylaxis of eye disorders such as ophthalmic diseases responsive to natriuretic peptides, retina disorders, glaucoma including primary open angle glaucoma (POAG), angle closure glaucoma, and congenital/developmental glaucoma, retinopathies, ocular trauma, optic neuropathies, ocular hypertension, elevated intraocular pressure, diabetic retinopathy, macular degeneration (AMD), age-related eye diseases, macular oedema, scleritis, uveitis, dry eye, corneal epithelial abrasion, corneal ulcer.

(143) Moreover, the antibody or fragment thereof according to the present invention is suitable for the treatment of bone and cartilage disorders such as bone and cartilage diseases responsive to natriuretic peptides, arthritis, degenerative diseases of cartilage tissue, osteoarthritis, cartilage degeneration, bone fractures, skeletal dysplasias, achondroplasia, osteoporosis, osteogenesis imperfecta, Paget disease of bone (PDB), metabolic bone disease, age-related bone diseases, osteomyelitis, osteonecrosis, rickets, osteomalacia, growth plate injuries and diseases, joint and bone replacement associated defects, Marfan syndrome, sports injuries, muscular dystrophies, Duchenne muscular dystrophy.

(144) Thus, in another aspect, the present invention relates to the use of the antibody or fragment thereof according to the present invention for treatment and/or prophylaxis of disorders, in particular the disorders mentioned above.

(145) In particular embodiments, the antibody or fragment thereof according to the present invention is for use in a method for treatment and/or prophylaxis of heart failure, angina pectoris, hypertension, pulmonary hypertension, ischaemias, vascular disorders, renal insufficiency, thromboembolic disorders, fibrotic disorders, skeletal and bone disorders, ocular disorders and arteriosclerosis.

(146) In another aspect, the present invention relates to the use of antibody or fragment thereof according to the present invention for production of a medicament for treatment and/or prophylaxis of disorders, especially of the aforementioned disorders.

(147) In particular embodiments, the present invention relates to the use of the antibody or fragment thereof according to the present invention for production of a medicament for treatment and/or prophylaxis of heart failure, angina pectoris, hypertension, pulmonary hypertension,

ischaemias, vascular disorders, renal insufficiency, thromboembolic disorders, fibrotic disorders, dementia illness, arteriosclerosis, skeletal and bone disorders, ocular disorders, dwarfism, achondroplasia and erectile dysfunction.

(148) In another aspect, the present invention relates to a method for treatment and/or prophylaxis of disorders, in particular the disorders mentioned above, using an effective amount of at least one antibody or fragment thereof according to the present invention.

(149) In particular embodiments, the present invention relates to a method for treatment and/or prophylaxis of heart failure, angina pectoris, hypertension, pulmonary hypertension, ischaemias, vascular disorders, renal insufficiency, thromboembolic disorders, fibrotic disorders, tumor and oncological diseases, skeletal and bone disorders, ocular disorders, dwarfism, achondroplasia and arteriosclerosis using an effective amount of at least one antibody or fragment thereof according to the present invention.

(150) An antibody of the invention or fragment thereof according to the present invention may be administered as the sole pharmaceutical agent or in combination with one or more additional therapeutic agents, and in some instances the antibody might itself be modified. For example, an antibody or fragment thereof could be conjugated to a chemical entity e.g., to further increase efficacy, stability and/or half-life. Particularly, the antibody or fragment thereof according to the present invention may be PEGylated and/or HESylated.

(151) Thus, in particular embodiments, the antibody or fragment thereof according to the present invention is used in combination with at least one additional therapeutic agent in a method of treatment, in particular for the above cited purposes.

(152) The present invention further provides pharmaceutical combinations comprising at least one antibody or fragment thereof according to the present invention and at least one additional therapeutic agent.

(153) Within the context of the present invention, the term “pharmaceutical combination” is used as known to persons skilled in the art, it being possible for such combination to be a fixed combination, a non-fixed combination or a kit-of-parts.

(154) Within the context of the present invention, the term “fixed combination” is used as known to persons skilled in the art and is defined as a combination wherein, for example, a first active ingredient, such as one or more antibody or fragment thereof according to the present invention, and a further active ingredient are present together in one unit dosage or in one single entity, e.g., a single dosage formulation. One example of a “fixed combination” is a pharmaceutical composition wherein a first active ingredient and a further active ingredient are present in admixture for simultaneous administration, such as in a formulation. Another example of a “fixed combination” is a pharmaceutical combination wherein a first active ingredient and a further active ingredient are present in one unit without being in admixture. The present invention thus provides such pharmaceutical compositions comprising at least one antibody or fragment thereof and at least one additional therapeutic agent, in particular for use in treatment and/or prophylaxis of the aforementioned disorders.

(155) Within the context of the present invention, the terms “non-fixed combination” and “kit-of-parts” are used as known to persons skilled in the art and are defined as a combination wherein a first active ingredient and a further active ingredient are present in more than one unit, e.g., in separate dosage formulations. One example of a non-fixed combination or kit-of-parts is a combination wherein the first active ingredient and the further active ingredient are present separately.

(156) It is possible for the components of the non-fixed combination or kit-of-parts to be administered separately, sequentially, simultaneously, concurrently or chronologically staggered.

(157) The antibody or fragment thereof according to the present invention may be administered simultaneously with, prior to or after said further therapeutically active agent. In the context of the present invention, the term “simultaneously with” means administration of the antibody or

fragment thereof according to the present invention and the at least one further therapeutically active agent on the same day, more particularly within 12 hours, more particularly within 2 hours.
(158) In particular embodiments, administration of the antibody or fragment thereof according to the present invention and the at least one further therapeutically active agent occurs within eight consecutive weeks, more particularly within one to six consecutive weeks. The antibody or fragment thereof according to the present invention and the at least one further therapeutically active agent may be administered via the same route or via different routes.
(159) The antibody or fragment thereof according to the present invention may for instance be combined with known agents of the same indication treatment group, such as agents used for the treatment and/or prophylaxis of diseases and/or conditions associated with hypertension, heart failure, pulmonary hypertension, COPD, asthma, cystic fibrosis, achondroplasia, hyperphosphatemia, chronic kidney disease (CKD), soft tissue calcification, chronic kidney disease associated calcification, non-chronic kidney disease associated calcification, media calcifications including Moenckeberg's medial sclerosis, atherosclerosis, intima calcification, CKD associated heart hypertrophy, CKD associated renal dystrophy, osteoporosis, post-menopausal osteoporosis, diabetes mellitus II, chronic renal disease, aging, hypophosphaturia, hyperparathyroidism, Vitamin D disorders, Vitamin K deficiency, Vitamin K-antagonist coagulants, Kawasaki disease, ACDC (arterial calcification due to deficiency of CD73), GACI (generalized arterial calcification of infancy), IBGC (idiopathic basal ganglia calcification), PXE (pseudoxanthoma elasticum), rheumatoid arthritis, Singleton-Merten syndrome, P-thalassemia, calciphylaxis, heterotrophic ossification, preterm placental calcification, calcification of the uterus, calcified uterine fibroids, morbus fahr, mircocalcification and calcification of the aortic valve.
(160) Preferred examples of suitable further therapeutic agents to be combined with the antibody or fragment thereof according to the present invention: organic nitrates and NO donors, for example sodium nitroprusside, nitroglycerin, isosorbide mononitrate, isosorbide dinitrate, molsidomine or SIN-1, and inhaled NO; compounds which inhibit the breakdown of cyclic guanosine monophosphate (cGMP), for example inhibitors of phosphodiesterases (PDE) 1, 2 and/or 5, especially PDE 5 inhibitors such as sildenafil, vardenafil, tadalafil, udenafil, desantafil, avanafil, mirodenafil, lodenafil or PF-00489791; antithrombotic agents, by way of example and with preference from the group of the platelet aggregation inhibitors, the anticoagulants or the profibrinolytic substances; hypotensive active ingredients, by way of example and with preference from the group of the calcium antagonists, angiotensin All antagonists, ACE inhibitors, NEP-inhibitors, vasopeptidase-inhibitors, endothelin antagonists, renin inhibitors, alpha-receptor blockers, beta-receptor blockers, mineralocorticoid receptor antagonists, rho-kinase-inhibitors and the diuretics; antiarrhythmic agents, by way of example and with preference from the group of sodium channel blocker, beta-receptor blocker, potassium channel blocker, calcium antagonists, If-channel blocker, digitalis, parasympatholytics (vagoliytics), sympathomimetics and other antiarrhythmics as adenosin, adenosine receptor agonists as well as vernakalant; positive-inotropic agents, by way of example cardiac glycoside (Dogoxin), beta-adrenergic and dopaminergic agonists, such as isoprenalin, adrenalin, noradrenalin, dopamin or dobutamin; vasopressin-rezeptor-antagonists, by way of example and with preference from the group of conivaptan, tolvaptan, lixivaptan, mozavaptan, satavaptan, SR-121463, RWJ 676070 or BAY 86-8050, as well as the compounds described in WO 2010/105770, WO2011/104322 and WO 2016/071212; active ingredients which alter lipid metabolism, for example and with preference from the group of the thyroid receptor agonists, PCSK9 inhibitors, cholesterol synthesis inhibitors such as, by way of example and preferably, HMG-CoA reductase inhibitors or squalene synthesis inhibitors, of ACAT inhibitors, CETP inhibitors, MTP inhibitors, PPAR-alpha, PPAR-gamma and/or PPAR-delta agonists, cholesterol absorption inhibitors, lipase inhibitors, polymeric bile acid adsorbents, bile acid reabsorption inhibitors and lipoprotein(a) antagonists. anti-inflammatory agents, for example and with preference from the group of the gluco-corticoids, such as, by way of example and

preferably, prednison, prednisolon, methylprednisolon, triamcinolon, dexamethason, beclomethason, betamethason, flunisolid, budesonid or fluticason as well as the non-steroidal anti-inflammatory agents (NSAIDs), by way of example and preferably, acetyl salicylic acid (aspirin), ibuprofen and naproxen, 5-amino salicylic acid-derivates, leukotriene-antagonists, TNF-alpha-inhibitors and chemokine-receptor antagonists, such as CCR1, 2 and/or 5 inhibitors; agents that inhibit the signal transductions cascade, for example and with preference from the group of the kinase inhibitors, by way of example and preferably, from the group of the tyrosine kinase and/or serine/threonine kinase inhibitors; agents, that inhibit the degradation and modification of the extracellular matrix, for example and with preference from the group of the inhibitors of the matrix-metalloproteases (MMPs), by way of example and preferably, inhibitors of chymasee, stromelysine, collagenases, gelatinases and aggrecanases (with preference from the group of MMP-1, MMP-3, MMP-8, MMP-9, MMP-10, MMP-11 and MMP-13) as well as of the metallo-elastase (MMP-12) and neutrophil-elastase (HNE), as for example sivelestat or DX-890; agents, that block the binding of serotonin to its receptor, for example and with preference antagonists of the 5-HT2b-receptor; anti-fibrotic agents, for example and with preference, nintedanib, pirfenidone, adenosine A2b receptor antagonists, sphingosine-1-phosphate receptor 3 (S1P3) antagonists, autotaxin-inhibitors, lysophosphatidic acid receptor 1 (LPA-1) and lysophosphatidic acid receptor 2 (LPA-2) antagonists, lysyloxidase (LOX) inhibitors, lysyloxidase-like-2 inhibitors, CTGF inhibitors, IL-13 antagonists, integrin antagonists, TGF-beta antagonists, inhibitors of wnt signaling, CCR2-antagonists; agents, that act as bronchodilators, for example and with preference antagonists of the 5-HT2b-receptor; β2(“beta two”)-adrenergic agonists (short- and long-acting), anticholinergics, and theophylline; agents that are antagonists of cytokines and chemokines, for example and with preference antagonists of TGF-beta, CTGF, IL-1, IL-4, IL-5, IL-6, IL-8, IL-13, IL-25, IL-33, TSLP and integrins; organic nitrates and NO-donators, for example and with preference sodium nitroprussid, nitro-glycerine, isosorbid mononitrate, isosorbid dinitrate, molsidomine or SIN-1, as well as inhaled NO; NO-independent, but heme-dependent stimulators of the soluble guanylate cyclase, for example and with preference the compounds described in WO 00/06568, WO 00/06569, WO 02/42301, WO 03/095451, WO 2011/147809, WO 2012/004258, WO 2012/028647 and WO 2012/059549; NO-independent and heme-independent activators of the soluble guanylate cyclase, for example and with preference the compounds described in WO 01/19355, WO 01/19776, WO 01/19778, WO 01/19780, WO 02/070462 and WO 02/070510; agents, that stimulate the synthesis of cGMP, for example sGC modulators, for example and with preference riociguat, cinaciguat, vericiguat; prostacyclin-analogs or IP receptor agonists, for example and with preference iloprost, beraprost, treprostinil, epoprostenol or Selexipag; endothelin receptor antagonists, for example and with preference Bosentan, Darusentan, Ambrisentan oder Sitaxsentan; agents, that inhibit soulble epoxidhydrolase (sEH), for example and with preference N,N′-Di-cyclohexyl urea, 12-(3-Adamantan-1-yl-ureido)-dodecanic acid or 1-Adamantan-1-yl-3-{5-[2-(2-ethoxyethoxy)ethoxy]pentyl}-urea; agents that interact with glucose metabolism, for example and with preference insuline, biguanide, thiazolidinedione, sulfonyl urea, acarbose, DPP4 inhibitors, GLP-1 analogs or SGLT-1 inhibitors; natriuretic peptides, for example and with preference Atrial Natriuretic Peptide (ANP, Carperitide), Brain Natriuretic Peptide (BNP, Nesiritide), C-Type Natriuretic Peptide (CNP) or urodilatin; natriuretic peptide derivatives, for example and with preference vosoritide, cenderitide, PL 3994 activators of the cardiac myosin, for example and with preference omecamtiv mecarbil (CK-1827452); calcium-sensitizers, for example and with preference levosimendan; agents that affect the energy metabolism of the heart, for example and with preference etomoxir, dichloroacetat, ranolazine or trimetazidine, full or partial adenosine A1 receptor agonists such as GS-9667 (formerly known as CVT-3619), capadenoson and neladenoson; agents that affect the heart rate, for example and with preference ivabradin.

(161) Antithrombotic agents are preferably understood to mean compounds from the group of the platelet aggregation inhibitors, the anticoagulants or the profibrinolytic substances.

(162) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a platelet aggregation inhibitor, by way of example and with preference aspirin, clopidogrel, prasugrel, ticagrelor, ticlopidin or dipyridamole.
(163) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a thrombin inhibitor, by way of example and with preference ximelagatran, dabigatran, melagatran, bivalirudin or clexane.
(164) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a GPIIb/IIIa antagonist such as, by way of example and with preference, tirofiban or abciximab.
(165) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a factor Xa inhibitor, by way of example and with preference rivaroxaban, DU-176b, apixaban, betrixaban, otamixaban, fidexaban, razaxaban, letaxaban, eribaxaban, fondaparinux, idraparinux, PMD-3112, darexab an (YM-150), KFA-1982, EMD-503982, MCM-17, MLN-1021, DX 9065a, DPC 906, JTV 803, SSR-126512 or SSR-128428.
(166) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with heparin or with a low molecular weight (LMW) heparin derivative.
(167) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a vitamin K antagonist, by way of example and with preference coumarin.
(168) Hypotensive agents are preferably understood to mean compounds from the group of the calcium antagonists, angiotensin All antagonists, ACE inhibitors, endothelin antagonists, renin inhibitors, alpha-receptor blockers, beta-receptor blockers, mineralocorticoid receptor antagonists, rho-kinase inhibitors and the diuretics.
(169) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a calcium antagonist, by way of example and with preference nifedipine, amlodipine, verapamil or diltiazem.
(170) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an alpha-1-receptor blocker, by way of example and with preference prazosin.
(171) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a beta-receptor blocker, by way of example and with preference propranolol, atenolol, timolol, pindolol, alprenolol, oxprenolol, penbutolol, bupranolol, metipranolol, nadolol, mepindolol, carazalol, sotalol, metoprolol, betaxolol, celiprolol, bisoprolol, carteolol, esmolol, labetalol, carvedilol, adaprolol, landiolol, nebivolol, epanolol or bucindolol.
(172) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an angiotensin All antagonist, by way of example and with preference losartan, candesartan, valsartan, telmisartan or embusartan or a dual angiotensin All antagonist/neprilysin-inhibitor, by way of example and with preference LCZ696 (valsartan/sacubitril).
(173) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an ACE inhibitor, by way of example and with preference enalapril, captopril, lisinopril, ramipril, delapril, fosinopril, quinopril, perindopril or trandopril.
(174) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an endothelin antagonist, by way of example and with preference bosentan, darusentan, ambrisentan or sitaxsentan.
(175) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a renin inhibitor, by way of example and with

preference aliskiren, SPP-600 or SPP-800.
(176) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a mineralocorticoid receptor antagonist, by way of example and with preference finerenone, spironolactone or eplerenone.
(177) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a loop diuretic, for example furosemide, torasemide, bumetanide and piretanide, with potassium-sparing diuretics, for example amiloride and triamterene, with aldosterone antagonists, for example spironolactone, potassium canrenoate and eplerenone, and also thiazide diuretics, for example hydrochlorothiazide, chlorthalidone, xipamide and indapamide.
(178) Lipid metabolism modifiers are preferably understood to mean compounds from the group of the CETP inhibitors, thyroid receptor agonists, cholesterol synthesis inhibitors such as HMG-CoA reductase inhibitors or squalene synthesis inhibitors, the ACAT inhibitors, MTP inhibitors, PPAR-alpha, PPAR-gamma and/or PPAR-delta agonists, cholesterol absorption inhibitors, polymeric bile acid adsorbents, bile acid reabsorption inhibitors, lipase inhibitors and the lipoprotein(a) antagonists.
(179) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a CETP inhibitor, by way of example and with preference dalcetrapib, anacetrapib, torcetrapib (CP-529 414), JJT-705 or CETP vaccine (Avant).
(180) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a thyroid receptor agonist, by way of example and with preference D-thyroxine, 3,5,3′-triiodothyronine (T3), CGS 23425 or axitirome (CGS 26214).
(181) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an HMG-CoA reductase inhibitor from the class of statins, by way of example and with preference lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin or pitavastatin.
(182) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a squalene synthesis inhibitor, by way of example and with preference BMS-188494 or TAK-475.
(183) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an ACAT inhibitor, by way of example and with preference avasimibe, melinamide, pactimibe, eflucimibe or SMP-797.
(184) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an MTP inhibitor, by way of example and with preference implitapide, BMS-201038, R-103757 or JTT-130.
(185) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a PPAR-gamma agonist, by way of example and with preference pioglitazone or rosiglitazone.
(186) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a PPAR-delta agonist, by way of example and with preference GW 501516 or BAY 68-5042.
(187) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a cholesterol absorption inhibitor, by way of example and with preference ezetimibe, tiqueside or pamaqueside.
(188) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a lipase inhibitor, a preferred example being orlistat.
(189) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a polymeric bile acid adsorbent, by way of example and with preference cholestyramine, colestipol, colesolvam, CholestaGel or colestimide.
(190) In particular embodiments, the antibody or fragment thereof according to the present

invention is administered in combination with a bile acid reabsorption inhibitor, by way of example and with preference ASBT (=IBAT) inhibitors, for example AZD-7806, S-8921, AK-105, BARI-1741, SC-435 or SC-635.
(191) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a lipoprotein(a) antagonist, by way of example and with preference, gemcabene calcium (CI-1027) or nicotinic acid.
(192) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a lipoprotein(a) antagonist, by way of example and with preference, gemcabene calcium (CI-1027) or nicotinic acid.
(193) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with sGC modulators, by way of example and with preference, riociguat, cinaciguat or vericiguat.
(194) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an agent affecting the glucose metabolism, by way of example and with preference, insulin, a sulfonyl urea, acarbose, DPP4 inhibitors, GLP-1 analogs or SGLT-1 inhibitors.
(195) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a TGFbeta antagonist, by way of example and with preference pirfenidone, nintedanib or fresolimumab.
(196) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a CCR2 antagonist, by way of example and with preference CCX-140.
(197) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a TNFalpha antagonist, by way of example and with preference adalimumab.
(198) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a galectin-3 inhibitor, by way of example and with preference GCS-100.
(199) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a Nrf-2 inhibitor, by way of example and with preference bardoxolone.
(200) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a BMP-7 agonist, by way of example and with preference THR-184.
(201) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a NOX1/4 inhibitor, by way of example and with preference GKT-137831.
(202) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a medicament which affects the vitamin D metabolism, by way of example and with preference calcitriol, alfacalcidol, doxercalciferol, maxacalcitol, paricalcitol, cholecalciferol or paracalcitol.
(203) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a cytostatic agent, by way of example and with preference cyclophosphamide.
(204) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an immunosuppressive agent, by way of example and with preference ciclosporin.
(205) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a phosphate binder, by way of example and with preference colestilan, sevelamer hydrochloride and sevelamer carbonate, Lanthanum and

lanthanum carbonate.
(206) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with renal proximal tubule sodium-phosphate co-transporter, by way of example and with preference, niacin or nicotinamide.
(207) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a calcimimetic for therapy of hyperparathyroidism.
(208) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with agents for iron deficit therapy, by way of example and with preference iron products.
(209) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with agents for the therapy of hyperurikaemia, by way of example and with preference allopurinol or rasburicase.
(210) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with glycoprotein hormone for the therapy of anaemia, by way of example and with preference erythropoietin.
(211) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with biologics for immune therapy, by way of example and with preference abatacept, rituximab, eculizumab or belimumab.
(212) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with vasopressin antagonists (group of the vaptanes) for the treatment of heart failure, by way of example and with preference tolvaptan, conivaptan, lixivaptan, mozavaptan, satavaptan or relcovaptan.
(213) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with Jak inhibitors, by way of example and with preference ruxolitinib, tofacitinib, baricitinib, CYT387, GSK2586184, lestaurtinib, pacritinib (SB1518) or TG101348.
(214) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with prostacyclin analogs for therapy of microthrombi.
(215) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an alkali therapy, by way of example and with preference sodium bicarbonate.
(216) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an mTOR inhibitor, by way of example and with preference everolimus or rapamycin.
(217) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an NHE3 inhibitor, by way of example and with preference AZD1722 or tenapanor.
(218) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with an eNOS modulator, by way of example and with preference sapropterin.
(219) In particular embodiments, the antibody or fragment thereof according to the present invention is administered in combination with a CTGF inhibitor, by way of example and with preference FG-3019.
(220) The antibody or fragment thereof for use in a method for treatment according to the present invention may be formulated in any conventional manner using one or more physiologically acceptable carriers or excipients. The antibody or fragment thereof according to the present invention may be administered by any suitable means, which can vary, depending on the type of disorder to be treated. Possible administration routes include enteral (e.g., oral), parenteral (e.g., intravenous, intra-arterial, intraperitoneal, intramuscular, subcutaneous, intracardiac, intraventricular, intrathecal, intramedullary, intralesional), intrapulmonary and intranasal

administration. In addition, an antibody or fragment thereof according to the present invention may be administered by pulse infusion, with, e.g., declining doses of the antibody or fragment thereof. Preferably, the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the condition is acute or chronic. The amount to be administered will depend on a variety of factors such as the clinical symptoms, sex, age, and/or weight of the individual, whether other drugs are administered, and others. The skilled artisan will recognize that the route of administration will vary depending on the disorder or condition to be treated.

(221) Methods of parenteral delivery include topical, intra-arterial, intratumoral, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration.

(222) In particular embodiments, the method for treatment comprises a single or multiple administrations of the antibody or fragment thereof or the pharmaceutical composition comprising the same. The single dose of the administrations may be the same or different. In particular, the method for treatment comprises 1, 2, 3, 4, 5 or 6 administrations of the antibody or fragment thereof according to the present invention, preferably wherein the multiple administrations occur within one to six consecutive months. The antibody or fragment thereof according to the present invention may for instance be administered every 3 to 4 days, every week, once every two weeks, or once every three weeks, depending on its half-life and clearance rate.

Description

SHORT DESCRIPTION OF FIGURES

(1) FIG. **1**: Mean plasma concentrations of TPP-10992 and TPP-5661 after intravenous administration of 5 mg/kg in rat.

(2) FIG. **2**: Mean plasma concentrations of TPP-12897 after intraperitoneal administration of 5 mg/kg in mice.

(3) FIG. **3**: Stability of ANP (A-C), TPP-10992 (D-F) and TPP-5661 (G-I) against proteolytic degradation. ANP, TPP-10992 and TPP-5661 activity were tested on the stable rat ANP receptor cell line directly (A, D, G), or after 4 h incubation at 37° C. with 0.6 μg/ml NEP (B, E, H) or 0.6 μg/ml IDE (C, F, I).

(4) FIG. **4**: Stability of BNP (A-C) and TPP-11155 (D-F) against proteolytic degradation. BNP and TPP-11155 activity were tested on the stable rat BNP receptor cell line directly (A, D), or after 4 h incubation at 37° C. with 0.6 μg/ml NEP (B, E) or 0.6 μg/ml IDE (C, F).

(5) FIG. **5**: Stability of CNP (A-C) and TPP-12897 (D-F) against proteolytic degradation. CNP and TPP-11155 activity were tested on the stable rat CNP receptor cell line directly (A, D), or after 4 h incubation at 37° C. with 0.6 μg/ml NEP (B, E) or 0.6 μg/ml IDE (C, F).

(6) FIG. **6**: ANP Peptide and TPP-10992 induced vasodilation dose-response curves in PE-contracted aortic rings. Concentration-response curves (0.0001-10 μM; n=3 Rats) to the ANP peptide (open circles) and TPP-10992 (closed circles) in endothelium-intact rat aortic rings contracted by phenylephrine (1 μM). Experimental values were calculated relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%. Potency of ANP peptide and TPP-10992 were −7.4 and −6.7 respectively (log EC.sub.50 values). Data represent the mean±S.E.M. of 2 experiments.

(7) FIG. **7**: ANP Peptide and TPP-5661 induced vasodilation dose-response curves in PE-contracted aortic rings. Concentration-response curves (0.0001-10 μM; n=3 Rats) to the ANP peptide (open circles) and TPP-5661 (closed circles) in endothelium-intact rat aortic rings contracted by phenylephrine (1 μM). Experimental values were calculated relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%.

Potency of ANP peptide and TPP-5661 were −7.4 and −6.5 respectively (log EC.sub.50 values). Data represent the mean±S.E.M. of 2 experiments.
(8) FIG. **8**: Hemodynamic effect of ANP in conscious rats. Rat ANP was given intraperitoneally at 0 hours. A 500 μg dose of ANP resulted in an approximately 25% drop in mean arterial blood pressure (MAP) with a duration of effect around 6-8 hours.
(9) FIG. **9**: Hemodynamic effect TPP-5661 in conscious rats. TPP-5661 was given intraperitoneally at 0 hours. A 15 mg/kg dose resulted in an approximately 20% reduction in mean arterial blood pressure (MAP) with maximum effect at 24-48 hours post application and a duration of effect greater than 6 days.
(10) FIG. **10**: Hemodynamic effect TPP-10992 in conscious rats. TPP-10992 was given intraperitoneally at 0 hours. A 30 mg/kg dose resulted in an approximately 20% reduction in mean arterial blood pressure (MAP) with maximum effect at 48 hours post application and a duration of effect greater than 6 days.
(11) FIG. **11**: Activity of BNP engrafted antibody constructs on hNPRA cells. The activity of purified compound samples on stable hNPRA-CHO k1 cells was assessed by comparison to reference sample TPP-5661 and TPP-5657. Samples were tested in dilution series in quadruplets.
(12) FIG. **12**: Different human IgG isotypes provide equally suitable antibody scaffolds. Exemplary activity determination of compounds 9, 33, 65, 91, 127 and 191 IgG1 (TPP-10294, TPP-10277, TPP-10279, TPP-10282, TPP-10269 and TPP-10355, respectively), IgG2 and IgG4 isotypes. The activity of purified compound samples on stable hNPRA-CHO k1 cells was assessed by comparison to reference samples compound 117 human IgG1 TPP-5661 and compound 209 human IgG1 TPP-5657. Samples were tested in dilution series in quadruplets.
(13) FIG. **13**: Equally suitable IgG antibody scaffolds originated from different species. Exemplary activity determination of compound 117 human IgG1 (TPP-5661) and compound 9 human IgG1 (TPP-10294) and their non-human IgG1 counterparts. The activity of purified compound samples on stable hNPRA-CHO k1 cells was assessed by comparison to reference sample compound 209 human IgG1 (TPP-5657). Samples were tested in dilution series in quadruplets.
(14) FIG. **14**: Equally suitable human IgG antibody scaffolds originated from different germline sequences. The activity of purified compound samples on stable hNPRA-CHO k1 cells was assessed by comparison to reference sample TPP-10992. Samples were tested in dilution series in quadruplets.
(15) FIG. **15**: Protective effects of TPP-12899 against LPS, IL-113 and thrombin induced endothelial barrier permeability as assessed by real-time impedance measurement.
(16) FIG. **16**: Therapeutic effects of TPP-13992 on survival (A), body weight gain (B), urinary protein/creatinine ratio (C) and left atrial weight (D); (n=8-12 (healthy control n=5), mean±SEM, One-Way ANOVA vs TPP-10155 (isotype specific control antibody).
(17) FIG. **17**: Hemodynamic assessment after Placebo, 0.1, 0.3 and 1.0 mg/kg of TPP-10992. TPP-10992 shows a dose-dependent and long-lasting (>5d) reduction in blood pressure.**p<0.01, ****p<0.0001 in comparison to placebo group using an One-way ANOVA test for repeated measurements followed by Tukey's multiple comparison test.
EXAMPLES
Example 1: Construction of Candidate TPP-5661
(18) Candidate TPP-5661 was designed by fusion of a heterologous amino acid sequence comprising a Ntls, wild type rat ANP and a Ctls to the C-terminus of HV 3-23 (SEQ ID NO 85) by substituting the two C-terminal residues of HV 3-23 by the two N-terminal residues of the heterologous amino acid sequence and to the N-terminus of IGHJ1 (SEQ ID NO 86) by substituting the nine N-terminal residues of IGHJ1 by the 9 C-terminal residues of the heterologous amino acid sequence. The corresponding full length heavy chain sequence of SEQ ID NO 67 further comprises amino acid sequence Constant-H (SEQ ID NO 87).
(19) Pairing of the full length heavy chain sequence of SEQ ID NO 67 harboring the inserted rat

ANP (rANP) with the full length light chain sequence of SEQ ID NO 66 built by combining sequences LV 1-40 (SEQ ID NO 88), IGLJ2 (SEQ ID NO 89) and Constant-L (SEQ ID NO 90) yields the full IgG candidate TPP-5661 (see Table 1).

(20) Shown below is the full length heavy chain sequence (SEQ ID NO 67); the incorporated heterologous amino acid sequence (Ntls-rANP-Ctls) is underlined; sequences derived from HV 3-23 and IGHJ1 are shown in bold:

(21) TABLE-US-00003

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVS
AISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC*TS*
VHQETKKYQSSPDGGSGGSLRRSSCFGGRIDRIGAQSGLGCNSFRYGSY
*SYTYNYEWHVDVWGQ***GTLVTVSS**ASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPG

(22) The designed and synthesized antibody construct was cloned according to well-known methods in the art and confirmed by DNA sequencing using plasmid specific oligonucleotides.

Example 2: Insertion of NPs within Antibody-CDRs Results in an Increased Serum Half-Life

(23) Determination of In Vivo Pharmacokinetic Parameters

(24) Pharmacokinetic parameters of TPP-10992 (SEQ ID NO 76 and SEQ ID NO 66) and TPP-5661 (SEQ ID NO 67 and SEQ ID NO 66) were determined after intravenous administration of 5 mg/kg to male Wistar rats (n=3). TPP-10992 and TPP-5661 were given as a bolus injection via the tail vein. Blood samples were collected from the jugular vein via previously implanted catheters in time intervals up to 14 days (336 hours). Generated EDTA-plasma was stored at −20° C. until further analysis.

(25) The quantification of TPP-10992 and TPP-5661 in plasma samples was performed employing an anti-human IgG ELISA (enzyme-linked immunosorbent assay) format. Pharmacokinetic parameters were calculated from plasma concentration time profiles using non-compartmental data analysis.

(26) Mean plasma concentrations of TPP-10992 and TPP-5661 after intravenous administration over time are graphically depicted in FIG. **1**.

(27) Mean clearance and terminal half-life of TPP-10992 and TPP-5661 are summarized in Table 2 below.

(28) TABLE-US-00004 TABLE 2 Mean clearance (CL) and terminal half-life (t.sub.1/2) of TPP-10992 and TPP-5661 after intravenous administration of 5 mg/kg in rat. TPP- Analyte TPP-5661 10992 CL [mL/h/kg] 0.62 0.27 t.sub.1/2 [h] 297 184

Determination of In Vivo Pharmacokinetic Parameters

(29) Pharmacokinetic parameters of TPP-12897 were determined after intraperitoneal administration to female Balb/c mice (n=3). Blood samples were collected from 15 minutes up to 72 hours post application. Generated EDTA-plasma was stored at −20° C. until further analysis. The quantification of TPP-12897 in plasma samples was performed by an anti-human IgG (Immunoglobulin G) ELISA format.

(30) Pharmacokinetic parameters were calculated from plasma concentration time profiles using non-compartmental data analysis.

(31) Mean plasma concentrations of TPP-12897 after intraperitoneal administration over time are graphically depicted in FIG. **2**.

(32) Mean area under the curve (AUC) and terminal half-life of TPP-12897 are summarized in Table 3 below.

(33) TABLE-US-00005 TABLE 3 Mean area under the curve (AUC) and terminal half-life (t.sub.1/2) of TPP- 12897 after intraperitoneal administration of 5 mg/kg in mice. Analyte TPP-12897 AUC [mg .Math. h/L] 7705 t.sub.1/2 [h] 194

Example 3: In Vitro, Ex Vivo and In Vivo Potency of NP Engrafted Antibodies

(34) Activity Data of ANP Engrafted Antibodies in NPR-A Receptor Cell Line

(35) A luminescence-based rat ANP receptor (NPR-A) cell line was generated as described previously (Wunder et al. (2013), *Eur J Pharmacol.* 698: 131). Accordingly, a fluorescence-based rat ANP receptor (NPR-A) cell line was generated by co-transfecting a CHO cell line, stably expressing the fluorescent calcium sensor protein GCaMP6, with plasmid constructs encoding CNGA2 (cGMP biosensor) and rat NPR-A.

(36) ANP receptor GCaMP6 cells were cultured for one day on black, clear-bottom 384-well microtiter plates (2500 cells/well). After removal of the cell culture medium reporter cells were loaded for 20 min with Tyrode (130 mM NaCl, 5 mM KCl, 2 mM CaCl.sub.2, 20 mM HEPES, 1 mM MgCl.sub.2, 4.8 mM NaHCO.sub.3 at pH 7.4) containing a black masking dye at 37° C. and 5% CO.sub.2. IBMX (0.2 mM) was used to prevent cGMP degradation by endogenous phosphodiesterases.

(37) Fluorescence measurements (3 min, kinetic mode) were directly started upon agonist addition. Receptor ligands were added in Tyrode containing a black masking dye and 0.1% BSA. Measurements were done on a FLIPR Tetra®.

(38) ANP (Bachem, H-2100) stimulated concentration-dependent fluorescence signals on the NPR-A cell line with an EC.sub.50 values of 0.22 nM. TPP-5661 and TPP-10992 stimulated the rat ANP receptor reporter cell line with EC.sub.50 values of 17 nM and 180 nM, respectively. The control antibody construct TPP-5657 did not significantly stimulate the NPR-A cell line (tested up to the max. concentration of 460 nM).

(39) To determine the sensitivity towards proteolytic degradation, the activity of receptor ligands was also characterized after 4 hours incubation with 0.6 μg/ml neutral endopeptidase (NEP, R&D Systems, 1182-ZNC) or 0.6 μg/ml insulin degrading enzyme (IDE, Merck, 407241-50UG) at 37° C.

(40) FIG. **3** graphically depicts the stability of ANP (A-C), TPP-10992 (D-F) and TPP-5661 (G-I) against proteolytic degradation. As shown in FIG. **3**, the natriuretic peptide ANP (Bachem, H-2100) showed high sensitivity towards degradation by NEP and IDE. In contrast, TPP-5661 and TPP-10992 showed high resistance to proteolytic degradation by NEP and IDE.

(41) Activity Data of BNP Engrafted Antibodies in NPR-A Receptor Cell Line

(42) A luminescence-based rat BNP receptor (NPR-A) cell line was generated as described previously (Wunder et al. (2013), *Eur J Pharmacol.* 698: 131). Accordingly, a fluorescence-based rat BNP receptor (NPR-A) cell line was generated by co-transfecting a CHO cell line, stably expressing the fluorescent calcium sensor protein GCaMP6, with plasmid constructs encoding CNGA2 (cGMP biosensor) and rat NPR-A.

(43) BNP receptor GCaMP6 cells were cultured for one day on black, clear-bottom 384-well microtiter plates (2500 cells/well). After removal of the cell culture medium reporter cells were loaded for 20 min with Tyrode (130 mM NaCl, 5 mM KCl, 2 mM CaCl.sub.2, 20 mM HEPES, 1 mM MgCl.sub.2, 4.8 mM NaHCO.sub.3 at pH 7.4) containing a black masking dye at 37° C. and 5% CO.sub.2. IBMX (0.2 mM) was used to prevent cGMP degradation by endogenous phosphodiesterases.

(44) Fluorescence measurements (3 min, kinetic mode) were directly started upon agonist addition. Receptor ligands were added in Tyrode containing a black masking dye and 0.1% BSA. Measurements were done on a FLIPR Tetra®.

(45) BNP (Bachem, H-5968) stimulated concentration-dependent fluorescence signals on the NPR-A cell line with an EC.sub.50 value of 2.9 nM. TPP-9902, TPP-11153, TPP-1154, TPP-11155, TPP-11156 and TPP-11157 stimulated the rat BNP receptor reporter cell line with EC.sub.50 values of

2.3 μM, >1.9 μM, 7 nM, 12 nM, 1.2 μM and 11 nM, respectively. The control antibody construct TPP-5657 did not significantly stimulate the NPR-A cell line (tested up to the max. concentration of 460 nM).
(46) To determine the sensitivity towards proteolytic degradation, the activity of receptor ligands was also characterized after 4 hours incubation with 0.6 μg/ml neutral endopeptidase (NEP, R&D Systems, 1182-ZNC) or 0.6 μg/ml insulin degrading enzyme (IDE, Merck, 407241-50UG) at 37° C. The natriuretic peptide BNP (Bachem, H-5968) showed high sensitivity towards degradation by NEP and IDE. In contrast, TPP-11155 and TPP-11157 showed high resistance to proteolytic degradation by NEP and IDE.
(47) FIG. **4** graphically depicts the stability of BNP (A-C) and TPP-11155 (D-F) against proteolytic degradation.
(48) Activity Data of CNP Engrafted Antibodies in NPR-B Receptor Cell Line
(49) A luminescence-based rat CNP receptor (NPR-B) reporter cell line was generated and luminescence measurements were performed as described previously (Wunder et al. (2013), Eur J Pharmacol. 698: 131).
(50) CNP receptor cells (2500 cells/well) were cultured for 1 day on opaque 384-well microtiter plates. After removal of the cell culture medium, cells were loaded for 3 h with 2.5 μg/ml coelenterazine in Ca.sup.2+-free Tyrode (130 mM NaCl, 5 mM KCl, 20 mM HEPES, 1 mM MgCl.sub.2, 4.8 mM NaHCO.sub.3 at pH 7.4) at 37° C. and 5% CO.sub.2. Receptor ligands were added for 10 min in Ca.sup.2+-free Tyrode containing 0.1% BSA. IBMX (0.2 mM) was used to prevent cGMP degradation by endogenous phosphodiesterases Immediately before adding calcium ions (final concentration 3 mM), luminescence measurements were started by using a charge-coupled device (CCD) camera in a light tight box. Luminescence was monitored continuously for 50 s.
(51) CNP (Bachem, H-1296) stimulated concentration-dependent luminescence signals on the rat NPR-B cell line with an EC.sub.50 value of 0.024 nM. TPP-9465, TPP-12377, TPP-12378, TPP-12897 and TPP-12899 stimulated the rat CNP receptor reporter cell line with EC.sub.50 values of 5.2 nM, 4.1 nM, 25 nM, 10 nM and 3.2 nM, respectively. TPP-12374 stimulated the rat CNP receptor reporter cell line with EC.sub.50 values of 150 nM, and TPP-12375, TPP-12376 showed only weak indication of activity.
(52) To determine the sensitivity towards proteolytic degradation, the activity of receptor ligands was also characterized after 4 hours incubation with 0.6 μg/ml neutral endopeptidase (NEP, R&D Systems, 1182-ZNC) or 0.6 μg/ml insulin degrading enzyme (IDE, Merck, 407241-50UG) at 37° C.
(53) In contrast to the natriuretic peptide CNP (Bachem, H-1296), TPP-12377, TPP-12897 and TPP-12899 showed high resistance to proteolytic degradation by NEP and IDE.
(54) FIG. **5** graphically depicts the stability of CNP (A-C) and TPP-12897 (D-F) against proteolytic degradation.
(55) Activity Data of ANP Engrafted Antibodies Determined with Isolated Rat Aortic Rings
(56) All experiments were conducted in accordance with institutional guidelines and approved by the local committee on animal experiments. Male Sprague-Dawley rats (weighing 250-300 g) were anesthetized with pentobarbital sodium (40 mg/kg i.p.), killed by decapitation, and exsanguinated. The thoracic aorta was excised and placed in ice-cold Krebs buffer of the following composition: 130 mM NaCl, 14.9 mM NaHCO.sub.3, 5.5 mM dextrose, 4.7 mM KCl, 1.18 mM KH.sub.2PO.sub.4, 1.17 mM MgSO.sub.47H.sub.2O, and 1.6 mM CaCl.sub.22H.sub.2O. The vessel was pinned in a Sylgard Petri dish filled with chilled Krebs' solution, cleaned of fat and connective tissue, and cut into ring segments of approximately 3 to 4 mm in length. Aortic rings were vertically mounted in 50-ml chambers (ADIinstruments) containing Krebs' solution at 37° C. continuously bubbled with a mixture of 95% O.sub.2 and 5% CO.sub.2. Changes in isometric force were recorded using a PowerLab data acquisition system (software Lab Chart 7.0)

(57) After the equilibration period, aortic rings were challenged with 80 mM KCl to check tissue viability. Next, the endothelial integrity of the preparations was determined by verifying the responsiveness to acetylcholine (ACh, 1 μM) in vessels pre-contracted with Phenylephrine (PE, 1 μM). After wash-out and a period of equilibration, Phenylephrine (PE, 1 μM) was used to induce contraction, thereafter natriuretic peptides and natriuretic peptide engrafted IgGs were evaluated for vasorelaxation. Rat ANP peptide (ADH-GM-10057T, Santai Labs) was used as a reference.
(58) As shown in FIG. **6**, both ANP peptide and TPP-10992 induced dose-dependent vasodilation in PE-contracted aortic rings.
(59) As shown in FIG. **7**, both ANP peptide and TPP-5661 induced dose-dependent vasodilation in PE-contracted aortic rings.
(60) Activity Data of ANP Engrafted Antibodies Obtained in Conscious Rats
(61) Blood pressure and heart rate were monitored in freely moving conscious animals by radiotelemetry (Data Sciences International). Female spontaneously hypertensive rats (SHR/N Crl BR, Charles River) with a body weight of 210-300 g were used for these studies. All animals were housed in individual cages at 22-24° C. ambient temperature and maintained on a 12-hour light/dark cycle with free access to standard laboratory rat chow and water ad libitum. Telemeter (HD-10, DSI) implantation was performed a minimum of 14 days before animals were used for blood pressure measurements. Surgery was performed under aseptic conditions. After shaving the abdominal wall, a midline abdominal incision was made, and the fluid-filled sensor catheter was inserted upstream into the exposed descending aorta between the iliac bifurcation and the renal arteries. According to the DSI guidelines the tip of the telemetric catheter was located caudal to the renal arteries and secured by tissue adhesive. The transmitter body was affixed to the inner peritoneal wall before closure of the abdomen. For postsurgical protection against infections and pain a single dosage of an antibiotic (Oxytetracyclin® 10%, 60 mg/kg s.c., 0.06 ml/100 g body weight, Beta-Pharma GmbH & Co, Germany) and analgesic were injected (Rimadyl®, 4 mg/kg s.c., Pfizer, Germany). Telemetric data acquisition was performed by DSI software was predefined to sample hemodynamic data for 10 seconds repeated every 5 minutes. Data collection was started at least 2 hours before drug administration and finished after completion of measurement cycles. Data are expressed as % of basal values±SEM of at least 4 animals per group. The basal value for each animal was calculated as the average of the values measured in two hours prior to substance application (7:00-9:00 AM). Data are then expressed as averages every half hour, starting 15 minutes post application. All animals were treated with a single intraperitoneal (ip) application of test substances dissolved in phosphate buffered saline (PBS). Drug administration took place at 9:00 AM (0 hours).
(62) The hemodynamic effect of ANP peptide is graphically depicted in FIG. **8**. The hemodynamic effect of TPP-5661 is graphically depicted in FIG. **9**. The hemodynamic effect of TPP-10992 is graphically depicted in FIG. **10**.
Example 4: Generation of Different NP Engrafted Antibody Constructs
(63) For constructs with a natriuretic peptide incorporation in a CDR region other than CDRH3 a presumably functionally neutral CDRH3 was designed by fusion of the three residues stretch Leu Thr Gly (IGHD7-27*01) to the C-terminus of HV 3-23 and the N-terminus of IGHJ1 (compare Example 1). The corresponding full length heavy chain sequence of SEQ ID NO 65 further comprises amino acid sequence Constant-H (SEQ ID NO 87).
(64) Pairing of the full length heavy chain sequence of SEQ ID NO 65 without any natriuretic peptide insertion with the full length light chain sequence of SEQ ID NO 66 described in Example 1 yields the synthetic and presumably neutral IgG negative control TPP-5657.
(65) The designed and synthesized antibody construct was cloned according to well-known methods in the art and confirmed by DNA sequencing using plasmid specific oligonucleotides.
(66) Starting from this antibody scaffold, the following ANP engrafted antibody constructs were generated.

(67) TABLE-US-00006 TABLE 4 Design of ANP engrafted antibody constructs SEQ ID SEQ ID NO NO # aa # aa Insertion comprised comprised N- C- Cmpd TPP Site in Ntls in Ctls term.sup.1 term.sup.2 1 TPP- CDRH1 6 2 13057 2 TPP- CDRH1 9 5 13056 3 TPP- CDRH1 12 8 13055 4 TPP- CDRH1 15 11 13054 5 TPP- CDRH1 18 10 12545 6 TPP- CDRH1 19 17 10454 7 TPP- CDRH1 19 17 10453 8 TPP- CDRH1 6 20 17 11172 9 TPP- CDRH1 23 14 10294 10 TPP- CDRH1 23 14 12547 11 TPP- CDRH1 6 24 13 11171 12 TPP- CDRH2 3 3 10841 13 TPP- CDRH2 7 7 10842 14 TPP- CDRH2 8 9 11009 15 TPP- CDRH2 10 11 11008 16 TPP- CDRH2 11 14 11018 17 TPP- CDRH2 12 8 10775 18 TPP- CDRH2 13 12 10767 19 TPP- CDRH2 9 14 9 11012 20 TPP- CDRH2 13 14 14 10 10774 21 TPP- CDRH2 14 11 11007 22 TPP- CDRH2 14 14 11179 23 TPP- CDRH2 13 14 15 10 10773 24 TPP- CDRH2 1 1 15 12 10770 25 TPP- CDRH2 15 14 10766 26 TPP- CDRH2 13 14 16 11 10772 27 TPP- CDRH2 16 11 11005 28 TPP- CDRH2 16 11 11006 29 TPP- CDRH2 16 14 11017 30 TPP- CDRH2 9 10 16 15 11169 31 TPP- CDRH2 16 15 11181 32 TPP- CDRH2 16 17 11182 33 TPP- CDRH2 9 10 17 11 10277 34 TPP- CDRH2 9 10 17 11 12553 35 TPP- CDRH2 9 10 17 11 12554 36 TPP- CDRH2 9 10 17 11 12555 37 TPP- CDRH2 9 10 17 11 12542 38 TPP- CDRH2 9 10 17 11 12543 39 TPP- CDRH2 9 10 17 11 12544 40 TPP- CDRH2 9 10 17 11 13058 41 TPP- CDRH2 9 10 17 11 13059 42 TPP- CDRH2 9 20 17 11 13060 43 TPP- CDRH2 9 20 17 11 13061 44 TPP- CDRH2 9 10 17 11 13062 45 TPP- CDRH2 9 10 17 11 13063 46 TPP- CDRH2 9 10 17 11 13064 47 TPP- CDRH2 9 10 17 11 13065 48 TPP- CDRH2 9 10 17 11 13066 49 TPP- CDRH2 9 10 17 11 12546 50 TPP- CDRH2 13 14 17 12 10452 51 TPP- CDRH2 4 5 17 12 10846 52 TPP- CDRH2 17 12 10852 53 TPP- CDRH2 6 17 13 10851 54 TPP- CDRH2 1 1 17 14 10769 55 TPP- CDRH2 17 14 10765 56 TPP- CDRH2 17 15 11180 57 TPP- CDRH2 17 17 11177 58 TPP- CDRH2 17 17 11178 59 TPP- CDRH2 17 18 17 17 11176 60 TPP- CDRH2 9 10 18 12 10278 61 TPP- CDRH2 4 5 18 13 10847 62 TPP- CDRH2 18 13 11004 63 TPP- CDRH2 2 3 18 13 10844 64 TPP- CDRH2 18 13 10853 65 TPP- CDRH2 9 10 18 13 10279 66 TPP- CDRH2 9 10 18 13 11170 67 TPP- CDRH2 9 18 13 11010 68 TPP- CDRH2 9 18 13 11011 69 TPP- CDRH2 18 14 10764 70 TPP- CDRH2 18 17 11183 71 TPP- CDRH2 16 17 18 17 11175 72 TPP- CDRH2 15 19 12 11016 73 TPP- CDRH2 15 19 14 11015 74 TPP- CDRH2 13 14 19 14 10451 75 TPP- CDRH2 1 1 19 14 10768 76 TPP- CDRH2 4 5 19 14 10848 77 TPP- CDRH2 19 14 11003 78 TPP- CDRH2 19 14 11002 79 TPP- CDRH2 2 3 19 14 10843 80 TPP- CDRH2 2 3 19 14 10845 81 TPP- CDRH2 19 14 10284 82 TPP- CDRH2 11 12 19 14 10446 83 TPP- CDRH2 19 14 10447 84 TPP- CDRH2 19 14 10854 85 TPP- CDRH2 15 19 15 11014 86 TPP- CDRH2 6 6 19 15 10849 87 TPP- CDRH2 6 19 15 10850 88 TPP- CDRH2 20 14 11013 89 TPP- CDRH2 1 1 20 15 10771 90 TPP- CDRH2 1 1 20 15 10287 91 TPP- CDRH2 20 15 10282 92 TPP- CDRH2 20 15 10285 93 TPP- CDRH2 20 15 10286 94 TPP- CDRH2 20 15 10283 95 TPP- CDRH2 9 10 20 15 11168 96 TPP- CDRH2 20 15 10857 97 TPP- CDRH2 20 15 10856 98 TPP- CDRH2 20 15 10855 99 TPP- CDRH3 10 3 10281 100 TPP- CDRH3 10 8 10280 101 TPP- CDRH3 12 10 10583 102 TPP- CDRH3 7 14 12 10582 103 TPP- CDRH3 7 8 15 18 10270 104 TPP- CDRH3 7 8 16 14 10264 105 TPP- CDRH3 16 14 10581 106 TPP- CDRH3 7 8 17 15 10263 107 TPP- CDRH3 7 8 17 18 10271 108 TPP- CDRH3 7 8 18 16 10262 109 TPP- CDRH3 7 8 18 18 10272 110 TPP- CDRH3 7 8 19 17 10261 111 TPP- CDRH3 19 17 10289 112 TPP- CDRH3 7 8 19 18 10273 113 TPP- CDRH3 7 8 20 17 10260 114 TPP- CDRH3 9 10 20 17 10275 115 TPP- CDRH3 20 18 10580 116 TPP- CDRH3 7 8 20 18 10274 117 TPP- CDRH3 7 8 20 18 5661 118 TPP- CDRH3 7 8 18 16 13226 119 TPP- CDRH3 7 8 18 16 13227 120 TPP- CDRH3 7 22 20 18 13228 121 TPP- CDRH3 7 22 20 18 13229 122 TPP- CDRH3 21 22 20 18 13230 123 TPP- CDRH3 21 22 20 18 13231 124 TPP- CDRH3 9 10 20 18 10276 125 TPP- CDRH3 20 18 10290 126 TPP- CDRH3 11 12 20 18 10445 127 TPP- CDRH3 7 8 20 18 10269 128 TPP- CDRH3 2 3 20 18 10288 129 TPP- CDRH3 7 8 20 19 10265 130 TPP- CDRH3 7 8 21 19 10268 131 TPP- CDRH3 19 22 20 10593 132 TPP- CDRH3 6 12 22 20 11174 133 TPP- CDRH3 7 8 22 20 10266 134 TPP- CDRH3 6 12 22 20 11173 135 TPP- CDRH3 11 12 22 20 10444 136 TPP- CDRH3 7 8 24 22 10267 137 TPP- CDRH3 11 12 24 22 10443 138 TPP- CDRL1 6 16 14 11163 139 TPP- CDRL1 19 13 10360 140 TPP- CDRL1 20 18

10462 141 TPP- CDRL1 21 19 10460 142 TPP- CDRL1 21 19 10461 143 TPP- CDRL1 6 6 21 19 11161 144 TPP- CDRL1 6 21 19 11162 145 TPP- CDRL1 23 14 10359 146 TPP- CDRL2 1 6 10824 147 TPP- CDRL2 5 10 10825 148 TPP- CDRL2 12 16 11019 149 TPP- CDRL2 13 17 11021 150 TPP- CDRL2 13 17 11020 151 TPP- CDRL2 14 14 10789 152 TPP- CDRL2 15 19 11022 153 TPP- CDRL2 4 5 16 15 10829 154 TPP- CDRL2 16 15 10835 155 TPP- CDRL2 16 16 10788 156 TPP- CDRL2 15 15 17 16 10571 157 TPP- CDRL2 4 5 17 16 10830 158 TPP- CDRL2 17 16 10790 159 TPP- CDRL2 19 17 16 10573 160 TPP- CDRL2 2 3 17 16 10827 161 TPP- CDRL2 19 17 16 10572 162 TPP- CDRL2 17 16 10836 163 TPP- CDRL2 6 17 17 10834 164 TPP- CDRL2 18 16 10787 165 TPP- CDRL2 4 5 18 17 10831 166 TPP- CDRL2 2 3 18 17 10828 167 TPP- CDRL2 2 3 18 17 10826 168 TPP- CDRL2 18 17 10837 169 TPP- CDRL2 19 17 10361 170 TPP- CDRL2 11 12 19 18 11023 171 TPP- CDRL2 19 18 10838 172 TPP- CDRL2 19 18 10840 173 TPP- CDRL2 19 18 10839 174 TPP- CDRL2 6 19 19 10832 175 TPP- CDRL2 6 19 19 10833 176 TPP- CDRL2 11 12 20 19 11024 177 TPP- CDRL3 2 2 10353 178 TPP- CDRL3 14 9 10780 179 TPP- CDRL3 16 10 10786 180 TPP- CDRL3 16 11 10779 181 TPP- CDRL3 16 13 10778 182 TPP- CDRL3 11 12 17 11 10783 183 TPP- CDRL3 18 12 10785 184 TPP- CDRL3 18 13 10776 185 TPP- CDRL3 18 13 10777 186 TPP- CDRL3 18 14 10784 187 TPP- CDRL3 11 12 19 13 10782 188 TPP- CDRL3 7 8 19 14 10352 189 TPP- CDRL3 19 14 10356 190 TPP- CDRL3 19 14 10354 191 TPP- CDRL3 19 14 10355 192 TPP- CDRL3 11 12 19 15 10781 193 TPP- CDRL3 7 8 20 14 10436 194 TPP- CDRL3 11 12 20 14 10440 195 TPP- CDRL3 20 14 10442 196 TPP- CDRL3 7 8 20 15 10351 197 TPP- CDRL3 7 8 20 15 10348 198 TPP- CDRL3 20 15 10358 199 TPP- CDLR3 6 12 21 15 11167 200 TPP- CDRL3 11 12 21 15 10438 201 TPP- CDRL3 11 12 21 15 10439 202 TPP- CDRL3 21 15 10441 203 TPP- CDRL3 7 8 21 16 10349 204 TPP- CDRL3 7 8 22 17 10350 205 TPP- CDRL3 11 12 23 17 10437 206 TPP- CDRL3 6 12 24 18 11166 207 TPP- CDRL3 6 6 24 20 10362 208 TPP- CDRL3 6 6 24 20 10363 209 TPP- no na n 5657 .sup.1The number of amino acid residues present between the respective N-terminal reference amino acid residue and the first amino acid of the inserted natriuretic peptide; .sup.2The number of amino acid residues present between the last amino acid of the inserted natriuretic peptide and the respective C-terminal reference amino acid residue

(68) TABLE-US-00007 Table 4 cont.: Design of ANP engrafted antibody constructs Cmpd TPP N-terminal sequence.sup.3 C-terminal sequence.sup.4 1 TPP- SGFTFSS YAM 13057 (SEQ ID NO: 92) 2 TPP- SGFTFGSGSG GSGSGM 13056 (SEQ ID NO: 93) (SEQ ID NO: 94) 3 TPP- SGFTFGSGSGSGS SGSGSGSGM 13055 (SEQ ID NO: 95) (SEQ ID NO: 96) 4 TPP- SGFTFGSGSGSGGSGG GGSGGSGSGSGM 13054 (SEQ ID NO: 97) (SEQ ID NO: 98) 5 TPP- SGFTFGSGSGSGSGGGSGG GSGSGGSGSGM 12545 (SEQ ID NO: 99) (SEQ ID NO: 100) 6 TPP- SPAVVYIEILDRHPDGGSGG GSGREVPISNGSGFVVAM 10454 (SEQ ID NO: 101) (SEQ ID NO: 102) 7 TPP- SGAVVYIEILDRHPDGGSGG GSGREVPISNGSGFVVAM 10453 (SEQ ID NO: 103) (SEQ ID NO: 102) 8 TPP- SSSDRSALLKSKLRALLTAPR GSGREVPISNGSGFVVAM 11172 (SEQ ID NO: 104) (SEQ ID NO: 102) 9 TPP- SGFTFGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGM 10294 (SEQ ID NO: 105) (SEQ ID NO: 106) 10 TPP- SGFTFGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGM 12547 (SEQ ID NO: 105) (SEQ ID NO: 106) 11 TPP- SGFTFSSDRSALLKSKLRALLTAPR GSGSGSGSGSGSGM 11171 (SEQ ID NO: 107) (SEQ ID NO: 108) 12 TPP- ISGS GGST 10841 (SEQ ID NO: 109) (SEQ ID NO: 110) 13 TPP- ISGSGSGS GSGSGGST 10842 (SEQ ID NO: 111) (SEQ ID NO: 112) 14 TPP- ISGSGSGSG GSSGSGSGST 11009 (SEQ ID NO: 113) (SEQ ID NO: 114) 15 TPP- ISGSGSGSGSG GSSGSGSGSGST 11008 (SEQ ID NO: 115) (SEQ ID NO: 116) 16 TPP- ISGSGSGSGSGG GEKEKEKVSTAVGST 11018 (SEQ ID NO: 117) (SEQ ID NO: 118) 17 TPP- ISGSAVVNGGSGG GKIAIGGST 10775 (SEQ ID NO: 119) (SEQ ID NO: 120) 18 TPP- ISGPNPNKNPNPGG GSNENPNPNPGST 10767 (SEQ ID NO:

121) (SEQ ID NO: 122) 19 TPP- ISGSVVVTSHGGSGG GGSGSGSGST 11012 (SEQ ID NO: 123) (SEQ ID NO: 124) 20 TPP- ISGSAVVNVRGGSGG GGDKIAIGGST 10774 (SEQ ID NO: 125) (SEQ ID NO: 126) 21 TPP- ISGSGSGSGSGSSGG GSSGSGSGSGST 11007 (SEQ ID NO: 127) (SEQ ID NO: 116) 22 TPP- ISGLAVQIRRGGSGG GGSGRETLTLYVGST 11179 (SEQ ID NO: 128) (SEQ ID NO: 129) 23 TPP- ISGSAVVNVRAGGSGG GGDKIAIGGST 10773 (SEQ ID NO: 130) (SEQ ID NO: 126) 24 TPP- ISGSYAMSWVRGGSGG GSYAMSWVRQGST 10770 (SEQ ID NO: 131) (SEQ ID NO: 132) 25 TPP- ISGPNPNKNPNPNPGG GSNPNENPNPNPGST 10766 (SEQ ID NO: 133) (SEQ ID NO: 134) 26 TPP- ISGSAVVNVRADGGSGG GSGDKIAIGGST 10772 (SEQ ID NO: 135) (SEQ ID NO: 136) 27 TPP- ISGSGSGSGSPDGGSGG GSSGSGSGSGST 11005 (SEQ ID NO: 137) (SEQ ID NO: 116) 28 TPP- ISGSGSGSGSGSGGSGG GSSGSGSGSGST 11006 (SEQ ID NO: 138) (SEQ ID NO: 116) 29 TPP- ISGSGSGSGSGSGGSGG GEKEKEKVSTAVGST 11017 (SEQ ID NO: 138) (SEQ ID NO: 118) 30 TPP- ISGSVVVTSHQAPGSGG GSGEKKKLKSLAYGST 11169 (SEQ ID NO: 139) (SEQ ID NO: 140) 31 TPP- ISGRYNILKIQKVGSGG GGSGEYLITYQIMGST 11181 (SEQ ID NO: 141) (SEQ ID NO: 142) 32 TPP- ISGRQLLFCRVTLGSGG GGSGEQAYPEYLITYGST 11182 (SEQ ID NO: 143) (SEQ ID NO: 144) 33 TPP- ISVVVTSHQAPGEGGSGG GEKKKLKSLAST 10277 (SEQ ID NO: 145) (SEQ ID NO: 146) 34 TPP- ISGVVTSHQAPGEGGSGG GEKKKLKSLAST 12553 (SEQ ID NO: 147) (SEQ ID NO: 146) 35 TPP- ISVVVTSHQAPGEGGSGG GEKKKLKSLGST 12554 (SEQ ID NO: 145) (SEQ ID NO: 148) 36 TPP- ISVVVTSHQAPGEGGSGG GEKKKLKSGGST 12555 (SEQ ID NO: 145) (SEQ ID NO: 149) 37 TPP- ISGVVTSHQAPGEGGSGG GEKKKLKSGGST 12542 (SEQ ID NO: 147) (SEQ ID NO: 149) 38 TPP- ISGSVTSHQAPGEGGSGG GEKKKLKSGGST 12543 (SEQ ID NO: 150) (SEQ ID NO: 149) 39 TPP- ISGSVTSHQAPGEGGSGG GEKKKGKSGGST 12544 (SEQ ID NO: 150) (SEQ ID NO: 151) 40 TPP- ISVVVTSHQAPGSGGSGG GEKKKLKSLAST 13058 (SEQ ID NO: 152) (SEQ ID NO: 146) 41 TPP- ISVVVTSHQAPTSGGSGG GEKKKLKSLAST 13059 (SEQ ID NO: 153) (SEQ ID NO: 146) 42 TPP- ISVVVTSHQSPTPGGSGG GGSTPLKSLAST 13060 (SEQ ID NO: 154) (SEQ ID NO: 155) 43 TPP- ISVVVTSHQAPGEGGSGG GGSTPLKSLAST 13061 (SEQ ID NO: 145) (SEQ ID NO: 155) 44 TPP- ISVVVTSHQAPGEGGSGG GSTPKLKSLAST 13062 (SEQ ID NO: 145) (SEQ ID NO: 156) 45 TPP- ISVVVTSHQSPTPGGSGG GEKKKLKSLAST 13063 (SEQ ID NO: 154) (SEQ ID NO: 146) 46 TPP- ISVVVTSHPTPGEGGSGG GEKKKLKSLAST 13064 (SEQ ID NO: 157) (SEQ ID NO: 146) 47 TPP- ISVVVTSHQAPSPGSTGG GEKKKLKSLAST 13065 (SEQ ID NO: 158) (SEQ ID NO: 146) 48 TPP- ISVVVTSHQANGSGGSGG GEKKKLKSLAST 13066 (SEQ ID NO: 159) (SEQ ID NO: 146) 49 TPP- ISVVVTSHQAPGEGGSGG GEKKKLKSLAST 12546 (SEQ ID NO: 145) (SEQ ID NO: 146) 50 TPP- ISGSAVVNVRAPDGGSGG GSKGDKIAIGGST 10452 (SEQ ID NO: 160) (SEQ ID NO: 161) 51 TPP- ISTSASLAITGPDGGSGG GSDRFSGSKSGST 10846 (SEQ ID NO: 162) (SEQ ID NO: 163) 52 TPP- ISGFILPIEVYPDGGSGG GSKVRFDYDLFST 10852 (SEQ ID NO: 164) (SEQ ID NO: 165) 53 TPP- ISSALLKSKLRALLTAPG GGSGSGSGSGSGST 10851 (SEQ ID NO: 166) (SEQ ID NO: 167) 54 TPP- ISGSYAMSWVRQAGGSGG GSSSYAMSWVRQGST 10769 (SEQ ID NO: 168) (SEQ ID NO: 169) 55 TPP- ISGPNPNKNPNPNPGSGG GSNPNENPNPNPGST 10765 (SEQ ID NO: 170) (SEQ ID NO: 134) 56 TPP- IHPLQNRWALWFFKGSGG GGSGNLRLISKFDTVT 11180 (SEQ ID NO: 171) (SEQ ID NO: 172) 57 TPP- ISGSVTIFSLATNEGSGG GGSGKTTWHRISVFGGST 11177 (SEQ ID NO: 173) (SEQ ID NO: 174) 58 TPP- IYLEGKIDYGEYMDGSGG GGSNVRRQATTIIADNIT 11178 (SEQ

ID NO: 175) (SEQ ID NO: 176) 59 TPP- ISGSVQGIINFEQKGSGG GGSGPVKVWGSIKGGGST 11176 (SEQ ID NO: 177) (SEQ ID NO: 178) 60 TPP- ISGVVVTSHQAPGEGGSGG GEKKKLKSLAGST 10278 (SEQ ID NO: 179) (SEQ ID NO: 180) 61 TPP- ISGTSASLAITGPDGGSGG GSDRFSGSKSGGST 10847 (SEQ ID NO: 181) (SEQ ID NO: 182) 62 TPP- ISGSGSGSGSGSPDGGSGG GSSGSGSGSGSGST 11004 (SEQ ID NO: 183) (SEQ ID NO: 184) 63 TPP- ISGTYISNVNHKPDGGSGG GSNTKVDKKVEGST 10844 (SEQ ID NO: 185) (SEQ ID NO: 186) 64 TPP- ISGGFILPIEVYPDGGSGG GSKVRFDYDLFGST 10853 (SEQ ID NO: 187) (SEQ ID NO: 188) 65 TPP- ISGSVVVTSHQAPGGGSGG GEKKKLKSLAYGST 10279 (SEQ ID NO: 189) (SEQ ID NO: 190) 66 TPP- ISGSVVVTSHQAPGGGSGG GEKPKPKPLAYGST 11170 (SEQ ID NO: 189) (SEQ ID NO: 191) 67 TPP- ISGSVVVTSHQAPGGGSGG GSSGSGSGSGSGST 11010 (SEQ ID NO: 189) (SEQ ID NO: 184) 68 TPP- ISGSVVVTSHQAPGGGSGG GSGSGSGSGSGGST 11011 (SEQ ID NO: 189) (SEQ ID NO: 192) 69 TPP- ISGPNPNKNPNPNPGGSGG GSNPNENPNPNPGST 10764 (SEQ ID NO: 193) (SEQ ID NO: 134) 70 TPP- ISGDIYLAINITNGEGSGG GGSGDIYLAINITNGEST 11183 (SEQ ID NO: 194) (SEQ ID NO: 195) 71 TPP- ISGSATKAVSVLKGDGSGG GGSGVQGIINFEQKGGST 11175 (SEQ ID NO: 196) (SEQ ID NO: 197) 72 TPP- ISGSVPKEKEKEKVSTAVGG GSGSGSGSGSGST 11016 (SEQ ID NO: 198) (SEQ ID NO: 199) 73 TPP- ISGSVPKEKEKEKVSTAVGG GSGSGSGSGSGSGST 11015 (SEQ ID NO: 198) (SEQ ID NO: 200) 74 TPP- ISGSSGAVVNVRAPDGGSGG GSKGDKIAIWTTGST 10451 (SEQ ID NO: 201) (SEQ ID NO: 202) 75 TPP- ISGSYAMSWVRQAPDGGSGG GSSSYAMSWVRQGST 10768 (SEQ ID NO: 203) (SEQ ID NO: 169) 76 TPP- ISGSTSASLAITGPDGGSGG GSDRFSGSKSGGGST 10848 (SEQ ID NO: 204) (SEQ ID NO: 205) 77 TPP- ISGSGSGSGSGSGPDGGSGG GSGSGSGSGSGSGST 11003 (SEQ ID NO: 206) (SEQ ID NO: 200) 78 TPP- ISGSGSGSGSGSSPDGGSGG GSYSGSGSGSGSGST 11002 (SEQ ID NO: 448) (SEQ ID NO: 208) 79 TPP- ISTQTYISNVNHKPDGGSGG GSNTKVDKKVEPKST 10843 (SEQ ID NO: 209) (SEQ ID NO: 210) 80 TPP- ISGSTYISNVNHKPDGGSGG GSNTKVDKKVEGGST 10845 (SEQ ID NO: 211) (SEQ ID NO: 212) 81 TPP- ISGPNPNPNPNPNPDGGSGG GSNPNPNPNPNPGST 10284 (SEQ ID NO: 213) (SEQ ID NO: 214) 82 TPP- ISAVQVKLELGHRPDGGSGG GSNHLRSEKLTFNST 10446 (SEQ ID NO: 215) (SEQ ID NO: 216) 83 TPP- ISGFILPIEVYFKPDGGSGG GSPRKVRFDYDLFST 10447 (SEQ ID NO: 217) (SEQ ID NO: 218) 84 TPP- ISGSGFILPIEVYPDGGSGG GSKVRFDYDLFGGST 10854 (SEQ ID NO: 219) (SEQ ID NO: 220) 85 TPP- ISGSVPKEKEKEKVSTAVGG GSYGSGSGSGSGSGST 11014 (SEQ ID NO: 198) (SEQ ID NO: 221) 86 TPP- ISDRSALLKSKLRALLTAPR GSDRSALLKSKLRAST 10849 (SEQ ID NO: 222) (SEQ ID NO: 223) 87 TPP- ISDRSALLKSKLRALLTAPG GGSGSGSGSGSGSGST 10850 (SEQ ID NO: 224) (SEQ ID NO: 225) 88 TPP- ISGSSDKTHTSPPSPDGGSGG GSKTHTSPPSPGGST 11013 (SEQ ID NO: 226) (SEQ ID NO: 227) 89 TPP- ISGSYAMSWVRQASPDGGSGG GSYSSYAMSWVRGGST 10771 (SEQ ID NO: 228) (SEQ ID NO: 229) 90 TPP- ISGSYAMSWVRQASPDGGSGG GSYSSYAMSWVRQGST 10287 (SEQ ID NO: 228) (SEQ ID NO: 230) 91 TPP- ISGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGST 10282 (SEQ ID NO: 231) (SEQ ID NO: 221) 92 TPP- ISGSPNPNPNPNPSPDGGSGG GSYPNPNPNPNPSGST 10285 (SEQ ID NO: 232) (SEQ ID NO: 233) 93 TPP- ISGPNPNKNPNPNSPDGGSGG GSYNPNENPNPNPGST 10286 (SEQ ID NO: 234) (SEQ ID NO: 235) 94 TPP- ISGPNPNPNPNPNSPDGGSGG GSYNPNPNPNPNPGST 10283 (SEQ ID NO: 236) (SEQ ID NO: 237) 95 TPP- ISGSVVVTSHQAPGGSGGSGG GSGEKKKLKSLAYGST 11168 (SEQ ID NO: 238) (SEQ ID NO: 140) 96 TPP- ISGSVVYIEILDRHPDGGSGG

GSGREVPISNGSGGST 10857 (SEQ ID NO: 239) (SEQ ID NO: 240) 97 TPP-
ISGAVVYIEILDRHPDGGSGG GSGREVPISNGSGGST 10856 (SEQ ID NO: 241) (SEQ
ID NO: 240) 98 TPP- ISPAVVYIEILDRHPDGGSGG GSGREVPISNGSGFST 10855 (SEQ
ID NO: 242) (SEQ ID NO: 243) 99 TPP- CAKSPDGGSGG GSYG 10281 (SEQ ID
NO: 244) (SEQ ID NO: 245) 100 TPP- CAKSPDGGSGG GSYQHWGQG 10280 (SEQ
ID NO: 244) (SEQ ID NO: 246) 101 TPP- CAKVHQETGGSGG GSWHVQHWGQG
10583 (SEQ ID NO: 247) (SEQ ID NO: 248) 102 TPP- CAKVHQETPDGGSGG
GSYEWHVQHWGQG 10582 (SEQ ID NO: 249) (SEQ ID NO: 250) 103 TPP-
CTSVHQETKKYQSSGG GSYSYTYNYEWHVDVWGQG 10270 (SEQ ID NO: 251)
(SEQ ID NO: 252) 104 TPP- CTSVHQETSSPDGGSGG GSYSYEWHVDVWGQG 10264
(SEQ ID NO: 253) (SEQ ID NO: 254) 105 TPP- CAKTHTSPPSPDGGSGG
GSSPPSPYFQHWGQG 10581 (SEQ ID NO: 255) (SEQ ID NO: 256) 106 TPP-
CTSVHQETKSSPDGGSGG GSYSNYEWHVDVWGQG 10263 (SEQ ID NO: 257) (SEQ
ID NO: 258) 107 TPP- CTSVHQETKKYQSSPDGG GSYSYTYNYEWHVDVWGQG 10271
(SEQ ID NO: 259) (SEQ ID NO: 252) 108 TPP- CTSVHQETKKSSPDGGSGG
GSYSYNYEWHVDVWGQG 10262 (SEQ ID NO: 260) (SEQ ID NO: 261) 109 TPP-
CTSVHQETKKYQSSGGSGG GSYSYTYNYEWHVDVWGQG 10272 (SEQ ID NO: 262)
(SEQ ID NO: 252) 110 TPP- CTSVHQETKKQSSPDGGSGG
GSYSYYNYEWHVDVWGQG 10261 (SEQ ID NO: 263) (SEQ ID NO: 264) 111 TPP-
CAKVHPNPNPNPNPDGGSGG GSNPNPNPNPHVDVWGQG 10289 (SEQ ID NO: 265)
(SEQ ID NO: 266) 112 TPP- CTSVHQETKKYQSSPDGGSG
GSYSYTYNYEWHVDVWGQG 10273 (SEQ ID NO: 267) (SEQ ID NO: 252) 113
TPP- CTSVHQETKKYQSSPDGGSGG GSYSYYNYEWHVDVWGQG 10260 (SEQ ID
NO: 268) (SEQ ID NO: 264) 114 TPP- CAKLTVVVTSHQAPGEGGSGG
GEKKKLKSLAYFQHWGQG 10275 (SEQ ID NO: 269) (SEQ ID NO: 270) 115 TPP-
CAKSSDKTHTSPPSPDGGSGG GSKTHTSPPSPYFQHWGQG 10580 (SEQ ID NO: 271)
(SEQ ID NO: 272) 116 TPP- CTSVHQETKKYQSSPDGGSGG
GSYSYTYNYEWHVDVWGQG 10274 (SEQ ID NO: 268) (SEQ ID NO: 252) 117
TPP- CTSVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVDVWGQG 5661 (SEQ ID
NO: 268) (SEQ ID NO: 252) 118 TPP- CAKVETKKYQSSPDGGSGG
GSYSYTYNYEVQHWGQG 13226 (SEQ ID NO: 273) (SEQ ID NO: 274) 119 TPP-
CAKVHTKKYQSSPDGGSGG GSYSYTYNYHVQHWGQG 13227 (SEQ ID NO: 275)
(SEQ ID NO: 276) 120 TPP- CAKLTVETKKYQSSPDGGSGG
GSYSYTYNYEWHVQHWGQG 13228 (SEQ ID NO: 277) (SEQ ID NO: 278) 121
TPP- CAKLTAETKKYQSSPDGGSGG GSYSYTYNYENYFQHWGQG 13229 (SEQ ID
NO: 279) (SEQ ID NO: 280) 122 TPP- CAKGITGTKKYQSSPDGGSGG
GSYSYTYNYAEYFQHWGQG 13230 (SEQ ID NO: 281) (SEQ ID NO: 282) 123
TPP- CAKGITGTKKYQSSPDGGSGG GSYDYVWGSYAYFQHWGQG 13231 (SEQ ID
NO: 281) (SEQ ID NO: 283) 124 TPP- CAKLTSVVVTSHQAPGGGSGG
GEKKKLKSLAYYFQHWGQG 10276 (SEQ ID NO: 284) (SEQ ID NO: 285) 125
TPP- CAKVHPNPNPNPNSPDGGSGG GSYNPNPNPNPHVDVWGQG 10290 (SEQ ID
NO: 286) (SEQ ID NO: 287) 126 TPP- CAKLTQVKLELGHRPDGGSGG
GSNHLRSEKLTYFQHWGQG 10445 (SEQ ID NO: 288) (SEQ ID NO: 289) 127 TPP-
CAKVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVQHWGQG 10269 (SEQ ID NO:
290) (SEQ ID NO: 278) 128 TPP- CAKTQTYISNVNHKPDGGSGG
GSNTKVDKKAEYFQHWGQG 10288 (SEQ ID NO: 291) (SEQ ID NO: 292) 129
TPP- CTSVHQETKKYQSSPDGGSGG GSYSYTTYNYEWHVDVWGQG 10265 (SEQ ID
NO: 268) (SEQ ID NO: 293) 130 TPP- CATSVHQETKKYQSSPDGGSGG
GSYSYTYNYEWHVDVHWGQG 10268 (SEQ ID NO: 294) (SEQ ID NO: 295) 131
TPP- CAKLTAEEWKKKYEKEKEKNKGS GGSGGSGGSGGAEYFQHWGQG 10593 (SEQ

ID NO: 296) (SEQ ID NO: 297) 132 TPP- CADSSDRSALLKSKLRALLTAPR GSNHLRSEKLTFNYFQHWGQG 11174 (SEQ ID NO: 298) (SEQ ID NO: 299) 133 TPP- CTSVHQETKKYQYQSSPDGGSGG GSYSYTYTYNYEWHVDVWGQG 10266 (SEQ ID NO: 300) (SEQ ID NO: 301) 134 TPP- CAKLTDRSALLKSKLRALLTAPR GSNHLRSEKLTFNYFQHWGQG 11173 (SEQ ID NO: 302) (SEQ ID NO: 299) 135 TPP- CAKLTAVQVKLELGHRPDGGSGG GSNHLRSEKLTFNYFQHWGQG 10444 (SEQ ID NO: 303) (SEQ ID NO: 299) 136 TPP- CTSVHQETKKYQSSYQSSPDGGSGG GSYSYTYSYTYNYEWHVDVWGQG 10267 (SEQ ID NO: 304) (SEQ ID NO: 305) 137 TPP- CAKLTAVQVKLELGHRAQPDGGSGG GSPVNHLRSEKLTFNYFQHWGQG 10443 (SEQ ID NO: 306) (SEQ ID NO: 307) 138 TPP- SSSNIGSKLRALLTAPR GSGSGGSGGSGSGYD 11163 (SEQ ID NO: 308) (SEQ ID NO: 309) 139 TPP- SGSGSGSGSGSGSPDGGSGG GSYGSGGSGSGSGD 10360 (SEQ ID NO: 29) (SEQ ID NO: 310) 140 TPP- SSLGQIQLTIRHSSPDGGSGG GSNKLIVVVHASRNLIGYD 10462 (SEQ ID NO: 311) (SEQ ID NO: 312) 141 TPP- SPLGQIQLTIRHSSQPDGGSGG GSRNKLIVVVHASRNLIAYD 10460 (SEQ ID NO: 313) (SEQ ID NO: 314) 142 TPP- SSLGQIQLTIRHSSQPDGGSGG GSRNKLIVVVHASRNLIGYD 10461 (SEQ ID NO: 315) (SEQ ID NO: 316) 143 TPP- SSSNIGSALLKSKLRALLTAPR GSSDRSALLKSKLRALLTAD 11161 (SEQ ID NO: 317) (SEQ ID NO: 318) 144 TPP- SSSNIGSALLKSKLRALLTAPR GSGSGGSGGSGGSGSGSGYD 11162 (SEQ ID NO: 317) (SEQ ID NO: 319) 145 TPP- SSSNIGSGSGSGSGSGSPDGGSGG GSYGSGGSGSGSGYD 10359 (SEQ ID NO: 320) (SEQ ID NO: 321) 146 TPP- YG NSNRPSG 10824 (SEQ ID NO: 322) 147 TPP- YGGSGS GSGSNSNRPSG 10825 (SEQ ID NO: 323) (SEQ ID NO: 324) 148 TPP- YGKTHTSPPSPGG GGKTHTSPPSPGNRPSG 11019 (SEQ ID NO: 325) (SEQ ID NO: 326) 149 TPP- YGSGSGSGSGSGGG GGKTHTSPPSPSGNRPSG 11021 (SEQ ID NO: 327) (SEQ ID NO: 328) 150 TPP- YGSKTHTSPPSPGG GGKTHTSPPSPSGNRPSG 11020 (SEQ ID NO: 329) (SEQ ID NO: 328) 151 TPP- YGSGSGSGSGGGSGG GSGGSGSGSGNRPSG 10789 (SEQ ID NO: 330) (SEQ ID NO: 331) 152 TPP- YGSSDKTHTSPPSPGG GGSGSGSGGSGSGSGNRPSG 11022 (SEQ ID NO: 332) (SEQ ID NO: 333) 153 TPP- YTSASLAITGPDGGSGG GSDRFSGSKSGNRPSG 10829 (SEQ ID NO: 334) (SEQ ID NO: 335) 154 TPP- YGFILPIEVYPDGGSGG GSKVRFDYDLFNRPSG 10835 (SEQ ID NO: 336) (SEQ ID NO: 337) 155 TPP- YGSGSGSGSGSGGGSGG GSGSGGSGSGSGNRPSG 10788 (SEQ ID NO: 338) (SEQ ID NO: 339) 156 TPP- YGVPKEKEKEKVSTAVGG GSAPLEVPKEKEKEKVG 10571 (SEQ ID NO: 340) (SEQ ID NO: 341) 157 TPP- YGTSASLAITGPDGGSGG GSDRFSGSKSGGNRPSG 10830 (SEQ ID NO: 342) (SEQ ID NO: 343) 158 TPP- YGGSGSGSGSGPDGGSGG GSGSGGSGSGSGNRPSG 10790 (SEQ ID NO: 344) (SEQ ID NO: 339) 159 TPP- YGSGSGSGSGSGSGGG GSYEKEKEKNKTLKNVG 10573 (SEQ ID NO: 345) (SEQ ID NO: 346) 160 TPP- YGTYISNVNHKPDGGSGG GSNTKVDKKVEGNRPSG 10827 (SEQ ID NO: 347) (SEQ ID NO: 348) 161 TPP- YGAEEWKKKYEKEKEKGG GSGSGSGSGSGSGSGSG 10572 (SEQ ID NO: 349) (SEQ ID NO: 350) 162 TPP- YGGFILPIEVYPDGGSGG GSKVRFDYDLFGNRPSG 10836 (SEQ ID NO: 351) (SEQ ID NO: 352) 163 TPP- YGSALLKSKLRALLTAPG GSDGSGGSGSGSGNRPSG 10834 (SEQ ID NO: 353) (SEQ ID NO: 354) 164 TPP- YGSGSGSGSGSGPDGGSGG GSGSGGSGSGSGNRPSG 10787 (SEQ ID NO: 355) (SEQ ID NO: 339) 165 TPP- YGGTSASLAITGPDGGSGG GSDRFSGSKSGGGNRPSG 10831 (SEQ ID NO: 356) (SEQ ID NO: 357) 166 TPP- YGGTYISNVNHKPDGGSGG GSNTKVDKKVEGGNRPSG 10828 (SEQ ID NO: 358) (SEQ ID NO: 359) 167 TPP- YTQTYISNVNHKPDGGSGG GSNTKVDKKVEPKNRPSG 10826 (SEQ ID NO: 360) (SEQ ID NO: 361) 168 TPP- YGGGFILPIEVYPDGGSGG GSKVRFDYDLFGGNRPSG 10837 (SEQ ID NO: 362) (SEQ ID NO: 363) 169 TPP-

YGSGSGSGSGSGSPDGGSGG GSYGSGGSGSGSGNRPSG 10361 (SEQ ID NO: 364) (SEQ ID NO: 365) 170 TPP- YGSQVKLELGHRAPDGGSGG GSVNHLRSEKLTSGNRPSG 11023 (SEQ ID NO: 366) (SEQ ID NO: 367) 171 TPP- YPAVVYIEILDRHPDGGSGG GSGREVPISNGSGFNRPSG 10838 (SEQ ID NO: 368) (SEQ ID NO: 369) 172 TPP- YGGVVYIEILDRHPDGGSGG GSGREVPISNGSGGNRPSG 10840 (SEQ ID NO: 370) (SEQ ID NO: 371) 173 TPP- YGAVVYIEILDRHPDGGSGG GSGREVPISNGSGGNRPSG 10839 (SEQ ID NO: 372) (SEQ ID NO: 371) 174 TPP- YGSRSALLKSKLRALLTAPR GSDGSGSGGSGSGSGNRPSG 10832 (SEQ ID NO: 373) (SEQ ID NO: 374) 175 TPP- YGSRSALLKSKLRALLTAPG GSDGSGSGGSGSGSGNRPSG 10833 (SEQ ID NO: 375) (SEQ ID NO: 374) 176 TPP- YGSAVQVKLELGHRPDGGSGG GSNHLRSEKLTFNSGNRPSG 11024 (SEQ ID NO: 376) (SEQ ID NO: 377) 177 TPP- CQS VVF 10353 178 TPP- CGSGSGSGPDGGSGG GSGSGSGSGF 10780 (SEQ ID NO: 378) (SEQ ID NO: 379) 179 TPP- CQSYDILPIEPDGGSGG GSRFDYDGVVF 10786 (SEQ ID NO: 380) (SEQ ID NO: 381) 180 TPP- CGSGSGSGSGPDGGSGG GSGSGSGSGSGF 10779 (SEQ ID NO: 382) (SEQ ID NO: 383) 181 TPP- CGSGSGSGSGSGGGSGG GSGSGSGSGSGSGF 10778 (SEQ ID NO: 384) (SEQ ID NO: 385) 182 TPP- CQSYDKLELGHPDGGSGG GSHLRSEKGVVF 10783 (SEQ ID NO: 386) (SEQ ID NO: 387) 183 TPP- CQSYDILPIEVYPDGGSGG GSKVRFDYDGVVF 10785 (SEQ ID NO: 388) (SEQ ID NO: 389) 184 TPP- CGSGSGSGSGSGPDGGSGG GSGSGSGSGSGSGF 10776 (SEQ ID NO: 390) (SEQ ID NO: 385) 185 TPP- CGSGSGSGSGSGSDGGSGG GSGSGSGSGSGSGF 10777 (SEQ ID NO: 391) (SEQ ID NO: 385) 186 TPP- CQSYDGFILPIEVYGGSGG GSKVRFDYDLFGVVF 10784 (SEQ ID NO: 392) (SEQ ID NO: 393) 187 TPP- CQSYDKLELGHRAPDGGSGG GSVNHLRSEKGVVF 10782 (SEQ ID NO: 394) (SEQ ID NO: 395) 188 TPP- CQVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVVF 10352 (SEQ ID NO: 396) (SEQ ID NO: 397) 189 TPP- CGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGF 10356 (SEQ ID NO: 398) (SEQ ID NO: 399) 190 TPP- CQSYDPNPNPNPNPDGGSGG GSNPNPNPNPSGVVF 10354 (SEQ ID NO: 400) (SEQ ID NO: 401) 191 TPP- CAAWNPNPNPNPNPNGGSGG GSNPNPNPNPNPNVF 10355 (SEQ ID NO: 402) (SEQ ID NO: 403) 192 TPP- CQSYDQVKLELGHRAGGSGG GSVNHLRSEKLTGVVF 10781 (SEQ ID NO: 404) (SEQ ID NO: 405) 193 TPP- CQSVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVVF 10436 (SEQ ID NO: 406) (SEQ ID NO: 397) 194 TPP- CQSYDQVKLELGHRPDGGSGG GSNHLRSEKLTGVVF 10440 (SEQ ID NO: 407) (SEQ ID NO: 408) 195 TPP- CQSYDGFILPIEVYPDGGSGG GSKVRFDYDLFGVVF 10442 (SEQ ID NO: 409) (SEQ ID NO: 393) 196 TPP- CTSVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVDVF 10351 (SEQ ID NO: 268) (SEQ ID NO: 410) 197 TPP- CQSVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVVVF 10348 (SEQ ID NO: 406) (SEQ ID NO: 411) 198 TPP- CGGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGGF 10358 (SEQ ID NO: 412) (SEQ ID NO: 413) 199 TPP- CQCQSYDSSALLKSKLRALLTAPR GSVNHLRSEKLTGVVF 11167 (SEQ ID NO: 414) (SEQ ID NO: 405) 200 TPP- CQSAVQVKLELGHRAPDGGSGG GSVNHLRSEKLTFNVF 10438 (SEQ ID NO: 415) (SEQ ID NO: 416) 201 TPP- CQSYDQVKLELGHRAPDGGSGG GSVNHLRSEKLTGVVF 10439 (SEQ ID NO: 417) (SEQ ID NO: 405) 202 TPP- CQSYDGFILPIEVYFPDGGSGG GSRKVRFDYDLFGVVF 10441 (SEQ ID NO: 418) (SEQ ID NO: 419) 203 TPP- CQSYVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVGVVF 10349 (SEQ ID NO: 420) (SEQ ID NO: 421) 204 TPP- CQSYDVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVSGVVF 10350 (SEQ ID NO: 422) (SEQ ID NO: 423) 205 TPP- CQSYDAVQVKLELGHRAPDGGSGG

GSVNHLRSEKLTFNGVVF 10437 (SEQ ID NO: 424) (SEQ ID NO: 425) 206 TPP-CQCQSYDSSDRSALLKSKLRALLTAPR GSGGSVNHLRSEKLTGVVF 11166 (SEQ ID NO: 426) (SEQ ID NO: 427) 207 TPP- CQSYDSSDRSALLKSKLRALLTAPR GSDRSALLKSKLRALLTAVVF 10362 (SEQ ID NO: 426) (SEQ ID NO: 428) 208 TPP- CQSYDSSDRSALLKSKLRALLTAPE GSDRSALLKSKLRALLTAVVF 10363 (SEQ ID NO: 429) (SEQ ID NO: 428) 209 TPP- 5657 .sup.3The N-terminal sequence corresponds to the nearest neighboring reference aa N-terminal from the inserted natriuretic peptide plus the amino acid stretch present between said reference aa and the first amino acid residue of the inserted natriuretic peptide .sup.4The C-terminal sequence corresponds the amino acid stretch present between the last amino acid residue of the inserted natriuretic peptide and the nearest neighboring reference aa C-terminal from the inserted natriuretic peptide plus and said reference aa

(69) In addition, the following BNP engrafted human IgG1 antibody constructs were generated starting from the antibody scaffold TPP-5657.

(70) TABLE-US-00008 TABLE 5 Design of BNP engrafted antibody constructs # aa # aa Corresp. Insertion N- C- ANP Cmpd TPP Site term.sup.1 term.sup.2 BNP Cpd.sup.3 B1 TPP- CTSVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVDVWGQG 18 Hum28aa #117 9902 (SEQ ID NO: 268) (SEQ ID NO: 252) B2 TPP- CTSVHQETKKYQSSPDGGSGGSG GGSYSYTYNYEWHVDVWGQG 19 Hum25aa 11153 (SEQ ID NO: 430) (SEQ ID NO: 431) B3 TPP- CTSVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVDVWGQG 18 Rat28aa #117 11154 (SEQ ID NO: 268) (SEQ ID NO: 252) B4 TPP- CTSVHQETKKYQSSPDGG SYSYTYNYEWHVDVWGQG 17 Rat32aa 11155 (SEQ ID NO: 259) (SEQ ID NO: 432) B5 TPP- ISGSVVVTSHQAPGGGSGG GEKKKLKSLAYGST 13 Hum28aa #65 11156 (SEQ ID NO: 189) (SEQ ID NO: 190) B6 TPP- ISGSVVVTSHQAPGGGSGG GEKKKLKSLAYGST 13 Rat28aa #65 11157 (SEQ ID NO: 189) (SEQ ID NO: 190) B7 TPP- SGFTFGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGM 14 Hum28aa #9 18029 (SEQ ID NO: 105) (SEQ ID NO: 106) B8 TPP- ISGS GGST 3 Hum28aa #12 18031 (SEQ ID NO: 109) (SEQ ID NO: 110) B9 TPP- ISGSGSGS GSGSGGST 7 Hum28aa #13 18032 (SEQ ID NO: 111) (SEQ ID NO: 112) B10 TPP- ISGSTYISNVNHKPDGGSGG GSNTKVDKKVEGGST 14 Hum28aa #80 18033 (SEQ ID NO: 211) (SEQ ID NO: 212) B11 TPP- ISGPNPNPNPNPNSPDGGSGG GSYNPNPNPNPNPGST 15 Hum28aa #94 18028 (SEQ ID NO: 236) (SEQ ID NO: 237) B12 TPP- CAKGITGTKKYQSSPDGGSGG GSYSYTYNYAEYFQHWGQG 18 Hum28aa #122 18034 (SEQ ID NO: 281) (SEQ ID NO: 282) B13 TPP- CAAWNPNPNPNPNPNGGSGG GSNPNPNPNPNPNVF 14 Hum28aa #191 18030 (SEQ ID NO: 402) (SEQ ID NO: 403) Cmpd TPP N-terminal sequence.sup.3 C-terminal sequence.sup.4 B1 TPP- CTSVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVDVWGQG 9902 B2 TPP- CTSVHQETKKYQSSPDGGSGGSG GGSYSYTYNYEWHVDVWGQG 11153 B3 TPP- CTSVHQETKKYQSSPDGGSGG GSYSYTYNYEWHVDVWGQG 11154 B4 TPP- CTSVHQETKKYQSSPDGG SYSYTYNYEWHVDVWGQG 11155 B5 TPP- ISGSVVVTSHQAPGGGSGG GEKKKLKSLAYGST 11156 B6 TPP- ISGSVVVTSHQAPGGGSGG GEKKKLKSLAYGST 11157 B7 TPP- SGFTFGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGM 18029 B8 TPP- ISGS GGST 18031 B9 TPP- ISGSGSGS GSGSGGST 18032 B10 TPP- ISGSTYISNVNHKPDGGSGG GSNTKVDKKVEGGST 18033 B11 TPP- ISGPNPNPNPNPNSPDGGSGG GSYNPNPNPNPNPGST 18028 B12 TPP- CAKGITGTKKYQSSPDGGSGG GSYSYTYNYAEYFQHWGQG 18034 B13 TPP- CAAWNPNPNPNPNPNGGSGG GSNPNPNPNPNPNVF 18030 .sup.1The number of amino acid residues present between the respective N-terminal reference amino acid residue and the first amino acid of the inserted natriuretic peptide; .sup.2The number of amino acid residues present between the last amino acid of

the inserted natriuretic peptide and the respective C-terminal reference amino acid residue .sup.3Corresponding ANP Cpd. refers to an ANP engrafted antibody construct with the same integration locus and comprising the same N-terminal and C-terminal sequence .sup.3The N-terminal sequence corresponds to the nearest neighboring reference aa N-terminal from the inserted natriuretic peptide plus the amino acid stretch present between said reference aa and the first amino acid residue of the inserted natriuretic peptide .sup.4The C-terminal sequence corresponds the amino acid stretch present between the last amino acid residue of the inserted natriuretic peptide and the nearest neighboring reference aa C-terminal from the inserted natriuretic peptide plus and said reference aa

(71) In addition, the following CNP engrafted human IgG1 antibody constructs were generated.

(72) TABLE-US-00009 TABLE 6 Design of CNP engrafted antibody constructs SEQ ID NO SEQ ID NO # aa # aa % Corresp. Insertion comprised comprised N- C- µg/ml purity ANP Cmpd TPP Site in Ntls in Ctls term.sup.1 term.sup.2 pcs pcs Cpd.sup.3 C1 TPP-9465 CDRH3 7 8 21 23 69-242 97 C2 TPP-12374 CDRL3 11 12 20 20 212 C3 TPP-12375 CDRH3 7 8 21 23 6 C4 TPP-12376 CDRH3 7 8 20 22 7 C5 TPP-12377 CDRH3 8 21 23 7 C6 TPP-12378 CDRH2 9 10 19 18 3 C13 TPP-18036 CDRH1 23 14 259 100 #9 C14 TPP-18038 CDRH2 3 3 0 #12 C15 TPP-18039 CDRH2 7 7 301 100 #13 C16 TPP-18040 CDRH2 2 3 19 14 300 100 #80 C17 TPP-18035 CDRH2 20 15 243 100 #94 C18 TPP-18041 CDRH3 21 22 20 18 287 100 #122 C19 TPP-18037 CDRL3 19 14 246 100 #191 Cmpds based on TPP-12377: Difference vs. TPP-12377 C7 TPP-12895 LC_G99E C8 TPP-12896 LC_G99L 101-131 C9 TPP-12897 LC_S98D 198-304 C10 TPP-12898 LC_S98G 147-258 C11 TPP-12899 LC_A33Y 165-197 82 C12 TPP-12900 LC_A33E 109-195 .sup.1The number of amino acid residues present between the respective N-terminal reference amino acid residue and the first amino acid of the inserted natriuretic peptide; .sup.2The number of amino acid residues present between the last amino acid of the inserted natriuretic peptide and the respective C-terminal reference amino acid residue .sup.3Corresponding ANP Cpd. refers to an ANP engrafted antibody construct with the same integration locus and comprising the same N-terminal and C-terminal sequence

(73) TABLE-US-00010 Table 6 cont.: Design of CNP engrafted antibody constructs Cmpd TPP N-terminal sequence.sup.3 C-terminal sequence.sup.4 C1 TPP-9465 (SEQ ID NO: 433) CTSVHQETKKYQSSPDGGSGGS (SEQ ID NO: 434) GSGGYGSYSYTYNYEWHVDVWGQG C2 TPP-12374 (SEQ ID NO: 435) CQSYDQVKLELGHRAGGSGGS (SEQ ID NO: 436) GSGGSGSVNHLRSEKLTGVVF C3 TPP-12375 (SEQ ID NO: 433) CTSVHQETKKYQSSPDGGSGGS (SEQ ID NO: 437) GSGGSGSYSYTYNYEWHVDVWGQG C4 TPP-12376 (SEQ ID NO: 268) CTSVHQETKKYQSSPDGGSGG (SEQ ID NO: 438) GGGSGSYSYTYNYEWHVDVWGQG C5 TPP-12377 (SEQ ID NO: 439) CTSVHQETKKYQSSPYKGANKK (SEQ ID NO: 437) GSGGSGSYSYTYNYEWHVDVWGQG C6 TPP-12378 (SEQ ID NO: 440) ISGSVVVTSHQAPGGGSGGS (SEQ ID NO: 441) GSGGSGEKKKLKSLAYGST .sup.3The N-terminal sequence corresponds to the nearest neighboring reference aa N-terminal from the inserted natriuretic peptide plus the amino acid stretch present between said reference aa and the first amino acid residue of the inserted natriuretic peptide of the inserted natriuretic peptide and the nearest .sup.4The C-terminal sequence corresponds the amino acid stretch present between the last amino acid residue neighboring reference aa C-terminal from the inserted natriuretic peptide plus and said reference aa

Example 5: In Vitro Activities of Generated Constructs

(74) All constructs were expressed transiently in HEK293 cells according to well-known methods in the art, targeting a cell density of about 2×10{circumflex over ( )}6 cells/ml, a total DNA concentration of about 1 µg/ml for the two plasmids encoding the light and heavy chain and a 5 day incubation for the expression.

(75) Raw compound samples (rcs) were expressed in a culture volume of 0.4 ml, and the

supernatant separated by centrifugation was directly used for testing. The compound concentration was assessed by an IgG-Fc quantification ELISA according to well-known methods in the art. Briefly, 1:1500 diluted supernatant and a 2-fold dilution series of Human Reference Serum (Bethyl, RS-110-4) starting with 400 ng/ml were immobilized in black Maxisorp 384 micro titer plates (MTP) coated with anti-human Fc [Sigma 12136] in a 1:440 dilution in 1× coating buffer (Candor, 121125) for 1 h, 37° C. After blocking with 100% SMART Block (Candor, 113125) anti-human Fc-HRP [Sigma, A0170] was applied in a 1:10000 dilution for the detection of antibodies in rcs and reference samples. Dose curves of the reference sample were used for the quantitative assessment of compound concentrations shown in Tables 7 and 8, column “µg/ml rcs”. All samples were applied in quadruplets.

(76) Isolated compound samples (ics) were generated by 1-step purification via protein-A from 6 ml expression culture and according to well-known methods in the art. Acid eluates were neutralized by addition of 8% (v/v) 1M Tris/HCl pH 9.0, quantified via absorption at 280 nm and normalized to a concentration of 125 nM.

(77) Purified compound samples (pcs) were generated by 2-step purification via protein-A and subsequent SEC in PBS buffer from expression culture of at least 35 ml. Values shown in Tables 7 and 8, column “µg/ml pcs”, refer to the compound concentration in the expression culture supernatant determined by analytical Protein A chromatography.

(78) All activities shown in Tables 7 and 8 were measured on cells with heterologous over expression of human NPRA (hNPRA) by use of a cGMP quantification assay conducted according to manufacturer's instructions (cisbio; 62GM2PEH). In brief, the assay quantifies cGMP in buffered solution or cell-culture supernatants based on the competition between cGMP produced by the cell as result of the NPRA stimulation through the (natriuretic peptide) sample and d2 labelled cGMP for binding to a Cryptate labelled antibody. Sample cGMP and d2 labeled cGMP compete for binding to a limited number of sites on Cryptate labeled anti-cGMP antibodies, and consequently, HTRF® specific fluorescent signal (i.e. energy transfer) is inversely proportional to the concentration of cGMP in the sample.

(79) Dose-response curve data were analyzed with GraphPad Prism (version 7.00 for Windows, GraphPad Software, La Jolla California USA) and EC50 were fitted according to Y=Bottom+(Top-Bottom)/(1+10{circumflex over ( )}((LogEC50−X)*HillSlope)) applying constraints for bottom, top and slope (shared value for all data sets of the respective experiment).

(80) The raw compound sample activity on stable hNPRA-CHO k1 cells was first assessed by comparison to the negative control TPP-5657 and to a positive sample, in particular TPP-5661. Controls and rcs were tested in quadruplets in two concentrations with a relative dilution factor of 5 aiming for a fluorescent signal (s) in the dynamic range of the assay. The assay window was defined as the difference in signal of inactive (max. signal, s_max) and highly active samples (min. signal, s_min), and for both compound concentrations the activity in % was calculated as 100* (s_max−s)/(s_max−s_min). Values listed in Table 7, columns “activity rcs” and “stdev activity rcs”, represent the average of the results for the two concentrations and the respective standard deviation. Rcs signals less than half of the signal of the reference compound TPP-5661 (36%) were assessed as not active (n.a.).

(81) The activity of several raw compound samples on stable hNPRA-CHO k1 cells was reassessed by comparison to reference sample TPP-5661 in 2.5-fold dilution series (8 concentrations) starting with a 5-fold dilution. The “log EC50” fit value as activity measure of the rcs was set in relation to the corresponding value of the reference rcs TPP-5661 by calculating the delta “log EC50”_compound−“log EC50”_TPP-5661; resulting values are listed in Table 7, column “rel. activity rcs”. Notably, the compound concentrations in the rcs was not considered, and consequently given values are influenced by compound activity and concentration in equal measure. All samples were applied in quadruplets.

(82) The activity of isolated compound samples on stable hNPRA-CHO k1 cells was assessed by

EC50 determination and comparison to reference sample TPP-5661. Samples were tested in 2.5-fold dilution series from 80 nM to 0.13 nM. The log EC50 fit value as activity measure of the ics was set in relation to the corresponding value of the reference ics TPP-5661 (−8.8) by calculating the delta log EC50_compound−log EC50_TPP-5661; resulting values are listed in Table 7, column “rel. log EC50 ics”. All samples were applied in quadruplets.

(83) The activity of purified compound samples on stable hNPRA-CHO k1 cells was assessed in multiple experiments by EC50 determination and comparison to reference sample TPP-5661. Samples were tested in dilution series at least in quadruplets. Values listed in Table 7, columns “rel. log EC50 pcs 1, 3 and 4” result from 5-fold dilution series (>4 concentrations) starting with 20 or 25 nM. Values listed in Table 7, column “rel. log EC50 pcs 2” result from 10-fold dilution series (4 concentrations) starting with 25 nM. Values listed in Table 7, columns “rel. log EC50 pcs 5, 6, 7, 8 and 9” result from 2.5-fold dilution series, with 8 or 12 concentrations starting with 40 to 200 nM, respectively. The log EC50 fit value as activity measure of the pcs was set in relation to the corresponding value of the reference TPP-5661 in the respective experiment (in average −9.2, standard deviation 0.5) by calculating the delta log EC50_compound−log EC50_TPP−5661.

(84) The activity of purified compound samples on transient hNPRA-HEK293 cells was assessed in three experiments by EC50 determination and comparison to reference sample TPP-5661. Samples were tested in 5-fold dilution series from 1000 nM to 0.013 nM. The log EC50 fit value as activity measure of the pcs was set in relation to the corresponding value of the reference TPP-5661 in the respective experiment (average of three experiments −9.0, standard deviation 0.4) by calculating the delta log EC50_compound−log EC50_TPP-5661; resulting values are listed in Table 7, columns “rel. log EC50*pcs”. All samples were applied in duplicates.

(85) A summarized qualitative assessment of the activity of compounds is given in the column “qualit. activity”, values listed in column “average rel. log EC50” were calculated as average of given rel. log EC50 values. Compounds showing an EC50 values>50-fold of the reference compound TPP-5661, meaning average rel. log EC50>1.7, were qualitatively assessed as not active (“−”), compounds showing a relative log EC50 between 0.7 and 1.7 were assessed as active (“+”), compounds showing a relative log EC50<0.7 were assessed as very active (“++”); highest activities were observed at relative log EC50 below −0.7 (“+++”). Compounds showing an activity in rcs without confirmation as ics or pcs are marked with an unified “y”, and compounds assessed as not active (“n.a.” in column “activity rcs”) probably due to their very low expression level (≤1 µg/ml, “n.e.” in column “µg/ml rcs”) are marked accordingly.

(86) TABLE-US-00011 TABLE 7 Expression levels and activities of atrial natriuretic peptide engrafted antibody constructs # # stdev rel. rel. rel. rel. rel. rel. rel. rel. rel. rel. rel. rel. rel. rel. rel. rel. average aa aa % activ- activ- activ- log- log- log- log- log- log- log- log- log- log- log- log- log- rel N- C- µg/ml µg/ml purity ity ity ity EC50 EC50 EC50 EC50 EC50 EC50 EC50 EC50 EC50 EC50* EC50* EC50* qualit. log- Compound term.sup.1 term.sup.2 rcs pcs pcs rcs rcs res ics pcs 1 pcs 2 pcs 3 pcs 4 pcs 5 pcs 6 pcs 7 pcs 8 pcs 9 pcs pcs pcs activity EC50 #1 6 2 n.d. 225 93 n.d. n.a. n.a. n.a. − n.a. #2 9 5 n.d. 179 96 n.d. n.a. n.a. n.a. − n.a. #3 12 8 n.d. 196 97 n.d. 0.2 0.0 0.0 ++ 0.1 #4 15 10 n.d. 214 96 n.d. −0.7 −0.8 −0.8 ++ −0.8 #5 18 10 n.d. 229 93 n.d. −0.7 −0.4 −0.4 −0.5 ++ −0.5 #6 19 17 1.6 150 76 33% 18% y #7 19 17 n.e 117 70 22% 20% y #9 23 14 4.8 226 97 41% 27% −0.2 0.0 −0.5 −0.6 −0.4 ++ −0.3 #10 23 14 n.d. 265 96 n.d. 0.9 0.2 0.1 0.0 ++ 0.3 #12 3 3 42.0 n.d. n.d. n.a. − #13 7 7 12.0 n.d. n.d. n.a. n.a. − n.a. #14 8 9 14.0 n.d. n.d. n.a. n.a. − n.a. #15 10 11 6.6 n.d. n.d. n.a. n.a. − n.a. #16 11 14 9.8 n.d. n.d. n.a. − #17 12 8 7.2 n.d. n.d. 53% 3% y #18 13 12 12.0 290 98 n.a. −0.2 0.9 + 0.9 #19 14 9 9.5 n.d. n.d. 48% 11% y #20 14 10 9.9 n.d. n.d. 64% 3% y #21 14 11 5.8 n.d. n.d. 38% 18% 0.7 ++ 0.7 #22 14 14 23.0 n.d. n.d. 75% 9% y #23 15 10 2.6 n.d. n.d. 60% 35% y #24 15 12 n.e 83 52 26% 22% 0.6 ++ 0.6 #25 15 14 14.0 n.d. n.d. 25% 5% −1.0 0.8 + 0.8 #26 16 11 2.0 181 98 n.a. 1.9 − #27 16 11 17.0 n.d. n.d. 37% 23% 1.4 + 1.4 #28 16 11 9.1 n.d. n.d. 50% 9% −0.9 0.5 ++ 0.5 #29 16 14 7.0 n.d. n.d. n.a. − #30 16 15 35.0 n.d. n.d. 88% 13% y #33 17 11 2.3 229 79 73% 32% −0.9

−0.1 −1.1 −0.7 −0.9 −0.5 −0.8 ++ −0.7 #34 17 11 n.d. 226 87 n.d. −0.2 ++ −0.2 #35 17 11 n.d. 215 86 n.d. −0.6 + −0.6 #36 17 11 n.d. 217 86 n.d. −0.1 ++ −0.1 #37 17 11 n.d. 244 86 n.d. −0.8 +++ −0.8 #38 17 11 n.d. 233 88 n.d. −0.5 ++ −0.5 #39 17 11 n.d. 208 86 n.d. −0.6 ++ −0.6 #40 17 11 n.d. 154 85 n.d. −0.5 −0.3 −0.6 + −0.5 #41 17 11 n.d. 106 79 n.d. n.d. #42 17 11 n.d. 112 97 n.d. −0.6 −0.7 −0.7 ++ −0.7 #43 17 11 n.d. 141 96 n.d. −0.6 −0.8 −0.6 + −0.7 #44 17 11 n.d. 166 93 n.d. −0.7 −0.8 −0.8 +++ −0.8 #45 17 11 n.d. 135 90 n.d. −0.4 −0.2 −0.5 ++ −0.4 #46 17 11 n.d. 166 92 n.d. −0.5 −0.4 −0.6 ++ −0.5 #47 17 11 n.d. 123 83 n.d. −0.3 −0.3 −0.7 ++ −0.4 #49 17 11 n.d. 203 84 n.d. −0.6 −0.2 −0.1 −0.6 ++ −0.4 #50 17 12 9.0 87 97 66% 21% −1.0 0.0 −0.4 ++ −0.2 #51 17 12 6.4 n.d. n.d. 44% 39% y #52 17 12 7.6 n.d. n.d. 26% 20% y #53 17 13 n.d. n.d. n.d. 69% 5% y #54 17 14 n.e 75 n.d. n.a. n.e #55 17 14 8.8 271 95 72% 40% −0.9 −0.9 −0.4 0.2 ++ −0.4 #57 17 17 13.0 n.d. n.d. 48% 1% y #58 17 17 33.0 n.d. n.d. 45% 9% y #59 17 17 31.0 n.d. n.d. 87% 4% −1.1 0.0 ++ 0.0 #60 18 12 4.1 367 90 53% 31% 0.0 −0.5 ++ −0.3 #61 18 13 n.e 334 97 n.a. 0.0 −0.9 ++ −0.5 #62 18 13 11.0 n.d. n.d. 36% 14% 1.5 + 1.5 #63 18 13 9.5 239 96 70% 22% −0.1 ++ −0.1 #64 18 13 5.3 n.d. n.d. 51% 18% y #65 18 13 1.2 253 86 69% 17% −0.5 −0.3 −1.2 −0.9 +++ −0.7 #66 18 13 38.0 n.d. n.d. 87% 4% −0.5 0.2 ++ 0.2 #67 18 13 4.8 n.d. n.d. 35% 17% −1.1 0.7 ++ 0.7 #68 18 13 9.2 n.d. n.d. 48% 13% y #69 18 14 7.1 n.d. n.d. 66% 11% −1.0 0.3 ++ 0.3 #70 18 17 n.e n.d. n.d. 30% 2% y #71 18 17 27.0 n.d. n.d. 89% 2% −0.5 −0.6 ++ −0.6 #72 19 12 11.0 n.d. n.d. 82% 0% −1.5 0.1 ++ 0.1 #73 19 14 9.4 n.d. n.d. 71% 0% −1.4 −0.2 ++ −0.2 #74 19 14 3.8 142 97 67% 7% −1.0 0.0 0.3 ++ 0.2 #75 19 14 4.4 50 49 95% 8% −0.4 ++ −0.4 #76 19 14 11.0 299 97 56% 2% 0.2 −0.8 ++ −0.3 #77 19 14 13.0 n.d. n.d. 38% 6% 1.1 + 1.1 #78 19 14 14.0 n.d. n.d. 55% 38% 1.0 + 1.0 #79 19 14 5.5 n.d. n.d. 67% 26% y #80 19 14 5.4 214 95 67% 19% −0.4 −0.8 ++ −0.6 #81 19 14 6.2 n.d. n.d. 64% 10% y #82 19 14 1.6 n.d. n.d. 57% 9% y #83 19 14 n.e n.d. n.d. 22% 40% y #84 19 14 11.0 n.d. n.d. 45% 30% y #85 19 15 4.5 n.d. n.d. 74% 27% −1.2 −0.9 +++ −0.9 #87 19 15 42.0 n.d. n.d. 90% 13% y #89 20 15 n.e 85 n.d. n.a. n.e #90 20 15 n.e 68 50 52% 18% 0.9 0.5 0.4 ++ 0.6 #91 20 15 3.4 275 99 30% 26% −0.3 0.3 −0.2 0.1 0.2 ++ 0.1 #92 20 15 2.3 n.d. n.d. n.a. −0.3 0.6 ++ 0.6 #93 20 15 4.1 286 98 70% 4% −0.8 −0.8 +++ −0.8 #94 20 15 12.0 318 98 83% 17% −0.8 −0.8 +++ −0.8 #95 20 15 38.0 n.d. n.d. 81% 16% y #96 20 15 7.3 n.d. n.d. n.a. − #97 20 15 8.3 n.d. n.d. 26% 17% y #98 20 15 7.7 n.d. n.d. 40% 14% y #99 10 3 44.0 257 100 n.a. n.a. n.a. − n.a. #100 10 8 88.0 188 99 n.a. n.a. n.a. − n.a. #101 12 10 83.0 n.d. n.d. n.a. − #102 14 12 16.0 n.d. n.d. 20% 7% y #103 15 18 7.7 101 85 50% 8% 0.7 −0.1 ++ 0.3 #104 16 14 15.0 137 98 n.a. 1.0 2.3 ? 1.7 #106 17 15 30.0 n.d. n.d. 20% 2% y #107 17 18 8.8 n.d. n.d. 50% 19% y #108 18 16 37.0 n.d. n.d. 68% 7% y #109 18 18 4.6 n.d. n.d. 62% 20% y #110 19 17 34.0 238 100 45% 25% −0.4 1.1 ++ 0.4 #111 19 17 41.0 296 98 80% 3% −0.4 0.2 ++ −0.1 #112 19 18 3.9 n.d. n.d. 31% 30% y #113 20 17 44.0 n.d. n.d. 52% 2% y #114 20 17 26.0 219 76 94% 4% 0.5 0.1 ++ 0.3 #116 20 18 9.4 247 84 71% 11% −0.3 −0.2 0.0 −0.2 0.0 −0.1 ++ −0.1 #117 20 18 12.9 238 92 36% 30% 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 + 0.0 #118 18 16 n.d. 191 n.d. n.d. −0.1 ++ −0.1 #119 18 16 n.d. 171 n.d. n.d. −0.2 ++ −0.2 #120 20 18 n.d. 200 n.d. n.d. −0.3 ++ −0.3 #121 20 18 n.d. 253 n.d. n.d. −0.2 ++ −0.2 #122 20 18 n.d. 115 n.d. n.d. −0.3 ++ −0.3 #123 20 18 n.d. 153 n.d. n.d. −0.1 ++ −0.1 #124 20 18 41.0 n.d. n.d. 77% 23% y #125 20 18 18.0 320 99 84% 16% −0.8 −0.1 ++ −0.5 #126 20 18 17.0 203 90 80% 2% y #127 20 18 35.0 119 92 95% 2% 0.5 −0.7 −0.5 −0.5 −0.5 ++ −0.3 #128 20 18 64.0 291 96 93% 9% y #129 20 19 28.0 n.d. n.d. 78% 12% y #130 21 19 33.0 n.d. n.d. 89% 5% y #132 22 20 7.5 n.d. n.d. 68% 20% y #133 22 20 20.0 n.d. n.d. 75% 14% y #134 22 20 9.7 n.d. n.d. 75% 3% y #135 22 20 30.0 158 88 67% 9% 0.1 3.5 ? 1.8 #136 24 22 15.0 n.d. n.d. 65% 13% y #137 24 22 61.0 n.d. n.d. 81% 23% y #139 19 13 n.e n.d. n.d. n.a. n.e #145 23 14 35.0 n.d. n.d. 73% 11% y #146 1 6 29.0 n.d. n.d. n.a. − #147 5 10 6.7 n.d. n.d. n.a. 0.8 + #151 14 14 13.0 n.d. n.d. 64% 23% −1.3 −0.4 ++ −0.4 #153 16 15 1.5 n.d. n.d. 40% 24% y #154 16 15 n.e n.d. n.d. 22% 21% y #155 16 16 7.8 n.d. n.d. 57% 17% −1.3 −0.7 ++ −0.7 #156 17 16 2.0 64 98 57% 33% −1.3 ++ #157 17 16 2.4 n.d. n.d. 23% 11% y #158 17 16 10.0 n.d. n.d. n.a. −0.6 ++ #159 17 16 4.9 n.d. n.d. 55% 22% −1.3 ++ #160 17 16 2.8 n.d. n.d. 34% 22% y #161

17 16 4.9 n.d. n.d. 34% 9% −0.7 ++ #162 17 16 n.e n.d. n.d. n.a. n.e #163 17 17 n.d. 84 85 n.d. 0.6 ++ 0.6 #164 18 16 3.0 n.d. n.d. n.a. −0.8 −0.1 ++ −0.1 #165 18 17 3.5 n.d. n.d. 53% 28% y #166 18 17 4.1 n.d. n.d. 20% 34% y #167 18 17 n.e 48 n.d. 44% 13% y #168 18 17 n.e n.d. n.d. n.a. n.e #169 19 17 2.8 78 99 20% 16% −0.1 −0.1 ++ −0.1 #170 19 18 n.e n.d. n.d. n.a. n.e #171 19 18 n.e n.d. n.d. n.a. n.e #172 19 18 n.e n.d. n.d. n.a. n.e #173 19 18 n.e n.d. n.d. n.a. n.e #174 19 19 n.d. 73 n.d. n.d. 1.3 + 1.3 #175 19 19 n.d. 105 82 n.d. 0.3 0.1 ++ 0.2 #176 20 19 n.e n.d. n.d. n.a. n.e #177 2 2 41.0 262 100 n.a. n.a. n.a. − n.a. #178 14 9 16.0 n.d. n.d. 67% 33% y #179 16 10 51.0 n.d. n.d. n.a. − #180 16 11 18.0 n.d. n.d. 37% 6% y #181 16 13 21.0 n.d. n.d. 79% 7% y #182 17 11 2.5 n.d. n.d. 73% 19% y #183 18 12 36.0 n.d. n.d. 21% 16% y #184 18 13 10.0 100 100 50% 3% y #185 18 13 17.0 81 100 72% 1% y #186 18 14 20.0 77 96 45% 22% y #187 19 13 35.0 217 95 85% 10% −1.1 +++ −1.1 #188 19 14 15.0 n.d. n.d. 77% 8% y #189 19 14 16.0 102 99 59% 4% y #190 19 14 41.0 n.d. n.d. 64% 13% y #191 19 14 29.0 248 99 94% 8% 0.1 −0.6 0.2 −0.3 ++ −0.2 #192 19 15 70.0 n.d. n.d. 90% 14% −1.0 −1.0 +++ −1.0 #193 20 14 2.7 n.d. n.d. 33% 37% y #194 20 14 32.0 98 97 75% 3% y #195 20 14 20.0 43 97 34% 12% y #196 20 15 n.e n.d. n.d. n.a. n.e #197 20 15 5.5 18 n.d. 54% 20% y #198 20 15 14.0 57 100 52% 21% y #199 21 15 7.3 n.d. n.d. 29% 12% y #200 21 15 33.0 97 n.d. 78% 7% y #201 21 15 51.0 134 97 87% 6% −0.3 0.1 −0.3 ++ −0.1 #202 21 15 16.0 29 n.d. 58% 3% y #203 21 16 3.1 17 n.d. 33% 2% y #204 22 17 5.7 20 n.d. 45% 17% y #205 23 17 34.0 n.d. n.d. 84% 19% y #206 24 18 11.0 n.d. n.d. 47% 33% y #207 24 20 12.0 115 95 59% 17% n.a. n.a. − n.a. #208 24 20 9.5 182 92 74% 11% 0.6 0.3 ++ 0.5 #209 na na 18.3 402 100 n.a. − Not determined (n.d.), not active (n.a.), not expressed (n.e.), not applicable (na) .sup.1The number of amino acid residues present between the respective N−terminal reference amino acid residue and the first amino acid of the inserted natriuretic peptide; .sup.2The number of amino acid residues present between the last amino acid of the inserted natriuretic peptide and the respective C−terminal reference amino acid residue

(87) No conclusive data for compounds #104 and #135 were obtained. 12 compounds (#54, #61, #89, #139, #162, #168, #170, #171, #172, #173, #176, #196) showed very low expression levels (≤1 μg/ml in rcs) and consequently no activity; activity of #61 was shown after compound preparation (pcs). 7 compounds (#7, #24, #70, #83, #90, #154, #167) showed in most cases low activity as rcs, although their expression level was very low; the activity of #24 and #90 was confirmed by compound preparation (pcs). No activity was observed in rcs of compounds #18, #26, #29, #92, #96, #101, #104, #158, #164, #179; however, the activity of compounds #18, #92, #158, #164 was shown using higher concentrations (rel. activity rcs); the lack of activity of #26 was thereby confirmed.

(88) TABLE-US-00012 TABLE 8 qualit. qualit. # aa # aa % activity activity Com- N- C- μg/ml purity on of corresp. pound TPP term.sup.1 term.sup.2 pcs pcs hNPRA ANP cpd..sup.3 B1 TPP-9902 20 18 304 96 ++ ++ B2 TPP-11153 22 19 202 99 + B3 TPP-11154 20 18 205 100 − ++ B4 TPP-11155 17 17 226 97 − B5 TPP-11156 18 13 115 75 ++ +++ B6 TPP-11157 18 13 125 93 − +++ B7 TPP-18029 23 14 265 99 + ++ B8 TPP-18031 3 3 260 99 − − B9 TPP-18032 7 7 262 99 − − B10 TPP-18033 19 14 310 98 + ++ B11 TPP-18028 20 15 74 99 + +++ B12 TPP-18034 20 18 315 97 ++ ++ B13 TPP-18030 19 14 304 100 + ++ #209 TPP-5657 na na 402 100 − − .sup.1The number of amino acid residues present between the respective N-terminal reference amino acid residue and the first amino acid of the inserted natriuretic peptide; .sup.2The number of amino acid residues present between the last amino acid of the inserted natriuretic peptide and the respective C-terminal reference amino acid residue .sup.3Corresponding ANP Cpd. refers to an ANP engrafted antibody construct with the same integration locus and comprising the same N-terminal and C-terminal sequence

(89) Purified compound samples were tested as described in Example 3 in quadruplets in dilution series on stable hNPRA-CHO k1 cells. The activities of BNP engrafted antibody constructs are graphically depicted in FIG. **11**.

(90) TPP-11156, TPP-9902 and TPP-11153 showed significant activity on hNPRA cells in contrast

to TPP-1154, TPP-11155, and TPP-11157. Opposed results were observed on rNPRA with EC50<20 nM for TPP-1154, TPP-11155, and TPP-11157, and EC50>1 μM for TPP-9902, TPP-11153, and TPP-11156 (see Example 3). This can be explained by the presence of a human BNP sequence in TPP-11156, TPP-9902 and TPP-11153, whereas TPP-1154, TPP-11155, and TPP-11157 comprise a rat BNP sequence (see Table 5). In contrast to all other human BNP engrafted antibody constructs, TPP-18031 and TPP-18032 with low numbers of additional amino acids N- and C-terminal to BNP showed no activity on hNPRA.

Example 6: Specific Linker Sequences are Particularly Advantageous for Achieving Good Homogeneity and Expression Levels

(91) The purity of purified compound samples (Example 5, Table 7, column “% purity pcs”) was determined by capillary Gel Electrophoresis according to manufacturer's instructions (LabChip GX, Caliper Life Sciences) under reduced conditions. The purity in % was calculated as sum of peak areas corresponding to the intact light and heavy chain relative to the sum of all peaks observed.

(92) Ntls sequences comprising a GS linker sequence, a PN linker sequence or the sequence of SEQ ID NOs 2, 4, 9, 11, 13 or 15 and Ctls sequences comprising a GS linker sequence, a PN linker sequence or the sequence of SEQ ID NOs 3, 5, 12, 14, 15 or 20 have proven particularly useful as they not only achieve high natriuretic peptide activities (provided that at least 12 amino acid residues are present between the respective N-terminal reference amino acid residue and the first amino acid of the inserted natriuretic peptide) but also good expression levels (in contrast to e.g., sequences used in compounds #186, #195 and #202) and (in contrast to e.g., sequences used in compounds 6 and 7) a low degree of inhomogeneity (see Table 9). With linker sequences comprising a GS linker sequence as well as linker sequences comprising a PN linker sequence very good purities of 98% in average were observed. Similarly good values were observed for compounds with linkers comprising sequences of SEQ ID NOs 2, 3, 4, 5, 9, 11, 12, 13, 14, 15 and 20. Notably, the Ntls having the sequence of SEQ ID NO 9 resulted only in combination with the Ctls having the sequence of SEQ ID NO 20 in compounds with very good purity; compounds with a combination of the Ntls having the sequence of SEQ ID NO 11 and the Ctls having the sequence of SEQ ID NO 12 showed only very good purity when SEQ ID NO 11 was flanked by Asp (D) on the N-terminal side and not by Thr (T) or Val (V) and when the SEQ ID NO 12 VNHLRSEKLT was flanked by Gly (G) on the C-terminal side but not by Tyr (Y) or Phe (F) as in compounds #126 and #135.

(93) TABLE-US-00013 TABLE 9 Ntls and Ctls effects on antibody purity (excerpt of Table 7) Inser- SEQ ID NO SEQ ID NO tion comprised comprised # aa # aa Cpd TPP Site in Ntls in Ctls N-term.sup.1 C-term.sup.2 2 TPP- CDRH1 GS GS 9 5 13056 3 TPP- CDRH1 GS GS 12 8 13055 4 TPP- CDRH1 GS GS 15 10 13054 5 TPP- CDRH1 GS GS 18 10 12545 9 TPP- CDRH1 GS GS 23 14 10294 10 TPP- CDRH1 GS GS 23 14 12547 91 TPP- CDRH2 GS GS 20 15 10282 99 TPP- CDRH3 GS GS 10 3 10281 100 TPP- CDRH3 GS GS 10 8 10280 169 TPP- CDRL2 GS GS 19 17 10361 184 TPP- CDRL3 GS GS 18 13 10776 185 TPP- CDRL3 GS GS 18 13 10777 189 TPP- CDRL3 GS GS 19 14 10356 198 TPP- CDRL3 GS GS 20 15 10358 18 TPP- CDRH2 PN PN 13 12 10767 55 TPP- CDRH2 PN PN 17 14 10765 93 TPP- CDRH2 PN PN 20 15 10286 94 TPP- CDRH2 PN PN 20 15 10283 111 TPP- CDRH3 PN PN 19 17 10289 125 TPP- CDRH3 PN PN 20 18 10290 191 TPP- CDRL3 PN PN 19 14 10355 63 TPP- CDRH2 2 3 18 13 10844 80 TPP- CDRH2 2 3 19 14 10845 128 TPP- CDRH3 2 3 20 18 10288 61 TPP- CDRH2 4 5 18 13 10847 76 TPP- CDRH2 4 5 19 14 10848 156 TPP- CDRL2 15 15 17 16 10571 42 TPP- CDRH2 9 20 17 11 13060 43 TPP- CDRH2 9 20 17 11 13061 44 TPP- CDRH2 9 20 17 11 13062 46 TPP- CDRH2 9 10 17 11 13064 45 TPP- CDRH2 9 10 17 11 13063 40 TPP- CDRH2 9 10 17 11 13058 47 TPP- CDRH2 9 10 17 11 13065 41 TPP- CDRH2 9 10 17 11 13059 201 TPP- CDRL3 11 12 21 15 10439 194 TPP- CDRL3 11 12 20 14

10440 187 TPP- CDRL3 11 12 19 13 10782 126 TPP- CDRH3 11 12 20 18 10445 135 TPP- CDRH3 11 12 22 20 10444 50 TPP- CDRH2 13 14 17 12 10452 74 TPP- CDRH2 13 14 19 14 10451 26 TPP- CDRH2 13 14 16 11 10772 6 TPP- CDRH1 19 17 10454 7 TPP- CDRH1 19 17 10453 186 TPP- CDRL3 18 14 10784 195 TPP- CDRL3 20 14 10442 202 TPP- CDRL3 21 15 10441 % µg/ml purity qualit. Cpd N-terminal seequence.sup.3 C-terminal sequence.sup.4 pcs pcs actvity 2 SGFTFGSGSG GSGSGM 179 96 - (SEQ ID NO: 93) (SEQ ID NO: 94) 3 SGFTFGSGSGSGS SGSGSGSGM 196 97 ++ (SEQ ID NO: 95) (SEQ ID NO: 96) 4 SGFTFGSGSGSGGSGG GGSGGSGSGSG 214 96 ++ (SEQ ID NO: 97) (SEQ ID NO: 39) 5 SGFTFGSGSGSGSGGGSGG GSGSGGSGSGM 229 93 ++ (SEQ ID NO: 99) (SEQ ID NO: 100) 9 SGFTFGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGM 226 97 ++ (SEQ ID NO: 105) (SEQ ID NO: 106) 10 SGFTFGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGM 265 96 ++ (SEQ ID NO: 105) (SEQ ID NO: 106) 91 ISGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGST 275 99 ++ (SEQ ID NO: 231) (SEQ ID NO: 221) 99 CAKSPDGGSGG GSYG 257 100 - (SEQ ID NO: 244) (SEQ ID NO: 245) 100 CAKSPDGGSGG GSYQHWGQG 188 99 - (SEQ ID NO: 244) (SEQ ID NO: 246) 169 YGSGSGSGSGSGSPDGGSGG GSYGSGGSGSGSGNRPSG 78 99 ++ (SEQ ID NO: 364) (SEQ ID NO: 365) 184 CGSGSGSGSGSGPDGGSGG GSGSGSGSGSGSGF 100 100 y (SEQ ID NO: 390) (SEQ ID NO: 385) 185 CGSGSGSGSGSGSDGGSGG GSGSGSGSGSGSGF 81 100 y (SEQ ID NO: 391) (SEQ ID NO: 385) 189 CGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGF 102 99 y (SEQ ID NO: 398) (SEQ ID NO: 399) 198 CGGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGGF 57 100 y (SEQ ID NO: 412) (SEQ ID NO: 413) 18 ISGPNPNKNPNPGG GSNENPNPNPGST 290 98 + (SEQ ID NO: 121) (SEQ ID NO: 122) 55 ISGPNPNKNPNPNPGSGG GSNPNENPNPNPGST 271 95 ++ (SEQ ID NO: 170) (SEQ ID NO: 134) 93 ISGPNPNKNPNPNSPDGGSGG GSYNPNENPNPNPGST 286 98 +++ (SEQ ID NO: 234) (SEQ ID NO: 235) 94 ISGPNPNPNPNPNSPDGGSGG GSYNPNPNPNPNPGST 318 98 +++ (SEQ ID NO: 236) (SEQ ID NO: 237) 111 CAKVHPNPNPNPNPDGGSGG GSNPNPNPNPHVDVWGQG 296 98 ++ (SEQ ID NO: 265) (SEQ ID NO: 266) 125 CAKVHPNPNPNPNSPDGGSGG GSYNPNPNPNPHVDVWGQG 320 99 ++ (SEQ ID NO: 286) (SEQ ID NO: 287) 191 CAAWNPNPNPNPNPNGGSGG GSNPNPNPNPNPNVF 248 99 ++ (SEQ ID NO: 402) (SEQ ID NO: 403) 63 ISGTYISNVNHKPDGGSGG GSNTKVDKKVEGST 239 96 ++ (SEQ ID NO: 185) (SEQ ID NO: 186) 80 ISGSTYISNVNHKPDGGSGG GSNTKVDKKVEGGST 214 95 ++ (SEQ ID NO: 211) (SEQ ID NO: 212) 128 CAKTQTYISNVNHKPDGGSGG GSNTKVDKKAEYFQHWGQG 291 96 y (SEQ ID NO: 291) (SEQ ID NO: 292) 61 ISGTSASLAITGPDGGSGG GSDRFSGSKSGGST 334 97 ++ (SEQ ID NO: 181) (SEQ ID NO: 182) 76 ISGSTSASLAITGPDGGSGG GSDRFSGSKSGGGST 299 97 ++ (SEQ ID NO: 204) (SEQ ID NO: 205) 156 YGVPKEKEKEKVSTAVGG GSAPLEVPKEKEKEKVG 64 98 ++ (SEQ ID NO: 340) (SEQ ID NO: 341) 42 ISVVVTSHQSPTPGGSGG GGSTPLKSLAST 112 97 ++ (SEQ ID NO: 154) (SEQ ID NO: 155) 43 ISVVVTSHQAPGEGGSGG GGSTPLKSLAST 141 96 ++ (SEQ ID NO: 145) (SEQ ID NO: 155) 44 ISVVVTSHQAPGEGGSGG GSTPKLKSLAST 166 93 +++ (SEQ ID NO: 145) (SEQ ID NO: 156) 46 ISVVVTSHPTPGEGGSGG GEKKKLKSLAST 166 92 ++ (SEQ ID NO: 157) (SEQ ID NO: 146) 45 ISVVVTSHQSPTPGGSGG GEKKKLKSLAST 135 90 ++ (SEQ ID NO: 154) (SEQ ID NO: 146) 40 ISVVVTSHQAPGSGGSGG GEKKKLKSLAST 154 85 ++ (SEQ ID NO: 152) (SEQ ID NO: 146) 47 ISVVVTSHQAPSPGSTGG GEKKKLKSLAST 123 83 ++ (SEQ ID

NO: 158) (SEQ ID NO: 146) 41 ISVVVTSHQAPTSGGSGG GEKKKLKSLAST 106 79 n.d. (SEQ ID NO: 153) (SEQ ID NO: 146) 201 CQSYDQVKLELGHRAPDGGSGG GSVNHLRSEKLTGVVF 134 97 ++ (SEQ ID NO: 417) (SEQ ID NO: 405) 194 CQSYDQVKLELGHRPDGGSGG GSNHLRSEKLTGVVF 98 97 y (SEQ ID NO: 407) (SEQ ID NO: 408) 187 CQSYDKLELGHRAPDGGSGG GSVNHLRSEKGVVF 217 95 +++ (SEQ ID NO: 394) (SEQ ID NO: 395) 126 CAKLTQVKLELGHRPDGGSGG GSNHLRSEKLTYFQHWGQG 203 90 y (SEQ ID NO: 288) (SEQ ID NO: 289) 135 CAKLTAVQVKLELGHRPDGGSGG GSNHLRSEKLTFNYFQHWGQG 158 88 ? (SEQ ID NO: 303) (SEQ ID NO: 299) 50 ISGSAVVNVRAPDGGSGG GSKGDKIAIGGST 87 97 ++ (SEQ ID NO: 160) (SEQ ID NO: 161) 74 ISGSSGAVVNVRAPDGGSGG GSKGDKIAIWTTGST 142 97 ++ (SEQ ID NO: 201) (SEQ ID NO: 202) 26 ISGSAVVNVRADGGSGG GSGDKIAIGGST 181 98 - (SEQ ID NO: 135) (SEQ ID NO: 136) 6 SPAVVYIEILDRHPDGGSGG GSGREVPISNGSGFVVAM 150 76 y (SEQ ID NO: 101) (SEQ ID NO: 102) 7 SGAVVYIEILDRHPDGGSGG GSGREVPISNGSGFVVAM 117 70 y (SEQ ID NO: 103) (SEQ ID NO: 102) 186 CQSYDGFILPIEVYGGSGG GSKVRFDYDLFGVVF 77 96 y (SEQ ID NO: 392) (SEQ ID NO: 393) 195 CQSYDGFILPIEVYPDGGSGG GSKVRFDYDLFGVVF 43 97 y (SEQ ID NO: 409) (SEQ ID NO: 393) 202 CQSYDGFILPIEVYFPDGGSGG GSRKVRFDYDLFGVVF 29 n.d. y (SEQ ID NO: 418) (SEQ ID NO: 419) .sup.1The number of amino acid residues present between the respective N-terminal reference amino acid residue and the first amino acid of the inserted natriuretic peptide; .sup.2The number of amino acid residues present between the last amino acid of the inserted natriuretic peptide and the respective C-terminal reference amino acid residue .sup.3The N-terminal sequence corresponds to the nearest neighboring reference aa N-terminal from the inserted natriuretic peptide plus the amino acid stretch present between said reference aa and the first amino acid residue of the inserted natriuretic peptide .sup.4The C-terminal sequence corresponds the amino acid stretch present between the last amino acid residue of the inserted natriuretic peptide and the nearest neighboring reference aa C-terminal from the inserted natriuretic peptide plus and said reference aa

Example 7: IgG1, IgG2 and IgG4 Isotypes Provide Equally Suitable Antibody Scaffolds

(94) Compounds 9, 33, 65, 91, 127 and 191 (human IgG1 TPP-10294, TPP-10277, TPP-10279, TPP-10282, TPP-10269 and TPP-10355, respectively) were generated as different IgG isotypes. Purified compound samples were tested as described in Example 3 in quadruplets in dilution series on stable hNPRA-CHO k1 cells. The activities of ANP engrafted human IgG2 and IgG4 isotype constructs (e.g. compound 9 IgG4 TPP-10992) are similar to their corresponding IgG1 isotype as graphically depicted in FIG. **12**.

Example 8: Human and Non-Human IgGs Provide Equally Suitable Antibody Scaffolds

(95) Compound 9 (human IgG1 TPP-10294 and IgG4 TPP-10992) and compound 117 (human IgG1 TPP-5665) were generated as non-human IgG isotypes. Purified compound samples were tested as described in Example 3 in quadruplets in dilution series on stable hNPRA-CHO k1 cells. ANP engrafted rat and mouse isotype constructs, e.g. compound 9 rat IgG1 TPP-13992, showed activities similar to their corresponding human IgG isotype (FIG. **13**).

Example 9: Human IgGs Comprising Varying Germline Sequences Provide Equally Suitable Antibody Scaffolds

(96) 22 additional ANP engrafted IgG4 antibodies (compounds A to S) were constructed. In each case ANP was incorporated within CDRH1. The heavy chains of these constructs comprise varying HV and CDRH3 sequences and were paired with varying lambda or kappa light chains. The structure of compounds A to S is summarized in Tables 10 and 11.

(97) TABLE-US-00014 TABLE 10 Design of ANP engrafted antibody constructs A to S Inser- tion # aa #aa Cmpd. Site N-term.sup.1 C-term.sup.2 N-terminal sequence.sup.3 C-terminal sequence.sup.4 A-P CDRH1 23 14 SGFTFGSGSGSGSGSGSPDGGSGG

GSYGSGSGSGSGSGM (SEQ ID NO: 105) (SEQ ID NO: 106) Q-S CDRH1 23 14 SGYSFGSGSGSGSGSGSPDGGSGG GSYGSGSGSGSGSGI (SEQ ID NO: 449) (SEQ ID NO: 450) .sup.1The number of amino acid residues present between the respective N-terminal reference amino acid residue and the first amino acid of the inserted natriuretic peptide; .sup.2The number of amino acid residues present between the last amino acid of the inserted natriuretic peptide and the respective C-terminal reference amino acid residue .sup.3The N-terminal sequence corresponds to the nearest neighboring reference aa N-terminal from the inserted natriuretic peptide plus the amino acid stretch present between said reference aa and the first amino acid residue of the inserted natriuretic peptide .sup.4The C-terminal sequence corresponds the amino acid stretch present between the last amino acid residue of the inserted natriuretic peptide and the nearest neighboring reference aa C-terminal from the inserted natriuretic peptide plus and said reference aa

(98) TABLE-US-00015 TABLE 11 Design of ANP engrafted antibody constructs A to S Cmpd TPP HV CDRH3 LV/KV Purity 9 TPP-10992 HV3-23 KLTGAEYFQHW LV1-40 99 (SEQ ID NO: 451) A TPP-13944 HV3-23 KLTGAEYFQHW LV2-14 98 (SEQ ID NO: 452) B TPP-13945 HV3-23 KLTGAEYFQHW LV3-21 93 (SEQ ID NO: 453) C TPP-13941 HV3-23 KDYGDYAEYFQHW LV1-40 100 (SEQ ID NO: 454) D TPP-13956 HV3-23 KLTGAEYFQHW KV1-5 99 (SEQ ID NO: 455) E TPP-13955 HV3-23 KLTGAEYFQHW KV3-20 99 (SEQ ID NO: 456) F TPP-13940 HV3-23 KVLRFLEWLLYAEYFQHW LV1-40 98 (SEQ ID NO: 457) G TPP-13939 HV3-23 KVQLERAEYFQHW LV1-40 100 (SEQ ID NO: 458) H TPP-13943 HV3-23 KYNRNHAEYFQHW LV1-40 54 (SEQ ID NO: 459) I TPP-13942 HV3-23 KYNWNDAEYFQHW LV1-40 99 (SEQ ID NO: 460) J TPP-14684 HV3-23 RGATFALDW KV3-20 97 (SEQ ID NO: 461) K TPP-13958 HV3-23 RGRLPDVW KV1-5 99 (SEQ ID NO: 462) L TPP-13957 HV3-23 RGRLPDVW KV3-20 97 (SEQ ID NO: 462) M TPP-13948 HV3-23 RGRLPDVW LV1-40 98 (SEQ ID NO: 462) N TPP-14289 HV3-23 RGRLPDVW LV1-47 (SEQ ID NO: 462) O TPP-13946 HV3-23 RGRLPDVW LV2-14 97 (SEQ ID NO: 462) P TPP-13947 HV3-23 RGRLPDVW LV3-21 96 (SEQ ID NO: 462) Q TPP-13952 HV5-51 RGRLPDVW LV2-14 99 (SEQ ID NO: 462) R TPP-13953 HV5-51 RGRLPDVW LV3-21 98 (SEQ ID NO: 462) S TPP-13962 HV5-51 RGRLPDVW KV1-5 99 (SEQ ID NO: 462)

(99) Purified compound samples of IgG scaffold constructs A to S comprising varying germline sequences were tested as described in Example 3 in quadruplets in dilution series on stable hNPRA-CHO k1 cells. Exemplary activity data are graphically depicted in FIG. **14**.

Example 10: CNP Engrafted IgG Protects Against Induced Endothelial Barrier Permeability

(100) Endothelial monolayer permeability was assayed by real-time impedance measurement with an xCELLigence RTCA system utilizing microtiter well plates covered with microelectrodes (E-Plates). Relative impedance changes are expressed as unitless Cell Index (CI) values.

(101) Primary Human Pulmonary Artery Endothelial Cells (HPAECs) were seeded at low passages in collagen pre-coated E-Plates. After tight monolayer and cell barrier formation with constant CI values, HPAECs were pre-treated with the indicated concentrations of compound TPP-12899 or the respective negative control antibody construct TPP-5657, followed by compromising the EC barrier with the disruptive agonists LPS (200 ng/ml), IL-1I3 (0.5 ng/ml), or thrombin (2 U/ml). CI were recorded every 10 min to monitor effects on cell growth and monolayer permeability. All cell indices were normalized at the last recording point before test substance application (=normalized CI).

(102) The experiments were performed with n=4 with 3 technical triplicates each. Results were expressed as mean±SEM. Data were statistically analyzed using one-way ANOVA followed by Sidak's multiple post-test; p-values<0.05 were considered as significant.

(103) FIG. **15** graphically depicts the effects on endothelial monolayer permeability expressed as

Cell Index values. As shown in FIG. **15**, pre-treatment of human endothelial monolayer cultures with TPP-12899 protected against induced endothelial barrier permeability in a dose-dependent manner. This was independent of the applied barrier disruptive agent; both, with fast and strong acting thrombin as well as with long-lasting pro-inflammatory stimuli LPS and IL-1I3 significant effects were observed. The respective negative control showed no effect.

Example 11: Long Term Effects of ANP Engrafted IgG in a Chronic Heart Failure Rat Model (TGR(mRenR2)27

(104) The TGR(mRenR2)27 rat model shows hypertension and endothelial dysfunction, as well as end-organ damage. Male renin-transgenic rats (Ganten D., Nature. 1990; 344(6266):541-4) at the age of 8 weeks were used. The nonselective inhibitor of nitric oxide synthetases L-NAME (Nω-Nitro-L-arginine methyl ester) was chronically administered via the drinking water (20 mg/1) in all study groups to induce endothelial dysfunction. TPP-13992, the rat IgG1 counterpart of TPP-10294 used in Example 8 and TPP-10155, a rat IgG1 isotype control antibody were administered once weekly intraperitoneally. Body weight and survival were assessed. The placebo group was treated with vehicle (PBS) and the healthy control group was treated with captopril-food from weaning on. Food and water were given ad libitum. Daily observation of the behavior and general health status of the animals was performed. At the end of the experiment (week 14), the rats were anaesthetized. The rats were then exsanguinated and the heart was removed from the thoracic cavity for analysis. Urine was collected at the end of the study to determine different urine parameters, e.g. urinary protein creatinine ratio. FIG. **16** graphically depicts the therapeutic effects of TPP-13992 on survival, body weight gain, urinary protein/creatinine ratio and left atrial weight.

Example 12: Hemodynamic Effects of ANP Engrafted IgG in Healthy Beagle Dogs

(105) The effects of TPP-10992 on cardiovascular and ECG parameters after single subcutaneous administration were assessed in a primary pharmacodynamic study in conscious telemetered beagle dogs.

(106) Telemetry devices (DSI™, USA) were surgically implanted to measure blood pressure as well as heart rate, followed by a recovery period to allow wound closure. On the day of the study, telemetry sensors were activated for continuous hemodynamic measurements. The transmitted signals were collected by telemetry receivers located in the animal facility. All collected data were processed by a data acquisition program and averaged over a predefined period of 12 h. As vehicle for TPP-10992 NaCl (0.9%) was used and doses of 0.1 mg/kg, 0.3 mg/kg and 1.0 mg/kg bodyweight were applied via subcutaneous injection.

(107) The results are graphically depicted in FIG. **17**. In healthy dogs, TPP-10992 showed a dose-dependent and long-lasting (>5d) reduction in blood pressure which was significant at 1.0 mg/kg s.c. (compared to placebo). No effects on heart rate were observable.

Claims

1. An antibody or a fragment thereof comprising a heterologous amino acid sequence incorporated within a CDR region of said antibody or fragment thereof, wherein said heterologous amino acid sequence comprises an N-terminal linker sequence (Ntls), a Brain Natriuretic Peptide (BNP), and a C-terminal linker sequence (Ctls), wherein the Ntls comprises at least 12 amino acid residues and the Ctls comprises at least 9 amino acid residues, wherein: (i) in case of incorporation of said heterologous amino acid sequence within CDRH1, the Ntls is present between amino acid residue HC res25 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between the last amino acid residue of said BNP and amino acid residue HC res35a according to Kabat, or (ii) in case of incorporation of said heterologous amino acid sequence within CDRH2, the Ntls is present between amino acid residue HC res51 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between amino acid residue HC res57 according to Kabat, or (iii) in case of incorporation of said heterologous amino acid sequence within CDRH3,

the Ntls is present between amino acid residue HC res92 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between amino acid residue HC res106 according to Kabat, or (iv) in case of incorporation of said heterologous amino acid sequence within CDRL1, the Ntls is present between amino acid residue LC res26 according to Kabat, and the Ctls is present between amino acid residue LC res 32 according to Kabat, or (v) in case of incorporation of said heterologous amino acid sequence within CDRL2, the Ntls is present between amino acid residue LC res49 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between amino acid residue LC res57 according to Kabat, or (vi) in case of incorporation of said heterologous amino acid sequence within CDRL3, the Ntls is present between amino acid residue LC res88 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between amino acid residue LC res98 according to Kabat, and wherein: (i) said Ntls and said Ctls each comprise a GS linker sequence, or (ii) said Ntls and said Ctls each comprise a PN linker sequence, or (iii) said Ntls comprises the sequence of any one of SEQ ID NOs: 1, 2, or 4 and said Ctls comprises the sequence of any one of SEQ ID NOs: 1, 3, or 5, or (iv) said Ntls and said Ctls each comprise the sequence of SEQ ID NO: 6, or (v) said Ntls comprises the sequence of SEQ ID NO: 7 and said Ctls comprises the sequence of SEQ ID NO: 8, or (vi) said Ntls comprises the sequence of SEQ ID NO: 9 and said Ctls comprises the sequence of SEQ ID NO: 10, or (vii) said Ntls comprises the sequence of SEQ ID NO: 11 and said Ctls comprises the sequence of SEQ ID NO: 12, or (viii) said Ntls comprises the sequence of SEQ ID NO: 13 and said Ctls comprises the sequence of SEQ ID NO: 14, or (ix) said Ntls and said Ctls each comprise the sequence of SEQ ID NO: 15; or (x) said Ntls comprises the sequence of SEQ ID NO: 9 and said Ctls comprises the sequence of SEQ ID NO: 20, or (xi) said Ntls comprises the sequence of SEQ ID NO: 21 and said Ctls comprises the sequence of SEQ ID NO: 22, and wherein said antibody or fragment thereof is of the class IgG.

2. The antibody or fragment thereof according to claim 1, wherein said BNP is a human BNP comprising the sequence of SEQ ID NO: 24 or a BNP comprising at least 95% sequence identity therewith.

3. The antibody or fragment thereof according to claim 1, wherein said Ntls further comprises an anchoring element A1 at its C terminal end and/or said Ctls further comprises an anchoring element A2 at its N terminal end, and wherein at least 60% of the amino acid residues of A1 and/or A2, when present, are selected from glycine and serine residues.

4. The antibody or fragment thereof according to claim 1, wherein: the amino acid stretch present between (i) amino acid residue HC res25 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRH1; or (ii) amino acid residue HC res51 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRH2; or (iii) amino acid residue HC res92 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRH3; or (iv) amino acid residue LC res26 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRL1; or (v) amino acid residue LC res49 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRL2; or (vi) amino acid residue LC res88 according to Kabat and the first amino acid residue of the BNP in case of an incorporation of said heterologous amino acid sequence within CDRL3 comprises the sequence of any one of SEQ ID NOs: 26 to 38; and wherein the amino acid stretch present between the last amino acid residue of said BNP and (i) amino acid residue HC res35a according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH1; or (ii) amino acid residue HC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH2; or (iii) amino acid residue HC res106 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH3; or (iv) amino acid residue

LC res 32 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL1; or (v) amino acid residue LC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL2; or (vi) amino acid residue LC res98 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL3 comprises the sequence of any one of SEQ ID NOs: 39 to 51.

5. The antibody or fragment thereof according to claim 1, wherein the amino acid stretch present between (i) amino acid residue HC res25 according to Kabat and amino acid residue HC res35a according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH1; or (ii) amino acid residue HC res51 according to Kabat and amino acid residue HC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH2; or (iii) amino acid residue HC res92 according to Kabat and amino acid residue HC res 106 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRH3; or (iv) amino acid residue LC res26 according to Kabat and amino acid residue LC res 32 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL1; or (v) amino acid residue LC res49 according to Kabat and amino acid residue LC res57 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL2; or (vi) amino acid residue LC res88 according to Kabat and amino acid residue LC res98 according to Kabat in case of an incorporation of said heterologous amino acid sequence within CDRL3 comprises the sequence of any one of SEQ ID NOs: 52 to 64.

6. The antibody or fragment thereof according to claim 1, further comprising at least one further natriuretic peptide, wherein said BNP and said at least one further natriuretic peptide are incorporated within at least two separate CDR regions, and wherein said at least one further natriuretic peptide is selected from ANP and CNP.

7. The antibody or fragment thereof according to claim 1, wherein the light chain comprises or consists of the amino acid sequence of SEQ ID NO: 66 and the heavy chain comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 442 to 444.

8. The antibody or fragment thereof according to claim 1, wherein said antibody fragment is selected from the group consisting of Fab, Fab′, Fab′-SH, F(ab′)2, Fv fragments, diabodies, single domain antibodies (Dabs), linear antibodies, single-chain antibody molecules (scFv), and disulfide-stabilized Fv antibody fragments (dsFv).

9. A composition comprising the antibody or fragment thereof according to claim 1 and a pharmaceutically acceptable carrier.

10. The antibody or fragment thereof according to claim 7, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 66 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 442.

11. The antibody or fragment thereof according to claim 7, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 66 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 443.

12. The antibody or fragment thereof according to claim 7, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 66 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 444.

13. The antibody or fragment thereof according to claim 1, wherein at least a portion of said CDR region is replaced by said heterologous amino acid sequence incorporated therein.

14. The antibody or fragment thereof according to claim 1, wherein: (i) the Ntls comprises the amino acid sequence of SEQ ID NO: 268 and the Ctls comprises the amino acid sequence of SEQ ID NO: 252; or (ii) the Ntls comprises the amino acid sequence of SEQ ID NO: 430 and the Ctls comprises the amino acid sequence of SEQ ID NO: 431; or (iii) the Ntls comprises the amino acid sequence of SEQ ID NO: 268 and the Ctls comprises the amino acid sequence of SEQ ID NO: 252; or (iv) the Ntls comprises the amino acid sequence of SEQ ID NO: 259 and the Ctls comprises the amino acid sequence of SEQ ID NO: 432; or (v) the Ntls comprises the amino acid sequence of

SEQ ID NO: 189 and the Ctls comprises the amino acid sequence of SEQ ID NO: 190; or (vi) the Ntls comprises the amino acid sequence of SEQ ID NO: 105 and the Ctls comprises the amino acid sequence of SEQ ID NO: 106; or (vii) the Ntls comprises the amino acid sequence of SEQ ID NO: 211 and the Ctls comprises the amino acid sequence of SEQ ID NO: 212; or (viii) the Ntls comprises the amino acid sequence of SEQ ID NO: 236 and the Ctls comprises the amino acid sequence of SEQ ID NO: 237; or (ix) the Ntls comprises the amino acid sequence of SEQ ID NO: 281 and the Ctls comprises the amino acid sequence of SEQ ID NO: 282; or (x) the Ntls comprises the amino acid sequence of SEQ ID NO: 402 and the Ctls comprises the amino acid sequence of SEQ ID NO: 403.

15. A nucleic acid or a mixture of nucleic acids encoding an antibody or fragment thereof, wherein the antibody or the fragment thereof comprises at least one a heterologous amino acid sequence incorporated within a CDR region of said antibody or the fragment thereof, wherein said heterologous amino acid sequence comprises an N-terminal linker sequence (Ntls), a Brain Natriuretic Peptide (BNP), and a C-terminal linker sequence (Ctls), wherein the Ntls comprises at least 12 amino acid residues and the Ctls comprises at least 9 amino acid residues, wherein: (i) in case of an incorporation of said heterologous amino acid sequence within CDRH1, the Ntls is present between amino acid residue HC res25 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between the last amino acid residue of said BNP and amino acid residue HC res35a according to Kabat, or (ii) in case of an incorporation of said heterologous amino acid sequence within CDRH2, the Ntls is present between amino acid residue HC res51 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between amino acid residue HC res57 according to Kabat, or (iii) in case of an incorporation of said heterologous amino acid sequence within CDRH3, the Ntls is present between amino acid residue HC res92 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between amino acid residue HC res106 according to Kabat, or (iv) in case of an incorporation of said heterologous amino acid sequence within CDRL1, the Ntls is present between amino acid residue LC res26 according to Kabat, and the Ctls is present between amino acid residue LC res 32 according to Kabat, or (v) in case of an incorporation of said heterologous amino acid sequence within CDRL2, the Ntls is present between amino acid residue LC res49 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between amino acid residue LC res57 according to Kabat, or (vi) in case of an incorporation of said heterologous amino acid sequence within CDRL3, the Ntls is present between amino acid residue LC res88 according to Kabat and the first amino acid residue of said BNP, and the Ctls is present between amino acid residue LC res98 according to Kabat; wherein: (i) said Ntls and said Ctls each comprise a GS linker sequence, or (ii) said Ntls and said Ctls each comprise a PN linker sequence, or (iii) said Ntls comprises the sequence of any one of SEQ ID NOs: 1, 2, or 4 and said Ctls comprises the sequence of any one of SEQ ID NOs: 1, 3, or 5, or (iv) said Ntls and said Ctls each comprise the sequence of SEQ ID NO: 6, or (v) said Ntls comprises the sequence of SEQ ID NO: 7 and said Ctls comprises the sequence of SEQ ID NO: 8, or (vi) said Ntls comprises the sequence of SEQ ID NO: 9 and said Ctls comprises the sequence of SEQ ID NO: 10, or (vii) said Ntls comprises the sequence of SEQ ID NO: 11 and said Ctls comprises the sequence of SEQ ID NO: 12, or (viii) said Ntls comprises the sequence of SEQ ID NO: 13 and said Ctls comprises the sequence of SEQ ID NO: 14, or (ix) said Ntls and said Ctls each comprise the sequence of SEQ ID NO: 15; or (x) said Ntls comprises the sequence of SEQ ID NO: 9 and said Ctls comprises the sequence of SEQ ID NO: 20, or xi) said Ntls comprises the sequence of SEQ ID NO: 21 and said Ctls comprises the sequence of SEQ ID NO: 22, and wherein said antibody or fragment thereof is of the class IgG.

16. A host cell comprising the nucleic acid or the mixture of nucleic acids according to claim 15.

17. A process for producing an antibody or fragment thereof, comprising culturing the host cell according to claim 16 under conditions suitable for expression of the antibody or fragment thereof.