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(54) Title: IMMUNOGLOBULIN CONSTRUCTS

(57) Abstract: The present invention relates to protein and polypeptide constructs that comprise single variable domains that are linked to an Fc portion. The immunoglobulin constructs comprise two polypeptide chains in which each polypeptide chain comprises two or more single variable domains that are linked, usually via a suitable hinge region or linker, to one or more constant domains that, together, form an Fc portion. The invention also relates to polypeptide chains that form part of such constructs and/or that can be used to form such constructs. The invention further relates to nucleotide sequences and nucleic acids that encode or can be used to express such constructs or polypeptide chains; to methods for producing such constructs and polypeptides chains; to compositions (and in particular pharmaceutical compositions) that comprise such constructs or polypeptide chains; and to uses of such constructs, polypeptide chains or compositions.

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Immunoglobulin constructs

The present invention relates to protein and polypeptide constructs that comprise single variable domains that are linked to an Fc portion.

The immunoglobulin single variable domains may for example be a domain antibody (or an immunoglobulin variable domain that is suitable for use as a domain antibody), a single domain antibody (or an immunoglobulin variable domain that is suitable for use as a single domain antibody), a "dAb" (or an immunoglobulin variable domain that is suitable for use as a dAb) or a Nanobody (as defined herein, and including but not limited to a V_{HH} sequence); or any suitable fragment of any one thereof.

The invention also relates to polypeptide chains that form part of such constructs and/or that can be used to form such constructs.

The invention further relates to nucleotide sequences and nucleic acids that encode or can be used to express such constructs or polypeptide chains; to methods for producing such constructs and polypeptides chains; to compositions (and in particular pharmaceutical compositions) that comprise such constructs or polypeptide chains; and to uses of such constructs, polypeptide chains or compositions.

Other aspects, embodiments, uses, applications and advantages of the invention will become clear from the further disclosure herein.

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Constructs comprising single variable domains that are linked to an Fc portion are known. For example, as described in EP 0 698 097 and in Hamers-Casterman et al. (Nature 1993, June 3; 363 (6428): 446-8), the naturally occurring “heavy chain antibodies” from Camelidae comprise naturally occurring single variable domains (called “V_{HH} domains”) that are linked via a hinge region to an Fc portion. Interestingly, as further described in these references, these heavy chain antibodies lack the C_H1 domain that is present in conventional 4-chain antibodies, with the V_{HH} being directly linked – via the hinge – to the C_H2 domain of the Fc portion.

EP 0 698 097 and its divisional application EP 1 621 554 also describe that V_{HH} domains can be linked to a human Fc portion. Reference is for example also made to

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WO 04/041862 which describes a fusion of a Nanobody against human TNF to the C_H1 deleted Fc portion of human IgG1.

WO 04/068820 describes so-called "dAb's" that are linked to an effector group. The examples given are constructs that comprise dAb's linked to the Fc of IgG1.

The constructs described in EP 1 621 554 and WO 04/068820 comprise two polypeptide chains, in which each polypeptide chain comprises one single variable domain that is linked, usually via a suitable hinge region or linker, to two constant domains that, in the final construct, form the Fc portion. These constructs bind to the intended antigen via the two antigen-binding sites that are formed by each of the single variable domains.

WO 02/056910 describes constructs in which binding domains that include at least one immunoglobulin variable region polypeptide are linked, via a suitable linker, to C_H2/C_H3 constant regions. In particular, the C_H2 and C_H3 regions of human IgG or human IgA are mentioned. The immunoglobulin variable region polypeptide may be all or a portion or fragment of heavy chain or light chain V-region, provided it is capable of specifically binding an antigen. WO 02/056910 further gives examples of constructs in which the immunoglobulin variable region polypeptide is comprised of an ScFv fragment. These constructs therefore comprise two polypeptide chains, in which each polypeptide chain comprises two variable domains (i.e. a V_H domain and a V_L domain that together form the ScFv fragment) that are linked to two constant domains (that together with the constant domains of the other polypeptide chain form an Fc portion). However, in these constructs the V_H and V_L domains that form the ScFv fragment need to interact so as together form a single antigen binding site, whereas in the constructs of the invention, each variable domain is a single variable domain (meaning that it forms a functional antigen binding unit or site without requiring interaction with another variable domain).

In one or more preferred embodiments the invention provides improved immunoglobulin constructs. In some particular embodiments the invention provides immunoglobulin constructs that have improved binding to a desired antigen and/or improved selectivity for a desired antigen.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

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According to a first aspect, the present invention relates to an immunoglobulin construct, comprising an Fc portion that is linked, optionally via a suitable linker or hinge region, to a pair of first single variable domains, which first single variable domains in turn are further linked, optionally via a suitable linker, to a pair of second single variable domains, wherein:

- both of the first single variable domains are directed against a first target, antigen epitope, antigenic determinant, part, domain or subunit; and
- both of the second single variable domains are directed against a second target, antigen, epitope, antigenic determinant, part, domain or subunit.

According to a second aspect, the present invention relates to a polypeptide, comprising two or three constant domains that are capable of forming (e.g. with the constant domains of a second such polypeptide chain) an Fc portion, or that form a monomeric Fc portion, which is linked, optionally via a suitable linker or hinge region, to a first single variable domain, which first single variable domain in turn is further linked, optionally via a suitable linker, to a second single variable domain, wherein:

- the first single variable domain is directed against a first target, antigen epitope, antigenic determinant, part, domain or subunit; and
- the second single variable domain is directed against a second target, antigen, epitope, antigenic determinant, part, domain or subunit.

According to a third aspect, the present invention relates to a nucleotide sequence or nucleic acid that encodes a construct according to the first aspect or a polypeptide according to the second aspect.

According to a fourth aspect, the present invention relates to a method for preparing a construct according to the first aspect, which method comprises joining two polypeptides according to the second aspect so as to form a construct according to the first aspect.

According to a fifth aspect, the present invention relates to a method for preparing a construct according to the first aspect, which method comprises co-expressing, in a suitable host or host organism, two polypeptides according to the second aspect so as to form a construct according to the first aspect.

According to a sixth aspect, the present invention relates to an isolated host cell or a non-human host that expresses a construct according to the first aspect or a polypeptide

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according to the second aspect, or that comprises a nucleotide sequence or nucleic acid according to the third aspect.

According to a seventh aspect, the present invention relates to a method for producing a construct according to the first aspect or a polypeptide according to the second aspect, said method comprising maintaining or cultivating a host or host cell according to the sixth aspect under conditions such that said host or host cell expresses or produces a construct according to the first aspect or a polypeptide according to the second aspect, respectively, and optionally further comprising isolating the construct according to the first aspect or the polypeptide according to the second aspect so expressed or produced.

According to an eighth aspect, the present invention relates to a composition comprising at least one construct according to the first aspect.

According to a ninth aspect, the present invention relates to use of a construct according to the first aspect, which is directed against a desired or intended target or antigen, in the preparation of a pharmaceutical composition for the prevention or treatment of a disease or disorder associated with said target or antigen.

According to a tenth aspect, the present invention relates to use of a construct according to the first aspect, which is directed against HER-2 antigen, in the preparation of a pharmaceutical composition for the prevention or treatment of cancer.

According to an eleventh aspect, the present invention relates to a method for preventing or treating a disease or disorder that is associated with a target or antigen, which method comprises administering, to a subject in need of such prevention or treatment, a therapeutically active amount of a construct according to the first aspect that is directed against said target or antigen, or administering a pharmaceutical composition comprising such a construct.

According to a twelfth aspect, the present invention relates to a method for preventing or treating cancer, which method comprises administering, to a subject in need of such prevention or treatment, a therapeutically active amount of a construct according to the first aspect that is directed against HER-2, or administering a pharmaceutical composition comprising such a construct.

Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an

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inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

The present invention provides immunoglobulin constructs that comprise two polypeptide chains (each, a "*polypeptide chain of the invention*"), in which each polypeptide chain comprises two or more single variable domains that are linked, usually via a suitable hinge region or linker, to one or more constant domains that, in the final construct, together form an Fc portion.

Thus, the constructs provided by the invention (which are also referred to herein as "*constructs of the invention*") generally comprise an Fc portion (as defined herein) in which each of the two polypeptide chains that form the Fc portion is linked, optionally via a suitable linker or hinge region, to two or more single variable domains (also as defined herein). Such constructs may for example be as described in EP 1 621 554 or WO 02/056910, but with one

or more additional single variable domains linked to each single variable domain that is already present in the constructs described in these references.

The polypeptide chains of the invention, and their use in forming the constructs of the invention, form further aspects of the invention. Also, in one specific aspect of the invention, as further described herein, these polypeptide chains of the invention may also be used as such (i.e. without interaction with another polypeptide chain and/or not as part of a construct of the invention).

Preferably, in the constructs of the invention, each polypeptide chain of the invention comprises two or three single variable domains, and more preferably only two single variable domains. In other words, the constructs of the invention preferably comprise a total of six single variable domains (i.e. three in each polypeptide chain) and more preferably a total of four single variable domains (i.e. two in each polypeptide chain).

Also, each polypeptide chain of the invention will usually comprise either two constant domains (for example, in case of an Fc portion that is derived from IgG, IgA or IgD) or three constant domains (for example, in case of an Fc portion that is derived from IgM or IgE), such that, in the final construct, the constant domains of the two polypeptide chains form an Fc portion, for example an Fc portion that is derived from IgG (e.g. IgG1, IgG2, IgG3 or IgG4), IgA, IgD, IgE or IgM, or a variant, analog, mutant, part or fragment thereof (including chimeric Fc portions), that may or may not have effector functions, as further described herein.

For the sake of convenience, and as these constructs are generally preferred in practice, the invention will now be described in more detail with reference to constructs that comprise a total of four single variable domains (i.e. two in each polypeptide chain) and four constant domains (i.e. two in each polypeptide chain), in which the variable domains are linked to each other via a suitable linker and are linked to the constant domains via a suitable linker or hinge region. However, it will be clear to the skilled person that the teaching of the present invention can equally be applied to constructs of the invention that comprise six or even more single variable domains (i.e. three or more variable domains in each polypeptide chain), and/or that comprise six constant domains (for example, in case of an Fc portion that is derived from IgM or IgE), and/or in which the constant domains are directly linked to each other and/or directly linked to the constant domains (for example, when the Fc portion is derived from IgE, a hinge region between the Fc portion and the variable domains may not be required).

In these preferred, but non-limiting constructs of the invention, each polypeptide chain of the invention comprises two constant domains that are capable, together with the constant domains that are present in the other polypeptide chain that forms part of the construct, to form an Fc portion (as defined herein). These constant domains are linked (optionally via a suitable linker or hinge region) to a "first" single variable domain, which first single variable domain is linked (again optionally via a suitable linker) to a "second" single variable domain (which may in turn be linked, again optionally via a suitable linker, to a "third" single variable domain, and so forth).

Constructs of the invention with four single variable domains and four constant domains (for example forming an Fc portion derived from an IgG or IgA, or an analog, mutant or variant thereof) are schematically shown in the non-limiting Figure 1. The constructs comprise two polypeptide chains (1) and (2), which each comprise two constant domains (7) and (8), a "first" single variable domain (3) and a "second" single variable domain (4). The first single variable domain (3) is linked, optionally via a suitable linker (5), to the second single variable domain (4), and is also linked to the constant domains, optionally (and usually) via a suitable linker or hinge region (6). The constant domains (7) and (8) of the polypeptide chain (1) and the corresponding constant domains (7) and (8) of the polypeptide chain (2) together form the Fc portion (9).

Constructs of the invention with four single variable domains and six constant domains (for example forming an Fc portion derived from IgE, or an analog, mutant or variant thereof) are schematically shown in the non-limiting Figure 2. The constructs comprise two polypeptide chains (1) and (2), which each comprise three constant domains (7), (8) and (10), a "first" single variable domain (3) and a "second" single variable domain (4). The first single variable domain (3) is linked, optionally via a suitable linker (5), to the second single variable domain (4), and is also linked to the constant domains, optionally (and usually) via a suitable linker or hinge region (6). The constant domains (7), (8) and (10) of the polypeptide chain (1) and the corresponding constant domains (7), (8) and (10) of the polypeptide chain (2) together form the Fc portion (9).

An example of a construct of the invention with more than four single variable domains is schematically shown in the non-limiting Figure 3. This Figure 3 shows a constructs of the invention with six single variable domains and four constant domains (for example forming an Fc portion derived from an IgG or IgA, or an analog, mutant or variant thereof). The construct comprise two polypeptide chains (1) and (2), which each comprise two

constant domains (7) and (8), a "first" single variable domain (3), a "second" single variable domain (4) and a "third" single variable domain (11). The first single variable domain (3) is linked, optionally via a suitable linker (5), to the second single variable domain (4), and is also linked to the constant domains, optionally (and usually) via a suitable linker or hinge region (6). The third first single variable domain (11) is linked, optionally via a suitable linker (12), to the second single variable domain (4). The constant domains (7) and (8) of the polypeptide chain (1) and the corresponding constant domains (7) and (8) of the polypeptide chain (2) together form the Fc portion (9). In constructs with more than six single variable domains, each chain (1) and (2) can contain one or more additional single variable domains (not shown), which can be linked to the third single variable domain (11) and to each other, again optionally via suitable linkers.

Figure 4 schematically shows a non-limiting example of a polypeptide of the invention, comprising two single variable domains and two constant domains. The polypeptide comprises two constant domains (7) and (8), a "first" single variable domain (3) and a "second" single variable domain (4). The first single variable domain (3) is linked, optionally via a suitable linker (5), to the second single variable domain (4), and is also linked to the constant domains, optionally (and usually) via a suitable linker or hinge region (6). As further described herein, such a polypeptide chain may be used to form a construct of the invention (i.e. with another polypeptide chain of the invention), but may also be used as such (i.e. without interaction with another polypeptide chain of the invention), for example when the Fc chain that is formed by the constant domains is monomeric (i.e. non self-associating).

In the constructs of the invention, all of the single variable domains that are present in the construct may each be directed against a different target, antigen, antigenic determinant or epitope. However, this is generally less preferred. Preferably, both of the "first" single variable domains that are present in each of the polypeptide chain are directed against the same target or antigen, and both of the "second" single variable domains that are present in each of the polypeptide chain are directed against the same target or antigen (and so on for the "third" and further single variable domains).

In this aspect of the invention, the first single variable domains and second single variable domains may be directed against a different target or antigen (such that the constructs of the invention are capable of simultaneously binding to two different targets or antigens); or may be directed against the same target or antigen (such that all single variable domains present in the construct are capable of binding to the same target or antigen).

As further described herein, when two or more single variable domains in a construct of the invention are capable of binding to the same target or antigen, they may bind to the same epitope, antigenic determinant, part, domain or subunit of the target or antigen, or to different epitopes, antigenic determinants, parts, domains or subunits of the target or antigen. For example, when the first and second binding domains are capable of binding to different targets or antigens, usually both of the first single variable domains will bind to the same epitope, antigenic determinant, part, domain or subunit of the first target or antigen, and both the second single variable domains will bind to the same epitope, antigenic determinant, part, domain or subunit of the second target or antigen (although the invention in its broadest sense is not limited thereto).

Also, when the first and second binding domains are capable of binding to the same target or antigen, usually both of the first single variable domains will bind to the same epitope, antigenic determinant, part, domain or subunit of the target or antigen, and both of the second single variable domains will bind to the same epitope, antigenic determinant, part, domain or subunit of the target or antigen (although the invention in its broadest sense is again not limited thereto, and for example also comprises constructs in which each of the single variable domains can bind to a different epitope, antigenic determinant, part, domain or subunit of the target or antigen). In the latter case, again as further described herein, the first single variable domains and the second single variable domains may bind to the same epitope, antigenic determinant, part, domain or subunit on the target or antigen, or may bind to different epitopes, antigenic determinants, parts, domains or subunits of the target or antigen.

Thus, in a preferred but non-limiting aspect of the invention, the constructs of the invention comprise an Fc portion that is linked (optionally via a suitable linker or hinge region) to a pair of first single variable domains (i.e. one linked to each polypeptide chain that forms the Fc portion, as further described herein), which are linked (optionally via a suitable linker) to a pair of second single variable domains, wherein the constructs and the single variable domains present therein are such that:

- both of the first single variable domains are directed against a first target, antigen epitope, antigenic determinant, part, domain or subunit; and
- both of the second single variable domains are directed against a second target, antigen, epitope, antigenic determinant, part, domain or subunit.

For example, in one aspect of the invention, the constructs of the invention and the single variable domains present therein may be such that:

- both of the first single variable domains are directed against a first target or antigen; and
- both of the second single variable domains are directed against a second target or antigen different from the first target or antigen.

As further described herein, such constructs of the invention may be capable of binding two different targets or antigens simultaneously.

In another aspect of the invention, the constructs of the invention and the single variable domains present therein may be such that:

- the first single variable domains and the second single variable domains are directed against the same target or antigen;
- both of the first single variable domains are directed against a first epitope, antigenic determinant, part, domain or subunit on said target or antigen; and
- both of the second single variable domains are directed against a second epitope, antigenic determinant, part, domain or subunit on said target or antigen which is the same as said first epitope, antigenic determinant, part, domain or subunit.

As further described herein, such constructs of the invention may be capable of binding to the relevant target or antigen with greater avidity than a comparable construct that comprises only one pair of single variable domains (e.g. as described in EP 1 621 554 or WO 04/068820)

In yet another aspect of the invention, the constructs of the invention and the single variable domains present therein may be such that:

- the first single variable domains and the second single variable domains are directed against the same target or antigen;
- both of the first single variable domains are directed against a first epitope, antigenic determinant, part, domain or subunit on said target or antigen; and
- both of the second single variable domains are directed against a second epitope, antigenic determinant, part, domain or subunit on said target or antigen which is different from said first epitope, antigenic determinant, part, domain or subunit.

Again, as further described herein, such constructs of the invention may be capable of binding to the relevant target or antigen with greater avidity than a comparable construct that comprises only one pair of single variable domains (e.g. as described in EP 1 621 554 or WO 04/068820).

For example, in such constructs, the first variable domains may be directed against an "interaction site" on the target or antigen (i.e. a site for ligand binding, a catalytic site, a cleavage site, a site for allosteric interaction, a site involved in homodimerization or heterodimerization of the target or antigen; or any other site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is involved in a biological action or mechanism of the target or antigen); and the second single variable domains may be directed against another site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is not an interaction site (or visa versa). A non-limiting example of such a construct is given in Figure 9A and SEQ ID NO: 9, which constructs comprises one Nanobody (121A2 p19+) that can bind to the p19 subunit of IL-23 and that can modulate (and in particular, reduce) binding of IL-23 to its receptor, and one Nanobody (81G2 p19-) that can bind to the p19 subunit of IL-23 but that does not modulate binding of IL-23 to its receptor.

Alternatively, the first variable domains may be directed against an interaction site on the target or antigen, and the second variable domains may be directed against different interaction site on the target or antigen. Besides binding to the target or antigen with increased avidity, such constructs have the further advantage that they are capable of modulating (as defined herein) two different mechanisms of action of the target or antigen simultaneously.

For example, in a specific, but non-limiting aspect, a construct of the invention may be directed against HER-2. For example, in a construct of the invention that is directed against HER-2

- the first single variable domains may be directed against the Herceptin® binding site on HER-2 (and in particular against domain IV of HER-2, and more in particular against the C-terminus of domain IV of HER-2) and/or be single variable domains that are capable of competing with Herceptin® for binding to HER-2; and the second single variable domains may be directed against another site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on HER-2 (or visa versa). These constructs are generally such that they are capable of modulating HER-2 via the mechanism of action of Herceptin®, and in addition may have one or more effector functions (depending on the Fc portion used);
- the first single variable domains may be directed against the Omnitarg® binding site on HER-2 (and in particular against domain II of HER-2, and more in particular against the middle of domain II of HER-2) and/or be single variable domains that are capable of

competing with Omnitarg® for binding to HER-2; and the second single variable domains may be directed against another site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on HER-2 (or visa versa). These constructs are generally such that they are capable of modulating HER-2 via the mechanism of action of Omnitarg®, and in addition may have one or more effector functions (depending on the Fc portion used);

- the first single variable domains may be directed against the Herceptin® binding site on HER-2 (and in particular against domain IV of HER-2, and more in particular against the C-terminus of domain IV of HER-2) and/or be single variable domains that are capable of competing with Herceptin® for binding to HER-2; and the second single variable domains may be directed against the Omnitarg® binding site on HER-2 (and in particular against domain II of HER-2, and more in particular against the middle of domain II of HER-2) and/or be single variable domains that are capable of competing with Omnitarg® for binding to HER-2. Such a construct is further preferably such that it is capable of (simultaneously) binding to both the the Omnitarg® binding site on HER-2 (and in particular against domain II of HER-2, and more in particular against the middle of domain II of HER-2) as well as the Herceptin® binding site on HER-2 (and in particular against domain IV of HER-2, and more in particular against the C-terminus of domain IV of HER-2), most preferably so as to allow binding with increased avidity and also intramolecular binding and/or recognition. These constructs are preferably further such that they are capable, as a single construct, to modulate HER-2 via both the mechanism of action of Herceptin® as well as the mechanism of action of Omnitarg®, and in addition may have one or more effector functions (depending on the Fc portion used).

For examples of single variable domains that can be used in such constructs, reference is made to the co-pending International patent application of Ablynx N.V. with the same filing date as the present application, entitled "*Amino acid sequences directed against HER2 and polypeptides comprising the same for the treatment of cancers and/or tumors*". Some non-limiting examples of such constructs are given in SEQ ID NO's: 1 to 8 and Figures 5 to 8.

Also, constructs of the invention that are directed against tumor cells or tumor antigens (such as HER-2) may comprise an Fc portion that is derived from IgG (e.g. an IgG1, IgG2, IgG3 or IgG4) or IgA (or, less preferred, even IgM, IgD); but according to one specific aspect of the invention comprise an "*IgE-derived Fc portion*" (i.e. an Fc portion that is derived from

IgE) as described in the co-pending International patent application of Ablynx N.V. entitled "*Constructs comprising single variable domains and an Fc portion derived from IgE*", which has the same filing date as the present application.

In yet another aspect of the invention, the constructs of the invention and the single variable domains present therein may be such that:

- the first single variable domains and the second single variable domains are directed against the same target or antigen, which is a target or antigen that comprises two or more subunits (i.e. a heteromeric target or antigen);
- both of the first single variable domains are directed against a first subunit on said target or antigen; and
- both of the second single variable domains are directed against a second subunit of said target or antigen which is different from said first subunit.

As further described herein, such constructs of the invention may not only be capable of binding to the relevant target or antigen with greater avidity than a comparable construct that comprises only one pair of single variable domains (e.g. as generally described in EP 1 621 554 or WO 04/068820, without reference to HER-2 as a possible target or antigen), but may also have improved selectivity, in particular when either the first and/or the second subunit is common between different heteromeric targets and antigens, as is for example the case for heterodimeric cytokines such as IL-12, IL-23, IL-27 and IL-35 (see Collson et al., Nature, Vol.450, 22 November 2007, 566), which share common subunits; and also for heteromeric (such as heterodimeric or heterotrimeric) receptors, such as the receptors for IL-12, IL-23 and IL-27, which share common receptor chains. Reference is made to the co-pending International patent application entitled "*Amino acid sequences directed against heterodimeric cytokines and/or their receptors and polypeptides comprising the same*" of Ablynx N.V., which has the same filing date as the present application.

Accordingly, in a specific, but non-limiting aspect, a construct of the invention may be directed against a heteromeric cytokine, and in particular against a heterodimeric cytokine of the IL-12 family, such as IL-12, IL-23, IL-27 or IL-35. For example, such a construct of the invention may comprise first single variable domains that are directed against IL12p40, IL12p35, IL23p19, EBI3 or IL27p28, and second single variable domains that are directed against the same subunit or against another subunit that is present in a heteromeric cytokine, and in particular against a heterodimeric cytokine of the IL-12 family.

When the first and single variable domains are directed against the same subunit, they may for example:

- be directed against p19 (in which case the construct may be selective for IL-23 compared to IL-12, IL-27 and IL-35). Such a construct may for example at least comprise, in each polypeptide chain, (i) two or more single variable domains that are capable of modulating binding of IL-23 to its receptor; (ii) two or more single variable domains that can bind to p19 but that are not capable of modulating binding of IL-23 to its receptor; or (iii) at least one single variable domain that is capable of modulating binding of IL-23 to its receptor and at least one single variable domains that can bind to p19 but that is not capable of modulating binding of IL-23 to its receptor;
- be directed against p35 (in which case the construct may be selective for IL-12 and IL-35 compared to IL-23 and IL-27). Such a construct may for example at least comprise, in each polypeptide chain, (i) two or more single variable domains that are capable of modulating binding of IL-12 and/or IL-35 to their respective receptors; (ii) two or more single variable domains that can bind to p35 but that are not capable of modulating binding of IL-12 and/or IL-35 to their respective receptors; or (iii) at least one single variable domain that is capable of modulating binding of IL-12 and/or IL-35 to their respective receptors and at least one single variable domains that can bind to p35 but that is not capable of modulating binding of IL-12 and/or IL-35 to their respective receptors;
- be directed against p28 (in which case the construct may be selective for IL-27 compared to IL-12, IL-23 and IL-35). Such a construct may for example at least comprise, in each polypeptide chain, (i) two or more single variable domains that are capable of modulating binding of IL-27 to its receptor; (ii) two or more single variable domains that can bind to p28 but that are not capable of modulating binding of IL-27 to its receptor; or (iii) at least one single variable domain that is capable of modulating binding of IL-27 to its receptor and at least one single variable domains that can bind to p28 but that is not capable of modulating binding of IL-27 to its receptor;
- be directed against p40 (in which case the construct may be selective for IL-12 and IL-23 compared to IL-27 and IL-35). Such a construct may for example at least comprise, in each polypeptide chain, (i) two or more single variable domains that are capable of modulating binding of IL-12 and/or IL-23 to their respective receptors; (ii) two or more single variable domains that can bind to p40 but that are not capable of modulating

binding of IL-12 and/or IL-23 to their respective receptors; or (iii) at least one single variable domain that is capable of modulating binding of IL-12 and/or IL-23 to their respective receptors and at least one single variable domains that can bind to p40 but that is not capable of modulating binding of IL-12 and/or IL-23 to their respective receptors;

- be directed against EBI3 (in which case the construct may be selective for IL-27 and IL-35 compared to IL-12 and IL-23). Such a construct may for example at least comprise, in each polypeptide chain, (i) two or more single variable domains that are capable of modulating binding of IL-27 and/or IL-35 to their respective receptors; (ii) two or more single variable domains that can bind to EBI3 but that are not capable of modulating binding of IL-27 and/or IL-35 to their respective receptors; or (iii) at least one single variable domain that is capable of modulating binding of IL-27 and/or IL-35 to their respective receptors and at least one single variable domains that can bind to EBI3 but that is not capable of modulating binding of IL-27 and/or IL-35 to their respective receptors;

and such constructs may bind with higher avidity to the relevant heterodimeric cytokine than a comparable construct that comprises only one pair of single variable domains (e.g. as generally described in EP 1 621 554 or WO 04/068820, without reference to heterodimeric cytokines as potential targets or antigens). A non-limiting example of such a construct is given in Figure 9A and SEQ ID NO: 9.

In a particularly preferred aspect, the first and second single variable domains are directed against different subunits. For example, the first single variable domains may be directed against a subunit chosen from p19, p35 and p28, and the second single variable domains may be directed against a subunit chosen from p40 and EBI3 (or visa versa). For example:

- a construct of the invention that is directed against IL-12 may comprise first single variable domains that are directed against IL12p40, and second single variable domains that are directed against IL12p35 (or visa-versa);
- a construct of the invention that is directed against IL-23 may comprise first single variable domains that are directed against IL12p40, and second single variable domains that are directed against IL23p19 (or visa-versa);

- a construct of the invention that is directed against IL-27 may comprise first single variable domains that are directed against EBI3, and second single variable domains that are directed against IL27p28 (or visa-versa);
- a construct of the invention that is directed against IL-35 may comprise first single variable domains that are directed against EBI3, and second single variable domains that are directed against IL12p35 (or visa-versa);

and such constructs may be more selective for the relevant heterodimeric cytokine than a comparable construct that comprises only one pair of single variable domains (e.g. as described in EP 1 621 554 or WO 04/068820) or that comprises two or more pairs of single variable domains against the same subunit. Non-limiting examples of such a construct are given in Figures 9B and 9C and in SEQ ID NO's: 10 and 11.

As further described herein, such constructs (and in particular, the linkers present therein) are further preferably such that they are capable of binding to both of the subunits against which they are directed, more preferably essentially simultaenously so as to allow binding with increased avidity and specificity.

For examples of single variable domains that can be used in such constructs against heterodimeric cytokines (as well as bispecific combinations of such single variable domains that are directed against different subunits), reference is made to the co-pending International patent application of Ablynx N.V. entitled "*Amino acid sequences directed against heterodimeric cytokines and/or their receptors and polypeptides comprising the same*" of Ablynx N.V., which has the same filing date as the present application.

The above constructs are generally capable of modulating the heterodimeric cytokine(s) against which they are directed. Also, depending on the Fc portion present (as further described herein), the above constructs against heterodimeric cytokines may or may not have one or more effector functions.

In the polypeptide chains that form the constructs of the invention, the constant domains are suitably linked to the single variable domains, either directly (which is less preferred), via a suitable hinge region or linker (which is most preferred), or even (although much less preferred) via a suitable constant domain (such as a $C_{\alpha}1$, $C_{\delta}1$, $C_{\mu}1$ or preferably C_H1 or $C_{\varepsilon}1$ domain, which may in turn be linked to the Fc portion via a suitable linker).

The hinge region or linker may be any suitable hinge region or linker, and suitable hinge regions and linkers, which will usually comprise or essentially consist of a suitable amino acid sequence, will be clear to the skilled person based on the disclosure herein. Some

preferred, but non-limiting examples include the hinge regions that naturally occur in immunoglobulins (such as the hinge region of IgG's or the hinge region from Camelid heavy chain antibodies, see for example EP 0 698 097 or the linkers described in WO 96/34103), suitable analogs, variants, homologs, parts or fragments thereof (including synthetic or semi-synthetic analogs, variants, homologs, parts or fragments, for example hinge regions that have been engineered so as not to contain cysteine residues, see for example WO 07/085814), as well as the synthetic and semi-synthetic linkers mentioned in WO 04/068820 and WO 02/056910, as well as the linkers that are mentioned in WO 06/122 825 for linking Nanobodies in multivalent Nanobody constructs. Also, a naturally occurring $C_{\varepsilon}1$ domain or a suitable variant thereof may be used as (or instead of) a hinge region. By means of non-limiting example, a nucleotide sequence encoding a hinge from a Camelid heavy chain antibody (see EP 0 698 097) is given in Table 1 (SEQ ID NO: 14) below.

In the polypeptide chains that form the constructs of the invention, the first and second (and optionally third and further) single variable domains are also suitably linked to each other, either directly (which is less preferred) or via a suitable hinge regions or linkers (which is most preferred), or even (although much less preferred) via a suitable constant domain.

Suitable linkers will be clear to the skilled person based on the disclosure herein, and include the linkers and hinge regions mentioned above for linking the single variable domains to the constant domains, the synthetic and semi-synthetic linkers mentioned in WO 04/068820 and WO 02/056910, the linkers that are mentioned in WO 06/122 825 for linking Nanobodies in multivalent Nanobody constructs, as well as the spacers and linkers that are used in the art to link antibody fragments or antibody domains. These include the linkers mentioned in the general background art cited herein, as well as for example linkers that are used in the art to construct diabodies or ScFv fragments.

For example, the linker may be a suitable amino acid sequence, and in particular amino acid sequences of between 1 and 60, preferably between 15 and 50, such as between 20 and 40 amino acid residues, such as about 25, 30 or 40 amino acid residues (depending on the amino acid composition of the linker). Some preferred examples of such amino acid sequences include gly-ser linkers, for example of the type $(gly_xser_y)_z$, such as (for example $(gly_4ser)_3$ or $(gly_3ser_2)_3$, as described in WO 99/42077 and the GS30, GS15, GS9 and GS7 linkers described in the applications by Ablynx mentioned herein (see for example WO 06/040153 and WO 06/122825), as well as hinge-like regions, such as the hinge regions of naturally occurring heavy chain antibodies or similar sequences (such as described in WO

94/04678). Some other particularly preferred linkers are the linkers GS9 (SEQ ID NO: 84 in WO 06/122825), and in particular the linkers GS30 (SEQ ID NO: 85 in WO 06/122825) and GS 35 (used in the constructs of SEQ ID NO's: 1 to 8).

Generally, the constructs of the invention, and in particular the linkers that link the first and second single variable domains, are preferably such that both the first single variable domains and the second single variable domains are capable of binding to their intended or respective target, antigen, epitope, antigenic determinant, part, domain or subunit, more preferably essentially simultaneously, so as to allow binding with higher avidity (and, where relevant, higher specificity or with other advantages that can be obtained through the use of the constructs of the invention). The skilled person will be able to choose suitable linkers for this purpose, optionally using in silico techniques for molecular modelling and/or a limited degree of experimentation. For an example of such in silico techniques for determining optimal linker length and design, reference is for example made to the co-pending International patent application of Ablynx N.V. with the same filing date as the present application, entitled "*Amino acid sequences directed against HER2 and polypeptides comprising the same for the treatment of cancers and/or tumors*" (see Example 19).

In further aspects, the invention relates to the polypeptide chains of the invention, and to nucleic acids encoding the same. Such polypeptide chains of the invention may further be as described herein for the construct of the invention, albeit that they consist of a single polypeptide chain with two or more single variable domains and one or more (such as two or three) constant domains that are capable (together with the constant domains of another polypeptide chain of the invention, to form an Fc portion (as further described herein).

For example, such polypeptide chains may:

- comprise a first single variable domain that is directed against a first target, antigen epitope, antigenic determinant, part, domain or subunit; and a second single variable domain that is directed against a second target, antigen, epitope, antigenic determinant, part, domain or subunit;
- comprise a first single variable domain that is directed against a first target or antigen; and a second single variable domain that is directed against a second target or antigen different from the first target or antigen;
- comprise a first single variable domain that is directed against a first epitope, antigenic determinant, part, domain or subunit on a target or antigen, and a second single variable

domain that is directed against the same epitope, antigenic determinant, part, domain or subunit of said target or antigen;

- comprise a first single variable domain that is directed against a first target or antigen, and a second single variable domain that is directed against the same target or antigen, but against a different epitope, antigenic determinant, part, domain or subunit of said target or antigen;
- comprise a first single variable domain that is directed against an interaction site (as described herein) on a target or antigen, and a second single variable domain that is directed against another site, epitope, antigenic determinant, part or domain of said target or antigen that is not an interaction site (or visa versa). Such a polypeptide chain may for example be directed against HER-2, and may then be as described herein for the anti-HER-2 constructs of the invention (but consisting of a single polypeptide chain);
- comprise a first single variable domain that is directed against an interaction site (as described herein) on a target or antigen, and a second single variable domain that is directed against another interaction site of said target or antigen. Such a polypeptide chain may for example be directed against HER-2, and may then be as described herein for the anti-HER-2 constructs of the invention (but consisting of a single polypeptide chain);
- comprise a first single variable domain that is directed against a first subunit of a heterodimeric target or antigen, and a second single variable domain that is directed against the same subunit of said target or antigen. Such a polypeptide chain may for example be directed against a heterodimeric cytokine, and may then be as described herein for the constructs of the invention that are directed against heterodimeric cytokine (but consisting of a single polypeptide chain);
- comprise a first single variable domain that is directed against a first subunit of a heterodimeric target or antigen, and a second single variable domain that is directed against a different subunit of said target or antigen. Such a polypeptide chain may for example be directed against a heterodimeric cytokine, and may then be as described herein for the constructs of the invention that are directed against heterodimeric cytokine (but consisting of a single polypeptide chain).

The polypeptide chains of the invention will usually be used as part of, and/or to form, a construct of the invention, as further described herein. However, one specific, but non-limiting, aspect, the constant domains used in a polypeptide chain of the invention may be

such that they allow the polypeptide chain of the invention to be used (i.e. for antigen-binding, for example for therapeutic purposes) as such, i.e. without forming part of a construct of the invention. Generally, according to this aspect of the invention, the constant domains may be naturally occurring, synthetic or semisynthetic analogs, variants, parts or fragments of constant domains that, when combined in a polypeptide chain of the invention, confer upon the polypeptide chain a reduced (or essentially no) tendency to self-associate into dimmers (i.e. compared to the constant domains that naturally occur in the native Fc portion). Such monomeric (i.e. not self-associating) Fc chain variants, or fragments thereof, will be clear to the skilled person. For example, Helm et al. (J Biol Chem 1996 271 7494), describe monomeric Fcε chain variants that can be used in the polypeptide chains of the invention.

Also, such monomeric Fc chain variants are preferably such that they are still capable of binding to the complement or the relevant Fc receptor(s) (depending on the Fc portion from which they are derived), and/or such that they still have some or all of the effector functions of the Fc portion from which they are derived (or at a reduced level still suitable for the intended use). Alternatively, in such a polypeptide chain of the invention, the monomeric Fc chain may be used to confer increased half-life upon the polypeptide chain, in which case the monomeric Fc chain may also have no or essentially no effector functions.

When two polypeptide chains of the invention form a construct of the invention, in practice, the first single variable domains will be essentially the same (i.e. be comprised of essentially the same amino acid sequence) in both polypeptide chains that form the construct of the invention, and the second single variable domain will be essentially the same in both polypeptide chains that form the construct of the invention (although the invention in its broadest sense is not limited thereto). Also, usually, in the practice of the invention, the constant domains that are present in both of the polypeptide chains that form the construct of the invention will be essentially the same (i.e. be comprised of essentially the same amino acid sequence). Thus, usually, both polypeptide chains that form the construct of the invention will comprise essentially the same first, second (and optionally third and further) single variable domains and essentially the same constant domains, so that the constructs of the invention will usually be comprised of two polypeptide chains that have essentially the same (variable and constant) domains and thus essentially the same sequence. However, the invention in its broadest sense is not limited thereto, and for example also comprises constructs in which either or both of the first and/or the second (and/or optionally the third or further) variable domains are not the same in the two polypeptide chains that from the

construct of the invention; as well as constructs in which the two polypeptide chains that form the construct of the invention comprise different hinge regions or linkers; and even constructs in which the two polypeptide chains that form the construct of the invention comprise different constant domains (as long as the constant domains of the two polypeptide chains are still capable of forming an Fc portion, as defined herein).

Thus, in another aspect, the invention relates to an immunoglobulin construct is directed against an intended or desired antigen, which immunoglobulin construct comprises an Fc portion and at least four (such as six and preferably four) single variable domains, which immunoglobulin construct is capable of binding to the desired or intended antigen with at least four (such as all six or all four) single variable domains.

According to this preferred but non-limiting aspect, when the single variable domains present in the construct are directed against the same target or antigen, they may be directed against the same antigenic determinant, epitope, part or domain on the target (in particular, for example, when the target is a multimeric target, so that the same antigenic determinant, epitope, part or domain occurs multiple times in the multimer), against different antigenic determinants, epitopes, parts or domains on the target, or (when the target is a heteromeric protein comprising two or more different subunits) against different subunits of the target.

Thus, according to this preferred but non-limiting aspect aspect, a construct of the invention that is directed against a desired or intended target or antigen may be comprised of two polypeptide chains, in which each polypeptide chain comprises one or more (and usually two or three) constant domains (that are capable, together with the constant domains that are present in the other polypeptide chain, to form an Fc portion), that is linked (optionally via a suitable linker or hinge region) to a "first" single variable domain that is directed against a first epitope, antigenic determinant, part, domain or subunit of the target or antigen, which first single variable domain is linked (again optionally via a suitable linker) to a "second" single variable domain that is directed against a second epitope, antigenic determinant, part, domain or subunit of the target or antigen (which may in turn be linked, again optionally via a suitable linker, to a "third" single variable domain that is directed against a third epitope, antigenic determinant, part, domain or subunit of the target or antigen, and so forth); in which the first and second (and optionally third and further) epitope, antigenic determinant, part, domain or subunit may be the same or different.

When the first and second (and optionally third or further) single variable domains are directed against the same epitope, antigenic determinant, part, domain or subunit, they may

have essentially the same sequence (which will usually be preferred in practice) or have different sequences. When the first and second (and optionally third or further) single variable domains are directed against different epitopes, antigenic determinants, parts, domains or subunits, they will as a rule have different sequences.

Also, again, in this preferred but non-limiting aspect, the first single variable domain will preferably be the essentially same (i.e. be comprised of essentially the same amino acid sequence) in both polypeptide chains that form the construct of the invention, the second single variable domain will preferably be essentially the same in both polypeptide chains that form the construct of the invention, and the constant domains will preferably be essentially the same in both polypeptide chains that form the construct of the invention (so that, again, the constructs of the invention will be comprised of two polypeptide chains that have essentially the same (variable and constant) domains and more in particular essentially the same sequence).

In one particularly preferred but non-limiting aspect of the invention, the constructs of the invention are directed towards a multimeric target or antigen (such as a dimeric target or trimeric target), and the first and second (and optionally third or further) single variable domains are directed against the same epitope, antigenic determinant, part or domain, and preferably have essentially the same sequence.

In another particularly preferred but non-limiting aspect of the invention, the constructs of the invention are directed towards a heteromeric target or antigen (for example comprised of two or three different subunits), and the first single variable domain is directed against a first subunit of the target or antigen and the second single variable domain is directed against a second subunit of the target or antigen.

In one highly specific aspect of the invention, the first single variable domains may be directed against different antigenic determinants, epitopes, parts or domains or subunits of the same target, and the second and further single variable domains may or may not be present. When the second and further single variable domains are not present, the constructs of this particular aspect of the invention only comprise two single variable domains, which are directed against different antigenic determinants, epitopes, parts or domains or subunits of the same target. Again, such a construct, and in particular the linkers or hinge region that link the single variable domains to the constant domain(s), is preferably such that both of the first single variable domains are capable of binding to their intended or respective epitope, antigenic determinant, part, domain or subunit, more preferably essentially simultaneously.

The constant domains that are present in each of the polypeptide chains that form a construct of the invention should be such that they are capable of forming an Fc portion with the constant domains that are present in the other polypeptide chain that forms the construct of the invention. As mentioned above, for this purpose, the constant domains that are present in both of the polypeptide chains that form the construct of the invention will in practice usually be essentially the same (i.e. be comprised of essentially the same amino acid sequence). For example, the constant domains may be C_H2 and C_H3 domains that are capable of forming an IgG Fc portion (e.g. an IgG1, IgG2, IgG3 or IgG4 Fc portion), $C_\alpha2$ and $C_\alpha3$ domains that are capable of forming an IgA Fc portion, $C_\delta2$ and $C_\delta3$ that are capable of forming an IgD Fc portion, $C_\mu2$, $C_\mu3$ and $C_\mu4$ domains that are capable of forming an IgM Fc portion; or $C_\varepsilon2$, $C_\varepsilon3$ and $C_\varepsilon4$ domains that are capable of forming an IgE Fc portion. The constant domains may also be such that they are capable of forming a chimeric Fc portion. Preferably, the constant domains are constant domains that are derived from human antibodies, so that the Fc portion is the Fc portion of a human IgG (e.g. an IgG1, IgG2, IgG3 or IgG4), IgA, IgM, IgD or IgE, or a chimeric constant domain that is fully comprised of human constant domains. By means of non-limiting example, nucleotide sequences encoding the CH_2 and CH_3 domains of human IgG1 are given in Table 1 (SEQ ID NOs: 12 and 13) below.

The constant domains may also be suitable parts, fragments, analogs, variants or mutants of the aforementioned constant domains, as long as they are capable of forming an Fc portion and preferably are further such that they are still capable of providing their desired effector functions. Again, the constant domains that form the Fc portion are preferably essentially comprised of human constant domains or of amino acid sequences that are derived from human constant domains.

The specific constant domain(s) and/or Fc portion chosen will depend upon the intended use and properties (such as effector functions) of the final construct, and may be suitably chosen so as to provide the desired properties (for example, binding and/or activating complement, the ability to trigger ADCC, the ability to trigger other immune responses, and/or other desired effector functions) or to avoid such properties. Based on the disclosure herein, a skilled person will be able to choose a suitable Fc portion for a specific purpose or application. Usually, IgG-derived Fc portions (such as IgG1, IgG2, IgG3 or IgG4-derived Fc portions) will be preferred. However, when the constructs of the invention are directed against tumor cells or tumor antigens (such as HER-2), they may also comprise an "*IgE-derived Fc*

portion" (i.e. an Fc portion that is derived from IgE) as described in the co-pending International patent application of Ablynx N.V. entitled "*Constructs comprising single variable domains and an Fc portion derived from IgE*", which has the same filing date as the present application.

In one specific, but non-limiting, aspect, the Fc portion may also (be used to) confer upon the constructs the invention an increased half-life in vivo. According to this aspect, the Fc portion may still have some or all of its effector functions (or have these effector functions, but at a reduced level still suitable for the intended use), but when the Fc portion is solely used to provide for increased half-life, also a suitable mutant, variant part or fragment of an Fc portion may be used that has no or essentially no effector functions. Half-life can generally be defined as the time taken for the serum concentration of the polypeptide to be reduce by 50%, in vivo, for example due to degradation of the ligand and/or clearance or sequestration of the ligand by natural mechanisms. Methods for pharmacokinetic analysis and determination of half-life are familiar to those skilled in the art. Details may be found in Kenneth, A et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and in Peters et al, Pharmacokinete analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2nd revised edition (1982). For example, the constructs or polypeptides of the invention may have a half-life in a mammal (such as a mouse, rat or monkey) and/or in man that is at least 25%, preferably at least 50%, more preferably at least 70%, such as about 80% or 90% or more, of a naturally occurring IgE in said mammal or in man.

The invention also relates to a protein or polypeptide that comprises or essentially consists of a construct of the invention.

The invention further relates to a polypeptide chain that forms part of a construct of the invention and/or that is capable of forming (or can be used to form) a construct of the invention (i.e. together with another such polypeptide chain), which. polypeptide chain is as further described herein.

The invention further relates to nucleotide sequences or nucleic acids that encode and/or that can be used to express a construct of the invention and/or a polypeptide chain of the invention. Such nucleotide sequences or nucleic acids (which are also generally referred to herein as "*nucleic acids of the invention*") may be as further described herein.

The invention further relates to methods for producing the constructs and polypeptides of the invention; to compositions (and in particular pharmaceutical compositions) that

comprise one or more constructs or polypeptide chains of the invention; and to uses of the constructs or polypeptide chains of the invention or of compositions comprising them. Such methods, compositions and uses may be as further described herein.

Other aspects, embodiments, uses, applications and advantages of the invention will be clear to the skilled person from the further disclosure herein.

It should also be noted that, unless specifically defined otherwise herein, all terms used in the present specification either have the meaning given to them in the prior art cited herein (and in particular given to them in WO 06/122786), or otherwise have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks, such as Sambrook et al, "Molecular Cloning: A Laboratory Manual" (2nd.Ed.), Vols. 1-3, Cold Spring Harbor Laboratory Press (1989); F. Ausubel et al, eds., "Current protocols in molecular biology", Green Publishing and Wiley Interscience, New York (1987); Lewin, "Genes II", John Wiley & Sons, New York, N.Y., (1985); Old et al., "Principles of Gene Manipulation: An Introduction to Genetic Engineering", 2nd edition, University of California Press, Berkeley, CA (1981); Roitt et al., "Immunology" (6th. Ed.), Mosby/Elsevier, Edinburgh (2001); Roitt et al., Roitt's Essential Immunology, 10th Ed. Blackwell Publishing, UK (2001); and Janeway et al., "Immunobiology" (6th Ed.), Garland Science Publishing/Churchill Livingstone, New York (2005), as well as to the further prior art cited herein, such as WO 04/068820 and WO 02/056910. Also, all methods, steps, techniques and manipulations that are not specifically described in detail in the present specification can be performed in a manner known per se to the skilled person, for which reference is again made to the prior art cited herein (including WO 04/068820 and WO 02/056910) as well as to the standard handbooks cited above.

In the context of the present invention, "modulating" or "to modulate" generally means either reducing or inhibiting the activity of, or alternatively increasing the activity of, a target or antigen, as measured using a suitable in vitro, cellular or in vivo assay. In particular, "modulating" or "to modulate" may mean either reducing or inhibiting the activity of, or alternatively increasing a (relevant or intended) biological activity of, a target or antigen, as measured using a suitable in vitro, cellular or in vivo assay (which will usually depend on the target or antigen involved), by at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to activity of the target or antigen in the same assay under the same conditions but without the presence of the construct of the invention.

As will be clear to the skilled person, "modulating" may also involve effecting a change (which may either be an increase or a decrease) in affinity, avidity, specificity and/or selectivity of a target or antigen for one or more of its ligands, binding partners, partners for association into a homomultimeric or heteromultimeric form, or substrates; and/or effecting a change (which may either be an increase or a decrease) in the sensitivity of the target or antigen for one or more conditions in the medium or surroundings in which the target or antigen is present (such as pH, ion strength, the presence of co-factors, etc.), compared to the same conditions but without the presence of the construct of the invention. As will be clear to the skilled person, this may again be determined in any suitable manner and/or using any suitable assay known per se, depending on the target or antigen involved.

"Modulating" may also mean effecting a change (i.e. an activity as an agonist, as an antagonist or as a reverse agonist, respectively, depending on the target or antigen and the desired biological or physiological effect) with respect to one or more biological or physiological mechanisms, effects, responses, functions, pathways or activities in which the target or antigen (or in which its substrate(s), ligand(s) or pathway(s) are involved, such as its signalling pathway or metabolic pathway and their associated biological or physiological effects) is involved. Again, as will be clear to the skilled person, such an action as an agonist or an antagonist may be determined in any suitable manner and/or using any suitable (in vitro and usually cellular or in vivo) assay known per se, depending on the target or antigen involved. In particular, an action as an agonist or antagonist may be such that an intended biological or physiological activity is increased or decreased, respectively, by at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to the biological or physiological activity in the same assay under the same conditions but without the presence of the construct of the invention.

Modulating may for example also involve allosteric modulation of the target or antigen; and/or reducing or inhibiting the binding of the target or antigen to one of its substrates or ligands and/or competing with a natural ligand, substrate for binding to the target or antigen. Modulating may also involve activating the target or antigen or the mechanism or pathway in which it is involved. Modulating may for example also involve effecting a change in respect of the folding or confirmation of the target or antigen, or in respect of the ability of the target or antigen to fold, to change its confirmation (for example, upon binding of a ligand), to associate with other (sub)units, or to disassociate. Modulating may for example

also involve effecting a change in the ability of the target or antigen to transport other compounds or to serve as a channel for other compounds (such as ions).

Modulating may be reversible or irreversible, but for pharmaceutical and pharmacological purposes will usually be in a reversible manner.

The single variable domains that are present in the constructs of the invention may be any variable domain that forms a single antigen binding unit. Generally, such single variable domains will be amino acid sequences that essentially consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively); or any suitable fragment of such an amino acid sequence (which will then usually contain at least some of the amino acid residues that form at least one of the CDR's, as further described herein). Such single variable domains and fragments are most preferably such that they comprise an immunoglobulin fold or are capable for forming, under suitable conditions, an immunoglobulin fold. As such, the single variable domain may for example comprise a light chain variable domain sequence (e.g. a V_L-sequence) or a suitable fragment thereof; or a heavy chain variable domain sequence (e.g. a V_H-sequence or V_{HH} sequence) or a suitable fragment thereof; as long as it is capable of forming a single antigen binding unit (i.e. a functional antigen binding unit that essentially consists of the single variable domain, such that the single antigen binding domain does not need to interact with another variable domain to form a functional antigen binding unit or site, as is for example the case for the variable domains that are present in for example conventional antibodies and ScFv fragments that need to interact with another variable domain – e.g. through a V_H/V_L interaction – to form a functional antigen binding domain).

For example, the single variable domain may be a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or a Nanobody (as defined herein, and including but not limited to a V_{HH} sequence); other single variable domains, or any suitable fragment of any one thereof. For a general description of (single) domain antibodies, reference is also made to the prior art cited above, as well as to EP 0 368 684. For the term "dAb's", reference is for example made to Ward et al. (Nature 1989 Oct 12; 341 (6242): 544-6), to Holt et al. (Trends Biotechnol., 2003, 21(11):484-490); as well as to for example WO 04/068820, WO 06/030220, WO 06/003388 and other published patent applications of Domantis Ltd. It should also be noted that, although less preferred in the context of the present invention because they

are not of mammalian origin, single domain antibodies or single variable domains can be derived from certain species of shark (for example, the so-called "IgNAR domains", see for example WO 05/18629).

In particular, the amino acid sequence of the invention may be a Nanobody™ or a suitable fragment thereof. *[Note: Nanobody®, Nanobodies® and Nanoclone® are trademarks of Ablynx N.V.]* For a further description of V_{HH}'s and Nanobodies, reference is made to the review article by Muyldermans in Reviews in Molecular Biotechnology (74(2001), 277-302); as well as to the following patent applications, which are mentioned as general background art: WO 94/04678, WO 95/04079 and WO 96/34103 of the Vrije Universiteit Brussel; WO 94/25591, WO 99/37681, WO 00/40968, WO 00/43507, WO 00/65057, WO 01/40310, WO 01/44301, EP 1134231 and WO 02/48193 of Unilever; WO 97/49805, WO 01/21817, WO 03/035694, WO 03/054016 and WO 03/055527 of the Vlaams Instituut voor Biotechnologie (VIB); WO 03/050531 of Algonomics N.V. and Ablynx N.V.; WO 01/90190 by the National Research Council of Canada; WO 03/025020 (= EP 1 433 793) by the Institute of Antibodies; as well as WO 04/041867, WO 04/041862, WO 04/041865, WO 04/041863, WO 04/062551, WO 05/044858, WO 06/40153, WO 06/079372, WO 06/122786, WO 06/122787 and WO 06/122825, by Ablynx N.V. and the further published patent applications by Ablynx N.V. Reference is also made to the further prior art mentioned in these applications, and in particular to the list of references mentioned on pages 41-43 of the International application WO 06/040153, which list and references are incorporated herein by reference. As described in these references, generally, Nanobodies (in particular V_{HH} sequences and partially humanized Nanobodies) can in particular be characterized by the presence of one or more "*Hallmark residues*" in one or more of the framework sequences.

The single variable domains used in the constructs of the invention may be directed against (as defined in WO 06/122 825) any suitable antigen. Examples of suitable antigens and of single variable domains directed against those antigens will be clear to the skilled person based on the disclosure herein, and for example include the antigens and single variable domains mentioned in the prior art cited herein as well as the antigens and "dAb's" mentioned and described in the patent applications of Domantis Ltd. or Domantis Inc..

The constructs of the invention may be prepared in any suitable manner known per se. Usually, such methods will either comprise joining two suitable polypeptide chains of the invention so as to form a construct of the invention (in which these polypeptide chains will usually be essentially identical in sequence, although the invention in its broadest sense is not

limited thereto). For the purposes of this method, the polypeptide chains of the invention may be prepared in any suitable manner, usually by suitably expressing, in a suitable host or host organism, a nucleic acid of the invention that encodes the desired polypeptide chain.

Alternatively, the constructs of the invention may be prepared by suitably co-expressing, in a suitable host or host organism, (suitable nucleic acids of the invention that encode) two suitable polypeptide chains of the invention (in which these polypeptide chains will again usually be essentially identical in sequence) so as to form a construct of the invention.

For the purposes of the above methods, the nucleic acids encoding the polypeptide chains of the invention may be prepared in any suitable manner, for example using PCR assembly using overlapping primers, by suitably linking (naturally occurring, synthetic or semi-synthetic) nucleotide sequences that encode the various parts of the desired polypeptide chain, or by de novo synthesis of the desired nucleic acid of the invention using an automated apparatus for synthesizing nucleic acid sequences with a predefined amino acid sequence. The particular codons used may also be chosen and/or optimized for the host or host organism to be used for the expression. Also, for the purposes of the invention, the nucleic acids of the invention may be provided and/or used in the form of a suitable genetic construct (such as a plasmid or expression vector), that may for example comprise – in addition to the nucleotide sequences that encodes the desired polypeptide chain – one or more regulatory elements and/or other suitable components of such constructs known per se. All this can be performed using methods and techniques known per se, for which reference is made to the prior art cited herein, such as WO 04/068820 and WO 02/056910. For example, nucleotide sequences encoding the desired single variable domains can be suitably linked to a nucleotide sequence that encodes the desired constant domains, optionally via nucleotide sequences that encode suitable linkers or hinge regions as described herein.

The further expression, production, purification and isolation (including any steps for joining the two polypeptide chains of the invention so as to form a construct of the invention, or co-expressing two polypeptide chains of the invention so as to form a construct of the invention) may be performed using techniques, vectors, host cells or host organisms known per se, for which reference is again made to for example WO 04/068820 and WO 02/056910.

In a further aspect, the invention relates to a host or host cell that expresses or is capable of expressing a construct of the invention or a polypeptide chain of the invention, or that contains a nucleotide sequence or nucleic acid that encodes a construct of the invention or

a polypeptide chain of the invention. The invention further relates to methods for producing a construct of the invention or a polypeptide chain of the invention, comprising maintaining or cultivating such a host or host cell under conditions such that said host or host cell expresses or produces a construct of the invention or a polypeptide chain of the invention, and optionally comprising isolating the construct of the invention or polypeptide chain of the invention so expressed or produced.

The invention further relates to compositions that comprise at least one construct of the invention or a polypeptide chain of the invention. Such a composition may in particular be a pharmaceutical composition that comprises at least one construct of the invention and one or more pharmaceutically acceptable carriers, adjuvants or excipients. Reference is again made to for example WO 04/068820 and WO 02/056910, as well as to the further prior art cited herein. Usually, such a pharmaceutical composition will be a (usually liquid and aqueous) composition that is suitable for injection or infusion, essentially similar to the formulations that are used for injection or infusion of conventional monoclonal antibodies, but containing one or more constructs of the invention instead of a conventional 4-chain monoclonal antibody.

The constructs and compositions of the invention may be used for any suitable purpose, mainly depending upon the antigen(s) against which the single variable domains that are present in the construct are directed. For example, when the single variable domains that are present in the construct are directed against a pharmaceutically relevant target or antigen, the constructs and compositions of the invention may be used in the prevention and/or treatment of diseases and disorders that are associated with said target or antigen, i.e. by suitably administering the constructs or compositions to a subject that is at risk of and/or suffering from said disease or disorder. Such diseases and disorders, and suitable routes of administration and treatment regimens, will be clear to the skilled person based on the disclosure herein. Generally, it is envisaged that constructs of the invention can be made that are analogous to conventional therapeutic monoclonal antibodies (i.e. directed against the same target or antigen, but only containing two polypeptide chains as described herein, in which the single variable domains in each chain are directed against the relevant target or antigen), and that such constructs (and pharmaceutical compositions comprising the same) can be used for the prevention and/or treatment of essentially the same diseases and disorders as these conventional monoclonals, using essentially similar routes of administration and treatment regimens, which can easily be determined by the treating physician.

Thus, in another aspect, the invention relates to (the use of) a construct of the invention that is directed against a desired or intended (therapeutically relevant) target or antigen (or of a pharmaceutical composition comprising the same) for the prevention or treatment of a disease or disorder associated with said target or antigen.

The invention further relates to the use of a construct of the invention that is directed against a desired or intended (therapeutically relevant) target or antigen in the preparation of a pharmaceutical composition for the prevention or treatment of a disease or disorder associated with said target or antigen.

The invention also relates to a method for preventing or treating a disease or disorder that is associated with a target or antigen, which method comprises administering, to a subject in need of such prevention or treatment, of a therapeutically active amount of a construct of the invention that is directed against said target or antigen (or of a pharmaceutical composition comprising the same).

As mentioned herein, in one specifically preferred, but non-limiting aspect, the construct of the invention is directed against HER-2. Such constructs of the invention are particularly suited for the prevention and/or treatment of various forms of cancer and tumors (including solid tumors), e.g. by slowing, stopping or reversing the growth of a tumor and/or by slowing, stopping or reversing the spread of metastases caused by the tumor; and it is envisaged that such constructs of the invention may have improved efficacy and/or other improved properties compared to conventional 4-chain monoclonal antibodies against HER-2, such as for example Herceptin® or Omnitarg ®. The efficacy of the constructs of the invention that are directed against HER-2 may be determined using suitable cellular assays (for example using a suitable tumor cell line) or animal models known per se, which may be suitably chosen by the skilled person.

Again, for use of the anti-HER-2 constructs of the invention in the prevention and/or treatment of cancer, suitable constructs of the invention, pharmaceutical compositions, routes of administration and treatment regimens will be clear to the skilled person based on the disclosure herein, and may for example also include suitable combination treatments with other cytostatic agents and/or with surgery and/or radiation treatment.

Thus, in another aspect, the invention relates to (the use of) a construct of the invention that is directed against HER-2 (or of a pharmaceutical composition comprising the same) for the prevention or treatment of cancer (and in particular, for the prevention or treatment of tumors that (over)express HER-2).

The invention further relates to the use of a construct of the invention that is directed against HER-2 in the preparation of a pharmaceutical composition for the prevention or treatment of cancer (and in particular, of tumors that express said tumor-associated target or antigen).

The invention also relates to a method for preventing or treating a cancer, which method comprises administering, to a subject in need of such prevention or treatment, of a therapeutically active amount of a construct of the invention that is directed against HER-2 or of a pharmaceutical composition comprising the same.

As mentioned herein, in another specifically preferred, but non-limiting aspect, the construct of the invention is directed against a heterodimeric cytokine, such as against IL-12, IL-23, IL-27 or IL-35. Such constructs of the invention are particularly suited for the prevention and/or treatment of a disease or disorder associated with said heterodimeric cytokine (i.e. with IL-12, IL-23, IL-27 and/or IL-35, respectively). For such diseases and disorders, reference is again made to the co-pending International patent application of Ablynx N.V. entitled "*Amino acid sequences directed against heterodimeric cytokines and/or their receptors and polypeptides comprising the same*" of Ablynx N.V., which has the same filing date as the present application. Such constructs may in particular be directed against IL-23, as further described herein.

Thus, in another aspect, the invention relates to (the use of) a construct of the invention that is directed against a heterodimeric cytokine (or of a pharmaceutical composition comprising the same), such as against IL-12, IL-23, IL-27 or IL-35, for the prevention or treatment of a disease or disorder associated with said heterodimeric cytokine (i.e. with IL-12, IL-23, IL-27 and/or IL-35, respectively).

The invention further relates to the use of a construct of the invention that is directed against a heterodimeric cytokine, such as against IL-12, IL-23, IL-27 or IL-35, in the preparation of a pharmaceutical composition for the prevention or treatment of a disease or disorder associated with said heterodimeric cytokine (i.e. with IL-12, IL-23, IL-27 and/or IL-35, respectively).

The invention also relates to a method for preventing or treating a disease or disorder that is associated with a heterodimeric cytokine, such as with IL-12, IL-23, IL-27 or IL-35, which method comprises administering, to a subject in need of such prevention or treatment, of a therapeutically active amount of a construct of the invention that is directed against a

heterodimeric cytokine, such as against IL-12, IL-23, IL-27 or IL-35 (or of a pharmaceutical composition comprising the same).

SEQ ID NO's 1 to 11, Figures 5 to 9 and Table 1 below give some non-limiting examples of amino acid sequences and nucleic acid sequences of polypeptide chains of the invention that can be used to form constructs of the invention:

- SEQ ID NO:1 and Figure 5A give an example of an amino acid sequence of a polypeptide chain of the invention comprising two different Nanobodies against HER-2 (i.e. 47D5 and 2D3, which together form a biparatopic Nanobody construct), that are linked via a hinge region (encoded by the nucleotide sequence of SEQ ID NO:14) to the CH_2 and CH_3 domains of human IgG1 (encoded by the nucleotide sequence of SEQ ID NO:12 and SEQ ID NO:13, respectively). In this construct, 47D5 and 2D3 are linked to each other via 35 a.a. Gly/Ser linker, which should allow both Nanobodies to bind to HER-2 (i.e. essentially simultaneously). The corresponding nucleotide sequence is given in SEQ ID NO:2 and Figure 5B.
- SEQ ID NO:3 and Figure 6A give another example of an amino acid sequence of a polypeptide chain of the invention comprising two different Nanobodies against HER-2 (i.e. 47D5 and 2D3, which together form a biparatopic Nanobody construct), that are linked via a hinge region (encoded by the nucleotide sequence of SEQ ID NO:14) to the CH_2 and CH_3 domains of human IgG1 (encoded by the nucleotide sequence of SEQ ID NO:12 and SEQ ID NO:13, respectively). In this construct, 47D5 and 2D3 are again linked to each other via 35 a.a. Gly/Ser linker, which should allow both Nanobodies to bind to HER-2 (i.e. essentially simultaneously); however, compared to the construct of SEQ ID NO:1/Figure 5A, the order of the 2 anti-HER-2 Nanobodies 47D5 and 2D3 is reversed. The corresponding nucleotide sequence is given in SEQ ID NO:4 and Figure 6B.
- SEQ ID NO:5 and Figure 7A give an example of an amino acid sequence of a polypeptide chain of the invention comprising two different Nanobodies against HER-2 (i.e. 47D5 and 2D3, which together form a biparatopic Nanobody construct), that are linked via a glycine-serine linker and the native $C_\varepsilon1$- $C_\varepsilon2$ linking sequence to the $C_\varepsilon2$, $C_\varepsilon3$ and $C_\varepsilon4$ domains of human IgE. In this construct, 47D5 and 2D3 are linked to each other via 35 a.a. Gly/Ser linker, which should allow both Nanobodies to bind to HER-2

(i.e. essentially simultaneously). The corresponding nucleotide sequence is given in SEQ ID NO:6 and Figure 7B.

- SEQ ID NO:7 and Figure 8A give another example of an amino acid sequence of a polypeptide chain of the invention comprising two different Nanobodies against HER-2 (i.e. 47D5 and 2D3, which together form a biparatopic Nanobody construct), that are linked via a glycine-serine linker and the native $C_\varepsilon1$- $C_\varepsilon2$ linking sequence to the $C_\varepsilon2$, $C_\varepsilon3$ and $C_\varepsilon4$ domains of human IgE. In this construct, 47D5 and 2D3 are again linked to each other via 35 a.a. Gly/Ser linker, which should allow both Nanobodies to bind to HER-2 (i.e. essentially simultaneously); however, compared to the construct of SEQ ID NO:5/Figure 7A, the order of the 2 anti-HER-2 Nanobodies 47D5 and 2D3 is reversed. The corresponding nucleotide sequence is given in SEQ ID NO:8 and Figure 8B.
- Figures 9A to 9C give examples of constructs of the invention that are directed against IL-23. The construct of Figure 9A (SEQ ID NO:9) comprises one Nanobody (121A2 p19+) that can bind to the p19 subunit of IL-23 and that can modulate (and in particular, reduce) binding of IL-23 to its receptor, and one Nanobody (81G2 p19-) that can bind to the p19 subunit of IL-23 but that does not modulate binding of IL-23 to its receptor. The construct of Figure 9B (SEQ ID NO:10) comprises one Nanobody (121A2 p19+) that can bind to the p19 subunit of IL-23 and that can modulate (and in particular, reduce) binding of IL-23 to its receptor, and one Nanobody (81E10 p40+) that can bind to the p40 subunit of IL-23 and that can modulate binding of IL-23 to its receptor. Because IL-12 shares the p40 subunit with IL-23, it is expected that this construct can also bind to IL-12 (but with less avidity) and that this construct shows selectivity for IL-23 compared to IL-12. The construct of Figure 9C (SEQ ID NO:11) comprises one Nanobody (81E10 p40+) that can bind to the p40 subunit of IL-23 and that can modulate binding of IL-23 to its receptor, and one Nanobody (80D10) that can bind to the p40 subunit of IL-23 but that does not modulate binding of IL-23 to its receptor. Because IL-12 shares the p40 subunit with IL-23, it is expected that this construct can also bind to IL-12, also with improved avidity.

Table 1: Non-limiting examples of amino acid sequences and nucleic acid sequences of polypeptide chains of the invention.

47D5-35GS-2D3-hinge-CH2-CH3; SEQ ID NO:1 kvqlvesggglvqpggslrlscaasgsifgfndmawyrqapgkqrelvalisrvgvtssadsvkgrftisrvnakdtvylqmn slkpedtavyycymdqrldgstlaywgqgtqvtvssggggsggggsggggsggggsggggsggggsggggsevqlves ggslvqpggslrlscaasgftfddyamswvrqvpgkglewvssinwsgthtdyadsvkgrftisrnnanntlylqmnslkse dtavyycaknwrdagttwfeksgsagqgtqvtvssEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
47D5-35GS-2D3-hinge-CH2-CH3; SEQ ID NO:2 aaggtgcagctggtggagtctgggggaggcttggtgcagcctgggggtctctgagactctcctgtgcagcctctggaagcatc ttcggtttcaatgacatggcctggtaccgccaggctccagggaagcagcgcgagttggtcgcactaattagtagggttggtgtcac aagttctgcagactccgtgaagggccgattcaccatctccagagtcaacgccaaggacacggtgtatctgcaaatgaacagcctg aaacctgaggatacggccgtctattattgttatatggatcagcgactcgacggtagtactttagcgtactggggccaggggaccca ggtcaccgtatcgagtgggggcgggggaagtggcggaggtgggtccggtggcggaggcagcggtggaggaggtagtggcg gtggcggtagtggcggtggcggcagtggaggcggaggatccgaagtgcagttagtcgagtcaggggtagcttggtgcagcc ggggggtagcctgcgcctgagctgcgccgcgagcggcttcaccttcgacgattatgcgatgtcatgggtcagacaggtccctgg taaagggcttgaatgggtttcctcaataaactggagcggcacccatacggattatgcggatagcgtgaaaggacgttttaccatta gccgcaataacgctaataacactctgtacctacaaatgaactcgctcaaatctgaagatactgctgtctactattgtgccaagaattg gcgtgacgcagggaccacatggtttgagaaatccggtagcgcgggccaaggcactcaggtgacagtgagcagcAgcccaaa tcttgtgacaaaactcacacatgcccaccgtgcccagCacctgaactcctgggggacccgtcagtcttcctcttccccccaaaac ccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaag ttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgt ggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccag ccccccatcgagaaaaccatctccaaagccaaagGgcagccccgagaaccacaggtgtacaccctgccccccatcccgggatga gctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg ggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtgg acaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc ctctccctgtctccgggtaaatga
2D3-35GS-47D5-hinge-CH2-CH3; SEQ ID NO:3 EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSSINWS GTHTDYADSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTW FEKSGSAGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGGLVQPGGSLRLSCAASGSIFGFNDMAWYRQAPGKQRELVALISRVG VTSSADSVKGRFTISRVNAKDTVYLQMNSLKPEDTAVYYCYMDQRLDGSTLAY WGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK

Table 1 (continued):

2D3-35GS-47D5-hinge-CH2-CH3; SEQ ID NO:4 gaggtgcagctggtggagtctgggggctccttggtgcagcctgggggggtctctgagactctcctgtgcagcctctggcttcacttttt gatgattatgccatgagctgggtccgacaggttccagggaaggggttggagtgggtttcatctattaattggtctggtactcacaca gactatgcagactccgtgaagggccgattcaccatctccagaaacaacgccaataacacgctgtatctacaaatgaacagtctga aatctgaggacacggccgtgtattactgtgcaaaaaactggagagacgcaggtactacctggttcgaaaagtccggctccgcgg gccaggggacccaggtcaccgtatcgagtgggggcgggggaagtggcggaggtgggtccggtggcggaggcagcggtgg aggaggtagtggcggtggcggtagtggcggtggcggcagtggaggcggaggatccgaggtgcagctggtggagtctgggg gaggcttggtgcagcctgggggtctctgagactctcctgtgcagcctctggaagcatcttcggtttcaatgacatggcctggtac cgccaggctccagggaagcagcgcgagttggtcgcactaattagtagggttggtgtcacaagttctgcagactccgtgaagggc cgattcaccatctccagagtcaacgccaaggacacggtgtatctgcaaatgaacagcctgaaacctgaggatacggccgtctatt attgttatatggatcagcgactcgacggtagtactttagcgtactggggccaggggacccaggtcaccgtctcctcaAgcccaaa tcttgtgacaaaactcacacatgcccaccgtgcccagCacctgaactcctgggggggaccgtcagtcttcctcttccccccaaaac ccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaag ttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgt ggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccag ccccccatcgagaaaaccatctccaaagccaaagGgcagccccgagaaccacaggtgtacaccctgccccccatcccgggatga gctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg ggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtgg acaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc ctctccctgtctccgggtaaatga
Her2 47D5-G/S-2D3-G/S-C2-3-4 IgE; SEQ ID NO:5 KVQLVESGGGLVQPGGSLRLSCAASGSIFGFNDMAWYRQAPGKQRELVALISRV GVTSSADSVKGRFTISRVNAKDTVYLQMNSLKPEDTAVYYCYMDQRLDGSTLAY WGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSSINWSGTHTDY ADSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFEKSGS AGQGTQVTVSSGGGSGGGSGGGSVDNKTFSVCSRDFTPPTVKILQSSCDGGGHFP PTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLSQKH WLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFDLFIRKSPTITCL VVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEG ETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQN FMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFI CRAVHEAASPSQTVQRAVSVNPGK

Table 1 (continued):

Her2 47D5-G/S-2D3-G/S-C2-3-4 IgE; SEQ ID NO:6 aaggtgcagctggtggagtctgggggaggcttggtgcagcctgggggtctctgagactctcctgtgcagcctctggaagcatc ttcggtttcaatgacatggcctggtaccgccaggctccagggaagcagcgcgagttggtcgcactaattagtagggttggtgtcac aagttctgcagactccgtgaagggccgattcaccatctccagagtcaacgccaaggacacggtgtatctgcaaatgaacagcctg aaacctgaggatacggccgtctattattgttatatggatcagcgactcgacggtagtactttagcgtactggggccaggggaccca ggtcaccgtatcgagtgggggcgggggaagtggcggaggtgggtccggtggcggaggcagcggtggaggaggtagtggcg gtggcggtagtggcggtggcggcagtggaggcggaggatccgaagtgcagttagtcgagtcaggggtagcttggtgcagcc ggggggtagcctgcgcctgagctgcgccgcgagcggcttcaccttcgacgattatgcgatgtcatgggtcagacaggtccctgg taaagggcttgaatgggtttcctcaataaactggagcggcacccatacggattatgcggatagcgtgaaaggacgttttaccatta gccgcaataacgctaataacactctgtacctacaaatgaactcgctcaaatctgaagatactgctgtctactattgtgccaagaattg gcgtgacgcagggaccacatggtttgagaaatccggtagcgcgggccaaggcactcaggtgacagtgagcagcGGCGG CGGCAGCGGCGGCGGCAGCGGCGGCGGCAGCgtcgacaacaaaacccttcagcgtctgctccagg gacttcacccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttcccccgaccatccagctcctgtgc ctcgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacgtggacttgtccaccgcc tctaccacgcaggagggtgagctggcctccacacaaagcgagctcaccctcagccagaagcactggctgtcagaccgcaccta cacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaagtgtgcagattccaacccgagaggggtgagcg cctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcacccag caaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcg caatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggggagacctaccagtgcagggtg acccaccccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgcccccggaagtctatgcgtt tgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcctgaggacatctcg gtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagaccaagggctccgg cttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggca gcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatga
Her2 2D3-G/S-47D5-G/S-C2-3-4 IgE; SEQ ID NO:7 EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSSINWS GTHTDYADSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTW FEKSGSAGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGGLVQPGGSLRLSCAASGSIFGFNDMAWYRQAPGKQRELVALISRVG VTSSADSVKGRFTISRVNAKDTVYLQMNSLKPEDTAVYYCYMDQRLDGSTLAY WGQGTQVTVSSGGGSGGGSGGGSVDNKTFSVCSRDFTPPTVKILQSSCDGGGHFP PTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLSQKH WLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFDLFIRKSPTITCL VVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEG ETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQN FMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFI CRAVHEAASPSQTVQRAVSVNPGK

Table 1 (continued):

Her2 2D3-G/S-47D5-G/S-C2-3-4 IgE; SEQ ID NO:8
gaggtgcagctggtggagtctgggggctccttggtgcagcctgggggtctctgagactctcctgtgcagcctctggcttcactttt gatgattatgccatgagctgggtccgacaggttccagggaaggggttggagtgggtttcatctattaattggtctggtactcacaca gactatgcagactccgtgaagggccgattcaccatctccagaaacaacgccaataacacgctgtatctacaaatgaacagtctga aatctgaggacacggccgtgtattactgtgcaaaaaactggagagacgcaggtactacctggttcgaaaagtccggctccgcgg gccaggggacccaggtcaccgtatcgagtgggggcgggggaagtggcggaggtgggtccggtggcggaggcagcggtgg aggaggtagtggcggtggcggtagtggcggtggcggcagtggaggcggaggatccgaggtgcagctggtggagtctgggg gaggcttggtgcagcctgggggtctctgagactctcctgtgcagcctctggaagcatcttcggtttcaatgacatggcctggtac cgccaggctccagggaagcagcgcgagttggtcgcactaattagtagggttggtgtcacaagttctgcagactccgtgaagggc cgattcaccatctccagagtcaacgccaaggacacggtgtatctgcaaatgaacagcctgaaacctgaggatacggccgtctatt attgttatatggatcagcgactcgacggtagtactttagcgtactggggccaggggacccaggtcaccgtctcctcaGGCGG CGGCAGCGGCGGCGGCAGCGGCGGCGGCAGCgtcgacaacaaaaccttcagcgtctgctccagg gacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttcccccgaccatccagctcctgtgc ctcgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacgtggacttgtccaccgcc tctaccacgcaggagggtgagctggcctccacacaaagcgagctcaccctcagccagaagcactggctgtcagaccgcaccta cacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattccaacccgagaggggtgagcg cctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcacccag caaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcg caatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggggagacctaccagtgcagggtg acccaccccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccggaagtctatgcgtt tgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcctgaggacatctcg gtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagaccaagggctccgg cttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggca gcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatga
121A2 (p19+) -35GS- 81G2 (p19-)-Hinge-CH2-CH3; SEQ ID NO:9
EVQLVESGGGLVQAGGSLRLSCAASGSIFNFNYMGWFRQAPGKEREFVAAIRWS GSSTYYADSVKGRFTISRDDAKNTVALQMNSLKPEDTAIYYCALRKGIPYSTSDR VIKGVNDYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSEVQLVESGGGLVQPGGSLRLSCIASGLPFSTKSMGWFRQAPGKEREFVARI SPGGTSRYYGDFVKGRFAISRDNAKNTTWLQMNSLKAEDTAVYYCASGERSTYI GSNYYRTNEYDYWGTGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
121A2 (p19+) –35GS- 81E10 (p40+)-Hinge-CH2-CH3; SEQ ID NO:10
EVQLVESGGGLVQAGGSLRLSCAASGSIFNFNYMGWFRQAPGKEREFVAAIRWS GSSTYYADSVKGRFTISRDDAKNTVALQMNSLKPEDTAIYYCALRKGIPYSTSDR VIKGVNDYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSEVQLVESGGGLVQAGGSLRLSCVASGRTFNTYGMGWFRQAPGKEREFVA ANNWSGGATSYADSVKGRFTISRDNAKNTVFLQMNTLKPEDTAVYYCAAADRG GGWLVVRENDYDYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Table 1 (continued):

81E10 (p40+) –35GS- 80D10 (p40-)-Hinge-CH2-CH3; SEQ ID NO:11 EVQLVESGGGLVQAGGSLRLSCVASGRTFNTYGMGWFRQAPGKEREFVAANNW SGGATSYADSVKGRFTISRDNAKNTVFLQMNTLKPEDTAVYYCAAADRGGGWL VVRENDYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSEVQLVESGGGLVQAGGSLRLSCAASGRTFEGNPMGWFRQASGKKREFVASI DWSGGITSYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAASARFGSG SYYDLMYDYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Human CH2; SEQ ID NO:12 Cacctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggt cacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatg ccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct gaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaag
Human CH3; SEQ ID NO:13 Ggcagccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcct ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcct cccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga
Hinge; SEQ ID NO:14 Agcccaaatcttgtgacaaaactcacacatgcccaccgtgcccag

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CLAIMS

1. Immunoglobulin construct, comprising an Fc portion that is linked, optionally via a suitable linker or hinge region, to a pair of first single variable domains, which first single variable domains in turn are further linked, optionally via a suitable linker, to a pair of second single variable domains, wherein:

- both of the first single variable domains are directed against a first target, antigen epitope, antigenic determinant, part, domain or subunit; and
- both of the second single variable domains are directed against a second target, antigen, epitope, antigenic determinant, part, domain or subunit.

2. Immunoglobulin construct according to claim 1, in which:

- both of the first single variable domains are directed against a first target or antigen; and
- both of the second single variable domains are directed against a second target or antigen different from the first target or antigen.

3. Immunoglobulin construct according to claim 1, in which:

- the first single variable domains and the second single variable domains are directed against the same target or antigen;
- both of the first single variable domains are directed against a first epitope, antigenic determinant, part, domain or subunit on said target or antigen; and
- both of the second single variable domains are directed against a second epitope, antigenic determinant, part, domain or subunit on said target or antigen which is the same as said first epitope, antigenic determinant, part, domain or subunit.

4. Immunoglobulin construct according to claim 1, in which:

- the first single variable domains and the second single variable domains are directed against the same target or antigen;
- both of the first single variable domains are directed against a first epitope, antigenic determinant, part, domain or subunit on said target or antigen; and

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- both of the second single variable domains are directed against a second epitope, antigenic determinant, part, domain or subunit on said target or antigen which is different from said first epitope, antigenic determinant, part, domain or subunit.

5. Immunoglobulin construct according to claim 1 or claim 4, which is directed against HER-2.

6. Immunoglobulin construct according to claim 5, in which the first single variable domains are directed against the trastuzumab binding site on HER-2 and/or are single variable domains that are capable of competing with trastuzumab for binding to HER-2; and the second single variable domains are directed against the pertuzumab binding site on HER-2 and/or are single variable domains that are capable of competing with pertuzumabfor binding to HER-2 (or vice versa).

7. Immunoglobulin construct according to claim 1, in which:

- the first single variable domains and the second single variable domains are directed against the same target or antigen, which is a target or antigen that comprises two or more subunits (i.e. a heteromeric target or antigen);
- both of the first single variable domains are directed against a first subunit on said target or antigen; and
- both of the second single variable domains are directed against a second subunit of said target or antigen which is different from said first subunit.

8. Immunoglobulin construct according to claim 7, which is directed against a heteromeric receptor.

9. Immunoglobulin construct according to claim 7, which is directed against a heterodimeric cytokine.

10. Immunoglobulin construct according to claim 9, in which the first single variable domains are directed against a subunit chosen from p19, p35 and p28, and the second single variable domains are directed against a subunit chosen from p40 and EBI3 (or vice versa).

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11. Immunoglobulin construct according to claim 9 or claim 10, which is directed against IL-23.

12. Immunoglobulin construct according to any one of claims 9 to 11, in which the first single variable domains are directed against IL12p40, and the second single variable domains are directed against IL23p19 (or vice versa).

13. Polypeptide, comprising two or three constant domains that are capable of forming (e.g. with the constant domains of a second such polypeptide chain) an Fc portion, or that form a monomeric Fc portion, which is linked, optionally via a suitable linker or hinge region, to a first single variable domain, which first single variable domain in turn is further linked, optionally via a suitable linker, to a second single variable domain, wherein:

- the first single variable domain is directed against a first target, antigen epitope, antigenic determinant, part, domain or subunit; and
- the second single variable domain is directed against a second target, antigen, epitope, antigenic determinant, part, domain or subunit.

14. Polypeptide according to claim 13, in which:

- the first single variable domain is directed against a first target or antigen; and
- the second single variable domain is directed against a second target or antigen different from the first target or antigen.

15. Polypeptide according to claim 13, in which:

- the first single variable domain and the second single variable domain are directed against the same target or antigen;
- the first single variable domain is directed against a first epitope, antigenic determinant, part, domain or subunit on said target or antigen; and
- the second single variable domain is directed against a second epitope, antigenic determinant, part, domain or subunit on said target or antigen which is the same as said first epitope, antigenic determinant, part, domain or subunit.

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16. Polypeptide according to claim 13, in which:

- the first single variable domain and the second single variable domain are directed against the same target or antigen;
- the first single variable domain is directed against a first epitope, antigenic determinant, part, domain or subunit on said target or antigen; and
- the second single variable domain is directed against a second epitope, antigenic determinant, part, domain or subunit on said target or antigen which is different from said first epitope, antigenic determinant, part, domain or subunit.

17. Polypeptide according to claim 13 or claim 16, which is directed against HER-2.

18. Polypeptide according to claim 17, in which the first single variable domain is directed against the trastuzumab binding site on HER-2 and/or is a single variable domain that is capable of competing with trastuzumab for binding to HER-2; and the second single variable domain is directed against the pertuzumab binding site on HER-2 and/or is a single variable domains that is capable of competing with pertuzumab for binding to HER-2 (or vice versa).

19. Polypeptide according to claim 13, in which:

- the first single variable domain and the second single variable domain are directed against the same target or antigen, which is a target or antigen that comprises two or more subunits (i.e. a heteromeric target or antigen);
- the first single variable domain is directed against a first subunit on said target or antigen; and
- the second single variable domain is directed against a second subunit of said target or antigen which is different from said first subunit.

20. Polypeptide according to claim 19, which is directed against a heteromeric receptor.

21. Polypeptide according to claim 19, which is directed against a heterodimeric cytokine.

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22. Polypeptide according to claim 21, in which the first single variable domain is directed against a subunit chosen from p19, p35 and p28, and the second single variable domain is directed against a subunit chosen from p40 and EBI3 (or vice versa)

23. Polypeptide according to claim 21 or claim 22, which is directed against IL-23.

24. Polypeptide according to any one of claims 21 to 23, in which the first single variable domain is directed against IL12p40, and the second single variable domain is directed against IL23p19 (or vice versa).

25. Nucleotide sequence or nucleic acid that encodes a construct according to any one of claims 1 to 12 or a polypeptide according to any one of claims 13 to 24.

26. Method for preparing a construct according to any one of claims 1 to 12, which method comprises joining two polypeptides according to any one of claims 13 to 24 so as to form a construct according to any one of claims 1 to 12.

27. Method for preparing a construct according to any one of claims 1 to 12, which method comprises co-expressing, in a suitable host or host organism, two polypeptides according to any one of claims 13 to 24 so as to form a construct according to any one of claims 1 to 12.

28. An isolated host cell or a non-human host that expresses a construct according to any one of claims 1 to 12 or a polypeptide according to any one of claims 13 to 24, or that comprises a nucleotide sequence or nucleic acid according to claim 25.

29. Method for producing a construct according to any one of claims 1 to 12 or a polypeptide according to any one of claims 13 to 24, said method comprising maintaining or cultivating a host or host cell according to claim 28 under conditions such that said host or host cell expresses or produces a construct according to any one

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of claims 1 to 12 or a polypeptide according to any one of claims 13 to 24, respectively, and optionally further comprising isolating the construct according to any one of claims 1 to 12 or the polypeptide according to any one of claims 13 to 24 so expressed or produced.

30. Composition comprising at least one construct according to any one of claims 1 to 12.

31. Composition according to claim 30, which is a pharmaceutical composition comprising at least one construct according to any one of claims 1 to 12 and one or more pharmaceutically acceptable carriers, adjuvants or excipients.

32. Construct according to any one of claims 1 to 12, which is directed against a desired or intended target or antigen, or pharmaceutical composition comprising such a polypeptide construct, when used in the prevention or treatment of a disease or disorder associated with said target or antigen.

33. Construct according to any one of claims 1 to 12, which is directed against HER-2, or pharmaceutical composition comprising such a polypeptide construct, when used in the prevention or treatment of cancer.

34. Use of a polypeptide construct according to any one of claims 1 to 12, which is directed against a desired or intended target or antigen, in the preparation of a pharmaceutical composition for the prevention or treatment of a disease or disorder associated with said target or antigen.

35. Use of a construct according to any one of claims 1 to 12, which is directed against HER-2 antigen, in the preparation of a pharmaceutical composition for the prevention or treatment of cancer.

36. Method for preventing or treating a disease or disorder that is associated with a target or antigen, which method comprises administering, to a subject in need of such prevention or treatment, a therapeutically active amount of a construct according

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to any one of claims 1 to 12 that is directed against said target or antigen, or administering a pharmaceutical composition comprising such a construct.

37. Method for preventing or treating cancer, which method comprises administering, to a subject in need of such prevention or treatment, a therapeutically active amount of a construct according to any one of claims 1 to 12 that is directed against HER-2, or administering a pharmaceutical composition comprising such a construct.

38. Immunoglobulin construct according to claim 1; polypeptide according to claim 13; nucleotide sequence or nucleic acid according to claim 25; method according to any one of claims 26, 27, 29, 36 or 37; an isolated host cell or a non-human host according to claim 28; composition according to claim 30; or use according to claim 34 or claim 36, substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

Figure 1

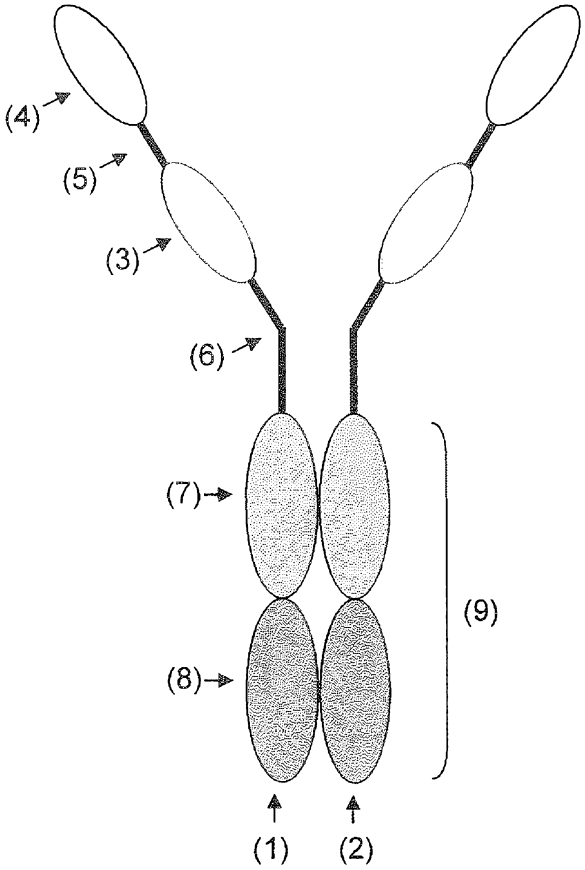

Figure 2

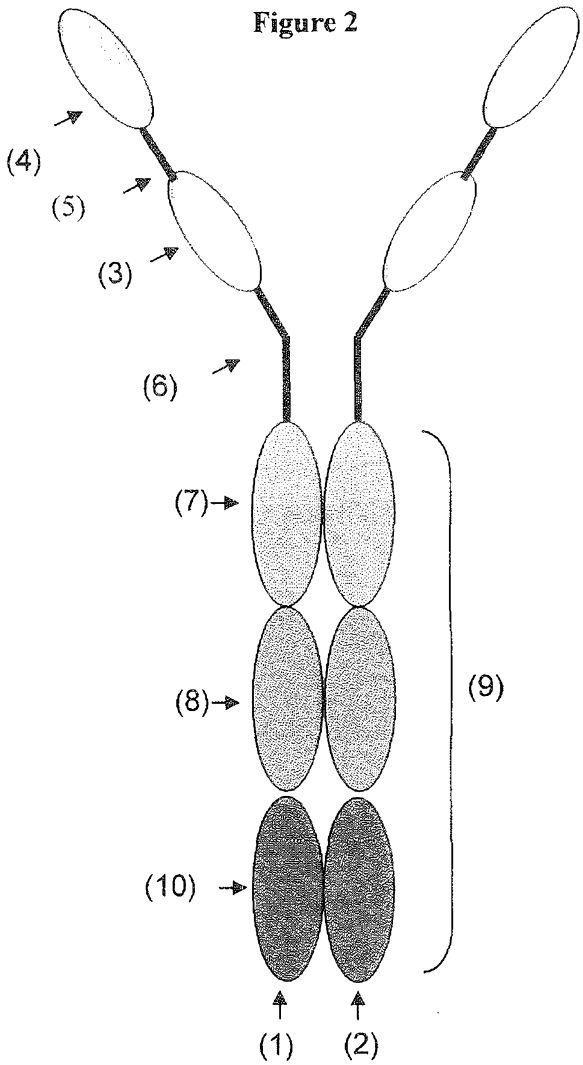

Figure 3

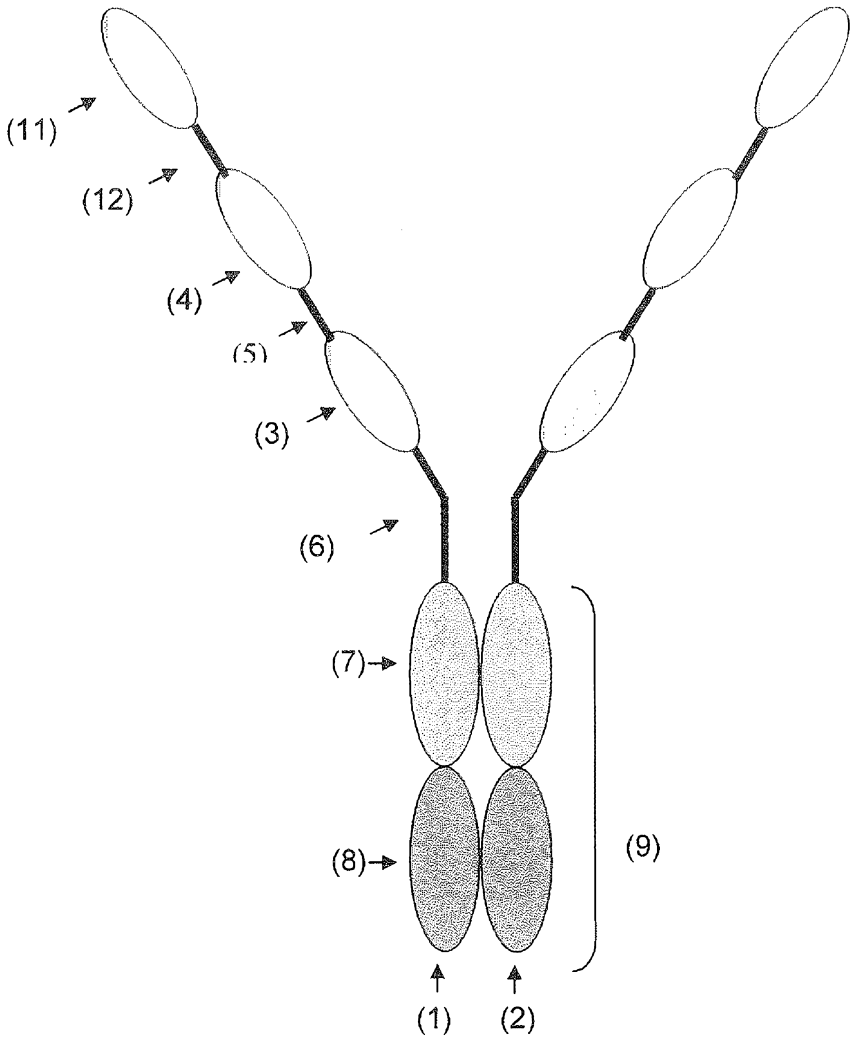

Figure 4

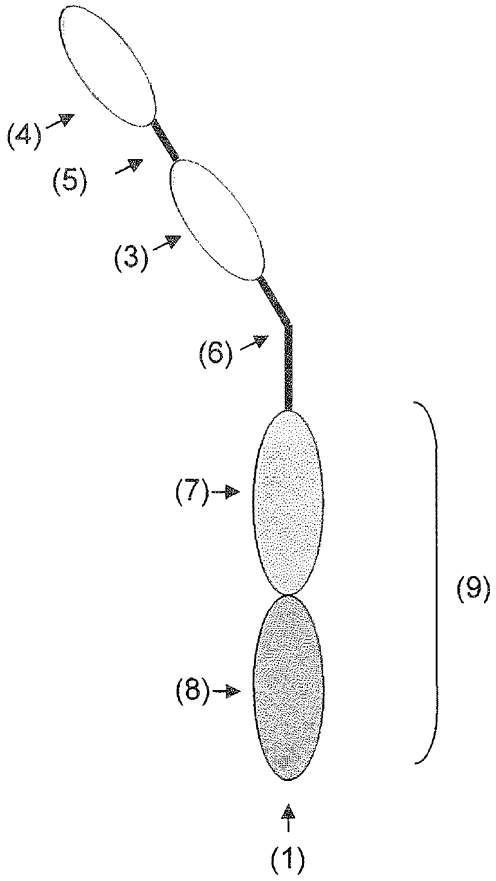

Figure 5A

>47D5-35GS-2D3-HINGE-CH2-CH3
KVQLVESGGGLVQPGGSLRLSCAASGSIFGFNDMAWYRQAPGKQRELVALISRVGV
TSSADSVKGRFTISRVNAKDTVYLQMNSLKPEDTAVYYCYMDQRLDGSTLAYWGQ
GTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV
QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSSINWSGTHTDYADSVKGRF
TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVS
SEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

Figure 5B

>47D5-35GS-2D3-HINGE-CH2-CH3
AAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTG
AGACTCTCCTGTGCAGCCTCTGGAAGCATCTTCGGTTTCAATGACATGGCCTGGT
ACCGCCAGGCTCCAGGGAAGCAGCGCGAGTTGGTCGCACTAATTAGTAGGGTTG
GTGTCACAAGTTCTGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGTCAA
CGCCAAGGACACGGTGTATCTGCAAATGAACAGCCTGAAACCTGAGGATACGGC
CGTCTATTATTGTTATATGGATCAGCGACTCGACGGTAGTACTTTAGCGTACTGGG
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GACGCAGGGACCACATGGTTTGAGAAATCCGGTAGCGCGGGCCAAGGCACTCAG
GTGACAGTGAGCAGCAgcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagCacctgaactcctgggg
ggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgt
gagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggag
cagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtc
tccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagGgcagccccgagaaccacaggtgtacaccctgc
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagt
gggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagc
tcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcaga
agagcctctccctgtctccgggtaaatga

Figure 6A

>2D3-35GS-47D5-HINGE-CH2-CH3
EVQLVESGGSLVQPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSSINWSGT
HTDYADSVKGRFTISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFEKS
GSAGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES
GGGLVQPGGSLRLSCAASGSIFGFNDMAWYRQAPGKQRELVALISRVGVTSSADSVK
GRFTISRVNAKDTVYLQMNSLKPEDTAVYYCYMDQRLDGSTLAYWGQGTQVTVSS
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GK

Figure 6B

>2D3-35GS-47D5-HINGE-CH2-CH3
GAGGTGCAGCTGGTGGAGTCTGGGGGGCTCCTTGGTGCAGCCTGGGGGGTCTCTGA
GACTCTCCTGTGCAGCCTCTGGCTTCACTTTTGATGATTATGCCATGAGCTGGGTC
CGACAGGTTCCAGGGAAGGGGTTGGAGTGGGTTTCATCTATTAATTGGTCTGGTA
CTCACACAGACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAAACAA
CGCCAATAACACGCTGTATCTACAAATGAACAGTCTGAAATCTGAGGACACGGCC
GTGTATTACTGTGCAAAAAACTGGAGAGACGCAGGTACTACCTGGTTCGAAAAGT
CCGGCTCCGCGGGCCAGGGGACCCAGGTCACCGTATCGAGTGGGGGCGGGGGAA
GTGGCGGAGGTGGGTCCGGTGGCGGAGGCAGCGGTGGAGGAGGTAGTGGCGGTG
GCGGTAGTGGCGGTGGCGGCAGTGGAGGCGGAGGATCCGAGGTGCAGCTGGTGG
AGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGCAGC
CTCTGGAAGCATCTTCGGTTTCAATGACATGGCCTGGTACCGCCAGGCTCCAGGG
AAGCAGCGCGAGTTGGTCGCACTAATTAGTAGGGTTGGTGTCACAAGTTCTGCAG
ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGTCAACGCCAAGGACACGGTGTA
TCTGCAAATGAACAGCCTGAAACCTGAGGATACGGCCGTCTATTATTGTTATATG
GATCAGCGACTCGACGGTAGTACTTTAGCGTACTGGGGCCAGGGGACCCAGGTC
ACCGTCTCCTCAAgcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagCacctgaactcctgggggggacc
gtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagcc
acgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagta
caacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaa
caaagccctcccagcccccatcgagaaaaccatctccaaagccaaagGgcagccccgagaaccacaggtgtacaccctgcccccca
tcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggag
agcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagag
cctctccctgtctccgggtaaatga

Figure 7A

>Her2 47D5-G/S-2D3-G/S-C2-3-4 IgE
KVQLVESGGGLVQPGGSLRLSCAASGSIFGFNDMAWYRQAPGKQRELVALISRVGV
TSSADSVKGRFTISRVNAKDTVYLQMNSLKPEDTAVYYCYMDQRLDGSTLAYWGQ
GTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGSLV
QPGGSLRLSCAASGFTFDDYAMSWVRQVPGKGLEWVSSINWSGTHTDYADSVKGRF
TISRNNANNTLYLQMNSLKSEDTAVYYCAKNWRDAGTTWFEKSGSAGQGTQVTVS
SGGGSGGGSGGGSVDNKTFSVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTP
GTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQG
HTFEDSTKKCADSNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRA
SGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTT
KTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHS
TTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

Figure 7B

>Her2 47D5-G/S-2D3-G/S-C2-3-4 IgE
aaggtgcagctggtggagtctgggggaggcttggtgcagcctgggggtctctgagactctcctgtgcagcctctggaagcatcttcg
gtttcaatgacatggcctggtaccgccaggctccagggaagcagcgcgagttggtcgcactaattagtagggttggtgtcacaagttct
gcagactccgtgaagggccgattcaccatctccagagtcaacgccaaggacacggtgtatctgcaaatgaacagcctgaaacctgag
gatacggccgtctattattgttatatggatcagcgactcgacggtagtactttagcgtactggggccaggggacccaggtcaccgtatcg
agtgggggcgggggaagtggcggaggtgggtccggtggcggaggcagcggtggaggaggtagtggcggtggcggtagtggcg
gtggcggcagtggaggcggaggatccgaagtgcagttagtcgagtcaggggggtagcttggtgcagccgggggggtagcctgcgcct
gagctgcgccgcgagcggcttcaccttcgacgattatgcgatgtcatgggtcagacaggtccctggtaaagggcttgaatgggtttcct
caataaactggagcggcacccatacggattatgcggatagcgtgaaaggacgttttaccattagccgcaataacgctaataacactctgt
acctacaaatgaactcgctcaaatctgaagatactgctgtctactattgtgccaagaattggcgtgacgcagggaccacatggtttgaga
aatccggtagcgcgggccaaggcactcaggtgacagtgagcagcGGCGGCGGCAGCGGCGGCGGCAGCG
GCGGCGGCAGCgtcgacaacaaaaccttcagcgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtc
ctgcgacggcggcgggcacttcccccgaccatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacatcacctgg
ctggaggacgggcaggtcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagct
caccctcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaa
gaagtgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccac
gatcacctgtctggtggtggacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaacc
actccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcga
gggggagacctaccagtgcagggtgacccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcg
tgctgccccggaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaactt
catgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaag
accaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgca
gtccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatga

Figure 8A

>Her2 2D3-G/S-47D5-G/S-C2-3-4 IgE
evqlvesggslvqpggslrlscaasgftfddyamswvrqvpgkglewvssinwsgthtdyadsvkgrftisrnnanntlylqmns
lksedtavyycaknwrdagttwfeksgsagqgtqvtvssggggsggggsggggsggggsggggsggggsevqlvesg
gglvqpggslrlscaasgsifgfndmawyrqapgkqrelvalisrvgvtssadsvkgrftisrvnakdtvylqmnslkpedtavyy
cymdqrldgstlaywgqgtqvtvssGGGSGGGSGGGSVDNKTFSVCSRDFTPPTVKILQSSCDGG
GHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLSQK
HWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFDLFIRKSPTITCLV
VDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQ
CRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDIS
VQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAAS
PSQTVQRAVSVNPGK

Figure 8B

>Her2 2D3-G/S-47D5-G/S-C2-3-4 IgE
gaggtgcagctggtggagtctgggggctccttggtgcagcctgggggtctctgagactctcctgtgcagcctctggcttcacttttgat
gattatgccatgagctgggtccgacaggttccagggaaggggttggagtgggtttcatctattaattggtctggtactcacacagactatg
cagactccgtgaagggccgattcaccatctccagaaacaacgccaataacacgctgtatctacaaatgaacagtctgaaatctgagga
cacggccgtgtattactgtgcaaaaaactggagagacgcaggtactacctggttcgaaaagtccggctccgcgggccaggggaccc
aggtcaccgtatcgagtgggggcgggggaagtggcggaggtgggtccggtggcggaggcagcggtggaggaggtagtggcggt
ggcggtagtggcggtggcggcagtggaggcggaggatccgaggtgcagctggtggagtctgggggaggcttggtgcagcctggg
gggtctctgagactctcctgtgcagcctctggaagcatcttcggtttcaatgacatggcctggtaccgccaggctccagggaagcagcg
cgagttggtcgcactaattagtagggttggtgtcacaagttctgcagactccgtgaagggccgattcaccatctccagagtcaacgcca
aggacacggtgtatctgcaaatgaacagcctgaaacctgaggatacggccgtctattattgttatatggatcagcgactcgacggtagta
ctttagcgtactggggccaggggacccaggtcaccgtctcctcaGGCGGCGGCAGCGGCGGCGGCAGCGGC
GGCGGCAGCgtcgacaacaaaacttcagcgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctg
cgacggcggcgggcacttcccccgaccatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacatcacctggctg
gaggacgggcaggtcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcac
cctcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaa
gtgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgat
cacctgtctggtggtggacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccact
ccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgagg
gggagacctaccagtgcagggtgacccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtg
ctgccccggaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttc
atgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaaga
ccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagt
ccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatga

Figure 9A

>121A2 (p19+) -35GS- 81G2 (p19-)-Hinge-CH2-CH3
EVQLVESGGGLVQAGGSLRLSCAASGSIFNFNYMGWFRQAPGKEREFVAAIRWSGSS
TYYADSVKGRFTISRDDAKNTVALQMNSLKPEDTAIYYCALRKGIPYSTSDRVIKGV
NDYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQ
LVESGGGLVQPGGSLRLSCIASGLPFSTKSMGWFRQAPGKEREFVARISPGGTSRYYG
DFVKGRFAISRDNAKNTTWLQMNSLKAEDTAVYYCASGERSTYIGSNYYRTNEYDY
WGTGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

Figure 9B

>121A2 (p19+) –35GS- 81E10 (p40+)-Hinge-CH2-CH3
EVQLVESGGGLVQAGGSLRLSCAASGSIFNFNYMGWFRQAPGKEREFVAAIRWSGSS
TYYADSVKGRFTISRDDAKNTVALQMNSLKPEDTAIYYCALRKGIPYSTSDRVIKGV
NDYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQ
LVESGGGLVQAGGSLRLSCVASGRTFNTYGMGWFRQAPGKEREFVAANNWSGGAT
SYADSVKGRFTISRDNAKNTVFLQMNTLKPEDTAVYYCAAADRGGGWLVVRENDY
DYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

Figure 9C

>81E10 (p40+) –35GS- 80D10 (p40-)-Hinge-CH2-CH3
EVQLVESGGGLVQAGGSLRLSCVASGRTFNTYGMGWFRQAPGKEREFVAANNWSG
GATSYADSVKGRFTISRDNAKNTVFLQMNTLKPEDTAVYYCAAADRGGGWLVVRE
NDYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQ
LVESGGGLVQAGGSLRLSCAASGRTFEGNPMGWFRQASGKKREFVASIDWSGGITSY
ADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAASARFGSGSYYDLMYDYW
GQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK