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(54) **Title**: IMMUNOGLOBULIN SINGLE VARIABLE DOMAINS DIRECTED AGAINST IGE

(57) **Abstract**: Polypeptides and protein constructs are provided that are directed against IgE. In a particular aspect, Nanobodies against IgE are described linked to a serum albumin binding peptide. Further described are nucleic acids that encode such polypeptides and protein constructs; compositions, and in particular pharmaceutical compositions, that comprise such polypeptides and protein constructs; and uses of such polypeptides, protein constructs and compositions.

IMMUNOGLOBULIN SINGLE VARIABLE DOMAINS DIRECTED AGAINST IGE

FIELD OF THE INVENTION

The present invention relates to polypeptides and protein constructs that are directed against IgE; to nucleic acids that encode such polypeptides and protein constructs; to compositions, and in particular pharmaceutical compositions, that comprise such polypeptides and protein constructs; and to uses of such polypeptides, protein constructs and compositions.

If any terms are not specifically defined herein, these terms have the meaning given to them in WO 09/068627. If any terms used herein are not specifically defined herein or in WO 09/068627, then they have their usual meaning in the art, for which reference is for example made to the standard handbooks.

BACKGROUND ART

The international application WO 04/041865 describes specific protein constructs that are directed against IgE that comprise at least one VHH or humanized VHH against IgE and at least one Nanobody that is directed against a serum protein such as (human) serum albumin, which because of the presence of the serum albumin-binding Nanobody have increased half-life *in vivo* compared to the corresponding VHH's against IgE alone. Examples of such bispecific anti-IgE/anti-serum albumin constructs are given in SEQ ID NO's: 22 to 24 of WO 04/041865.

The international application WO 04/041867 describes VHH's that are directed against IgE (see for example SEQ ID NO's: 1 to 11 of WO 04/041867). WO 04/041867 further mentions that these VHH's may be humanized and may be suitably linked to one or more VHH's that are directed against a serum protein such as serum albumin, to provide a protein construct that has increased half-life as well as the favorable properties that are associated with VHH's and Nanobodies.

The international application WO 06/122787 describes a number of Nanobodies against (human) serum albumin. These Nanobodies include the Nanobody called Alb-1 (SEQ ID NO: 52 in WO 06/122787) and humanized variants thereof, such as Alb-8 (SEQ ID NO: 62 in WO 06/122787; SEQ ID NO: 174 herein). *[Nanobody® and Nanobodies® are trademarks of Ablynx N.V.].*

As of the date of first filing of the present application, the use of Nanobodies against (human) serum albumin for extending the half-life of therapeutic moieties such as Nanobodies has been validated by means of clinical trials. For example, the safety, tolerability, immunogenicity and pharmacokinetics (PK) of ALX-0141, a protein construct that comprises two Nanobodies against RANK-L and one Nanobody against human serum albumin, has been confirmed in phase I clinical trials (data presented by Ablynx N.V. on May 27, 2011 at the Annual European Congress of Rheumatology (EULAR) in London). Also, numerous published patent applications of Ablynx N.V. give examples of constructs with increased half-life that comprise one or more Nanobodies against a therapeutic target and one or more Nanobodies against serum albumin (such as Alb-8). Reference is for example made to WO 04/041862, WO 06/122786, WO 08/020079, WO 08/142164, WO 09/068627 and WO 09/147248.

Although it has been established that the use of Nanobodies against (human) serum albumin is a good and broadly applicable way of extending the half-life of Nanobodies and other therapeutic entities, the present inventors have found that when representative Nanobodies according to WO 06/122787 are applied to extending the half-life of Nanobodies that are directed to IgE (in the manner generally described in WO 04/041865 and WO 04/041867), that the constructs thus obtained, even though they are sufficiently biologically active against IgE and have a half-life that is suitable for therapeutic applications, have some properties that would benefit from further improvement.

In particular, it has been found that that polypeptides that comprise a Nanobody against IgE and Alb-8, a representative serum albumin-binding Nanobody according to WO 06/122787, have a limited storage stability that leaves room for further improvement (see for example the results presented in the Experimental Part herein).

SUMMARY OF THE INVENTION

Thus, the invention generally has the objective of providing improved polypeptides and (other) protein constructs that comprise at least one immunoglobulin single variable domain (such as a Nanobody) against IgE and that have an increased half-life *in vivo* (as further described herein, and compared to the immunoglobulin single variable domain against IgE per se) that are not associated with the same problem(s) as have now been found to occur when anti-IgE constructs of the type generally described in WO 04/041865 and WO 04/041867 are used.

The published international applications WO 08/068280, WO 09/127691, US application US 2011/0243954 and WO 2011/095545 describe peptides that are directed against human serum albumin and that that can be linked to therapeutic moieties (such as Nanobodies against a therapeutic target) so as provide said therapeutic moiety with increased half-life. The half-life extension technology described in these international applications is also known as the NExpedite™ technology *[NExpedite™ is a trademark of Ablynx N.V.]*. WO 08/068280 also generally mentions that the therapeutic moiety may be a Nanobody against IgE as described in WO 04/041867).

Surprisingly, it has been found that the use of the serum albumin-binding peptides described in WO 08/068280, in WO 09/127691, in US 2011/0243954 and in particular in WO 2011/095545 for extending the half-life of Nanobodies against IgE is not associated with the problems that occur when anti-IgE/anti-serum albumin polypeptides and constructs of the type generally described in WO 04/041865 and WO 04/041867 are used. In particular, it has been found that constructs that comprise one or more Nanobodies against IgE and one or more albumin binding peptides as described in WO 08/068280, in WO 09/127691, in US 2011/0243954 and in particular in WO 2011/095545, have an improved storage stability (as for example determined by the SE-HPLC experiments described in Example 16) compared to comparable half-life extended anti-IgE polypeptides in which a representative albumin-binding Nanobody according to WO 06/122787 is used. Reference is again made to the data and results presented in the Experimental Part herein.

In addition to solving these problems with the known anti-IgE constructs (which problems have as yet not been recognized in the prior art), in establishing the present invention, the inventors have also generated a number of immunoglobulin single variable domains against IgE that have certain advantages compared to the known Nanobodies against IgE (such as those described in WO 04/041865 and WO 04/041867). These improved Nanobodies against IgE can, for example and with advantage, be used as "building blocks" to provide the half-life extended anti-IgE protein constructs described herein. These anti-IgE immunoglobulin single variable domains (or "ISV's) and polypeptides and protein constructs comprising the same form further aspects of the invention.

Furthermore, by combining the improved anti-IgE building block disclosed herein with the half-life extended peptides described in WO 08/068280, in WO 09/127691, in US

2011/0243954 and in particular in WO 2011/095545, the inventors have also provided a range of different half-life extended anti-IgE proteins constructs with improved properties compared to the half-life extended anti-IgE constructs described in WO 04/041865, and these improved protein constructs and polypeptides form further aspects of the invention.

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

DETAILED DESCRIPTION

Thus, in a first aspect, the invention relates to a polypeptide, protein, construct or compound (also collectively referred to herein as "*anti-IgE polypeptides of the invention*" or "*polypeptides of the invention*") that comprises or essentially consists of:

a) one or more (such as one or two) immunoglobulin single variable domains (ISV's) that are directed against IgE (which can be as further described herein, and are preferably according to the preferred aspects described herein); and

b) one or more (such as one or two, including without limitation a tandem repeat of) peptides directed against (human) serum albumin which are as described in WO 08/068280, in WO 09/127691, in US 2011/0243954 and in particular in WO 2011/095545 (which preferably are according to the preferred aspects described herein);

suitably linked to each other either directly or via one or more suitable linkers or spacers.

Such a polypeptide, protein, construct or compound (and/or the one or more ISV's against IgE present therein) may in particular be such that they are capable of modulating, and in particular inhibiting or blocking (fully or partially) the interaction between (human) IgE and the (human) Fc(epsilon)RI (the high affinity IgE receptor), for example as measured in a suitable assay, such as one of the assays used in the Experimental Part below (for example, the assay used in Example 7). For example, it may be such that it inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M.

Such a polypeptide, protein, construct or compound (and/or the one or more ISV's against IgE present therein) may also be such (or in addition be such) that they are capable of

modulating, and in particular inhibiting or blocking (fully or partially) that interaction between (human) IgE and the (human) Fc(epsilon)RII (the low affinity IgE receptor), for example as measured in a suitable assay, such as one of the assays used in the Experimental Part below (for example, the assay used in Example 8). For example, it may be such that it inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M.

Also, any such polypeptide, protein, construct or compound (and/or the one or more ISV's against IgE present therein) may be such that it has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

IgE binding ISV

The one or more immunoglobulin single variable domains or "ISV's" against IgE that are present in the IgE constructs of the invention can be any suitable immunoglobulin single variable domain that is directed against IgE. As such the ISV against IgE can for example be a domain antibody, single domain antibody, dAb™, Nanobody, VHH, IgNAR domain or other suitable immunoglobulin single variable domain that is directed against IgE. Preferably, said one or more ISV's against IgE are Nanobodies (such as VHH's, humanized VHH's or camelized VH's, such as camelized human VH's).

The one or more ISV's (and in particular Nanobodies) against IgE present in the anti-IgE polypeptides of the invention may in particular be ISV's or Nanobodies that are cross-reactive between the human IgE Fc sequence of SEQ ID NO: 172 and the and IgE Fc sequence from cynomolgus monkey given in SEQ ID NO: 173. In particular, such ISV's may have an affinity (as determined by a suitable technique, such as Biacore, for example using the protocol described in Example 12) for cynomolgus IgE that is at least 1%, for example 5%, such as at least 10%, for example at least 25% such as at least 50% or more of the affinity of the ISV for human IgE. For example, the one or more ISV's (and in particular Nanobodies) against IgE present in the anti-IgE polypeptides of the invention may have an affinity for human IgE (as determined using

Biacore, for example using the protocol described in Example 12) that is better than 50nM, preferably better than 25nM, (KD1 value – see Example 12) and/or better than 5 nM, preferably better than 2 nM, such as better than 1 nM (KD2 value – see again Example 12); and an affinity for cyno IgE that is better than 50 nM, such as better than 30nM (KD1 value) and/or better than 5 nM, preferably better than 2nM, such as better than 1 nM (KD2 value).

Some non-limiting examples of Nanobodies against IgE that can be used in the anti-IgE polypeptides of the invention are the anti-IgE Nanobodies described in WO 04/041865 or WO 04/041867, such as VHH#2C3, VHH#4G12, VHH#2C1, VHH#2H3, VHH#2D12, VHH#2G4, VHH#4C5, VHH#4A2, VHH#2D4, VHH#2B6 or VHH#2H11 (see Table 14 and SEQ ID NO's: 1 to 11 from WO 04/041867), or humanized and/or sequence optimized variants of the same. Other Nanobodies against IgE that can be used in the anti-IgE polypeptides of the invention are the anti-IgE Nanobodies described herein, such as 39B02 (SEQ ID NO: 114), 39D8 (SEQ ID NO: 115), 42D7 (SEQ ID NO: 116), 42G5(SEQ ID NO: 117), 36G5 (SEQ ID NO: 118) or humanized and/or sequence-optimized version of the same, and in particular 39D11 (SEQ ID NO: 119), a "39D11-type sequence" (as defined herein) and in particular a "39D11-like sequence" (as defined herein), such as a humanized and/or sequence-optimized variant of 39D11, for example of the variants of SEQ ID NO's: 120 to 133, of which IGE026 (SEQ ID NO: 128, also referred to herein as IGE0045 or IgE66G02) is a particularly preferred example. Another particularly preferred example of such a building block (in particular when it is present at the N-terminal end) is IGE026 with a E to D mutation at position 1. This building block is also referred to herein as IgE66G02(E1D) or IgE66G02^{E1D} (SEQ ID NO: 129).

The amino acid sequence of 39D11 is (with the CDR's underlined and in bold. These CDR's are also given in SEQ ID NO's: 134, 136 and 137, respectively):

EVQLVESGGGLVQPGGSLRLSCAASGFTFG**SYDMS**WVRQAPGKGPEWVS**SIDT GGDITHYADSVKG**RFTISRDNANNMLYLQMNSLKPEDTAVYWCAT**DEDYALG PNEYDY**YGQGTQVTVSS [SEQ ID NO: 119]

One preferred humanized and sequence-optimized variant of 39D11 (which has also been affinity matured compared to 39D11) is IGE026 (also called IgE0045). The amino acid sequence of IGE026 is (with the CDR's underlined and in bold. These CDR's are also given in SEQ ID

NO's: 135, 136 and 138, respectively; it being understood that CDR2 is the same for 39D11 and IGE026):

EVQLLESGGGLVQPGGSLRLSCAASGFTFG**NYDMA**WVRQAPGKRPEWVS**SIDT**
GGDITHYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYWCAT**DEEYALGP**
NEFDYYGQGTLVTVSS [SEQ ID NO: 128]

As defined herein, a "*39D11-type sequence*", "*39D11-type ISV*" or "*39D11-type building block*" in its broadest sense is an immunoglobulin single variable domain, and in particular a Nanobody, that is such that (i) it competes with 39D11 for binding to (human) IgE (for example, in a suitable binding assay, such as a BIAcore assay, for example the BIAcore assay set up or Alphascreen assay essentially as described in Example 9, but using 39D11 instead of omalizumab); and/or (ii) it binds to the same epitope on (human) IgE as 39D11; and/or (iii) it cross-blocks (as defined in WO 09/068627) the binding of 39D11 to IgE. 39D11-type sequences also preferably are cross reactive between human IgE and cyno IgE, as further described herein. An "39D11-type sequence", "39D11-type ISV" or "39D11-type building block" is preferably further such that it (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M; and/or (iii) has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

The "39D11-type sequence" as such also forms part of the present invention.

As defined herein, a "*39D11-like sequence*", "*39D11-like sequence*", "*39D11-like ISV*" or "*39D11-like building block*" is defined as an ISV (as described herein) that comprises:

a) a CDR1 which comprises or essentially consists of either (i) the amino acid sequence SYDMS (SEQ ID NO: 134) and/or the amino acid sequence NYDMA (SEQ ID NO: 135) or

(ii) an amino acid sequence that has only 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence SYDMS (SEQ ID NO: 134) and/or the amino acid sequence NYDMA (SEQ ID NO: 135); and/or

b) a CDR2 which comprises or essentially consists of either (i) the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136); and/or

c) a CDR3 which comprises or essentially consists of either (i) the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and/or the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and/or the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and/or the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138);

in which the framework sequences present in such an ISV are as further described herein, and in which CDR1, CDR2 and CDR3 are preferably such that the 39D11-like ISV (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M; and/or (iii) has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

Preferably, in such a 39D11-like sequence, CDR1 and CDR2 are as defined under a) and b), respectively; or CDR1 and CDR3 are as defined under a) and c), respectively; or CDR2 and CDR3 are as defined under b) and c), respectively. More preferably, in such a 39D11-like sequence, CDR1, CDR2 and CDR3 are all as defined under a), b) and c), respectively. Again, in such an 39D11-like sequence, CDR1, CDR2 and CDR3 are preferably such that the 39D11-like ISV: (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M; and/or (iii) has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

For example, in such an 39D11-like sequence: CDR1 may comprise or essentially consist of the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably of the amino acid sequence NYDMA (SEQ ID NO: 135) (with CDR2 and CDR3 being as defined under b) and c), respectively); and/or CDR2 may comprise or essentially consist of the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136) (with CDR1 and CDR3 being as defined under a) and c), respectively); and/or CDR3 may comprise or essentially consist of the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably of the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138) (with CDR1 and CDR2 being as defined under a) and b), respectively). Particularly, when an 39D11-like sequence is according to this aspect: CDR1 may comprise or essentially consist of the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably of the amino acid sequence NYDMA (SEQ ID NO: 135) and CDR2 may comprise or essentially consist of the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136) (with CDR3 being as defined under c) above); and/or CDR1 may comprise or essentially consist of the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably of the amino acid sequence NYDMA (SEQ ID NO: 135) and CDR3 may comprise or essentially consist of the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably of the amino acid

sequence DEEYALGPNEFDY (SEQ ID NO: 138) (with CDR2 being as defined under b) above); and/or CDR2 may comprise or essentially consist of the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136) and CDR3 may comprise or essentially consist of the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably of the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138) (with CDR1 being as defined under a) above). Again, in such 39D11-like sequences, CDR1, CDR2 and CDR3 are preferably such that the 39D11-like ISV (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M; and/or (iii) has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

In a particularly preferred 39D11-like sequence: CDR1 comprises or essentially consists of the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably the amino acid sequence NYDMA (SEQ ID NO: 135), CDR2 comprises or essentially consists of the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136); and CDR3 comprises or essentially consists of the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138).

In a specifically preferred aspect, a "*39D11-like sequence*", "*39D11-like ISV*" or "*39D11-like building block*" is an ISV that comprises:

d) a CDR1 which is either (i) the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably the amino acid sequence NYDMA (SEQ ID NO: 135) or (ii) an amino acid sequence that has only 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence SYDMS (SEQ ID NO: 134) and/or the amino acid sequence NYDMA (SEQ ID NO: 135); and/or

e) a CDR2 which is either (i) the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for

example at least 90% or more than 95% sequence identity with the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136); and/or

f) a CDR3 which is either (i) the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and/or the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and/or the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138);

in which the framework sequences present in such an ISV are as further described herein, and in which CDR1, CDR2 and CDR3 are preferably such that the 39D11-like ISV (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M; and/or (iii) has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

Preferably, in a 39D11-like sequence according to this specifically preferred aspect, CDR1 and CDR2 are as defined under d) and e), respectively; or CDR1 and CDR3 are as defined under d) and f), respectively; or CDR2 and CDR3 are as defined under e) and f), respectively. More preferably, in such a 39D11-like sequence, CDR1, CDR2 and CDR3 are all as defined under d), e) and f), respectively. Again, in such an 39D11-like sequence, CDR1, CDR2 and CDR3 are preferably such that the 39D11-like ISV has (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay

described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M; and/or (iii) has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

For example, in a 39D11-like sequence according to this specifically preferred aspect: CDR1 is the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably the amino acid sequence NYDMA (SEQ ID NO: 135) (with CDR2 and CDR3 being as defined under e) and f), respectively); and/or CDR2 is the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136) (with CDR1 and CDR3 being as defined under d) and f), respectively); and/or CDR3 is the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138) (with CDR1 and CDR2 being as defined under d) and e), respectively). Particularly, when an 39D11-like sequence is according to this aspect: CDR1 is the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably the amino acid sequence NYDMA (SEQ ID NO:135) and CDR2 is the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136) (with CDR3 being as defined under f) above); and/or CDR1 is the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably the amino acid sequence NYDMA (SEQ ID NO: 135) and CDR3 is the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138) (with CDR2 being as defined under e) above); and/or CDR2 is the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136) and CDR3 is the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138) (with CDR1 being as defined under d) above). Again, in such 39D11-like sequences, CDR1, CDR2 and CDR3 are preferably such that the 39D11-like ISV (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M,

better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M; and/or (iii) has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

In a particularly preferred 39D11-like sequence: CDR1 is the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably the amino acid sequence NYDMA (SEQ ID NO: 135), CDR2 is the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136); and CDR3 is the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138).

In all the 39D11-like sequence described herein, the FR1, FR2, FR3 and FR4 sequences, respectively, are preferably such that taken together, these framework sequences have at least 80%, such as at least 85%, for example at least 90%, such as at least 95% sequence identity with the framework sequences of 39D11 (SEQ ID NO's: 139, 141, 143 and 145, respectively) and/or the framework sequences of IGE026 (SEQ ID NO's: 140, 142, 144 and 146, respectively). For example and without limitation, each of the FR1, FR2, FR3 and FR4 sequences in a 39D11-like sequence may have between 0 and 7, such as between 0 and 5, such as 1, 2, 3 or 4 (suitable) amino acid differences with the FR1, FR2, FR3 and FR4 sequence, respectively, of 39D11 and/or with the FR1, FR2, FR3 and FR4 sequence, respectively, of IGE026. Other suitable framework sequences may essentially be as described on pages 273 to 291 of WO 09/068627 (described in WO 09/068627 for Nanobodies against IL-23). Reference is for example made to Tables A-10 to A-25 of WO 09/068627, as well as the various humanizing substitutions described in Tables A-6 to A-9 of WO 09/068627.

Again, the combination of CDR's and frameworks present in a given sequence are preferably such that the resulting 39D11-like ISV (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA

assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M; and/or (iii) has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

In one specific aspect, a 39D11-like sequence is an ISV that has at least 70%, such at least 80%, for example at least 85%, such as at least 90% or more than 95% sequence identity with the amino acid sequence 39D11 (SEQ ID NO: 119). For example, in an 39D11-like sequence according to this aspect, the CDR's may be according to the specifically preferred aspect described above, and may in particularly (but without limitation) be SYDMS (SEQ ID NO: 134) and preferably NYDMA (SEQ ID NO: 135) (CDR1); SIDTGGDITHYADSVKG (SEQ ID NO: 136) (CDR2); and DEDYALGPNEYDY (SEQ ID NO: 137) and preferably DEEYALGPNEFDY (SEQ ID NO: 138) (CDR3). Again, preferably, the combination of CDR's and frameworks present in such a 39D11-like ISV are preferably such that the resulting 39D11-like ISV (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M; and/or (iii) has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

In one particular aspect, any 39D11-like sequences may be a humanized and/or sequence-optimized variant of 39D11, as further described herein. These may for example and without limitation contain (any suitable combination of) one or more, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and essentially up to all, of the following mutations compared to the sequence of 39D11: E1D, V5L, E6Q, F29Y, S31N, S35A or S36G, G44R, N73K, M77T or M77L, K83R, W91A, D97E, Y100eF and/or Q108L (with the numbering according to Kabat, see for example Tables A-5 to

A-8 of WO 08/020079; and with each letter denominating an amino acid residue in accordance with the standard one-letter amino acid code, for which reference is made to Table A-2 of WO 08/020079). In another aspect, these may for example and without limitation contain (any suitable combination of) one or more, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and essentially up to all, of the following mutations compared to the sequence of 39D11: E1D, V5L, E6Q, F29Y, S31N, S35A or S35G, G44R, N75K, M77T or M77L, K83R, W91Y, D97E, Y100eF and/or Q108L (with the numbering according to Kabat, see for example Tables A-5 to A-8 of WO 08/020079; and with each letter denominating an amino acid residue in accordance with the standard one-letter amino acid code, for which reference is made to Table A-2 of WO 08/020079). As will be clear to the skilled person, some of these mutations are in the CDR's and may improve affinity of the ISV of IgE, potency, activity, and/or other biological properties of the 39D11-like sequence (compared to 39D11). Other suitable humanizing and/or sequence-optimizing substitutions will be clear to the skilled person, for example from the further disclosure herein and/or from comparing the sequence of 39D11 with a human VH sequence (for which reference is again for example made to Tables A-5 to A-8 of WO 08/020079).

In a preferred aspect, the 39D11-like sequence is selected from any of SEQ ID NO's: 120-133 or an ISV that has at least 70%, such at least 80%, for example at least 85%, such as at least 90% or more than 95% sequence identity with any of SEQ ID NO's: 120-133. Preferred 39D11-like sequences are SEQ ID NO's: 128 and 129.

39D11-like sequences also preferably are cross reactive between human IgE and cyno IgE, as further described herein.

As already mentioned, generally, any "39D11-like sequence", "39D11-like sequence", "39D11-like ISV" or "39D11-like building block" is preferably such that it: (i) inhibits the HuIgE/HuFc(epsilon)RI interaction (for example, in the ELISA assay described in Example 7; in the Alphascreen assay described in Example 6) with an IC50 value better than 8.10^{-10} M, preferably better than 6.10^{-10} M, such as better than 10^{-10}M, better than 5.10^{-11}M, better than 2.10^{-11}M, or even better than 10^{-11}M; and/or (ii) inhibits the HuIgE/HuFc(epsilon)RII interaction (for example, in the ELISA assay described in Example 8) with an IC50 value better than 5.10^{-8}M, preferably better than 2.10^{-8}M, such as better than 10^{-8}M, better than 5.10^{-9}M, better than 2.10^{-9}M, better than 10^{-9}M, better than 5.10^{-10}M, or better than 2.10^{-10} M.

Also, any "39D11-like sequence", "39D11-like sequence", "39D11-like ISV" or "39D11-like building block" is preferably such that it has an IC50 value in the degranulation assay described in Example 11 better than 100 nM, preferably better than 50 nM, more preferably better than 20nM, such as better than 5nM, better than 1 nM, or even better than 0.5 nM.

The "39D11-like sequence" as such also forms part of the present invention.

The ISV against IgE, and in particular the "39D11-type sequence" and the "39D11-like sequence", may or may not further comprise one or more other groups, residues, moieties or binding units. If present, such further groups, residues, moieties or binding units may or may not provide further functionality to the immunoglobulin single variable domain (and/or to the polypeptide in which it is present) and may or may not modify the properties of the immunoglobulin single variable domain.

For example, such further groups, residues, moieties or binding units may be one or more additional amino acid sequences, such that the polypeptide is a (fusion) protein or (fusion) polypeptide.

As described above, additional binding units can be linked to form multispecific polypeptides.

In the polypeptides described above, the one, two or more immunoglobulin single variable domains and the one or more groups, residues, moieties or binding units may be linked directly to each other and/or via one or more suitable linkers or spacers. For example, when the one or more groups, residues, moieties or binding units are amino acid sequences, the linkers may also be amino acid sequences, so that the resulting polypeptide is a fusion (protein) or fusion (polypeptide).

The one or more further groups, residues, moieties or binding units may be any suitable and/or desired amino acid sequences. The further amino acid sequences may or may not change, alter or otherwise influence the (biological) properties of the fusion polypeptide, and may or may not add further functionality to the fusion polypeptide. Preferably, the further amino acid sequence is such that it confers one or more desired properties or functionalities to the fusion polypeptide.

Example of such amino acid sequences will be clear to the skilled person, and may generally comprise all amino acid sequences that are used in peptide fusions based on conventional antibodies and fragments thereof (including but not limited to ScFv's and single

domain antibodies). Reference is for example made to the review by Holliger and Hudson (Nature Biotechnology 23: 1126-1136, 2005).

For example, such an amino acid sequence may be an amino acid sequence that increases the half-life, the solubility, or the absorption, reduces the immunogenicity or the toxicity, eliminates or attenuates undesirable side effects, and/or confers other advantageous properties to and/or reduces the undesired properties of the (fusion) polypeptide, compared to the ISV *per se*. Some non-limiting examples of such amino acid sequences are serum proteins, such as human serum albumin (see for example WO 00/27435) or haptenic molecules (for example haptens that are recognized by circulating antibodies, see for example WO 98/22141).

In one specific aspect of the invention, a polypeptide is prepared that has an increased half-life, compared to the corresponding ISV against IgE. Examples of polypeptides of the invention that comprise such half-life extending moieties for example include, without limitation, polypeptides in which the immunoglobulin single variable domains are suitable linked to one or more serum proteins or fragments thereof (such as (human) serum albumin or suitable fragments thereof) or to one or more binding units that can bind to serum proteins (such as, for example, Domain antibodies, amino acids that are suitable for use as a domain antibody, single domain antibodies, amino acids that are suitable for use as a single domain antibody, "dAb"'s, amino acids that are suitable for use as a dAb, or Nanobodies) that can bind to serum proteins such as serum albumin (such as human serum albumin), serum immunoglobulins (such as IgG), transferrin or one of the other serum proteins listed in WO 04/003019; polypeptides in which the immunoglobulin single variable domain is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more immunoglobulin single variable domains are suitable linked to one or more small proteins or peptides that can bind to serum proteins (such as, without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746 or WO 02/076489). Reference is also made to the dAb's described in WO 03/002609 and WO 04/003019 and to Harmsen *et al.* (Vaccine 23: 4926-42, 2005; to EP 0368684, as well as to WO 08/028977, WO 08/043821, WO 08/043822 by Ablynx N.V. and WO 08/068280, WO 09/127691, US 2011/0243954 and WO 2011/095545.

According to a specific, but non-limiting aspect of the invention, the polypeptides of the invention may contain, besides the one, two or more immunoglobulin single variable domains against IgE, at least one peptide (as further defined) against human serum albumin.

Generally, the polypeptides of the invention with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding immunoglobulin single variable domain or polypeptide of the invention *per se*.

Generally, the polypeptides of the invention with increased half-life preferably have a half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the half-life of the corresponding immunoglobulin single variable domain or polypeptide of the invention *per se*.

In another preferred, but non-limiting aspect, such polypeptides of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

The further amino acid residues may or may not change, alter or otherwise influence other (biological) properties of the (fusion) polypeptide and may or may not add further functionality to the (fusion) polypeptide. For example, such amino acid residues:

a) can comprise an N-terminal Met residue, for example as result of expression in a heterologous host cell or host organism.

b) may form a signal sequence or leader sequence that directs secretion of the (fusion) polypeptide from a host cell upon synthesis (for example to provide a pre-, pro- or prepro- form of the (fusion) polypeptide, depending on the host cell used to express the(fusion) polypeptide). Suitable secretory leader peptides will be clear to the skilled person, and may be as further described herein. Usually, such a leader sequence will be linked to the N-terminus of the (fusion) polypeptide, although the invention in its broadest sense is not limited thereto;

c) may form a "tag", for example an amino acid sequence or residue that allows or facilitates the purification of the (fusion) polypeptide, for example using affinity techniques directed

against said sequence or residue. Thereafter, said sequence or residue may be removed (e.g. by chemical or enzymatical cleavage) to provide the polypeptide (for this purpose, the tag may optionally be linked to the amino acid sequence or polypeptide sequence via a cleavable linker sequence or contain a cleavable motif). Some preferred, but non-limiting examples of such residues are multiple histidine residues, glutathione residues and a myc-tag such as AAAEQKLISEEDLNGAA (SEQ ID NO: 193);

d) maybe one or more amino acid residues that have been functionalized and/or that can serve as a site for attachment of functional groups. Suitable amino acid residues and functional groups will be clear to the skilled person and include, but are not limited to, the amino acid residues and functional groups mentioned herein for the derivatives of the polypeptides of the invention.

Accordingly, the one or more ISVs may be used as a "binding unit", "binding domain" or "building block" (these terms are used interchangeable) for the preparation of a (fusion) polypeptide, which may optionally contain one or more further binding unit (*i.e.*, against the same or another epitope on IgE and/or against one or more other antigens, proteins or targets than IgE).

The ISVs comprising the CDR sequences as described herein are particularly suited for use as building block or binding unit for the preparation of the (fusion) polypeptides.

Accordingly, the present invention also relates to the use of the ISVs as described herein in preparing a multivalent polypeptide. The method for the preparation of a multivalent polypeptide will comprise the linking of an ISV to at least one further binding unit, optionally via one or more linkers. The ISV as defined herein is then used as a binding domain or binding unit in providing and/or preparing the multivalent, polypeptide comprising two (*e.g.*, in a bivalent polypeptide), three (*e.g.*, in a trivalent polypeptide) or more (*e.g.*, in a multivalent polypeptide) binding units. In this respect, the IVS as described herein may be used as a binding domain or binding unit in providing and/or preparing a multivalent, such as bivalent or trivalent polypeptide comprising two, three or more binding units.

Serum albumin binding peptide

The serum albumin binding peptide that may be present in the anti-IgE polypeptides of the invention may generally be as described in WO 08/068280, in WO 09/127691, and in

particular as described in WO 2011/095545. Thus, for a general description of these peptides, reference is made to these applications.

As mentioned in these applications, these peptides may have a total length of between 5 and 50, preferably between 7 and 40, more preferably between 10 and 35, such as about 15, 20, 25 or 30 amino acid residues and are such that they can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493 (numbering as described in Example 8 of WO 09/127691). These applications also mention that these peptides can be linked or fused to a therapeutic moiety, compound, protein or other therapeutic entity in order to increase the half-life thereof.

WO 08/068280 describes a number of such peptides, including the amino acid sequence AASYSDYDVFGGGTDFGP (SEQ ID NO: 19), which is called "17D12" in WO 08/068280 and which is listed in WO 08/068280 as SEQ ID NO: 3.

WO 09/127691 describes a number of improved variants of 17D2 which generally have at least 50%, preferably at least 65 %, more preferably at least 70%, even more preferably at least 75%, such as at least 80%, such as at least 90%, but not 100%, sequence identity (as defined herein) with the amino acid sequence of SEQ ID NO: 19 and which can bind better to human serum albumin than the amino acid sequence of SEQ ID NO: 19.

In particular, some of the preferred peptides according to WO 09/127691 comprise:

(i) an Arg (R) residue, in particular an Arg (R) residue that is capable of forming a hydrogen bond with the amino acid residues Asn (N) 133 and Asn (N) 135 of human serum albumin and/or capable of forming electrostatic interactions with the main-chain oxygen atoms of the Pro (P) 134 and Leu (L) 136 residues of human serum albumin; and/or

(ii) a Trp (W) residue, in particular a Trp (W) residue that is capable of forming electrostatic interactions with the Arg (R) 138 residue of human serum albumin; and/or

(iii) the sequence motif GGG;

and preferably at least any two and more preferably all three of (i), (ii) and (iii).

In particular, some of the exemplary peptides described in WO 09/127691 may for example contain one or more of the following features:

(i) the sequence motif RXWD, in which X may be any amino acid sequence but is preferably W, Y, F, S or D; and/or

(ii) the sequence motif GGG, preferably the sequence motif FGGG (SEQ ID NO: 24), more preferably the sequence motif DVFGGG (SEQ ID NO: 33), and in particular the sequence motif DVFGGGT (SEQ ID NO: 37);

and most preferably both these sequence motifs (i) and (ii).

WO 2011/095545 describes some even further improved variants of the peptides described in WO 09/127691, which compared to the peptides described in WO 09/127691 further comprise, upstream (as defined in WO 2011/095545) of the Arg (R) residue mentioned above (which according to the numbering used in WO 08/068280, WO 09/127691 and WO 2011/095545 is position 3), and in particular upstream of the RXWD motif mentioned above, a stretch of amino acid residues of between 2 and 10 amino acid residues, which comprises at least one hydrophobic and/or aromatic amino acid residue (and for the remainder one or more further suitable amino acid residues, as for example exemplified in WO 2011/095545), in which said at least one hydrophobic amino acid residue may in particular be chosen from L, I, V and/or M and/or in which said at least one aromatic amino acid residue may in particular be chosen from W, Y and/or F. Preferably, said stretch of amino acids upstream of position 3 is preferably such that at least one of said hydrophobic and/or aromatic amino acid residues can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493. In particular, albumin binding peptides described in WO 2011/095545 may contain, upstream of position 3, a stretch of amino acid residues of between 2 and 10 amino acid residues, which comprises at least one W residue and/or at least one Y residue, such that at least one of said W or Y residues can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493. More in particular, said stretch of amino acid residues may comprise (i) at least two W residues; (ii) at least two Y residues; and/or (iii) at least one W residue and at least one Y residue, such that at least one of said W or Y residues can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493. Some non-limiting examples of such stretches of amino acid residues that may be present upstream of position 3 are given in SEQ ID NO's: 72 to 92 (SEQ ID NO's: 78 to 98 of WO 2011/095545).

WO 2011/095545 further describes that it is possible, and may even be advantageous, in the peptides describes therein: (i) to replace an aspartate (D) residue at position 8 by a threonine (T) residue (for example, but without limitation, in amino acid sequences of the invention that do not contain a threonine residue at position 14); and/or (ii) to replace the threonine (T) residue at position 14 with another amino acid residue, such as (for example and without limitation) A, N or D.

For example, some sequence motifs that may be present in the albumin-binding peptides according to WO 2011/095545 are:

- a stretch of amino acid residues upstream of position 3 that is as described in WO 2011/095545 (see also the preceding paragraphs herein). This may for example be one of the sequences of SEQ ID NO's: 78 to 98 of WO 2011/095545 (SEQ ID NO's: 72 to 92 herein), or a sequence that has 2 or only 1 "amino acid difference" - as defined in WO 2011/095545 - with at least one of these sequences;

in combination with one of the following sequence motifs:

- the amino acid sequence RXWDXDVFGGG (SEQ ID NO: 23 of WO 2011/095545; SEQ ID NO: 93 herein), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;
- the amino acid sequence RXWDXDVFGGGT (SEQ ID NO: 24 of WO 2011/095545; SEQ ID NO: 94 herein), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;
- the amino acid sequence RXWDXDVFGGGTP (SEQ ID NO: 25 of WO 2011/095545; SEQ ID NO: 95 herein), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;
- the amino acid sequence RXWDXDVFGGGTPG (SEQ ID NO: 26 of WO 2011/095545; SEQ ID NO: 96 herein), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;

- the amino acid sequence RXWDXDVFGGGTPGG (SEQ ID NO: 27 of WO 2011/095545; SEQ ID NO: 97 herein), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;
- an amino acid sequence chosen from RYWDYDVFGGG (SEQ ID NO: 28 of WO 2011/095545; SEQ ID NO: 98 herein); RDWDFDVFGGG (SEQ ID NO: 29 of WO 2011/095545; SEQ ID NO: 99 herein); RSWDFDVFGGG (SEQ ID NO: 30 of WO 2011/095545; SEQ ID NO: 100 herein) or RYWDFDVFGGG (SEQ ID NO: 31 of WO 2011/095545; SEQ ID NO: 101 herein); and in particular chosen from RDWDFDVFGGG (SEQ ID NO: 28 of WO 2011/095545; SEQ ID NO: 98 herein); RSWDFDVFGGG (SEQ ID NO: 29 of WO 2011/095545; SEQ ID NO: 99 herein) or RYWDFDVFGGG (SEQ ID NO: 30 of WO 2011/095545; SEQ ID NO: 100 herein);
- an amino acid sequence chosen from RYWDYDVFGGGT (SEQ ID NO: 32 of WO 2011/095545; SEQ ID NO: 102 herein); RDWDFDVFGGGT (SEQ ID NO: 33 of WO 2011/095545; SEQ ID NO: 103 herein); RSWDFDVFGGGT (SEQ ID NO: 34 of WO 2011/095545; SEQ ID NO: 104 herein) or RYWDFDVFGGGT (SEQ ID NO: 35 of WO 2011/095545; SEQ ID NO: 105 herein); and in particular chosen from RDWDFDVFGGGT (SEQ ID NO: 33 of WO 2011/095545; SEQ ID NO: 103 herein); RSWDFDVFGGGT (SEQ ID NO: 34 of WO 2011/095545; SEQ ID NO: 104 herein) or RYWDFDVFGGGT (SEQ ID NO: 35 of WO 2011/095545; SEQ ID NO: 105 herein);
- an amino acid sequence chosen from RYWDYDVFGGGTP (SEQ ID NO: 36 of WO 2011/095545; SEQ ID NO: 106 herein); RDWDFDVFGGGTP (SEQ ID NO: 37 of WO 2011/095545; SEQ ID NO: 107 herein); RSWDFDVFGGGTP (SEQ ID NO: 38 of WO 2011/095545; SEQ ID NO: 108 herein) or RYWDFDVFGGGTP (SEQ ID NO: 39 of WO 2011/095545; SEQ ID NO: 109 herein); and in particular chosen from RDWDFDVFGGGTP (SEQ ID NO: 37 of WO 2011/095545; SEQ ID NO: 107 herein); RSWDFDVFGGGTP (SEQ ID NO: 38 of WO 2011/095545; SEQ ID NO: 108 herein) or RYWDFDVFGGGTP (SEQ ID NO: 39 of WO 2011/095545; SEQ ID NO: 109 herein);
- an amino acid sequence chosen from RYWDYDVFGGGTPV (SEQ ID NO: 40 of WO 2011/095545; SEQ ID NO: 110 herein); RDWDFDVFGGGTPV (SEQ ID NO: 41 of WO 2011/095545; SEQ ID NO: 111 herein); RSWDFDVFGGGTPV (SEQ ID NO: 42 of WO

2011/095545; SEQ ID NO: 112 herein) or RYWDFDVFGGGTPV (SEQ ID NO: 43 of WO 2011/095545; SEQ ID NO: 113 herein); and in particular chosen from RDWDFDVFGGGTPV (SEQ ID NO: 41 of WO 2011/095545; SEQ ID NO: 111 herein); RSWDFDVFGGGTPV (SEQ ID NO: 42 of WO 2011/095545; SEQ ID NO: 112 herein) or RYWDFDVFGGGTPV (SEQ ID NO: 43 of WO 2011/095545; SEQ ID NO: 113 herein).

Thus, some other (non-limiting) sequence motifs that may be present in the albumin-binding peptides according to WO 2011/095545:

- an amino acid sequence according to any of sequence motifs of SEQ ID NO's: 24 to 27 of WO 2011/095545 (SEQ ID NO's: 94 to 97 herein) or 32 to 43 of WO 2011/095545 (SEQ ID NO's: 102 to 113 herein), in which the threonine (T) residue at position 14 has been replaced by another amino acid residue (preferably but without limitation, A, N or D);
- an amino acid sequence according to any of sequence motifs of SEQ ID NO's: 23 to 43 of WO 2011/095545 (SEQ ID NO's: 93 to 113 herein), in which the aspartate (D) at position 8 has been replaced by a threonine (T);
- an amino acid sequence according to any of sequence motifs of SEQ ID NO's: 24 to 27 of WO 2011/095545 (SEQ ID NO's: 94 to 97 herein) or 32 to 43 of WO 2011/095545 (SEQ ID NO's: 102 to 113 herein), in which (i) the threonine (T) residue at position 14 has been replaced by another amino acid residue (preferably but without limitation, A, N or D), and (ii) the aspartate (D) at position 8 has been replaced by a threonine (T).

As also described in WO 2011/095545, all these sequence motifs may contain one or more other suitable substitutions, such as (for example and without limitation) one or more of the substitutions listed in Table I of WO 2011/095545.

WO 2011/095545 and WO 09/127691 also describe that two or more (such as two) of the peptides described therein (which may be the same or different) may be linked to each other (either directly or via a suitable linker, for example a GS-linker such as the 9GS linker of SEQ ID NO: 164 herein) in order to provide a "tandem repeat" of two (or more) such serum albumin-binding peptides. These applications also mention that these peptides can be linked or fused to a therapeutic moiety, compound, protein or other therapeutic entity in order to increase the half-life thereof.

As mentioned, the serum albumin binding peptide or peptides that is/are present in the anti-IgE polypeptides of the invention may generally be as described in WO 08/068280, in WO 09/127691, and in particular as described in WO 2011/095545.

Thus, in one aspect, the serum albumin-binding peptide(s) that is/are present in the anti-IgE polypeptides of the invention may be a peptide with a total length of between 5 and 50, preferably between 7 and 40, more preferably between 10 and 35, such as about 15, 20, 25 or 30 amino acid residues (as a single repeat), which is such that it can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493 (numbering as described in Example 8 of WO 09/127691).

In particular, the serum albumin-binding peptide(s) that is/are present in the anti-IgE polypeptides of the invention (as a single repeat or tandem repeat) may be such a peptide that has at least 50%, preferably at least 65 %, more preferably at least 70%, even more preferably at least 75%, such as at least 80%, such as at least 90%, but not 100%, sequence identity (as defined herein) with the amino acid sequence of SEQ ID NO: 19 and that can bind better to human serum albumin than the amino acid sequence of SEQ ID NO: 19.

More in particular, the serum albumin-binding peptide(s) that is/are present in the anti-IgE polypeptides of the invention (as a single repeat or tandem repeat) may be such a peptide that comprises:

(i) an Arg (R) residue, in particular an Arg (R) residue that is capable of forming a hydrogen bond with the amino acid residues Asn (N) 133 and Asn (N) 135 of human serum albumin and/or capable of forming electrostatic interactions with the main-chain oxygen atoms of the Pro (P) 134 and Leu (L) 136 residues of human serum albumin; and/or

(ii) a Trp (W) residue, in particular a Trp (W) residue that is capable of forming electrostatic interactions with the Arg (R) 138 residue of human serum albumin; and/or

(iii) the sequence motif GGG;

and preferably further comprises:

(iv) upstream of said Arg (R) residue: a stretch of amino acid residues of between 2 and 10 amino acid residues, which comprises at least one hydrophobic and/or aromatic amino acid residue (and for the remainder one or more further suitable amino acid residues), in which said at least one hydrophobic amino acid residue may in particular be chosen from L, I, V

and/or M and/or in which said at least one aromatic amino acid residue may in particular be chosen from W, Y and/or F. In particular, said stretch of amino acids upstream of position 3 is preferably such that at least one of said hydrophobic and/or aromatic amino acid residues can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493; and may in particular contain, upstream of position 3, a stretch of amino acid residues of between 2 and 10 amino acid residues, which comprises at least one W residue and/or at least one Y residue, such that at least one of said W or Y residues can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493; and may more in particular comprise (i) at least two W residues; (ii) at least two Y residues; and/or (iii) at least one W residue and at least one Y residue, such that at least one of said W or Y residues can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493. For example, in the serum albumin-binding peptide(s) that is/are present in the anti-IgE polypeptides of the invention (as a single repeat or tandem repeat), the stretch of amino acids upstream of position 3 may be chosen from SEQ ID NO's: 78 to 98 of WO 2011/095545 (SEQ ID NO's: 72 to 92 herein).

Even more in particular, the serum albumin-binding peptide(s) that is/are present in the anti-IgE polypeptides of the invention (as a single repeat or tandem repeat) may be a peptide:

a) with a total length of between 5 and 50, preferably between 7 and 40, more preferably between 10 and 35, such as about 15, 20, 25 or 30 amino acid residues (as a single repeat); and

b) which is such that it can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493; and

c) which has at least 50%, preferably at least 65 %, more preferably at least 70%, even more preferably at least 75%, such as at least 80%, such as at least 90%, but not 100%, sequence identity (as defined herein) with the amino acid sequence of SEQ ID NO: 19; and

d) which is such that can bind better to human serum albumin than the amino acid sequence of SEQ ID NO: 19; and

e) which contains one or more of the following sequence motifs: DYDV (SEQ ID NO: 20), YDVF (SEQ ID NO: 21), DVFG (SEQ ID NO: 22), VFGG (SEQ ID NO: 23), FGGG (SEQ ID NO: 24) and/or GGGT (SEQ ID NO: 25), and in particular one of the sequence motifs DYDVF (SEQ ID NO: 26), YDVFG (SEQ ID NO: 27), DVFGG (SEQ ID NO: 28), VFGGG (SEQ ID NO: 29), FGGGT (SEQ ID NO: 30), DYTVF (SEQ ID NO: 44), YTVFG (SEQ ID NO: 45) or TVFGG (SEQ ID NO: 46); and more in particular one of the sequence motifs DYDVFG (SEQ ID NO: 31), YDVFGG (SEQ ID NO: 32), DVFGGG (SEQ ID NO: 33), VFGGGT (SEQ ID NO: 34), DYTVFG (SEQ ID NO: 47), YTVFGG (SEQ ID NO: 48) or TVFGGG (SEQ ID NO: 49), and even more in particular one of the sequence motifs DYDVFGG (SEQ ID NO: 35), YDVFGGG (SEQ ID NO: 36), DVFGGGT (SEQ ID NO: 37); DYTVFGG (SEQ ID NO: 50), YTVFGGG (SEQ ID NO: 51), TVFGGGT, (SEQ ID NO: 52), DVFGGGA (SEQ ID NO: 53), DVFGGGN (SEQ ID NO: 54), DVFGGGD (SEQ ID NO: 55), TVFGGGA (SEQ ID NO: 56), TVFGGGN (SEQ ID NO: 57) or TVFGGGD (SEQ ID NO: 58), such as one of the sequence motifs DYDVFGGG (SEQ ID NO: 38), YDVFGGGT (SEQ ID NO: 39); DYDVFGGGT (SEQ ID NO: 40); DYTVFGGG (SEQ ID NO: 59), YTVFGGGT (SEQ ID NO: 60), YDVFGGGA (SEQ ID NO: 61), YDVFGGGN (SEQ ID NO: 62), YDVFGGGD (SEQ ID NO: 63); YTVFGGGA (SEQ ID NO: 64), YTVFGGGN (SEQ ID NO: 65) or YTVFGGGD (SEQ ID NO: 66);

f) which contains, upstream of the sequence motif referred to under e), for example at positions 3 to 6, a sequence motif RXWD, in which X may be any amino acid sequence but is preferably W, Y, F, S or D; and/or

g) which contains upstream of said sequence motif RXWD: a stretch of amino acid residues of between 2 and 10 amino acid residues, which comprises at least one hydrophobic and/or aromatic amino acid residue (and for the remainder one or more further suitable amino acid residues), in which said at least one hydrophobic amino acid residue may in particular be chosen from L, I, V and/or M and/or in which said at least one aromatic amino acid residue may in particular be chosen from W, Y and/or F. In particular, said stretch of amino acids upstream of position 3 is preferably such that at least one of said hydrophobic and/or aromatic amino acid residues can bind (in)to a subpocket in (human) serum albumin that

comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493; and may in particular contain, upstream of position 3, a stretch of amino acid residues of between 2 and 10 amino acid residues, which comprises at least one W residue and/or at least one Y residue, such that at least one of said W or Y residues can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493; and may more in particular comprise (i) at least two W residues; (ii) at least two Y residues; and/or (iii) at least one W residue and at least one Y residue, such that at least one of said W or Y residues can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493. For example, in the serum albumin-binding peptide(s) that is/are present in the anti-IgE polypeptides of the invention (as a single repeat or tandem repeat), the stretch of amino acids upstream of position 3 may be chosen from SEQ ID NO's: 78 to 98 of WO 2011/095545 (SEQ ID NO's: 72 to 92).

Even more in particular, the serum albumin-binding peptide(s) that is/are present in the anti-IgE polypeptides of the invention (as a single repeat or tandem repeat) may be a peptide:

i) with a total length of between 5 and 50, preferably between 7 and 40, more preferably between 10 and 35, such as about 15, 20, 25 or 30 amino acid residues (as a single repeat); and

ii) which is such that it can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493; and

iii) which has at least 50%, preferably at least 65 %, more preferably at least 70%, even more preferably at least 75%, such as at least 80%, such as at least 90%, but not 100%, sequence identity (as defined herein) with the amino acid sequence of SEQ ID NO: 19; and

iv) which is such that can bind better to human serum albumin than the amino acid sequence of SEQ ID NO: 19;

and comprises

v) a stretch of amino acid residues upstream of position 3 that is as described in WO 2011/095545 and in particular as described under g) above. This may for example be one

of the sequences of SEQ ID NO's: 72 to 92 herein, or a sequence that has 2 or only 1 "amino acid difference" - as defined in WO 2011/095545 - with at least one of these sequences;

in combination with one of the following sequence motifs:

vi) the amino acid sequence RXWDXDVFGGG (SEQ ID NO: 93), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;

vii) the amino acid sequence RXWDXDVFGGGT (SEQ ID NO: 94), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;

viii) the amino acid sequence RXWDXDVFGGGTP (SEQ ID NO: 95), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;

ix) the amino acid sequence RXWDXDVFGGGTPG (SEQ ID NO: 96), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;

x) the amino acid sequence RXWDXDVFGGGTPGG (SEQ ID NO: 97), in which the first (from the N-terminal end) amino acid residue indicated by X is chosen from Y, S or D; and the second amino acid residue indicated by X is chosen from Y or F;

xi) an amino acid sequence chosen from RYWDYDVFGGG (SEQ ID NO: 98); RDWDFDVFGGG (SEQ ID NO: 99); RSWDFDVFGGG (SEQ ID NO: 100) or RYWDFDVFGGG (SEQ ID NO: 101); and in particular chosen from RDWDFDVFGGG (SEQ ID NO: 98); RSWDFDVFGGG (SEQ ID NO: 99) or RYWDFDVFGGG (SEQ ID NO: 100);

xii) an amino acid sequence chosen from RYWDYDVFGGGT (SEQ ID NO: 102); RDWDFDVFGGGT (SEQ ID NO: 103); RSWDFDVFGGGT (SEQ ID NO: 104) or RYWDFDVFGGGT (SEQ ID NO: 105); and in particular chosen from RDWDFDVFGGGT (SEQ ID NO: 103); RSWDFDVFGGGT (SEQ ID NO: 104) or RYWDFDVFGGGT (SEQ ID NO: 105);

xiii) an amino acid sequence chosen from RYWDYDVFGGGTP (SEQ ID NO: 106); RDWDFDVFGGGTP (SEQ ID NO: 107); RSWDFDVFGGGTP (SEQ ID NO: 108) or

RYWDFDVFGGGTP (SEQ ID NO: 109); and in particular chosen from RDWDFDVFGGGTP (SEQ ID NO: 107); RSWDFDVFGGGTP (SEQ ID NO: 108) or RYWDFDVFGGGTP (SEQ ID NO: 109);

xiv) an amino acid sequence chosen from RYWDYDVFGGGTPV (SEQ ID NO: 110); RDWDFDVFGGGTPV (SEQ ID NO: 111); RSWDFDVFGGGTPV (SEQ ID NO: 112) or RYWDFDVFGGGTPV (SEQ ID NO: 113); and in particular chosen from RDWDFDVFGGGTPV (SEQ ID NO: 111); RSWDFDVFGGGTPV (SEQ ID NO: 112) or RYWDFDVFGGGTPV (SEQ ID NO: 113 herein).

Thus, some other (non-limiting) sequence motifs that may be present in the albumin-binding peptides according to WO 2011/095545:

i) an amino acid sequence according to any of sequence motifs of SEQ ID NO's: 94 to 97 or 102 to 113, in which the threonine (T) residue at position 14 has been replaced by another amino acid residue (preferably but without limitation, A, N or D);

ii) an amino acid sequence according to any of sequence motifs of SEQ ID NO's: 93 to 113, in which the aspartate (D) at position 8 has been replaced by a threonine (T);

iii) an amino acid sequence according to any of sequence motifs of SEQ ID NO's: 94 to 97or SEQ ID NO's: 102 to 113, in which (i) the threonine (T) residue at position 14 has been replaced by another amino acid residue (preferably but without limitation, A, N or D), and (ii) the aspartate (D) at position 8 has been replaced by a threonine (T).

Some preferred, but non-limiting examples of such serum albumin-binding peptide(s) that may be present in the anti-IgE polypeptides of the invention (as a single repeat or tandem repeat) are the sequences of SEQ ID NO's: 2 to 115 or 147 to 157 of WO 09/127691.

Some non-limiting examples of such serum albumin-binding peptide(s) that may be present in the anti-IgE polypeptides of the invention (as a single repeat or tandem repeat) are the sequences of SEQ ID NO's: 54 to 74 or 103 to 108 of WO 2011/095545.

Some especially preferred, but non-limiting examples of such serum albumin-binding peptide(s) that may be present in the anti-IgE polypeptides of the invention (as a single repeat or tandem repeat) are the sequences of SEQ ID NO's: 1 to 8. SEQ ID NO's: 9 to 18 are some preferred, but non-limiting examples of tandem repeats of such peptides that can be present in the anti-IgE polypeptides of the invention.

Polypeptide of the invention

In the anti-IgE polypeptides of the invention, the one or more (such as one or two) ISV's against IgE (and in particular Nanobodies against IgE, such as 39D11-type sequences and in particular 39D11-like sequences) and the one or more (such as one or two) serum albumin binding peptides may be suitably linked to each other, either directly or via one or more suitable spacers or linkers. For example, the linker may be a "GS linker" (i.e. a linker essentially consisting of G and S residues) such as one of the linkers of SEQ ID NO's: 162 to 171, of which the 9GS, 20GS, 30GS and 35GS linkers may be particularly suited. In some cases, the use of a 20GS linker may be preferred over a 9GS linker, because in some instances, albumin-binding peptides that are linked via a 20GS linker show improved (*i.e.*, less) susceptibility for (proteolytic) degradation than albumin-binding peptides that are linked via a 9GS linker.

For example and without limitation, anti-IgE polypeptides of the invention may comprise:

- a single ISV against IgE (and in particular a Nanobody against IgE, such as an 39D11-type sequence and in particular a 39D11-like sequences) and a single albumin binding peptide as described herein; which may be linked directly to each other or via a suitable linker;
- a single ISV against IgE (and in particular a Nanobody against IgE, such as an 39D11-type sequence and in particular a 39D11-like sequences) and a tandem repeat of two single albumin binding peptides as described herein (which may the same or different, and which may be linked directly to each other or via a suitable linker, such as the 9GS linker of SEQ ID NO: 164); which may be linked directly to each other or via a suitable linker;
- a single ISV against IgE (and in particular a Nanobody against IgE, such as an 39D11-type sequence and in particular a 39D11-like sequences) and two single albumin binding peptides as described herein (which may be as described herein, and which may the same or different); which may be linked directly to each other or via one or more suitable linkers;
- two ISV's against IgE (and in particular a Nanobody against IgE, such as an 39D11-type sequence and in particular a 39D11-like sequences), which may be the same or different, and a single albumin binding peptide as described herein; which may be linked directly to each other or via one or more suitable linkers;
- two ISV's against IgE (and in particular a Nanobody against IgE, such as an 39D11-type sequence and in particular a 39D11-like sequences), which may be the same or different, and

a tandem repeat of two single albumin binding peptide as described herein (which may the same or different, and which may be linked directly to each other or via a suitable linker); which may be linked directly to each other or via one or more suitable linkers;

- two ISV's against IgE (and in particular a Nanobody against IgE, such as an 39D11-type sequence and in particular a 39D11-like sequences), which may be the same or different, and two single albumin binding peptides as described herein (which may be as described herein, and which may the same or different); which may be linked directly to each other or via one or more suitable linkers.

Some non-limiting examples of such anti-IgE polypeptides of the invention can be schematically represented as follows, in which "[peptide]" represents a serum albumin binding peptide as described herein, "[peptide-peptide]" represents a tandem repeat of two serum albumin binding peptide as described herein (which may be the same or different, and which may be linked directly or via a suitable linker), and "[anti-IgE]" represents a ISV against IgE (and in particular a Nanobody against IgE, such as an 39D11-type sequence and in particular a 39D11-like sequences), and " - " represents a suitable linker (which is optional), with the N-terminus on the left hand side and the C-terminus on the right hand side. It should be noted that when the polypeptides schematically shown below comprise two serum albumin binding peptides or two ISV's against IgE, respectively, that these may be the same or different.

[peptide] - [anti-IgE]

[peptide-peptide] - [anti-IgE]

[anti-IgE] - [peptide]

[anti-IgE] - [peptide-peptide]

[peptide] - [anti-IgE] - [peptide]

[peptide] - [anti-IgE] - [anti-IgE]

[peptide-peptide] - [anti-IgE] - [anti-IgE]

[anti-IgE] - [anti-IgE] - [peptide]

[anti-IgE] - [anti-IgE] - [peptide-peptide]

[anti-IgE] - [peptide] - [anti-IgE]

[anti-IgE] - [peptide-peptide] - [anti-IgE]

[peptide]- [anti-IgE] - [anti-IgE] - [peptide]

[anti-IgE] - [peptide]- [anti-IgE] - [peptide]

[peptide]- [anti-IgE] - [peptide] - [anti-IgE]

[peptide]- [anti-IgE] – [peptide] - [anti-IgE] - [peptide]

As will be clear from the above, the anti-albumin peptide(s) may be at the N-terminus, may be at the C-terminus, or may be part of the linker; or, when more than one albumin-binding peptide is present, any suitable combination thereof. In one preferred, but non-limiting aspect, the albumin-binding peptides are at the C-terminus (compare for example the results given in Example 15).

Preferably, the anti-IgE polypeptides of the invention contain one or two, and more preferably only one ISV against IgE, and only one serum albumin binding peptide (or one tandem repeat of two serum albumin binding peptides).

As mentioned, the ISV against IgE present in these polypeptides and constructs is preferably a Nanobody against IgE, such as an 39D11-type sequence and in particular an 39D11-like sequences, of which IGE026/IGE0045 (SEQ ID NO: 128) is a particularly preferred example. The peptide against serum albumin is preferably chosen from either the "single repeat" peptides of SEQ ID NO's: 1 to 8 or the "tandem repeat" peptides of SEQ ID NO's: 9 to 18. The linker(s) present (if any) are preferably chosen from SEQ ID NO's: 162 to 171, with 9GS, 20GS, 30GS and 35GS being particularly preferred.

In one specific, but non-limiting aspect, a polypeptide of the invention is such that, when it is tested in the storage stability assay described in Example 16, that the pre-peak on SE-HPLC for the polypeptide after 1 month storage at 25°C (under the further conditions given in Example 16) is less than 10%, preferably less than 5%; and/or that the pre-peak on SE-HPLC for the polypeptide after 1 month storage at 40°C (under the further conditions given in Example 16) is less than 20%, preferably less than 10%. Reference is for example made to the comparative results in Table 19.

Some preferred, but non-limiting examples of anti-IgE polypeptides of the invention are given in SEQ ID NO's: 147 to 161 (see also Table A-9). The invention also relates to a polypeptide selected from any of SEQ ID NO's: 147 to 161, or a polypeptide which has at least 80%, such as at least 85%, for example at least 90%, such as at least 95% sequence identity with any of SEQ ID NO's: 147 to 161.

Of these IGE109 (IGE66G02^{E1D}-20GS-NEXP001-9GS- NEXP002) (SEQ ID NO: 155) is particularly preferred. Thus, one specific aspect of the invention relates to a polypeptide which has at least 80%, such as at least 85%, for example at least 90%, such as at least 95% sequence identity with SEQ ID NO: 155. Such a polypeptide is preferably as further described herein, and preferably according to the preferred aspects mentioned herein.

As already mentioned herein, 39D11 and its variants as described herein (such as the 39D11-type sequences and in particular 39D11-like sequences) may also generally have a number of advantages compared to the anti-IgE Nanobodies that have been described in the art, such as those described in WO 04/041867. These will generally become clear to the skilled person based on the disclosure and data presented herein, and for example and without limitation can be:

- 39D11, 39D11-type sequences and the 39D11-like sequences are cross-reactive between human IgE and cynomolgus IgE (as further described herein);
- 39D11, 39D11-type sequences and the 39D11-like sequences may have improved potency (see for example the data presented in the Experimental Part);
- 39D11, 39D11-type sequences and the 39D11-like sequences may block interaction of Human and cyno IgE both with the high affinity IgE receptor (Fc(epsilon)RI) and the low affinity receptor (Fc(epsilon)RII) (see Examples 6, 7 and 8 respectively); and generally have a desirable "balance" between affinity for the human receptor(s) and the cyno receptor(s); and/or
- 39D11, 39D11-type sequences and the 39D11-like sequences have a desirable "balance" between affinity for the human high affinity IgE receptor and the low affinity IgE receptor (see again the data presented in the Experimental Part);

or any combination of any or all of the foregoing.

This means that 39D11, the 39D11-type sequences and in particular the 39D11-like sequences described herein may not only be used in the anti-IgE polypeptides of the invention described herein (i.e. in constructs comprising at least one such building block and at least one albumin-binding peptide according to WO 08/068280, WO 09/127691 and WO 2011/095545), but also with advantage either by itself or as a component or building block for other polypeptides, proteins or other compounds or constructs. For example and without limitation,

39D11, the 39D11-type sequences and in particular the 39D11-like sequences described herein may be used in polypeptides of the type described in WO 04/041867 and/or WO 04/041865 (*i.e.*, instead of one of the anti-IgE Nanobodies described therein). Such polypeptides may also be half-life extended, for example through pegylation or the use of a serum albumin binding Nanobody (including those described in WO 04/041867, WO 04/041865 or WO 06/122787, although it should then be noted that such polypeptides may have a limited stability under storage, as described herein).

Thus, in some other aspects, the invention further relates to:

- a protein or polypeptide that comprises or essentially consists of at least one (such as one or two) 39D11, a 39D11-type sequence (as defined herein) and in particular of a 39D11-like sequence (as defined herein); and more in particular a protein or polypeptide that comprises or essentially consists of at least one (such as one or two) 39D11-like sequence (as defined herein) that is a humanized and/or sequence-optimized variant of 39D11 such as one of the variants of SEQ ID NO's: 120 to 133 (of which SEQ ID NO's: 128 and 129 are particularly preferred examples). Such a protein or polypeptide may for example also comprise two or three such ISV's against IgE (which may be the same or different), which may be linked to each other either directly or via one or more suitable linkers. It may also be half-life extended (for example through pegylation) or otherwise modified (for example, through some of the modifications generally described in WO 09/068627);
- a (fusion) protein, polypeptide, construct or compound that comprises or essentially consists of at least one (such as one or two) (i) 39D11, a 39D11-type sequence (as defined herein) and in particular of a 39D11-like sequence (as defined herein); and more in particular a protein or polypeptide that comprises or essentially consists of at least one (such as one or two) 39D11-like sequences (as defined herein) that is a humanized and/or sequence-optimized variant of 39D11 such as one of the variants of SEQ ID NO's: 120 to 133 (of which SEQ ID NO's: 128 and 129 are particularly preferred examples); and (ii) at least one other amino acid sequence, protein, (poly)peptide, binding domain, binding unit, group, residue or moiety; suitably linked to each other via one or more suitable linkers or spacers. For example, such as further amino acid sequence may be another ISV (such as another Nanobody) that is directed against IgE (for example, against the same or a different part or epitope on IgE) or against another target (such as for example serum albumin or another serum protein so as to provide for

increased half-life or against another therapeutic target so as to provide a bispecific construct). Such polypeptides etc. may for example generally be as described in WO 04/041867 and/or WO 04/041865 (or as described in WO 09/068627, but comprising an ISV against IgE instead of the ISV's against subunits of IL-23 used in WO 09/068627) and may for example be bivalent (or multivalent), biparatopic (multiparatopic) and/or bispecific (or multispecific) constructs (as these terms are generally defined in WO 09/068627). Again, in such a protein or polypeptide, the 39D11-like sequence may generally be as further described herein, and is preferably according to the preferred aspects described herein. Some non-limiting examples of bispecific anti-IgE/anti-albumin bispecific Nanobody constructs comprising 39D11-like sequences are given in SEQ ID NO's: 175 to 182 (Table A-10).

In another aspect, the invention relates to a nucleic acid that encodes one of the amino acid sequences or polypeptides against IgE that are described herein. Such a nucleic acid may for example be in the form of a genetic construct, as for example generally described in WO 04/041867, WO 04/041865 or WO 09/068627.

The invention further relates to methods for preparing the polypeptides, nucleic acids, host cells, and compositions described herein.

The polypeptides and nucleic acids of the invention can be prepared in a manner known per se, as will be clear to the skilled person from the further description herein. For example, the polypeptides of the invention can be prepared in any manner known per se for the preparation of antibodies and in particular for the preparation of antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments). Some preferred, but non-limiting methods for preparing the polypeptides and nucleic acids include the methods and techniques described herein.

The polypeptides of the invention can generally be prepared by a method which comprises at least the step of suitably linking the immunoglobulin single variable domain against IgE to one or more further binding units, such as the one or more peptides against serum albumin, optionally via the one or more suitable linkers. The immunoglobulin single variable domains and peptides (and linkers) can be coupled by any method known in the art and as further described herein. Preferred techniques include the linking of the nucleic acid sequences that encode the immunoglobulin single variable domains and peptides (and linkers) to prepare a genetic construct that expresses the polypeptide. Techniques for linking amino acids or nucleic acids will

be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook *et al.* and Ausubel *et al.*, mentioned above, as well as the Examples below.

Polypeptides of the invention can thus be prepared by a method which generally comprises at least the steps of providing a nucleic acid that encodes a polypeptide of the invention, expressing said nucleic acid in a suitable manner, and recovering the expressed polypeptide of the invention. Such methods can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the methods and techniques further described herein.

Accordingly, the method for producing a polypeptide of the invention may comprise the following steps:

- the expression, in a suitable host cell or host organism (also referred to herein as a "*host of the invention*") or in another suitable expression system of a nucleic acid that encodes said polypeptide of the invention (also referred to herein as a "*nucleic acid of the invention*"),

optionally followed by:

- isolating and/or purifying the polypeptide of the invention thus obtained.

In particular, such a method may comprise the steps of:

- cultivating and/or maintaining a host of the invention under conditions that are such that said host of the invention expresses and/or produces at least one polypeptide of the invention;

optionally followed by:

- isolating and/or purifying the polypeptide of the invention thus obtained.

Accordingly, the present invention also relates to a nucleic acid or nucleotide sequence that encodes an ISV or polypeptide as described herein (also referred to as "*nucleic acid of the invention*"). A nucleic acid of the invention can be in the form of single or double stranded DNA or RNA, and is preferably in the form of double stranded DNA. For example, the nucleotide sequences of the invention may be genomic DNA, cDNA or synthetic DNA (such as DNA with a codon usage that has been specifically adapted for expression in the intended host cell or host organism).

According to one embodiment of the invention, the nucleic acid of the invention is in essentially isolated from, as defined herein. The nucleic acid of the invention may also be in the form of, be present in and/or be part of a vector, such as for example a plasmid, cosmid or YAC, which again may be in essentially isolated form.

The nucleic acids of the invention can be prepared or obtained in a manner known per se, based on the information on the ISV or polypeptides as described herein, and/or can be isolated from a suitable natural source. Also, as will be clear to the skilled person, to prepare a nucleic acid of the invention, also several nucleotide sequences, such as at least two nucleic acids encoding an immunoglobulin single variable domain or a monovalent polypeptide and for example nucleic acids encoding one or more linkers can be linked together in a suitable manner.

Techniques for generating the nucleic acids of the invention will be clear to the skilled person and may for instance include, but are not limited to, automated DNA synthesis; site-directed mutagenesis; combining two or more naturally occurring and/or synthetic sequences (or two or more parts thereof), introduction of mutations that lead to the expression of a truncated expression product; introduction of one or more restriction sites (e.g. to create cassettes and/or regions that may easily be digested and/or ligated using suitable restriction enzymes), and/or the introduction of mutations by means of a PCR reaction using one or more "mismatched" primers. These and other techniques will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook *et al.* and Ausubel *et al.*, mentioned above, as well as the Examples below.

The nucleic acid of the invention may also be in the form of, be present in and/or be part of a genetic construct, as will be clear to the person skilled in the art. Such genetic constructs generally comprise at least one nucleic acid of the invention that is optionally linked to one or more elements of genetic constructs known per se, such as for example one or more suitable regulatory elements (such as a suitable promoter(s), enhancer(s), terminator(s), etc.) and the further elements of genetic constructs referred to herein. Such genetic constructs comprising at least one nucleic acid of the invention will also be referred to herein as "*genetic constructs of the invention*".

The genetic constructs of the invention may be DNA or RNA, and are preferably double-stranded DNA. The genetic constructs of the invention may also be in a form suitable for transformation of the intended host cell or host organism, in a form suitable for integration into the genomic DNA of the intended host cell or in a form suitable for independent replication, maintenance and/or inheritance in the intended host organism. For instance, the genetic constructs of the invention may be in the form of a vector, such as for example a plasmid, cosmid, YAC, a viral vector or transposon. In particular, the vector may be an expression vector,

i.e. a vector that can provide for expression in vitro and/or in vivo (e.g. in a suitable host cell, host organism and/or expression system).

In a preferred but non-limiting embodiment, a genetic construct of the invention comprises:

a) at least one nucleic acid of the invention; operably connected to

b) one or more regulatory elements, such as a promoter and optionally a suitable terminator; and optionally also

c) one or more further elements of genetic constructs known per se;

in which the terms "regulatory element", "promoter", "terminator" and "operably connected" have their usual meaning in the art (as further described herein); and in which said "further elements" present in the genetic constructs may for example be 3'- or 5'-UTR sequences, leader sequences, selection markers, expression markers/reporter genes, and/or elements that may facilitate or increase (the efficiency of) transformation or integration. These and other suitable elements for such genetic constructs will be clear to the skilled person, and may for instance depend upon the type of construct used; the intended host cell or host organism; the manner in which the nucleotide sequences of the invention of interest are to be expressed (e.g. via constitutive, transient or inducible expression); and/or the transformation technique to be used. For example, regulatory sequences, promoters and terminators known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments) may be used in an essentially analogous manner.

Preferably, in the genetic constructs of the invention, said at least one nucleic acid of the invention and said regulatory elements, and optionally said one or more further elements, are "operably linked" to each other, by which is generally meant that they are in a functional relationship with each other. For instance, a promoter is considered "operably linked" to a coding sequence if said promoter is able to initiate or otherwise control/regulate the transcription and/or the expression of a coding sequence (in which said coding sequence should be understood as being "under the control of" said promoter). Generally, when two nucleotide sequences are operably linked, they will be in the same orientation and usually also in the same reading frame. They will usually also be essentially contiguous, although this may also not be required.

The nucleic acids of the invention and/or the genetic constructs of the invention may be used to transform a host cell or host organism, *i.e.,* for expression and/or production of the ISV

or polypeptide as described herein. Suitable hosts or host cells will be clear to the skilled person, and may for example be any suitable fungal, prokaryotic or eukaryotic cell or cell line or any suitable fungal, prokaryotic or eukaryotic organism, for example:

- a bacterial strain, including but not limited to gram-negative strains such as strains of *Escherichia coli*; of *Proteus*, for example of *Proteus mirabilis*; of *Pseudomonas*, for example of *Pseudomonas fluorescens*; and gram-positive strains such as strains of *Bacillus*, for example of *Bacillus subtilis* or of *Bacillus brevis*; of *Streptomyces*, for example of *Streptomyces lividans*; of *Staphylococcus*, for example of *Staphylococcus carnosus*; and of *Lactococcus*, for example of *Lactococcus lactis*;
- a fungal cell, including but not limited to cells from species of *Trichoderma*, for example from *Trichoderma reesei*; of *Neurospora*, for example from *Neurospora crassa*; of *Sordaria*, for example from *Sordaria macrospora*; of *Aspergillus*, for example from *Aspergillus niger* or from *Aspergillus sojae*; or from other filamentous fungi;
- a yeast cell, including but not limited to cells from species of *Saccharomyces*, for example of *Saccharomyces cerevisiae*; of *Schizosaccharomyces*, for example of *Schizosaccharomyces pombe*; of *Pichia*, for example of *Pichia pastoris* or of *Pichia methanolica*; of *Hansenula*, for example of *Hansenula polymorpha*; of *Kluyveromyces*, for example of *Kluyveromyces lactis*; of *Arxula*, for example of *Arxula adeninivorans*; of *Yarrowia*, for example of *Yarrowia lipolytica*;
- an amphibian cell or cell line, such as *Xenopus oocytes*;
- an insect-derived cell or cell line, such as cells/cell lines derived from lepidoptera, including but not limited to *Spodoptera* SF9 and Sf21 cells or cells/cell lines derived from *Drosophila*, such as Schneider and Kc cells;
- a plant or plant cell, for example in tobacco plants; and/or
- a mammalian cell or cell line, for example a cell or cell line derived from a human, a cell or a cell line from mammals including but not limited to CHO-cells, BHK-cells (for example BHK-21 cells) and human cells or cell lines such as HeLa, COS (for example COS-7) and PER.C6 cells;

as well as all other hosts or host cells known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments), which will be clear to the skilled person. Reference is also made to the general

background art cited hereinabove, as well as to for example WO 94/29457; WO 96/34103; WO 99/42077; Frenken *et al.* (Res Immunol. 149: 589-99, 1998); Riechmann and Muyldermans (1999), supra; van der Linden (J. Biotechnol. 80: 261-70, 2000); Joosten *et al.* (Microb. Cell Fact. 2: 1, 2003); Joosten *et al.* (Appl. Microbiol. Biotechnol. 66: 384-92, 2005); and the further references cited herein.

In another aspect, the invention relates to a host or host cell that expresses (or that under suitable circumstances is capable of expressing) one of the ISV's, amino acid sequences or polypeptides against IgE that are described herein; and/or that contains a nucleic acid encoding the same. Some preferred but non-limiting examples of such hosts or host cells can be as generally described in WO 04/041867, WO 04/041865 or WO 09/068627. For example, polypeptides comprising at least one anti-IgE building block as described herein and at least one albumin-binding peptide according to WO 08/068280, WO 09/127691 and WO 2011/095545 may with advantage be expressed, produced or manufactured in a yeast strain, such as a strain of *Pichia pastoris*. Reference is also made to WO 2011/095545, which also describes the expression/production in *Pichia* and other hosts/host cells of polypeptides that comprise the albumin binding peptides of WO 2011/095545.

The ISV's and polypeptides as described herein may also be expressed as so-called "intrabodies", as for example described in WO 94/02610, WO 95/22618 and US 7,004,940; WO 03/014960; in Cattaneo and Biocca ("Intracellular Antibodies: Development and Applications" Landes and Springer-Verlag, 1997); and in Kontermann (Methods 34: 163-170, 2004).

Suitable techniques for transforming a host or host cell of the invention will be clear to the skilled person and may depend on the intended host cell/host organism and the genetic construct to be used. Reference is again made to the handbooks and patent applications mentioned above.

After transformation, a step for detecting and selecting those host cells or host organisms that have been successfully transformed with the nucleotide sequence/genetic construct of the invention may be performed. This may for instance be a selection step based on a selectable marker present in the genetic construct of the invention or a step involving the detection of the ISV or polypeptide as described herein, *e.g.*, using specific antibodies.

The transformed host cell (which may be in the form or a stable cell line) or host organisms (which may be in the form of a stable mutant line or strain) form further aspects of the present invention.

Preferably, these host cells or host organisms are such that they express, or are (at least) capable of expressing (*e.g.*, under suitable conditions), an ISV or polypeptide as described herein (and in case of a host organism: in at least one cell, part, tissue or organ thereof). The invention also includes further generations, progeny and/or offspring of the host cell or host organism of the invention, which may for instance be obtained by cell division or by sexual or asexual reproduction.

To produce/obtain expression of the ISV's and polypeptides as described herein, the transformed host cell or transformed host organism may generally be kept, maintained and/or cultured under conditions such that the (desired) ISV or polypeptide is expressed/produced. Suitable conditions will be clear to the skilled person and will usually depend upon the host cell/host organism used, as well as on the regulatory elements that control the expression of the (relevant) nucleotide sequence of the invention. Again, reference is made to the handbooks and patent applications mentioned above in the paragraphs on the genetic constructs of the invention.

Generally, suitable conditions may include the use of a suitable medium, the presence of a suitable source of food and/or suitable nutrients, the use of a suitable temperature, and optionally the presence of a suitable inducing factor or compound (*e.g.*, when the nucleotide sequences of the invention are under the control of an inducible promoter); all of which may be selected by the skilled person. Again, under such conditions, the ISV's or polypeptides may be expressed in a constitutive manner, in a transient manner, or only when suitably induced.

It will also be clear to the skilled person that ISV or polypeptide may (first) be generated in an immature form (as mentioned above), which may then be subjected to post-translational modification, depending on the host cell/host organism used. Also, the ISV or polypeptide may be glycosylated, again depending on the host cell/host organism used.

The ISV or polypeptide as described herein may then be isolated from the host cell/host organism and/or from the medium in which said host cell or host organism was cultivated, using protein isolation and/or purification techniques known per se, such as (preparative) chromatography and/or electrophoresis techniques, differential precipitation techniques, affinity techniques (*e.g.*, using a specific, cleavable amino acid sequence fused with the polypeptide of

the invention) and/or preparative immunological techniques (i.e. using antibodies against the ISV or polypeptide to be isolated).

The invention further relates to a product or composition containing or comprising at least one ISV, amino acid sequence, polypeptide, protein, construct or other compound as described herein (i.e. directed against IgE/comprising at least one ISV against IgE as described herein), and optionally one or more further components of such compositions known *per se*, *i.e.*, depending on the intended use of the composition. Such a product or composition may for example be a pharmaceutical composition (as described herein), a veterinary composition or a product or composition for diagnostic use (as also described herein). Some preferred but non-limiting examples of such products or (pharmaceutical) compositions may generally be as described in WO 04/041867, WO 04/041865, WO 09/068627 or WO 2011/095545.

The invention also relates to the use of an ISV, amino acid sequence, polypeptide, protein, construct or other compound as described herein, or of a composition comprising the same, in (methods or compositions for) modulating (as generally defined in WO 09/068627) IgE and/or one or more biological actions, mechanisms or responses associated with IgE, either *in vitro* (*e.g.*, in an *in vitro* or cellular assay) or *in vivo* (*e.g.*, in an a single cell or in a multicellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a disease or disorder associated with IgE, with increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE), or more generally any disease or disorder associated with and/or mediated by IgE.

The invention also relates to methods for modulating IgE and/or one or more biological actions, mechanisms or responses associated with IgE, either *in vitro* (*e.g.*, in an *in vitro* or cellular assay) or *in vivo* (*e.g.*, in a single cell or multicellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a disease associated with IgE, with increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE), which method comprises at least the step of contacting IgE with an ISV, amino acid sequence, polypeptide, protein, construct or other compound as described herein, or with a composition comprising the same, in a manner and in an amount suitable to modulate IgE (or the intended or desired action(s), mechanism(s) or response(s) associated with IgE).

The invention also relates to the use of an ISV, amino acid sequence, polypeptide, protein, construct or other compound as described herein in the preparation of a composition (such as, without limitation, a pharmaceutical composition or preparation as further described herein) for modulating IgE or one or more biological actions, mechanisms or responses associated with IgE, either *in vitro* (*e.g.,* in an *in vitro* or cellular assay) or *in vivo* (*e.g.,* in a single cell or multicellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a disease or disorder associated with IgE, with increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE).

The invention also relates to an ISV, amino acid sequence, polypeptide, protein, construct or other compound as described herein for use in therapy.

In particular, the invention also relates to the use of an ISV, amino acid sequence, polypeptide, protein, construct or other compound as described herein for the treatment of a disease or disorder that can be prevented or treated by administering, to a subject in need thereof, of (a pharmaceutically effective amount of) an ISV, amino acid sequence, polypeptide, protein, construct or other compound as described herein (or a suitable composition comprising the same).

More in particular, the invention relates to an ISV, amino acid sequence, polypeptide, protein, construct or other compound as described herein for use in therapy of a disease or disorder associated with IgE, with increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE, or more generally any disease or disorder associated with and/or mediated by IgE. Such "IgE mediated" diseases and disorders will be clear to the skilled person and may for example include, without limitation: conditions such as asthma, allergic rhinitis, hay fever, conjunctivitis, eczema, utricaria, food allergies and other allergies, including serious and/or life-threatening allergic reactions such as those to insect bites or stings, snake bites etc., as well as to allergic reaction to medication; and more generally any disease or disorder associated with anaphylactic hypersensitivity and/or (atopic) allergy.

For example, a monoclonal against IgE called omalizumab (Xolair®) is currently marketed by Genentech for IgE mediated disorders. It is envisaged that the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds, as well as compositions comprising the same, can be used in the prevention or treatment of the same diseases and

disorders that omalizumab has been approved for prior to the priority date of the present invention and/or will be approved for after the priority date of the present application. From the further description and data presented herein, it will also be clear to the skilled person that the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds, as well as compositions comprising the same may have substantial advantages over omalizumab (see for example the Experimental Part, in which omalizumab (obtained from a commercial source) and a Fab obtained through papain digest of commercial omalizumab were used as reference compounds). Generally, for pharmaceutical use, the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein may be formulated as a pharmaceutical preparation or compositions comprising at least one ISV or polypeptide as described herein and at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally one or more further pharmaceutically active polypeptides and/or compounds. By means of non-limiting examples, such a formulation may be in a form suitable for oral administration, for parenteral administration (such as by intravenous, intramuscular or subcutaneous injection or intravenous infusion), for topical administration, for administration by inhalation, by a skin patch, by an implant, by a suppository, etc. Such suitable administration forms - which may be solid, semi-solid or liquid, depending on the manner of administration - as well as methods and carriers for use in the preparation thereof, will be clear to the skilled person, and are further described herein.

Generally, the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein can be formulated and administered in any suitable manner known per se, for which reference is for example made to the general background art cited above (and in particular to WO 04/041862, WO 04/041863, WO 04/041865, WO 04/041867 and WO 08/020079) as well as to the standard handbooks, such as Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Company, USA (1990), Remington, the Science and Practice of Pharmacy, 21th Edition, Lippincott Williams and Wilkins (2005); or the Handbook of Therapeutic Antibodies (S. Dubel, Ed.), Wiley, Weinheim, 2007 (see for example pages 252-255).

For example, the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein may be formulated and administered in any manner known per se for conventional antibodies and antibody fragments (including ScFv's and diabodies) and other

pharmaceutically active proteins. Such formulations and methods for preparing the same will be clear to the skilled person, and for example include preparations suitable for parenteral administration (for example intravenous, intraperitoneal, subcutaneous, intramuscular, intraluminal, intra-arterial or intrathecal administration) or for topical (*i.e.*, transdermal or intradermal) administration.

Preparations for parenteral administration may for example be sterile solutions, suspensions, dispersions or emulsions that are suitable for infusion or injection. Suitable carriers or diluents for such preparations for example include, without limitation, those mentioned on page 143 of WO 08/020079. Usually, aqueous solutions or suspensions will be preferred.

Thus, the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein may be systemically administered, *e.g.,* orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the ISV's, amino acid sequences, Nanobodies and polypeptides of the invention may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of the ISV, amino acid sequence, Nanobody or polypeptide as described herein. Their percentage in the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of the ISV, amino acid sequence, Nanobody or polypeptide as described herein in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain binders, excipients, disintegrating agents, lubricants and sweetening or flavouring agents, for example those mentioned on pages 143-144 of WO 08/020079. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the ISV's, amino acid sequences, Nanobodies and polypeptides as described herein, sucrose or fructose as a

sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described may be incorporated into sustained-release preparations and devices.

Preparations and formulations for oral administration may also be provided with an enteric coating that will allow the constructs of the invention to resist the gastric environment and pass into the intestines. More generally, preparations and formulations for oral administration may be suitably formulated for delivery into any desired part of the gastrointestinal tract. In addition, suitable suppositories may be used for delivery into the gastrointestinal tract.

The ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein may also be administered intravenously or intraperitoneally by infusion or injection, as further described on pages 144 and 145 of WO 08/020079.

For topical administration, the ISV's, amino acid sequences, Nanobodies and polypeptides as described herein may be applied in pure form, *i.e.,* when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid, as further described on page 145 of WO 08/020079.

Generally, the concentration of the ISV's, amino acid sequences, Nanobodies and polypeptides as described herein in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein required for use in treatment will vary not only with the particular ISV, amino acid sequence, polypeptide, protein or construct selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced

administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

An administration regimen could include long-term, daily treatment. By "long-term" is meant at least two weeks and preferably, several weeks, months, or years of duration. Necessary modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See Remington's Pharmaceutical Sciences (Martin, E.W., ed. 4), Mack Publishing Co., Easton, PA. The dosage can also be adjusted by the individual physician in the event of any complication.

In another aspect, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder associated with IgE, with increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence, polypeptide, protein, construct or compound as described herein, and/or of a pharmaceutical composition comprising the same.

In the context of the present invention, the term "prevention and/or treatment" not only comprises preventing and/or treating the disease, but also generally comprises preventing the onset of the disease, slowing or reversing the progress of disease, preventing or slowing the onset of one or more symptoms associated with the disease, reducing and/or alleviating one or more symptoms associated with the disease, reducing the severity and/or the duration of the disease and/or of any symptoms associated therewith and/or preventing a further increase in the severity of the disease and/or of any symptoms associated therewith, preventing, reducing or reversing any physiological damage caused by the disease, and generally any pharmacological action that is beneficial to the patient being treated.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

The invention relates to a method for the prevention and/or treatment of at least one disease or disorder that is associated with IgE, with increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE, with its biological or pharmacological activity, and/or with the biological pathways or signalling in which IgE is

involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein, and/or of a pharmaceutical composition comprising the same. In particular, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder that can be treated by modulating IgE, its biological or pharmacological activity, and/or the biological pathways or signalling in which IgE is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein, and/or of a pharmaceutical composition comprising the same. In particular, said pharmaceutically effective amount may be an amount that is sufficient to modulate IgE, its biological or pharmacological activity, and/or the biological pathways or signalling in which IgE is involved; and/or an amount that provides a level of an ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein that is sufficient to modulate IgE, its biological or pharmacological activity, and/or the biological pathways or signalling in which IgE is involved.

The invention furthermore relates to a method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering an ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein to a patient, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein, and/or of a pharmaceutical composition comprising the same.

More in particular, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder chosen from the group consisting of the diseases and disorders listed herein, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein, and/or of a pharmaceutical composition comprising the same.

In another aspect, the invention relates to a method for immunotherapy, and in particular for passive immunotherapy, which method comprises administering, to a subject suffering from or at risk of the diseases and disorders mentioned herein, a pharmaceutically active amount of an ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein, and/or of a pharmaceutical composition comprising the same.

In the above methods, the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein and/or the compositions comprising the same can be administered in any suitable manner, depending on the specific pharmaceutical formulation or composition to be used. Thus, the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein and/or the compositions comprising the same can for example be administered orally, intraperitoneally (*e.g.,* intravenously, subcutaneously, intramuscularly, or via any other route of administration that circumvents the gastrointestinal tract), intranasally, transdermally, topically, by means of a suppository, by inhalation, again depending on the specific pharmaceutical formulation or composition to be used. The clinician will be able to select a suitable route of administration and a suitable pharmaceutical formulation or composition to be used in such administration, depending on the disease or disorder to be prevented or treated and other factors well known to the clinician.

The ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein and/or the compositions comprising the same are administered according to a regime of treatment that is suitable for preventing and/or treating the disease or disorder to be prevented or treated. The clinician will generally be able to determine a suitable treatment regimen, depending on factors such as the disease or disorder to be prevented or treated, the severity of the disease to be treated and/or the severity of the symptoms thereof, the specific ISV, amino acid sequence, polypeptide, protein, construct or compound to be used, the specific route of administration and pharmaceutical formulation or composition to be used, the age, gender, weight, diet, general condition of the patient, and similar factors well known to the clinician.

Generally, the treatment regimen will comprise the administration of one or more ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein, or of one or more compositions comprising the same, in one or more pharmaceutically effective amounts or doses. The specific amount(s) or doses to administer can be determined by the clinician, again based on the factors cited above.

Generally, for the prevention and/or treatment of the diseases and disorders mentioned herein and depending on the specific disease or disorder to be treated, the potency of the specific ISV, amino acid sequence, polypeptide, protein, construct or compound to be used, the specific route of administration and the specific pharmaceutical formulation or composition used, the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described

herein will generally be administered in an amount between 1 gram and 0.01 microgram per kg body weight per day, preferably between 0.1 gram and 0.1 microgram per kg body weight per day, such as about 1, 10, 100 or 1000 microgram per kg body weight per day, either continuously (*e.g.*, by infusion), as a single daily dose or as multiple divided doses during the day. The clinician will generally be able to determine a suitable daily dose, depending on the factors mentioned herein. It will also be clear that in specific cases, the clinician may choose to deviate from these amounts, for example on the basis of the factors cited above and his expert judgment. Generally, some guidance on the amounts to be administered can be obtained from the amounts usually administered for comparable conventional antibodies or antibody fragments against the same target administered via essentially the same route, taking into account however differences in affinity/avidity, efficacy, biodistribution, half-life and similar factors well known to the skilled person.

Usually, in the above methods, a single ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein is used. It is however within the scope of the invention to use two or more ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein.

The ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein may also be used in combination with one or more further pharmaceutically active compounds or principles, *i.e.*, as a combined treatment regimen, which may or may not lead to a synergistic effect. Again, the clinician will be able to select such further compounds or principles, as well as a suitable combined treatment regimen, based on the factors cited above and his expert judgement.

In particular, the ISV's, amino acid sequences, polypeptides, proteins, constructs or other compounds as described herein may be used in combination with other pharmaceutically active compounds or principles that are or can be used for the prevention and/or treatment of the diseases and disorders cited herein, as a result of which a synergistic effect may or may not be obtained. Examples of such compounds and principles, as well as routes, methods and pharmaceutical formulations or compositions for administering them will be clear to the clinician.

When two or more substances or principles are to be used as part of a combined treatment regimen, they can be administered via the same route of administration or via different routes of

administration, at essentially the same time or at different times (*e.g.*, essentially simultaneously, consecutively, or according to an alternating regime). When the substances or principles are to be administered simultaneously via the same route of administration, they may be administered as different pharmaceutical formulations or compositions or part of a combined pharmaceutical formulation or composition, as will be clear to the skilled person.

Also, when two or more active substances or principles are to be used as part of a combined treatment regimen, each of the substances or principles may be administered in the same amount and according to the same regimen as used when the compound or principle is used on its own, and such combined use may or may not lead to a synergistic effect. However, when the combined use of the two or more active substances or principles leads to a synergistic effect, it may also be possible to reduce the amount of one, more or all of the substances or principles to be administered, while still achieving the desired therapeutic action. This may for example be useful for avoiding, limiting or reducing any unwanted side-effects that are associated with the use of one or more of the substances or principles when they are used in their usual amounts, while still obtaining the desired pharmaceutical or therapeutic effect.

The effectiveness of the treatment regimen used according to the invention may be determined and/or followed in any manner known per se for the disease or disorder involved, as will be clear to the clinician. The clinician will also be able, where appropriate and on a case-by-case basis, to change or modify a particular treatment regimen, so as to achieve the desired therapeutic effect, to avoid, limit or reduce unwanted side-effects, and/or to achieve an appropriate balance between achieving the desired therapeutic effect on the one hand and avoiding, limiting or reducing undesired side effects on the other hand.

Generally, the treatment regimen will be followed until the desired therapeutic effect is achieved and/or for as long as the desired therapeutic effect is to be maintained. Again, this can be determined by the clinician.

In another aspect, the invention relates to the use of an ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one disease or disorder that is associated with IgE, with increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE; and/or for use in one or more of the methods of treatment mentioned herein.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

The invention also relates to the use of an ISV, amino acid sequence, polypeptide, protein, construct or compound as described herein in the preparation of a pharmaceutical composition for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering an ISV, amino acid sequence, Nanobody or polypeptide of the invention to a patient.

More in particular, the invention relates to the use of an to be used ISV, amino acid sequence, Nanobody or polypeptide as described herein in the preparation of a pharmaceutical composition for the prevention and/or treatment of a disease or disorder that is associated with IgE, with increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE, and in particular for the prevention and treatment of one or more of such diseases and disorders listed herein.

Again, in such a pharmaceutical composition, the one or more ISV's, amino acid sequences, Nanobodies or polypeptides as described herein may also be suitably combined with one or more other active principles, such as those mentioned herein.

Definitions

Unless indicated or defined otherwise, all terms used 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.* (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 general background art cited herein.

Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks and the general background art mentioned herein and to the further references cited therein; as well as to for example the following reviews Presta (Adv. Drug Deliv. Rev. 58 (5-6): 640-56, 2006), Levin and Weiss (Mol. Biosyst. 2(1): 49-57, 2006), Irving *et al.* (J. Immunol. Methods 248(1-2): 31-45, 2001), Schmitz *et al.* (Placenta 21 Suppl. A: S106-12, 2000), Gonzales *et al.* (Tumour Biol. 26(1): 31-43, 2005), which describe techniques for protein engineering, such as affinity maturation and other techniques for improving the specificity and other desired properties of proteins such as immunoglobulins.

The term "sequence" as used herein (for example in terms like "immunoglobulin sequence", "antibody sequence", "variable domain sequence", "V_{HH} sequence", "polypeptide sequence" or "protein sequence"), should generally be understood to include both the relevant amino acid sequence as well as nucleic acids or nucleotide sequences encoding the same, unless the context requires a more limited interpretation.

A nucleic acid or amino acid is considered to be "(in) (essentially) isolated (form)" - for example, compared to the reaction medium or cultivation medium from which it has been obtained - when it has been separated from at least one other component with which it is usually associated in said source or medium, such as another nucleic acid, another protein/polypeptide, another biological component or macromolecule or at least one contaminant, impurity or minor component. In particular, a nucleic acid or amino acid is considered "(essentially) isolated" when it has been purified at least 2-fold, in particular at least 10-fold, more in particular at least 100-fold, and up to 1000-fold or more. A nucleic acid or amino acid that is "in (essentially) isolated form" is preferably essentially homogeneous, as determined using a suitable technique, such as a suitable chromatographical technique, such as polyacrylamide-gel electrophoresis.

When a nucleotide sequence or amino acid sequence is said to "comprise" another nucleotide sequence or amino acid sequence, respectively, or to "essentially consist of" another nucleotide sequence or amino acid sequence, this may mean that the latter nucleotide sequence or amino acid sequence has been incorporated into the first mentioned nucleotide sequence or amino acid sequence, respectively, but more usually this generally means that the first mentioned nucleotide sequence or amino acid sequence comprises within its sequence a stretch of

nucleotides or amino acid residues, respectively, that has the same nucleotide sequence or amino acid sequence, respectively, as the latter sequence, irrespective of how the first mentioned sequence has actually been generated or obtained (which may for example be by any suitable method described herein). By means of a non-limiting example, when a polypeptide is said to comprise an immunoglobulin single variable domain, this may mean that said immunoglobulin single variable domain sequence has been incorporated into the sequence of the polypeptide, but more usually this generally means that the polypeptide contains within its sequence the sequence of the immunoglobulin single variable domains irrespective of how said polypeptide has been generated or obtained. Also, when a nucleic acid or nucleotide sequence is said to comprise another nucleotide sequence, the first mentioned nucleic acid or nucleotide sequence is preferably such that, when it is expressed into an expression product (e.g. a polypeptide), the amino acid sequence encoded by the latter nucleotide sequence forms part of said expression product (in other words, that the latter nucleotide sequence is in the same reading frame as the first mentioned, larger nucleic acid or nucleotide sequence).

By a polypeptide that "essentially consist of" an ISV is meant that the immunoglobulin single variable domain either is exactly the same as the polypeptide or corresponds to the polypeptide which has a limited number of amino acid residues, such as 1-20 amino acid residues, for example 1-10 amino acid residues and preferably 1-6 amino acid residues, such as 1, 2, 3, 4, 5 or 6 amino acid residues, added at the amino terminal end, at the carboxy terminal end, or at both the amino terminal end and the carboxy terminal end of the immunoglobulin single variable domain.

For the purposes of comparing two or more amino acid sequences, the percentage of "sequence identity" between a first amino acid sequence and a second amino acid sequence (also referred to herein as "amino acid identity") may be calculated by dividing [the number of amino acid residues in the first amino acid sequence that are identical to the amino acid residues at the corresponding positions in the second amino acid sequence] by [the total number of amino acid residues in the first amino acid sequence] and multiplying by [100%], in which each deletion, insertion, substitution or addition of an amino acid residue in the second amino acid sequence - compared to the first amino acid sequence - is considered as a difference at a single amino acid residue (position), *i.e.*, as an "amino acid difference" as defined herein. Alternatively, the degree of sequence identity between two amino acid sequences may be calculated using a known

computer algorithm for sequence alignment such as NCBI Blast v2.0, using standard settings. Some other techniques, computer algorithms and settings for determining the degree of sequence identity are for example described in WO 04/037999, EP 0967284, EP 1085089, WO 00/55318, WO 00/78972, WO 98/49185 and GB 2357768. Usually, for the purpose of determining the percentage of "sequence identity" between two amino acid sequences in accordance with the calculation method outlined hereinabove, the amino acid sequence with the greatest number of amino acid residues will be taken as the "first" amino acid sequence, and the other amino acid sequence will be taken as the "second" amino acid sequence.

Also, in determining the degree of sequence identity between two amino acid sequences, the skilled person may take into account so-called "conservative" amino acid substitutions, which can generally be described as amino acid substitutions in which an amino acid residue is replaced with another amino acid residue of similar chemical structure and which has little or essentially no influence on the function, activity or other biological properties of the polypeptide. Such conservative amino acid substitutions are well known in the art, for example from WO 04/037999, GB 335768, WO 98/49185, WO 00/46383 and WO 01/09300; and (preferred) types and/or combinations of such substitutions may be selected on the basis of the pertinent teachings from WO 04/037999 as well as WO 98/49185 and from the further references cited therein.

Such conservative substitutions preferably are substitutions in which one amino acid within the following groups (a) – (e) is substituted by another amino acid residue within the same group: (a) small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro and Gly; (b) polar, negatively charged residues and their (uncharged) amides: Asp, Asn, Glu and Gln; (c) polar, positively charged residues: His, Arg and Lys; (d) large aliphatic, nonpolar residues: Met, Leu, Ile, Val and Cys; and (e) aromatic residues: Phe, Tyr and Trp. Particularly preferred conservative substitutions are as follows: Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

When comparing two amino acid sequences, the term "amino acid difference" refers to an insertion, deletion or substitution of a single amino acid residue on a position of the first sequence, compared to the second sequence; it being understood that two amino acid sequences

can contain one, two or more such amino acid differences. More particularly, in the amino acid sequences and/or polypeptides as described herein, the term "amino acid difference" refers to an insertion, deletion or substitution of a single amino acid residue on a position of the specified CDR sequence, compared to the CDR sequence under consideration.

The "amino acid difference" can be any one, two, three, four, five, six or maximal seven substitutions, deletions or insertions, or any combination thereof, that either improve the properties of the polypeptide or that at least do not detract too much from the desired properties or from the balance or combination of desired properties of the polypeptide as described herein. In this respect, the resulting polypeptide of the invention should at least bind IgE with the same, about the same, or a higher affinity compared to the polypeptide comprising the one or more CDR sequences without the one, two, three, four, five, six or maximal seven substitutions, deletions or insertions, said affinity as measured by surface plasmon resonance.

In this respect, the amino acid sequence under consideration may be an amino acid sequence that is derived from the specified amino acid sequence according by means of affinity maturation using one or more techniques of affinity maturation known *per se*.

For example, and depending on the host organism used to express the polypeptide, such deletions and/or substitutions may be designed in such a way that one or more sites for post-translational modification (such as one or more glycosylation sites) are removed, as will be within the ability of the person skilled in the art.

A polypeptide (such as an immunoglobulin, an antibody, an immunoglobulin single variable domain, a polypeptide of the invention, or generally an antigen binding molecule or a fragment thereof) that can "bind to" or "specifically bind to", that "has affinity for" and/or that "has specificity for" a certain epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is said to be "against" or "directed against" said epitope, antigen or protein or is a "binding" molecule with respect to such epitope, antigen or protein, or is said to be "anti"-epitope, "anti"-antigen or "anti"-protein (*e.g.*, "anti"-IgE).

The terms "(cross)-block", "(cross)-blocked", "(cross)-blocking", "competitive binding", "(cross)-compete", "(cross)-competing" and "(cross)-competition" are used interchangeably herein to mean the ability of an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent to interfere with the binding of other immunoglobulins, antibodies, immunoglobulin single variable domains, polypeptides or binding

agents to a given target. The extent to which an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent is able to interfere with the binding of another to the target, and therefore whether it can be said to cross-block according to the invention, can be determined using competition binding assays. One particularly suitable quantitative cross-blocking assay uses a BIAcore instrument which can measure the extent of interactions using surface plasmon resonance technology. Another suitable quantitative cross-blocking assay uses an ELISA-based approach to measure competition between immunoglobulins, antibodies, immunoglobulin single variable domains, polypeptides or other binding agents in terms of their binding to the target.

The following generally describes a suitable BIAcore assay for determining whether an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent cross-blocks or is capable of cross-blocking according to the invention. It will be appreciated that the assay can be used with any of the immunoglobulins, antibodies, immunoglobulin single variable domains, polypeptides or other binding agents described herein. The BIAcore instrument (for example the BIAcore 3000) is operated in line with the manufacturer's recommendations. Thus in one cross-blocking assay, the target protein (e.g. IgE) is coupled to a CM5 BIAcore chip using standard amine coupling chemistry to generate a surface that is coated with the target. Typically 200-800 resonance units of the target would be coupled to the chip (an amount that gives easily measurable levels of binding but that is readily saturable by the concentrations of test reagent being used). Two test binding agents (termed A* and B*) to be assessed for their ability to cross- block each other are mixed at a one to one molar ratio of binding sites in a suitable buffer to create the test mixture. When calculating the concentrations on a binding site basis the molecular weight of a binding agent is assumed to be the total molecular weight of the binding agent divided by the number of target binding sites on that binding agent. The concentration of each binding agent in the test mix should be high enough to readily saturate the binding sites for that binding agent on the target molecules captured on the BIAcore chip. The binding agents in the mixture are at the same molar concentration (on a binding basis) and that concentration would typically be between 1.00 and 1.5 micromolar (on a binding site basis). Separate solutions containing A* alone and B* alone are also prepared. A* and B* in these solutions should be in the same buffer and at the same concentration as in the test mix. The test mixture is passed over the target-coated BIAcore chip and the total amount of

binding recorded. The chip is then treated in such a way as to remove the bound binding agents without damaging the chip-bound target. Typically this is done by treating the chip with 30 mM HCl for 60 seconds. The solution of A* alone is then passed over the target-coated surface and the amount of binding recorded. The chip is again treated to remove all of the bound binding agents without damaging the chip-bound target. The solution of B* alone is then passed over the target-coated surface and the amount of binding recorded. The maximum theoretical binding of the mixture of A* and B* is next calculated, and is the sum of the binding of each binding agent when passed over the target surface alone. If the actual recorded binding of the mixture is less than this theoretical maximum then the two binding agents are said to cross-block each other. Thus, in general, a cross-blocking immunoglobulin, antibody immunoglobulin single variable domain, polypeptide or other binding agent according to the invention is one which will bind to the target in the above BIAcore cross-blocking assay such that during the assay and in the presence of a second immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent the recorded binding is between 80% and 0.1% (e.g. 80% to 4%) of the maximum theoretical binding, specifically between 75% and 0.1% (e.g. 75% to 4%) of the maximum theoretical binding, and more specifically between 70% and 0.1% (e.g. 70% to 4%) of maximum theoretical binding (as just defined above) of the two immunoglobulins, antibodies, immunoglobulin single variable domains, polypeptides or binding agents in combination. The BIAcore assay described above is a primary assay used to determine if immunoglobulins, antibodies, immunoglobulin single variable domains, polypeptide or other binding agents cross-block each other according to the invention. On rare occasions particular immunoglobulins, antibodies, immunoglobulin single variable domains, polypeptides or other binding agents may not bind to a target coupled via amine chemistry to a CM5 BIAcore chip (this usually occurs when the relevant binding site on the target is masked or destroyed by the coupling to the chip). In such cases cross-blocking can be determined using a tagged version of the target, for example a N-terminal His-tagged version. In this particular format, an anti-His antibody would be coupled to the Biacore chip and then the His-tagged target would be passed over the surface of the chip and captured by the anti-His antibody. The cross blocking analysis would be carried out essentially as described above, except that after each chip regeneration cycle, new His-tagged target would be loaded back onto the anti-His antibody coated surface. In addition to the example given using N-terminal His-tagged target, C-terminal His-tagged target

could alternatively be used. Furthermore, various other tags and tag binding protein combinations that are known in the art could be used for such a cross-blocking analysis (e.g. HA tag with anti-HA antibodies; FLAG tag with anti-FLAG antibodies; biotin tag with streptavidin).

The following generally describes an ELISA assay for determining whether an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent directed against a target (*e.g.,* IgE) cross-blocks or is capable of cross-blocking as defined herein. It will be appreciated that the assay can be used with any of the immunoglobulins, antibodies, immunoglobulin single variable domains, polypeptides or other binding agents described herein. The general principal of the assay is to have an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or binding agent that is directed against the target coated onto the wells of an ELISA plate. An excess amount of a second, potentially cross-blocking, anti-target immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide is added in solution (i.e. not bound to the ELISA plate). A limited amount of the target is then added to the wells. The coated immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide and the immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide in solution compete for binding of the limited number of target molecules. The plate is washed to remove excess target that has not been bound by the coated immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide and to also remove the second, solution phase immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide as well as any complexes formed between the second, solution phase immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide and target. The amount of bound target is then measured using a reagent that is appropriate to detect the target. An immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide in solution that is able to cross-block the coated immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide will be able to cause a decrease in the number of target molecules that the coated immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide can bind relative to the number of target molecules that the coated immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide can bind in the absence of the second, solution phase, immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide. In the instance where the first immunoglobulin, antibody, immunoglobulin single

variable domain or polypeptide, *e.g.,* an Ab-X, is chosen to be the immobilized immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide, it is coated onto the wells of the ELISA plate, after which the plates are blocked with a suitable blocking solution to minimize non-specific binding of reagents that are subsequently added. An excess amount of the second immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide, i.e. Ab-Y, is then added to the ELISA plate such that the moles of Ab-Y target binding sites per well are at least 10 fold higher than the moles of Ab-X target binding sites that were used, per well, during the coating of the ELISA plate. Target is then added such that the moles of target added per well are at least 25-fold lower than the moles of Ab-X target binding sites that were used for coating each well. Following a suitable incubation period the ELISA plate is washed and a reagent for detecting the target is added to measure the amount of target specifically bound by the coated anti-target immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide (in this case Ab-X). The background signal for the assay is defined as the signal obtained in wells with the coated immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide (in this case Ab-X), second solution phase immunoglobulin single variable domain or polypeptide (in this case Ab-Y), target buffer only (*i.e.,* without target) and target detection reagents. The positive control signal for the assay is defined as the signal obtained in wells with the coated immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide (in this case Ab-X), second solution phase immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide buffer only (*i.e.,* without second solution phase immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide), target and target detection reagents. The ELISA assay may be run in such a manner so as to have the positive control signal be at least 6 times the background signal. To avoid any artefacts (e.g. significantly different affinities between Ab-X and Ab-Y for the target) resulting from the choice of which immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide to use as the coating immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide and which to use as the second (competitor) immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide, the cross-blocking assay may to be run in two formats: 1) format 1 is where Ab-X is the immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide that is coated onto the ELISA plate and Ab-Y is the competitor immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide that is in solution and 2) format

2 is where Ab-Y is the immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide that is coated onto the ELISA plate and Ab-X is the competitor immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide that is in solution. Ab-X and Ab-Y are defined as cross-blocking if, either in format 1 or in format 2, the solution phase anti-target immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide is able to cause a reduction of between 60% and 100%, specifically between 70% and 100%, and more specifically between 80% and 100%, of the target detection signal (*i.e.*, the amount of target bound by the coated immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide) as compared to the target detection signal obtained in the absence of the solution phase anti-target immunoglobulin, antibody, immunoglobulin single variable domain or polypeptide (*i.e.*, the positive control wells).

Other methods for determining whether an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent directed against a target cross-blocks, is capable of cross-blocking, competitively binds or is cross-competitive as defined herein are described e.g. in Xiao-Chi Jia et al. (Journal of Immunological Methods 288: 91–98, 2004), Miller et al. (Journal of Immunological Methods 365: 118–125, 2011) and/or the methods described herein (see e.g. Examples 6, 7, 8, 9, 10).

The "half-life" of a polypeptide of the invention can generally be defined as described in paragraph o) on page 57 of WO 08/020079 and as mentioned therein refers to the time taken for the serum concentration of the polypeptide to be reduced by 50%, *in vivo*, for example due to degradation of the polypeptide and/or clearance or sequestration of the polypeptide by natural mechanisms. The *in vivo* half-life of a polypeptide of the invention can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally be as described in paragraph o) on page 57 of WO 08/020079. As also mentioned in paragraph o) on page 57 of WO 08/020079, the half-life can be expressed using parameters such as the t1/2-alpha, t1/2-beta and the area under the curve (AUC). Reference is for example made to the standard handbooks, such as Kenneth *et al.* (Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists, John Wiley & Sons Inc, 1986) and M Gibaldi and D Perron ("Pharmacokinetics", Marcel Dekker, 2nd Rev. Edition, 1982). The terms "increase in half-life" or "increased half-life" are also as defined in paragraph

o) on page 57 of WO 08/020079 and in particular refer to an increase in the t1/2-beta, either with or without an increase in the t1/2-alpha and/or the AUC or both.

Unless indicated otherwise, the term "immunoglobulin" - whether used herein to refer to a heavy chain antibody or to a conventional 4-chain antibody - is used as a general term to include both the full-size antibody, the individual chains thereof, as well as all parts, domains or fragments thereof (including but not limited to antigen-binding domains or fragments such as V_{HH} domains or V_H/V_L domains, respectively).

The term "domain" (of a polypeptide or protein) as used herein refers to a folded protein structure which has the ability to retain its tertiary structure independently of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins, and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain.

The term "immunoglobulin domain" as used herein refers to a globular region of an antibody chain (such as *e.g.*, a chain of a conventional 4-chain antibody or of a heavy chain antibody), or to a polypeptide that essentially consists of such a globular region. Immunoglobulin domains are characterized in that they retain the immunoglobulin fold characteristic of antibody molecules, which consists of a two-layer sandwich of about seven antiparallel beta-strands arranged in two beta-sheets, optionally stabilized by a conserved disulphide bond.

The term "immunoglobulin variable domain" as used herein means an immunoglobulin domain essentially consisting of four "framework regions" which are referred to in the art and herein below as "framework region 1" or "FR1"; as "framework region 2" or "FR2"; as "framework region 3" or "FR3"; and as "framework region 4" or "FR4", respectively; which framework regions are interrupted by three "complementarity determining regions" or "CDRs", which are referred to in the art and herein below as "complementarity determining region 1" or "CDR1"; as "complementarity determining region 2" or "CDR2"; and as "complementarity determining region 3" or "CDR3", respectively. Thus, the general structure or sequence of an immunoglobulin variable domain can be indicated as follows: FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4. It is the immunoglobulin variable domain(s) that confer specificity to an antibody for the antigen by carrying the antigen-binding site.

The term "immunoglobulin single variable domain", interchangeably used with "single variable domain" and "ISV", defines molecules wherein the antigen binding site is present on,

and formed by, a single immunoglobulin domain. This sets immunoglobulin single variable domains apart from "conventional" immunoglobulins or their fragments, wherein two immunoglobulin domains, in particular two variable domains, interact to form an antigen binding site. Typically, in conventional immunoglobulins, a heavy chain variable domain (VH) and a light chain variable domain (VL) interact to form an antigen binding site. In this case, the complementarity determining regions (CDRs) of both VH and VL will contribute to the antigen binding site, i.e. a total of 6 CDRs will be involved in antigen binding site formation.

In view of the above definition, the antigen-binding domain of a conventional 4-chain antibody (such as an IgG, IgM, IgA, IgD or IgE molecule; known in the art) or of a Fab fragment, a F(ab')2 fragment, an Fv fragment such as a disulphide linked Fv or a scFv fragment, or a diabody (all known in the art) derived from such conventional 4-chain antibody, would normally not be regarded as an immunoglobulin single variable domain, as, in these cases, binding to the respective epitope of an antigen would normally not occur by one (single) immunoglobulin domain but by a pair of (associating) immunoglobulin domains such as light and heavy chain variable domains, *i.e.,* by a VH-VL pair of immunoglobulin domains, which jointly bind to an epitope of the respective antigen.

In contrast, immunoglobulin single variable domains are capable of specifically binding to an epitope of the antigen without pairing with an additional immunoglobulin variable domain. The binding site of an immunoglobulin single variable domain is formed by a single VH, VHH or VL domain. Hence, the antigen binding site of an immunoglobulin single variable domain is formed by no more than three CDRs.

As such, the single variable domain may be a light chain variable domain sequence (*e.g.,* a VL-sequence) or a suitable fragment thereof; or a heavy chain variable domain sequence (*e.g.,* a VH-sequence or VHH 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).

In one embodiment of the invention, the immunoglobulin single variable domains are heavy chain variable domain sequences (*e.g.,* a VH-sequence); more specifically, the immunoglobulin single variable domains can be heavy chain variable domain sequences that are

derived from a conventional four-chain antibody or heavy chain variable domain sequences that are derived from a heavy chain antibody.

For example, the immunoglobulin single variable domain may be a (single) domain antibody (or an amino acid that is suitable for use as a (single) domain antibody), a "dAb" or dAb (or an amino acid that is suitable for use as a dAb) or a Nanobody (as defined herein, and including but not limited to a VHH); other single variable domains, or any suitable fragment of any one thereof.

In particular, the immunoglobulin single variable domain may be a Nanobody® (as defined herein) or a suitable fragment thereof. [Note: Nanobody®, Nanobodies® and Nanoclone® are registered trademarks of Ablynx N.V.] For a general description of Nanobodies, reference is made to the further description below, as well as to the prior art cited herein, such as e.g. described in WO 08/020079 (page 16).

"VHH domains", also known as VHHs, V_HH domains, VHH antibody fragments, and VHH antibodies, have originally been described as the antigen binding immunoglobulin (variable) domain of "heavy chain antibodies" (*i.e.*, of "antibodies devoid of light chains"; Hamers-Casterman *et al*. Nature 363: 446-448, 1993). The term "VHH domain" has been chosen in order to distinguish these variable domains from the heavy chain variable domains that are present in conventional 4-chain antibodies (which are referred to herein as "V_H domains" or "VH domains") and from the light chain variable domains that are present in conventional 4-chain antibodies (which are referred to herein as "V_L domains" or "VL domains"). For a further description of VHH's and Nanobodies, reference is made to the review article by Muyldermans (Reviews in Molecular Biotechnology 74: 277-302, 2001), 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, Nanobodies (in particular VHH 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. A further description of the Nanobodies, including humanization and/or camelization of Nanobodies, as well as other modifications, parts or fragments, derivatives or "Nanobody fusions", multivalent constructs (including some non-limiting examples of linker sequences) and different modifications to increase the half-life of the Nanobodies and their preparations can be found e.g. in WO 08/101985 and WO 08/142164. For a further general description of Nanobodies, reference is made to the prior art cited herein, such as *e.g.,* described in WO 08/020079 (page 16).

"Domain antibodies", also known as "Dab"s, "Domain Antibodies", and "dAbs" (the terms "Domain Antibodies" and "dAbs" being used as trademarks by the GlaxoSmithKline group of companies) have been described in *e.g.,* EP 0368684, Ward *et al.* (Nature 341: 544-546, 1989), Holt *et al.* (Tends in Biotechnology 21: 484-490, 2003) and WO 03/002609 as well as for example WO 04/068820, WO 06/030220, WO 06/003388 and other published patent applications of Domantis Ltd. Domain antibodies essentially correspond to the VH or VL domains of non-camelid mammalians, in particular human 4-chain antibodies. In order to bind an epitope as a single antigen binding domain, *i.e.,* without being paired with a VL or VH domain, respectively, specific selection for such antigen binding properties is required, e.g. by using libraries of human single VH or VL domain sequences. Domain antibodies have, like VHHs, a molecular weight of approximately 13 to approximately 16 kDa and, if derived from fully human sequences, do not require humanization for e.g. therapeutical use in humans.

It should also be noted that, although less preferred in the context of the present invention because they are not of mammalian origin, single variable domains can be derived from certain species of shark (for example, the so-called "IgNAR domains", see for example WO 05/18629).

Thus, in the meaning of the present invention, the term "immunoglobulin single variable domain" or "single variable domain" comprises polypeptides which are derived from a non-human source, preferably a camelid, preferably a camelid heavy chain antibody. They may be humanized, as previously described. Moreover, the term comprises polypeptides derived from

non-camelid sources, e.g. mouse or human, which have been "camelized", as *e.g.,* described in Davies and Riechmann (FEBS 339: 285-290, 1994; Biotechnol. 13: 475-479, 1995; Prot. Eng. 9: 531-537, 1996) and Riechmann and Muyldermans (J. Immunol. Methods 231: 25-38, 1999).

The amino acid residues of a VHH domain are numbered according to the general numbering for V_H domains given by Kabat *et al.* ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, MD, Publication No. 91), as applied to VHH domains from Camelids, as shown *e.g.,* in Figure 2 of Riechmann and Muyldermans (J. Immunol. Methods 231: 25-38, 1999). Alternative methods for numbering the amino acid residues of V_H domains, which methods can also be applied in an analogous manner to VHH domains, are known in the art. However, in the present description, claims and figures, the numbering according to Kabat applied to VHH domains as described above will be followed, unless indicated otherwise.

It should be noted that - as is well known in the art for V_H domains and for VHH domains - the total number of amino acid residues in each of the CDRs may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering). This means that, generally, the numbering according to Kabat may or may not correspond to the actual numbering of the amino acid residues in the actual sequence. The total number of amino acid residues in a VH domain and a VHH domain will usually be in the range of from 110 to 120, often between 112 and 115. It should however be noted that smaller and longer sequences may also be suitable for the purposes described herein.

Determination of CDR regions may also be done according to different methods. In the CDR determination according to Kabat, FR1 of a VHH comprises the amino acid residues at positions 1-30, CDR1 of a VHH comprises the amino acid residues at positions 31-35, FR2 of a VHH comprises the amino acids at positions 36-49, CDR2 of a VHH comprises the amino acid residues at positions 50-65, FR3 of a VHH comprises the amino acid residues at positions 66-94, CDR3 of a VHH comprises the amino acid residues at positions 95-102, and FR4 of a VHH comprises the amino acid residues at positions 103-113.

Immunoglobulin single variable domains such as Domain antibodies and Nanobodies (including VHH domains) can be subjected to humanization. In particular, humanized

immunoglobulin single variable domains, such as Nanobodies (including VHH domains) may be immunoglobulin single variable domains that are as generally defined for in the previous paragraphs, but in which at least one amino acid residue is present (and in particular, in at least one of the framework residues) that is and/or that corresponds to a humanizing substitution. Potentially useful humanizing substitutions can be ascertained by comparing the sequence of the framework regions of a naturally occurring V_{HH} sequence with the corresponding framework sequence of one or more closely related human V_H sequences, after which one or more of the potentially useful humanizing substitutions (or combinations thereof) thus determined can be introduced into said V_{HH} sequence (in any manner known per se, as further described herein) and the resulting humanized V_{HH} sequences can be tested for affinity for the target, for stability, for ease and level of expression, and/or for other desired properties. In this way, by means of a limited degree of trial and error, other suitable humanizing substitutions (or suitable combinations thereof) can be determined by the skilled person based on the disclosure herein. Also, based on the foregoing, (the framework regions of) an immunoglobulin single variable domain, such as a Nanobody (including VHH domains) may be partially humanized or fully humanized.

Immunoglobulin single variable domains such as Domain antibodies and Nanobodies (including VHH domains and humanized VHH domains), can also be subjected to affinity maturation by introducing one or more alterations in the amino acid sequence of one or more CDRs, which alterations result in an improved affinity of the resulting immunoglobulin single variable domain for its respective antigen, as compared to the respective parent molecule. Affinity-matured immunoglobulin single variable domain molecules of the invention may be prepared by methods known in the art, for example, as described by Marks *et al.* (Biotechnology 10:779-783, 1992), Barbas *et al.* (Proc. Nat. Acad. Sci, USA 91: 3809-3813, 1994), Shier *et al.* (Gene 169: 147-155, 1995), Yelton *et al.* (Immunol. 155: 1994-2004, 1995), Jackson *et al.* (J. Immunol. 154: 3310-9, 1995), Hawkins *et al.* (J. Mol. Biol. 226: 889 896, 1992), Johnson and Hawkins (Affinity maturation of antibodies using phage display, Oxford University Press, 1996).

The process of designing/selecting and/or preparing a polypeptide, starting from an immunoglobulin single variable domain such as a Domain antibody or a Nanobody, is also referred to herein as "formatting" said immunoglobulin single variable domain; and an immunoglobulin single variable domain that is made part of a polypeptide is said to be

"formatted" or to be "in the format of" said polypeptide. Examples of ways in which an immunoglobulin single variable domain can be formatted and examples of such formats will be clear to the skilled person based on the disclosure herein; and such formatted immunoglobulin single variable domain form a further aspect of the invention.

For example, and without limitation, one or more immunoglobulin single variable domains may be used as a "binding unit", "binding domain" or "building block" (these terms are used interchangeable) for the preparation of a polypeptide, which may optionally contain one or more immunoglobulin single variable domain or peptide that can serve as a binding unit (*i.e.*, against the same or another epitope on IgE and/or against one or more other antigens, proteins or targets than IgE).

Monovalent polypeptides comprise or essentially consist of only one binding unit (such as *e.g.*, immunoglobulin single variable domains). Polypeptides that comprise two or more binding units (such as *e.g.*, immunoglobulin single variable domains) will also be referred to herein as "multivalent" polypeptides, and the binding units/immunoglobulin single variable domains present in such polypeptides will also be referred to herein as being in a "multivalent format". For example a "bivalent" polypeptide may comprise two immunoglobulin single variable domains, optionally linked via a linker sequence, whereas a "trivalent" polypeptide may comprises three immunoglobulin single variable domains, optionally linked via two linker sequences; etc..

In a multivalent polypeptide, the two or more immunoglobulin single variable domains may be the same or different, and may be directed against the same antigen or antigenic determinant (for example against the same part(s) or epitope(s) or against different parts or epitopes) or may alternatively be directed against different antigens or antigenic determinants; or any suitable combination thereof. Polypeptides that contain at least two binding units (such as *e.g.*, immunoglobulin single variable domains) in which at least one binding unit is directed against a first antigen (*i.e.*, IgE) and at least binding unit is directed against a second antigen (*i.e.*, different from IgE) will also be referred to as "multispecific" polypeptides, and the binding units (such as *e.g.*, immunoglobulin single variable domains) present in such polypeptides will also be referred to herein as being in a "multispecific format". Thus, for example, a "bispecific" polypeptide of the invention is a polypeptide that comprises at least one immunoglobulin single variable domain directed against a first antigen (*i.e.*, IgE) and at least one further

immunoglobulin single variable domain directed against a second antigen (*i.e.*, different from IgE), whereas a "trispecific" polypeptide of the invention is a polypeptide that comprises at least one immunoglobulin single variable domain directed against a first antigen (*i.e.*, IgE), at least one further immunoglobulin single variable domain directed against a second antigen (*i.e.*, different from IgE) and at least one further immunoglobulin single variable domain directed against a third antigen (*i.e.*, different from both IgE and the second antigen); etc.

Other aspects, embodiments, advantages and applications of the invention will also become clear from the further description herein. The invention will now be further illustrated by means of the non-limiting Experimental Part and Figures, in which:

FIGURE LEGENDS

Figure 1: Sequence alignment of the various humanized and sequence-optimized variants of 39D11 compared to 39D11.

Figure 2: Sequence alignment of the various humanized and sequence-optimized variants of 39D11 compared to the humanized variant IgE009.

Figure 3: Study design of the PK/PD study.

Figure 4: Free IgE plasma levels. Levels of free IgE overtime after a single injection of either vehicle, IgE109 or Xolair. Data on the graph represent the median value for each group of animals.

EXPERIMENTAL PART

Example 1: Immunizations

Four llamas (No. 002 , No. 004, No. 193, No. 197) were 4-6 times immunized with 50-100 microgram doses of human IgE (Scripps Laboratories, San Diego, CA, Cat# I0224) (llamas 002 and 004) or 50-100 microgram human IgE kappa chain (Diatec, Oslo, Norway). The intervals between the immunizations varied between 1-5 weeks. Proteins were formulated in Stimmune adjuvant (Cedi Diagnostics, Lelystad, The Netherlands) for animals 002 and 004, or Complete Freund's adjuvant (CFA) or incomplete Freund's adjuvant (IFA) (Difco, Becton Dickinson, Franklin Lakes, NJ) for animals 193 and 197.

Sera from blood samples of llamas 002, 004, 193 and 197 were obtained prior to immunization, during the immunization protocol and after completion of the immunizations.

Human IgE was coated onto Nunc Maxisorb plates at 2 microgram/ml, blocked with 1% casein in PBS and incubated with serial dilutions of pre- and post-immune llama sera. Plate-immobilized llama IgG was detected using HRP conjugated goat-anti-llama IgG (Bethyl Labs, Montgomery, TX) and TMB chromogen according to standard methods. Comparison of optical density values clearly indicated immunization induced a humoral immune response against IgE in all four animals.

Peripheral blood mononuclear cells were prepared from the blood samples using Ficoll-Hypaque according to the manufacturer's instructions. Total RNA extracted from these cells and from lymph nodes was used as starting material for RT-PCR to amplify Nanobody encoding gene fragments. These fragments were cloned into phagemid vector derived of pUC119, containing the LacZ promoter, a coliphage pIII protein coding sequence, a resistance gene for ampicillin or carbenicillin, and a multicloning site harbours several restriction sites. In frame with the Nanobody® coding sequence, the vector codes for a C-terminal c-myc tag and a (His)6 tag. The signal peptide is the gen3 leader sequence which translocates the expressed Nanobody® to the periplasm. Phage was prepared according to standard protocols (Phage Display of Peptides and Proteins: A Laboratory Manual, Academic Press; 1st edition (October 28, 1996)) and stored after filter sterilization at 4°C until further use. In total, 4 phage libraries were constructed (002, 004, 193 and 197), with library sizes between $3.5x10^6$ and $28x10^6$, and a percentage of insert ranging from 96 to 100%.

Example 2: Selections of IgE binding Nanobodies

A cyno IgE-Fc fragment was produced and used as a tool to identify IgE-specific Nanobodies which were human and cyno IgE cross-reactive. RNA was extracted from peripheral blood monomorphonuclear cells from 2 male and 2 female cynomolgus monkeys. The cyno IgE sequence (SEQ ID NO: 173) was amplified by RT-PCR using different primers (SEQ ID NO's: 189 to 192). The obtained fragments were cloned in a cloning vector and sequenced. The cyno IgE c(epsilon)2-c(epsilon)3-c(epsilon)4 fragment was cloned in an expression vector derived from pCIneo which contained the human cytomegalovirus (CMV) immediate-early enhancer/promoter region, the SV40 late polyadenylation signal, a resistance gene for ampicillin or carbenicillin, a multicloning site and the murine Ig kappa light chain leader sequence. In frame with the cynomolgus IgE-Fc coding sequence, the vector coded for a C-terminal (His)10

tag. The cis-acting viral DNA element, oriP locus allowed for episomal replication in HEK-EBNA cells, expressing the Epstein-Barr virus nuclear antigen-1. Culture supernatant was harvested and cyno IgE Fc was purified using cation exchange (Source 30S) followed by affinity purification (His TrapTMFF) and desalting (Desalting 26/10 Hiprep, all columns from GE Healthcare).

An Fc-fusion of the human high affinity IgE receptor Fc(epsilon)RIα (gene bank P12319) was produced as a tool. The human FcεRIα was synthetized and cloned in an expression vector as a fusion with a human IgG Fc fragment. Stable transfected CHO cells (ATCC) were generated and culture supernatant was harvested. The human Fc(epsilon)RI(alpha)Fc fragment was then purified using protein A (Mabselect sure; GE Healthcare).

Phage display was used to enrich IgE-specific Nanobodies. Phages were prepared according to standard methods from libraries obtained from llamas No. 002, 004, 193 and 197. Phage libraries were used for two rounds (R1/R2) of selection on plate-immobilized cyno IgE Fc. Cyno IgE c(epsilon)2-c(epsilon)3-c(epsilon)4-Fc was immobilized at concentrations varying from 0.2 to 2 microgram/ml on Nunc Maxisorp ELISA plates. Plate-immobilized phages were retrieved using trypsin elution or Fc(epsilon)RIα elution. Outputs of R1/R2 selections were analyzed for enrichment factor (# phage present in eluate relative to control). Based on these parameters, the best selections were chosen for further analysis. Individual colonies were picked and grown in 96 deep well plates (1 ml volume) and induced by adding IPTG for Nanobody expression. Periplasmic extracts were prepared according to standard methods. Nanobodies were expressed as fusion proteins containing C-terminal both the c-myc as well as the 6His tags.

Example 3: Screening for IgE blocking Nanobodies in AlphaScreen assay

In order to screen for human/cyno cross-reactive IgE blocking Nanobodies, periplasmic extracts were analyzed for their ability to block the interaction of human IgE (Diatec, Oslo, Norway) or cyno IgE c(epsilon)2-c(epsilon)3-c(epsilon)4 with human Fc(epsilon)RI(alpha)-Fc. To this end, two alphascreen assays (Amplified Luminiscent Proximity Homogeneous Assay; Perkin Elmer, Waltman, MA) were set up. In brief, 0.05 nM biotinylated hIgE or 0.1 nM biotinylated cyno IgE c(epsilon)2-c(epsilon)3-c(epsilon)4 was captured by streptavidin Donor beads and 0.05 nM Fc(epsilon)RI was captured by anti-hFc-Acceptor beads.

Diluted periplasmic extracts of individual Nanobody clones were analysed. The omalizumab Fab fragment, known to inhibit the human IgE/Fc(epsilon)RI(alpha)Fc interaction, was used as a positive control. Assays were read in an Envision alphascreen option fitted multimode reader (Perkin Elmer, Waltman, MA). Individual clones were scored as putative HuIgE/HuFc(epsilon)RI(alpha)Fc interaction or cyno IgE c(epsilon)2-c(epsilon)3-c(epsilon)4/HuFc(epsilon)RI(alpha)Fc interaction inhibiting if the presence of the periplasmic extract decreased the fluorescent signal of the acceptor beads. All four libraries contained both non-blocking and blocking clones when tested on human IgE, but only library 197 retrieved cyno cross-reactive neutralizing Nanobodies. Cyno cross-reactive inhibiting Nanobodies were selected and sequenced. Sequence analysis revealed 1 Family (named family 12) which was subdivided into two subfamilies 12.1 (comprising clones 39D11, 39D8, 42D7 and 42G5; SEQ ID NO's: 119, 115, 116 and 117, respectively) and 12.2 (clone 36G5; SEQ ID NO: 118) and a unique clone (39B02; SEQ ID NO: 114). The corresponding DNA sequences are given in SEQ ID NO's: 183 to 188. This low diversity indicates that it is very difficult to generate and identify cyno-cross reactive Nanobodies which block the interaction of IgE with Fc(epsilon)RI(alpha).

Example 4: Off-rate determination of IgE binding Nanobodies

Off-rate constants (koff) of individual inhibitory Nanobody clone periplasmic extracts were determined by surface plasmon resonance on a Biacore T100 instrument. Human IgE (Diatec, Oslo, Norway) was amine-coupled to a CM5 sensor chip at a density of 3100 RU. Remaining reactive groups were inactivated using ethanolamine. Nanobody binding and off-rate was assessed at a single dilution of periplasmic extract. The omalizumab Fab fragment was tested at a single concentration of 100nM. Each sample was injected 2 minutes at a flow rate of 45 µl/min to allow binding to chip-bound antigen. Next, binding buffer without periplasmic extracts was sent over the chip at the same flow rate to allow spontaneous dissociation of bound Nanobody. Analyte remaining bound after the monitored dissociation phase was removed by injecting regeneration solution (4.5M $MgCl_2$). Data of the best representatives per subfamily and the unique clone 39B2 (all selected for purification and more detailed analysis) are shown in Table 1.

Table 1: Off rate values of Nanobodies (periplasmic extracts).

Clone	Off rate Hu IgE (s^{-1})	Off rate cyno IgE (s^{-1})
39D8	2.24E-03	1.22E-03
42D7	2.25E-03	1.09E-03
39D11	2.39E-03	1.32E-03
36G5	1.11E-02	3.28E-03
42G5	2.83E-02	5.76E-03
39B2	7.96E-02	5.69E-04

Example 5: IgE-binding Nanobody expression and purification

Selected Nanobodies were expressed in the periplasmic space of *E.coli* as c-myc, His6-tagged proteins in a culture volume of 250 mL. Expression was induced in high density cultures by addition of 1 mM IPTG and allowed to continue for 4h at 37°C. After spinning the cell cultures, periplasmic extracts were prepared by freeze-thawing the pellets and resuspension in dPBS. These extracts were used as starting material for affinity chromatography using HisTrap crude columns (GE Healthcare). Nanobodies were eluted from the column with 250 mM imidazole and subsequently desalted towards dPBS.

Example 6: Inhibition of HuIgE or cyno IgE Fc (ce2-ce3-ce4) /HuFc(epsilon)RI(alpha)Fc interaction in Alphascreen

Serial dilutions of purified Nanobodies were then analyzed for their ability to block the interaction of HuIgE or cyno IgE Fc(ce2-ce3-ce4) with HuFc(epsilon)RI(alpha)Fc using the same alphascreen assays as described above. Data are shown in Tables 2 and 3 and indicate all Nanobodies except 39B2 have a similar or better potency compared to the positive control Fab (obtained by papain digestion of commercially obtained omalizumab).

Table 2: IC50 values for competition between the human IgE receptor Fc(epsilon)RI(alpha) and anti-IgE Nanobodies to bind human IgE, their 95% confidence intervals (CI95) and percentage inhibition at 250 nM Nanobody as determined by Alphascreen. NA: not applicable.

Clone	IC50 (M)	CI95	% inhibition at 250 nM Nanobody
39D8	4.191e-010	3.985e-010 to 4.408e-010	97.1
39D11	4.412e-010	4.087e-010 to 4.764e-010	97.1
42D7	5.977e-010	5.190e-010 to 6.885e-010	97.2
Human IgE	3.008e-010	2.311e-010 to 3.915e-010	98.6
Reference Fab	9.223e-010	8.634e-010 to 9.853e-010	99.0
36G5	1.423e-009	1.133e-009 to 1.789e-009	94.7
42G5	4.346e-009	3.192e-009 to 5.917e-009	96.0
39B2	No bottom level reached at 250 nM Nb	NA	51.8
Human IgE	2.005e-010	1.582e-010 to 2.541e-010	99.1
Reference Fab	6.187e-010	4.773e-010 to 8.021e-010	98.2

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Table 3: IC50 values for competition between the human IgE receptor Fc(epsilon)RI(alpha) and anti-IgE Nanobodies to bind cyno IgE Fc (Ce2-ce3-ce4), their 95% confidence intervals (CI95) and percentage inhibition at 250 nM Nanobody as determined by Alphascreen. NA: not applicable.

Clone	IC50 (M)	CI95	% inhibition at 250 nM Nanobody
36G5	5,835e-010	4.648e-010 to 7.325e-010	88.1
39B2	No bottom level reached at 250 nM Nb	NA	38.0
42G5	9,595e-010	8.047e-010 to 1.144e-009	91.2
cyno-IgE	9,197e-010	7.528e-010 to 1.123e-009	99.5
Reference Fab	1,385e-009	1.190e-009 to 1.611e-009	99.3
39D8	7,420e-011	6.598e-011 to 8.345e-011	93.6
39D11	1,194e-010	9.836e-011 to 1.450e-010	93.5
42D7	1,265e-010	1.013e-010 to 1.579e-010	92.7
cyno-IgE	2,822e-010	2.133e-010 to 3.733e-010	96.5
Reference Fab	1,303e-009	8.692e-010 to 1.954e-009	97.7

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Example 7: Inhibition of HuIgE or cyno IgE/ HuFc(epsilon)RI interaction in ELISA

Nanobodies were analyzed for their ability to block the interaction of HuIgE (Diatec, Oslo, Norway) or cyno IgE (plasma) with Fc(epsilon)RI. To this end, ELISA plates were coated with Fc(epsilon)RI (0.1 microgram/ml), then blocked. Serial Nanobody dilutions were pre-incubated with 50 pM HuIgE or 1/50 cyno plasma dilution, followed by addition of the pre-incubated mixture to the Fc(epsilon)RI coated ELISA plates. Binding of HuIgE or cyno IgE to the immobilized Fc(epsilon)RI was detected by using goat anti-human IgE-HRP (KPL, Gaithersburg, MD). Presence of putative IgE/ Fc(epsilon)RI interaction inhibitors would result in decreased OD signals. The results are shown in Tables 4 and 5.

Table 4: IC50 values for competition between Fc(epsilon)RI(alpha) and anti-IgE Nanobodies to bind human IgE Fc (Ce2-ce3-ce4), their 95% confidence intervals and percentage inhibition at 500 nM Nanobody as determined by ELISA.

clone	HuIgE		
	IC50 (M)	CI95	% inhibition by 500 nM Nanobody
39D8	5.504e-010	4.312e-010 to 7.025e-010	99.4
42D7	9.588e-010	8.439e-010 to 1.089e-009	99.6
39D11	5.107e-010	4.302e-010 to 6.063e-010	99.5
36G5	2.962e-009	2.534e-009 to 3.463e-009	98.6
39B2	3.942e-008	2.694e-008 to 5.768e-008	69.3
Reference Fab	1.168e-009	1.005e-009 to 1.358e-009	99.1
Reference mAb	2.568e-010	1.588e-010 to 4.154e-010	97.7
42G5	8.377e-009	6.729e-009 to 1.043e-008	98.1

Table 5: IC50 values for competition between the human IgE receptor Fc(epsilon)RI(alpha) and anti-IgE Nanobodies to bind cyno IgE Fc (Ce2-ce3-ce4), their 95% confidence intervals and percentage inhibition at 500 nM Nanobody as determined by ELISA. NA: not applicable.

clone	CynoIgE		
	IC50 (M)	CI95	% inhibition by 500 nM Nanobody
39D8	1.800e-009	1.445e-009 to 2.242e-009	99.7
39D11	5.199e-009	3.541e-009 to 7.633e-009	99.8
42D7	4.564e-009	4.046e-009 to 5.149e-009	92.0
42G5	7.536e-009	4.591e-009 to 1.237e-008	92.5
Omalizumab	5.085e-009	4.097e-009 to 6.311e-009	90.5
Reference Fab	3.782e-008	2.900e-008 to 4.932e-008	87.4
36G5	3.005e-008	2.564e-008 to 3.521e-008	80.3
39B2	No competition	NA	0

Example 8: Inhibition of HuIgE/HuFc(epsilon)RII interaction in ELISA

Nanobodies were analyzed for their ability to block the interaction of HuIgE (Diatec, Oslo, Norway) with recombinant Fc(epsilon)RII (R&D Systems, Minneapolis, MN). To this end, ELISA plates were coated with Fc(epsilon)RII, then blocked. Serial Nanobody dilutions were pre-incubated with 20 nM HuIgE, followed by addition of the pre-incubated mixture to the Fc(epsilon)RII coated ELISA plates. Binding of HuIgE to the immobilized Fc(epsilon)RII was detected by using goat anti-human IgE-HRP (KPL, Gaithersburg, MD). Presence of putative HuIgE/ Fc(epsilon)RII interaction inhibitors would result in decreased OD signals. The data are shown in Table 6 and indicate that all three tested Nanobodies block IgE binding to Fc(epsilon)RII. Nanobodies 39D11 and 36G5 had a similar potency and efficacy (100% block) as compared to Reference Fab, whereas Nanobody 39B2 was 10-fold less potent and only reached around 80% block at 500 nM Nanobody concentration.

Table 6: IC50 values for competition between the human IgE receptor Fc(epsilon)RII and anti-IgE Nanobodies to bind human IgE, their 95% confidence intervals as determined by ELISA.

Clone	IC50 (M)	CI95
39D11	1.291e-008	6.846e-009 to 2.436e-008
36G5	1.996e-008	1.115e-008 to 3.571e-008
39B2	1.527e-007	6.977e-008 to 3.344e-007
Reference Fab	1.547e-008	9.281e-009 to 2.580e-008

Example 9: Inhibition of HuIgE/Omalizumab interaction in Alphascreen

Nanobodies were analyzed for their ability to block the interaction of HuIgE (Diatec, Oslo, Norway) with Omalizumab. To this end, an alphascreen assay (Perkin Elmer, Waltham, MA) was set up. In brief, serial dilution series of individual Nanobodies were incubated with 0.1nM biotinylated huIgE, 0.1nM Omalizumab, streptavidin coated donor beads and anti-human Fc Nanobody coupled acceptor beads. The Reference Fab fragment, known to inhibit the HuIgE/Omalizumab interaction, was used as a positive control. Assays were read in an Envision alphascreen option fitted multimode reader (Perkin Elmer, Waltham, MA). Individual clones were scored as putative HuIgE/Omalizumab interaction inhibitors if their presence decreased the fluorescent signal of the acceptor beads. The results are shown in Table 7 and indicate that all tested Nanobodies except 39B2 compete with Omalizumab for IgE binding. The bottom plateau levels of the Nanobody inhibition curves are significantly higher (% inhibition maximum 79.9%) in comparison to that of Reference Fab or IgE (% inhibition maximum 96.9 and 99.6, respectively), suggesting that the Omalizumab en Nanobody epitopes are only partially overlapping.

Table 7: IC50 values for competition between omalizumab and anti-IgE Nanobodies to bind human IgE, their 95% confidence intervals and percentage inhibition at 250 nM Nanobody as determined by Alphascreen. NA: not applicable.

Clone	IC50 (M)	CI95	% inhibition by 250 nM Nanobody
36G5	6,363e-009	3.854e-009 to 1.051e-008	62.9
39B2	Not bottom plateau reached	NA	9.3
42G5	2.486e-008	1.520e-008 to 4.065e-008	61.5
39D8	1.482e-009	1.300e-009 to 1.691e-009	79.9
39D11	1.511e-009	1.342e-009 to 1.702e-009	79.6
42D7	1.723e-009	1.511e-009 to 1.964e-009	79.1
IgE	5.189e-010	4.388e-010 to 6.137e-010	96.9
Reference Fab	2.686e-009	2.548e-009 to 2.832e-009	99.6

Example 10: Inhibition of HuIgE/HuFc(epsilon)RI and HuFc(epsilon)RII interaction in FACS

Nanobodies 39D11 and 36G5 were tested in FACS competition assay for their ability to inhibit IgE-binding to Fc(epsilon)RI on RBL Fc(epsilon)RI(alpha) stable transfectants and Fc(epsilon)RII on Raji cells. To this end serial dilutions of Nanobody were added to the cells followed by addition of human IgE (7.5 nM for RBL Fc(epsilon)RI assay; 10 nM for Raji cell assay). Presence of putative interaction inhibitors would result in decreased MCF signals. The data in Table 8 indicate that both Nanobodies block IgE binding to Fc(epsilon)RI and Fc(epsilon)RII comparable to the positive control Reference Fab. All three tested molecules reached more than 90% inhibition at 1 μM concentrations.

Table 8: IC50 values for competition between anti-IgE Nanobodies and Fc(epsilon)RI or Fc(epsilon)RII (expressed on transfected RBL and endogenously expressed on Raji cells, respectively) to bind human IgE and their 95% confidence intervals as determined by FACS.

clone	RBL- Fc(epsilon)RI(alpha)		Raji- Fc(epsilon)RII(alpha)	
	IC50 (M)	CI95	IC50 (M)	CI95
Ref Fab	8.386e-009	7.753e-009 to 9.071e-009	8.767e-009	6.131e-009 to 12.54e-009
39D11	4.952e-009	4.475e-009 to 5.479e-009	10.19e-009	3.660e-009 to 28.35e-009
36G05	9.543e-009	8.418e-009 to 10.82e-009	35.83e-009	13.27e-009 to 96.76e-009

Example 11: Inhibition in degranulation assay

Assay setup was based on a protocol supplied with xCELLigence RTCA instrument (Roche). The System measures electrical impedance across interdigitated micro-electrodes integrated on the bottom of tissue culture E-Plates. The real time impedance measurement provides quantitative information about the morphological status of the cells, including degranulation, without incorporation of labels. RBL cells transfected with Fc(epsilon)RIalpha were overnight incubated in E-plates and sensitized with anti-DNP chimeric IgE (50ng/ml; Serotec) for 1 hr. Then, degranulation was triggered by adding NIP-BSA (50 ng/ml; Biosearch Technologies). The activation/degranulation of the basophils can be detected in real time on the xCELLigence System measuring impedance (measure every minute during 8 hrs). The maximum cell index signal per well was used to calculate EC50 and IC50 values. A correlation was shown with histamine release in supernatant (LDN histamine Research ELISA).

Nanobodies 39D08, 39D11, 36G5 and 39B2 were tested in degranulation assay for their ability to inhibit IgE-mediated degranulation. To this end serial dilutions of Nanobody or control antibody were pre-incubated with human IgE and then added to the cells. Results are shown in Table 9. Nanobody 39B2 only reached 25% block of degranulation at a Nanobody concentration of 500 nM, whereas the other 3 Nanobodies and Reference Fab blocked more than 95% at this concentration. The potency of the Nanobodies was 3- to 40-fold weaker in comparison with the Reference Fab control.

Table 9: IC50 values for blocking IgE-mediated degranulation of RBL-Fc(epsilon)RI(alpha) transfected cells by anti-IgE Nanobodies or control Fab, and their 95% confidence intervals as determined by impedance measurement.

clone	IC50 (nM)	CI95 (nM)
Omalizumab	2.858	1.662 to 4.917
39D08	18.52	14.00 to 24.50
39D11	18.72	12.38 to 28.32
36G5	112.2	43.61 to 288.4
Reference Fab	5.768	3.900 to 8.530

Example 12: Affinity determination

Affinity constants of individual Nanobodies were determined by surface plasmon resonance (SPR) on a Biacore 3000 instrument. In brief, Human IgE (Diatec, Oslo, Norway) or cyno IgE Fc (Ce2-ce3-ce4) was amine-coupled to a CM5 sensor chip. Remaining reactive groups were inactivated using ethanolamine. Nanobodies were injected at different concentrations. Each sample was injected at a flow rate of 45 µl/min to allow for binding to chip-bound antigen, followed by binding buffer without Nanobody at the same flow rate to allow for spontaneous dissociation. Analyte remaining bound after the monitored dissociation phase was removed by injecting regeneration solution (4.5M $MgCl_2$). Binding curves at different concentrations were used to calculate the kinetic parameters k_{on}-values (k_a), k_{off}-values (k_d) and K_D. Kinetic parameters were determined using heterogeneous ligand fit since a two phase interaction was observed. The obtained results are presented in Table 10.

Table 10: Kinetic parameters for binding of anti-IgE Nanobodies to human IgE and cyno IgE Fc.

clone	IgE	Type	KD (nM)	k_a (1/Ms)	k_d (1/s)
39D11	Human IgE	KD1 (52%)	15.3	$5.81^{E}5$	8.9^{E}-3
		KD2 (48%)	0.7	$5.58^{E}6$	3.9^{E}-3
	Cyno IgE Fc	KD1 (46%)	26.1	$5.34^{E}5$	1.4^{E}-2
		KD2 (54%)	0.58	$7.02^{E}6$	4.1^{E}-3
36G5	Human IgE	KD1 (28%)	96	$6.00^{E}5$	5.7^{E}-2
		KD2 (72%)	10	$4.76^{E}6$	4.9^{E}-2
	Cyno IgE Fc	KD1 (46%)	54	$1.69^{E}6$	9.2^{E}-2
		KD2 (54%)	6	$1.13^{E}7$	6.7^{E}-2

Example 13: Sequence optimization and affinity maturation of 39D11

A. Sequence optimization: sequence analysis

The IgE-specific Nanobody 39D11 was the most potent cyno cross-reactive Nanobody identified during screening. It was selected and further pursued for sequence optimization and affinity maturation.

Based on alignment of 39D11 sequence with VH3-23/JH5 human germline, it was decided to generate 4 variants: IGE009, IGE010, IGE011 and IGE012 (SEQ ID NO's: 120 to 123, respectively). All variants include the three mutations V5L, K83R and Q108L. In variants IGE011 and IGE012, a unique mutation W91Y is included. In all four variants, the methionine at position 77 is substituted by threonine (IGE009 and IGE011) or leucine (IGE010 and IGE012). Sequence alignment of sequence optimized variants with 39D11 is shown in Figure 1.

B. Characterization of four 39D11 humanization variants in potency assays

The four variants were first tested in the degranulation assay to evaluate the effect of introduced mutations on the ability to inhibit IgE-mediated degranulation. Table 11 shows obtained calculated IC50 values. These results indicated that the introduced mutation W91Y is detrimental for potency and results in a higher off rate, excluding variants IGE011 and IGE012. Variant IGE009 was selected for affinity maturation as it has the highest percentage framework identity with human germline sequences.

Table 11: Potency analysis of 39D11 humanization variants measured in a competition Alphascreen, off rate determination and a degranulation assay.

	Alphascreen		Off rate (s^{-1})	Degranulation	
	IC50 (nM)	CI95		IC50 (nM)	CI95
39D11	0.417	0.491 to 0.698	k_d1:1.6^{E}-2 (73.3%) k_d2: 1.1^{E}-2 (26.7%)	4.,250	3.022 to 5.978
IGE009	0.343	0.211 to 0.557	k_d1: 1.2^{E}-2 (77.2%) k_d2: 1.2^{E}-2 (22.8%)	5.289	3.275 to 8.542
IGE010	0.351	0.229 to 0.536	k_d1: 8.6^{E}-3 (79.6%) k_d2: 1.4^{E}-2 (20.4%)	5.844	4.628 to 7.379
IGE011	1.336	0.879 to 2.029	k_d: 9.81^{E}-2	103.7	70.93 to 151.7
IGE012	1.236	0.772 to 1.977	k_d: 1.23^{E}-1	78.24	44.46 to 137.7

C. Affinity maturation of IGE009 by error prone PCR methodology

An error-prone library was generated by amplifying IGE009 under error-prone conditions and cloning the fragments into a phage display vector. The obtained library was subjected to multiple rounds of in solution panning using gradually decreasing concentrations (10-0.001 nM) of biotinylated human IgE. Outputs from the phage selections were analyzed for off rate on ProteOn. Up to 6-fold improved off-rates relative to 39D11 were measured for the best clones. Sequence analysis identified mutations (both in the frameworks and the CDR's) with positive effect on off-rate. These were: E6Q; F29Y; G30D; S31N or S31P; S35G or S35A; G44R; N75K; D97E and Y100eF.

These mutations were included in a combinatorial library. The obtained library was again subjected to in solution panning using 1 nM biotinylated human IgE. Outputs from the phage selections were analyzed for off rate on ProteOn and sequences were analysed. Sequence analysis and off rate analysis were combined and as a result, 7 variants (IGE025-IGE030, SEQ ID NO's: 127 to 133) containing different combinations of beneficial mutations were designed and expressed in *Pichia* as fusions (SEQ ID NO's: 177 to 182) with the HSA-specific Nanobody ALB8 to improve the in vivo half-life of the construct. As control, similar Nb-9GS-ALB8 formats were made containing the parental IgE-specific Nanobody 39D11 or its humanized variant IGE009 (constructs IGE020 and IGE019 respectively, SEQ ID NO's: 175 and 176).

Alignment of the affinity matured variants with 39D11 en IGE009 is shown in Figures 1 and 2 respectively.

D. Off rate determination on BIAcore of 10 affinity matured bispecific variants

Off rates were analysed on BIAcore. Binding profiles and off rates are shown in Table 12. Off rate analysis on BIAcore shows decreased off rates for affinity matured variants compared to IGE009 (up to 25-fold on human IgE and up to 15-fold on cyno IgE-Fc). The binding patterns are still biphasic but the percentage of the fast off rate is significantly reduced after affinity maturation (certainly on human IgE).

Table 12: Kinetic parameters for binding of affinity matured formatted anti-IgE Nanobodies to human IgE and cyno IgE-Fc.

Construct	Off rate (1/S)	
	Human IgE	**Cyno IgE-Fc**
IGE009	k_d 1: 1.32^E-2 (58%)	k_d 1: 1.54^E-2 (58.5%)
	k_d 2: 2.53^E-3	k_d 2: 2.01^E-3
IGE019 (IGE009/ALB8)	k_d 1: 1.34^E-2 (51.6%)	k_d 1: 1.52^E-2 (51.9%)
	k_d 2: 3.00^E-3	k_d 2: 2.21^E-3
IGE025/ALB8	k_d 1: 1.05^E-2 (20.4%)	k_d 1: 1.82^E-2 (27.9%)
	k_d 2: 1.02^E-3	k_d 2: 9.74^E-4
IGE026/ALB8	k_d 1: 9.78^E-3 (3.3%)	k_d 1: 8.9^E-3 (10.1%)
	k_d 2: 1.18^E-4	k_d 2: 1.24^E-4
IGE027/ALB8	k_d 1: 7.44^E-3 (4.8%)	k_d 1: 8.46^E-3 (13.2%)
	k_d 2: 1.14^E-4	k_d 2: 1.82^E-4
IGE028/ALB8	k_d 1: 9.65^E-3 (3.7%)	k_d 1: 6.55^E-3 (12.0%)
	k_d 2: 1.31^E-4	k_d 2: 1.38^E-4
IGE029/ALB8	k_d 1: 1.06^E-2 (7.8%)	k_d 1: 9.66^E-3 (23.5%)
	k_d 2: 3.13^E-4	k_d 2: 4.8^E-4
IGE030/ALB8	k_d 1: 1.31^E-2 (3.8%)	k_d 1: 8.57^E-3 (15.3%)
	k_d 2: 1.57^E-4)	k_d 2: 2.26^E-4

E. In vitro potency analysis in degranulation assay of affinity matured bispecific variants

Affinity matured variants were tested in degranulation assay in the presence or absence of purified human serum albumin (Sigma, A8763). Calculated IC50s and CI95 are shown in Table 13. The obtained results clearly indicate that a number of variants outperform Omalizumab in degranulation assay. Binding of HSA does not influence the potency of the Nanobody constructs.

Table 13: IC50 values for blocking IgE-mediated degranulation of RBL-Fc(epsilon)RI(alpha) transfected cells by anti-IgE Nanobodies or control Mab/Fab, and their 95% confidence intervals as determined by impedance measurement.

construct	Without HSA		With 25 μM HSA	
	IC50 (nM)	CI95		
omalizumab	0.8179	0.4745 to 1.410	1,151	0.6501 to 2.037
Reference Fab	4.618	3.799 to 5.612	3.688	2.103 to 6.466
IGE009	8.883	6.610 to 11.94	9.803	6.520 to 14.74
IGE019 (IGE009/ALB8)	10.23	6.293 to 16.63	9.139	6.909 to 12.09
IGE025/ALB8	1.795	1.462 to 2.202	2.361	1.169 to 4.769
IGE026ALB8	0.2480	0.1854 to 0.3316	0.3102	0.2727 to 0.3528
IGE027/ALB8	0.2473	0.2079 to 0.2941	0.1922	0.1409 to 0.2622
IGE028/ALB8	0.3814	0.3226 to 0.4510	0.5225	0.3684 to 0.7413
IGE029/ALB8	0.2646	0.2376 to 0.2947	0.2702	0.2058 to 0.3547
IGE030/ALB8	0.2161	0.1691 to 0.2761	0.2050	0.1865 to 0.2255

F. Affinity determination of IGE026 and IGE027 using KinExA approach

The affinity of Nanobodies IGE026, IGE027 and 39D11 was determined on human IgE in solution using KinExA technology. The obtained affinity of the Nanobodies IGE026 and IGE027 was 30 to 50-fold improved compared to that of 39D11 (Table 14).

Table 14: KD values for IgE binding.

construct	KD (pM)	CI95 (pM)
39D11	624	350-788
IGE026	19	13-25
IGE027	12	5-6

G. Binding affinity to serum albumin

Binding of formatted Nanobodies to serum albumin was analysed by SPR (Biacore 3000) in conditions with low density albumin immobilization (500 RU). A titration series of Nanobodies (2.5 to 200 nM) was injected at a flow rate 45 µl/min. Affinity constants were determined and are listed in Table 15. The HSA-specific Nanobody ALB8 was included for comparison. In general, a lower affinity was observed.

Table 15: KD values of formatted Nanobodies to serum albumin

	Human serum albumin			Cyno serum albumin		
	ka (1/Ms)	kd (1/s)	KD (nM)	ka (1/Ms)	kd (1/s)	KD (nM)
ALB8	3.15E+05	1.93E-03	6.12	3.28E+05	1.89E-03	5.76
IGE025/ALB8	9.15E+04	5.94E-03	64.9	1.11E+05	5.87E-03	53
IGE026ALB8	1.12E+05	5.86E-03	52.3	1.03E+05	5.78E03	56.3
IGE027/ALB8	1.09E+05	5.42E-03	49.6	9.99E+04	5.37E-03	53.8
IGE030/ALB8	1.14E+05	5.31E-03	46.4	1.04E+05	5.27E-03	50.6

Example 14: Expression and purification of IGE Nanobodies with NExpedite peptide fusion

Examples 1 to 13 above illustrate the favourable properties of the anti-IgE Nanobodies (including 39D11, the 39D11-type sequences and the 39D11-like sequences) per se or when used as building blocks in order to provide multivalent, multispecific and/or biparatopic constructs.

In the Examples 14 to 17, the anti-IgE Nanobodies of the invention are combined with the half-life extending peptides that are preferred according to the invention.

According to this preferred aspect of the invention, instead of using ALB8 for improvement of in vivo half-life of the IgE Nanobodies, the IgE binding Nanobody building block of IGE026/ALB8, i.e. IgE66G02 (SEQ ID NO's: 128 or 129), was genetically fused at its

N- or C-terminus to one or more HSA binding NExpedite peptides via a short or long GlySer-linker, resulting in 15 variants (IGE100-105, IGE107-113, EXP476, EXP483, SEQ ID NO's: 147 to 161). The constructs were cloned in an in-house *Pichia pastoris* expression vector derived from pPICZa (Invitrogen) which contains the AOX1 promotor for tightly regulated, methanol induced expression of Nanobodies® in Pichia pastoris, a resistance gene for Zeocin™, a multicloning site and the alpha-factor secretion signal. Expression and purification of the constructs was performed essentially as described in Example 20 of US 2011/0243954.

Example 15: Characterization of IGE-NExpedite Nanobodies

The influence of the N-or C-terminal NExpedite peptide(s) on the IgE-blocking characteristics of Nanobody IgE66G02 was investigated as well as the influence of the Nanobody on the HSA binding characteristics of the NExpedite peptide(s).

A. Inhibition of HuIgE/HuFc(epsilon)RI(alpha)Fc interaction in Alphascreen

Serial dilutions of purified Nanobodies with NExpedite peptide fusion were analyzed for their ability to block the interaction of HuIgE with HuFc(epsilon)RI(alpha)Fc using the same Alphascreen assay as described above (Example 6), in the presence or absence of HSA. Data are shown in Table 16. All Nanobody-NExpedite constructs have similar potency compared to the corresponding monovalent anti-IgE Nanobody building block, i.e. IgE66G02 (referred to as IgE045 in Table 16). In the presence of HSA however, there is a 4 to 12 fold increase in IC50 for all construct containing the NExpedite moiety at N-terminus of the Nanobody. The negative effect of HSA on the potency varies depending on (i) the linker length between the NExpedite peptide(s) and the Nanobody and (ii) the number of NExpedite peptides. In contrast, there was no influence of HSA binding observed for the Nanobody-NExpedite fusions in which the NExpedite moiety is at the C-terminus of the Nanobody. Nevertheless, all constructs have a better potency compared to the positive control Fab.

Table 16: IC50 values for competition between the human IgE receptor Fc(epsilon)RI(alpha) and IGE-NExpedite Nanobodies to bind human IgE, as determined in AlphaScreen. 95% CI: 95% confidence interval.

	without HSA		With 5μM HSA	
	global IC50 (M)	95% CI (M)	global IC50 (M)	95% CI (M)
IGE045	9.55E-12	[8.8E-12 - 1.0E-11]	1.03E-11	[9.5E-12 - 1.1E-11]
IGE026/ALB-8	3.31E-11	[3.0E-11 - 3.6E-11]	3.51E-11	[3.2E-11 - 3.8E-11]
EXP476	1.31E-11	[1.2E-11 - 1.4E-11]	1.41E-11	[1.3E-11 - 1.5E-11]
EXP483	1.17E-11	[1.1E-11 - 1.2E-11]	9.55E-12	[9.0E-12 - 1.0E-11]
IGE100	1.00E-11	[8.9E-12 - 1.1E-11]	1.00E-11	[8.9E-12 - 1.1E-11]
IGE101	1.00E-11	[9.1E-12 - 1.1E-11]	1.00E-11	[9.2E-12 - 1.1E-11]
IGE102	7.24E-12	[6.6E-12 - 7.9E-12]	6.17E-11	[5.6E-11 - 6.8E-11]
IGE103	1.20E-11	[1.1E-11 - 1.3E-11]	4.36E-11	[4.0E-11 - 4.8E-11]
IGE104	6.46E-12	[5.9E-12 - 7.1E-12]	7.94E-11	[7.2E-11 - 8.7E-11]
IGE105	6.92E-12	[6.4E-12 - 7.5E-12]	6.03E-11	[5.6E-11 - 6.5E-11]
IGE107	1.32E-11	[1.2E-11 - 1.4E-11]	1.29E-11	[1.2E-11 - 1.4E-11]
IGE109	1.58E-11	[1.5E-11 - 1.7E-11]	1.91E-11	[1.7E-11 - 2.1E-11]
Reference Fab	5.61E-10	[5.2E-10 - 6.0E-10]	5.78E-10	[5.4E-10 - 6.1E-10]

B. Inhibition in degranulation assay

All IGE-NExpedite Nanobodies were tested in a degranulation assay as described above in the presence or absence of HSA (see Example 11). Data are shown in Table 17. The obtained results are in agreement with data obtained in the HuIgE/HuFc(epsilon)RI(alpha)Fc Alphascreen. Except for IGE103, all compounds have similar potency compared to that of IgE045. Binding of HSA does not influence the potency of the Nanobody when the NExpedite peptide is at its C-terminus. The negative effect of HSA complexation on the potency of IGE102-103-104 and 105 is also observed in this degranulation assay, though the effect is less pronounced.

Table 17: IC50 values for blocking IgE-mediated degranulation of RBL-Fc(epsilon)RI(alpha) transfected cells by IGE-NExpedite Nanobodies or control Mab and their 95% confidence intervals as determined by impedance measurement.

	without HSA		with 25µM HSA	
	global IC50 (M)	**95% CI (M)**	**global IC50 (M)**	**95% CI (M)**
IGE045	2.28E-10	[2.1E-10 - 2.5E-10]	1.84E-10	[1.7E-10 - 2.0E-10]
IGE026/ALB-8	3.18E-10	[2.9E-10 - 3.4E-10]	1.87E-10	[1.7E-10 - 2.0E-10]
EXP476	1.80E-10	[1.5E-10 - 2.1E-10]	2.27E-10	[2.0E-10 - 2.5E-10]
EXP483	2.59E-10	[2.3E-10 - 2.9E-10]	2.03E-10	[1.8E-10 - 2.2E-10]
IGE100	1.80E-10	[1.6E-10 - 2.0E-10]	1.56E-10	[1.3E-10 - 1.8E-10]
IGE101	2.21E-10	[2.0E-10 - 2.5E-10]	2.21E-10	[2.0E-10 - 2.5E-10]
IGE102	3.28E-10	[2.6E-10 - 4.1E-10]	1.02E-09	[8.6E-10 - 1.2E-09]
IGE103	8.57E-10	[7.0E-10 - 1.0E-09]	1.25E-09	[1.1E-09 - 1.4E-09]
IGE104	1.71E-10	[1.3E-10 - 2.3E-10]	1.82E-09	[1.6E-09 - 2.1E-09]
IGE105	2.09E-10	[1.3E-10 - 3.2E-10]	5.69E-10	[4.1E-10 - 7.9E-10]
IGE107	1.94E-10	[1.6E-10 - 2.4E-10]	1.72E-10	[1.5E-10 - 2.0E-10]
IGE109	2.91E-10	[2.4E-10 - 3.4E-10]	2.62E-10	[2.1E-10 - 3.2E-10]
Reference Mab	9.90E-10	[9.1E-10 - 1.1E-09]	6.27E-10	[5.7E-10 - 6.8E-10]

C. Inhibition of HSA/NExpedite interaction in Alphascreen

For all IGE-NExpedite Nanobodies, the influence of the Nanobody on the HSA binding characteristics of the NExpedite peptide(s) was investigated in Alphascreen. In brief, acceptor beads (Perkin Elmer, Waltham, MA, USA) were conjugated with EXP413, i.e. a construct comprising SEQ ID NO: 2112 of WO 09/068625 (hereinafter referred to as "Nb2112" linked to EXP89D3 via a 9 GS linker (i.e. Nb2112-9GS-EXP89D3) according to manufacturer instructions. Purified IGE-NExpedite Nanobodies were incubated with 3.4pM bio-HSA, in presence or absence of hIgE, prior to the addition of EXP413 conjugated acceptor beads and streptavidin donor beads (Perkin Elmer). Fluorescence was measured by reading plates on the EnVision Multilabel Plate Reader (Perkin Elmer) using an excitation wavelength of 680 nm and an emission wavelength of 520 nm. Decrease in fluorescence signal indicates that the binding of

bio-HSA to Nb2112-9GS-EXP89D03 is blocked by IGE-NExpedite Nanobody. As a positive control, EXP413, EXP486 (Nb2112-9GS-EXP89D3-9GS-EXP89D3) and EXP553 (Nb2112-20GS-NEXP001-9GS-NEXP002) were included in the assays. The data are shown in Table 18. If a single NExpedite peptide is at the N-terminus of the IGE Nanobody (IGE102, IGE103), the IC50 is markedly increased, suggesting the Nanobody hampers binding of the NExpedite peptide to HSA. Note that the IC50's for IGE104 and IGE105, which have two NExpedite peptides fused at the N-terminus of the IGE Nanobody, are similar to the IC50 of EXP486, however these data are inconclusive since there is no clear potency difference observed between EXP413 and EXP486 in this Alphascreen assay. In contrast, when the NExpedite moiety is at the C-terminus of the IGE Nanobody, the IC50's are comparable to the IC50's of their respective Nb2112-NExpedite controls: compare EXP476 and IGE100 with EXP413, compare EXP483, IGE101 and IGE107 with EXP486, compare IGE109 with EXP553. Furthermore, binding of hIgE to the Nanobody does not influence the HSA binding of the NExpedite peptide(s).

Table 18: IC50 values for competition between Nb2112-9GS-EXP89D3 and IGE-NExpedite Nanobodies to HSA and their 95% confidence intervals, as determined in AlphaScreen.

	without hIgE		+5µM hIgE	
	global IC50 (M)	**95% CI (M)**	**global IC50 (M)**	**95% CI (M)**
EXP413	3.09E-09	[2.9E-09 - 3.3E-09]	na	na
EXP486	4.72E-09	[4.3E-09 - 5.1E-09]	na	na
EXP553	1.29E-08	[1.1E-08 - 1.6E-08]	na	na
EXP483	2.59E-10	[2.3E-10 - 2.9E-10]	2.03E-10	[1.8E-10 - 2.2E-10]
EXP476	3.27E-09	[3.1E-09 - 3.4E-09]	3.67E-09	[3.4E-09 - 4.0E-09]
EXP483	3.90E-09	[3.7E-09 - 4.1E-09]	3.21E-09	[2.9E-09 - 3.5E-09]
IGE100	2.51E-09	[2.4E-09 - 2.7E-09]	3.16E-09	[3.0E-09 - 3.3E-09]
IGE101	3.16E-09	[2.8E-09 - 3.6E-09]	3.16E-09	[2.8E-09 - 3.6E-09]
IGE102	2.56E-08	[2.2E-08 - 2.9E-08]	nd	nd
IGE103	1.83E-08	[1.7E-08 - 2.0E-08]	nd	nd
IGE104	5.66E-09	[4.5E-09 - 7.1E-09]	nd	nd
IGE105	3.30E-09	[2.9E-09 - 3.7E-09]	nd	nd
IGE107	4.03E-09	[3.7E-09 - 4.4E-09]	4.73E-09	[4.3E-09 - 5.2E-09]
IGE109	1.32E-08	[1.2E-08 - 1.5E-08]	1.30E-08	[1.1E-08 - 1.5E-08]

Example 16: Storage stability: comparison of IgE-NExpedite constructs and construct comprising an albumin-binding Nanobody

The storage stability of the representative IgE-NExpedite constructs was compared with corresponding constructs in which the albumin-binding NExpedite peptide was replaced with an albumin-binding Nanobody (Alb-8). The constructs used were IGE026/ALB-8 (SEQ ID NO: 178) and IGE109 (SEQ ID NO: 155).

The constructs were formulated at 50mg/ml in D-PBS buffer and stored for 1 month at the indicated temperature in plastic PCR tubes in the dark.

After that, the percentage of pre-peak (corresponding to the amount of dimer formed) was determined using SE-HPLC. For IgE026/ALB-8, SE-HPLC was performed using aBioSep SEC-2000 column (Phenomenex) and D-PBS as running buffer at a flow rate of 0.2 ml/min. 10µg material was injected and data was analysed using Chromeleon software. For IGE109, SE-HPLC was performed using a SEC-5 column (Agilent) and 10mM Na-Phosphate (pH 7.5), 125mM Arginine.HCl, 10% Ethanol as running buffer at a flow rate of 0.5 ml/min. 10µg material was injected and data was analysed using Chromeleon software. The results are shown in Table 19.

Table 19: Percentage of pre-peak on SE-HPLC after 1 month storage at indicated temperatures.

Construct	**Temperature (°C)**		
	5	**25**	**40**
IGE026/ALB8	0.0	11.7	30.3
IGE109	0.0	0.7	1.5

Storage stability was also determined for three other IgE-NExpedite constructs, namely EXP483 (SEQ ID NO: 161), IGE100 (SEQ ID NO: 147) and IGE101 (SEQ ID NO: 148). The same SE-HPLC protocol was used as for IGE109 above. The results are shown in Table 20.

Table 20: Percentage of pre-peak on SE-HPLC after storage at indicated temperatures.

Construct	Concentration	Weeks	Temperature (°C)		
			5	25	40
EXP483	50 mg/ml (in D-PBS)	1	1.0	1.1	1.4
EXP483	50 mg/ml (in D-PBS)	2	1.0	1.1	2.1
EXP483	50 mg/ml (in D-PBS)	4	1.6	3.1	5.9
EXP483	50 mg/ml (in D-PBS)	8	2.2	5.0	7.9
Construct	**Concentration**	**Weeks**	**Temperature (°C)**		
			5	**25**	**40**
IgE100	59 mg/ml (in D-PBS)	1	1.0	1.2	4.1
IgE100	59 mg/ml (in D-PBS)	2	0.8	2.8	4.6
IgE100	59 mg/ml (in D-PBS)	4	1.2	4.1	4.1
Construct	**Concentration**	**Weeks**	**Temperature (°C)**		
			5	**25**	**40**
IgE101	44 mg/ml (in D-PBS)	1	1.0	1.0	1.2
IgE101	44 mg/ml (in D-PBS)	2	0.9	1.0	1.4
IgE101	44 mg/ml (in D-PBS)	4	1.1	1.6	2.0

Example 17: *In vivo* PK/PD analysis of IgE109 in cynomolgus monkey

PK, PD and immunogenicity markers were measured at different time points after a single administration of IgE or Xolair in cynomolgus monkeys, screened for moderate to high IgE plasma levels. Modelling analysis resulting in the determination of the *in vivo* K_D is performed using a published model for Xolair (Hayashi *et al*, British Journal of Clinical Pharmacology, 2006).

A. Study design

In this study, 6 groups of 3 cynomolgus monkeys received a single administration of either vehicle, IgE109 or Xolair (Table 21). A single administration of vehicle, different amounts

of IgE109 (0.5 and 1 mg/kg) or Xolair (0.5 and 1 mg/kg) were administered either subcutaneously (s.c.) or intravenously (i.v.) as indicated in Table 21.

Prior to the start of the study, levels of plasma IgE were determined in all the animals of the study. After the single administration of vehicle, IgE109 or Xolair, the animals were monitored daily and blood samples were collected from the femoral vein at different pre-determined time points (see Figure 3).

Table 21: Animal treatment groups for the PK/PD study. NA: not applicable. S.c.: subcutaneous injection, i.v.: intravenous injection. The dose volume for each animal is based on the most recent body weight measurement.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Drug	Vehicle	IgE109	IgE109	IgE109	Xolair	Xolair
Amount (mg/kg)	NA	0.5	0.5	1	0.5	1
Route of Administration	s.c.	s.c.	i.v.	i.v.	i.v.	i.v.
Dosage volume (mL/kg)	1	1	1	1	1	1

B. The effect of anti-IgE Nanobody on free IgE levels

A decrease in free IgE levels was observed upon IgE109 administration for all concentrations as early as 1 hour after drug administration compared to constant free IgE levels of the vehicle group (Figure 4). A decrease in free IgE levels was also observed in the two Xolair groups.

Modelling analysis based on pharmacokinetics (PK, free and total IgE109 and total Xolair) and pharmacodynamics (PD, free and total IgE) markers will allow the determination of the *in vivo* K_D of both IgE109 and Xolair.

The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced herein.

SEQUENCES

Table A-1: IgE sequences.

Name	SEQ ID NO:	SEQUENCE
human IgE Fc	172	Dnktfsvcsrdftpptvkilqsscdggghfpptiqllclvsgytpgtinitwledgqvmdvdlstas ttqegelastqseltlsqkhwlsdrtytcqvtyqghtfedstkkcadsnprgvsaylsrpspfdlfirk sptitclvvdlapskgtvnltwsrasgkpvnhstrkeekqrngtltvtstlpvgtrdwiegetyqcrv thphlpralmrsttktsgpraapevyafatpewpgsrdkrtlacliqnfmpedisvqwlhnevql pdarhsttqprktkgsgffvfsrlevtraeweqkdeficravheaaspsqtvqravsvnpgk
Cyno IgE Fc	173	VNKTFGVSSRNFTPPTVKILQSSCDDDGHFPPTIQLLCLISGYTP GAINVTWLENGQVMKVNSPTPPATQEGELASTQSEFTLAQKH WLSDRTYTCQVTYQGTTYNDSTKKCADSNPRGVSAYLSRPSPF DLFISKSPTITCLVVDLAPSKETVNLTWSRASGKPVPHIPATEKK QRNGTLTVTSILPVVTQDWIEGETYQCRVTHPHLPRALVRSMT KTSGPRAAPEVYVFATPEKLESRDKRTLACLIQNFMPEDISVQW LHSDVQLPDARHSVTQPRKTKGSGFFVFSRLEVTKAEWEQKDE FICRAVHEAASPSWIVQQAVSVNPGK

Table A-2: Amino acid sequences of IgE binding Nanobodies.

Name	SEQ ID NO:	SEQUENCE
39B02	114	EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGK GPEWVSAINSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSL KPEDTAVYYCSTDLGPWIEGTMEEYEYEYWGQGTQVTVSS
39D8	115	EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGK GPEWVSSIDTGGDITHYADSVKGRFTISRDNANNMLYLQMNSLK PGDTAVYWCATDEDYALGPNEYDYYGQGTQVTVSS
42D7	116	EVQLVESGGGLVQPGGPLRLSCAASGFTFGSYDMSWVRQAPGK GPEWVSSIDTGGDITHYADSVKGRFTISRDNANNMLYLQMNSLK PEDTAVYWCATDEDYALGPNEYDYYGQGTQVTVSS
42G5	117	EVQLVESGGGLVRPGGSLRLSCAASGFTFGNYDMSWVRQAPGK GPEWVSSIDTGGDITHYADPVKGRFTISRDNAANTLYLQMNSLK PEDTAVYYCATDEDYALGPNEYDYYGQGTQVTVSS
36G5	118	EVQLVESGGGLVQPGGSLRLSCVASGFTFGSYDMSWVRQAPGK RPEWVSAINSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLK PEDTAVYYCATDEDYALGPNEYDYYGQGTQVTVSS
39D11	119	EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGK GPEWVSSIDTGGDITHYADSVKGRFTISRDNANNMLYLQMNSLK PEDTAVYWCATDEDYALGPNEYDYYGQGTQVTVSS

Table A-3: DNA sequence of IgE binding Nanobodies.

Name	SEQ ID NO:	SEQUENCE
39B02	183	GAGGTGCAATTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTT CTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCGGAAGCTATGAC ATGAGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCCCGAGTGGGTCTCAG CTATTAATAGTGGTGGTGGTAGCACATACTATGCAGACTCCGTGAAGGG CCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAA ATGAACAGCCTGAAACCTGAGGACACGGCCGTGTATTACTGTTCAACAG ACCTCGGACCGTGGATAGAAGGTACCATGGAGGAATATGAGTATGAGTA CTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCAGCGGCCGCA
39D11	184	GAGGTGCAATTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTT CTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCGGAAGCTATGAC ATGAGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCCCGAGTGGGTCTCAT CTATTGATACTGGTGGTGATATCACACACTACGCAGACTCCGTGAAGGGC CGATTCACCATCTCCAGAGACAACGCCAACAATATGTTGTATCTGCAAAT GAACAGCCTGAAACCTGAGGACACGGCCGTGTATTGGTGTGCAACAGAT GAGGACTACGCGTTGGGACCGAATGAGTATGACTACTACGGCCAGGGGA CCCAGGTCACCGTCTCCTCAGCGGCCGCA
39D8	185	GAGGTGCAATTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTT CTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCGGAAGCTATGAC ATGAGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCCCGAGTGGGTCTCAT CTATTGATACTGGTGGTGATATCACACACTACGCAGACTCCGTGAAGGGC CGATTCACCATCTCCAGAGACAACGCCAACAATATGTTGTATCTGCAAAT GAACAGCCTGAAACCTGGGGACACGGCCGTGTATTGGTGTGCAACAGAT GAGGACTACGCGTTGGGACCGAATGAGTATGACTACTACGGCCAGGGGA CCCAGGTCACCGTCTCCTCAGCGGCCGCA
42D7	186	GAGGTGCAATTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTC CTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCGGAAGCTATGAC ATGAGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCCCGAGTGGGTCTCAT CTATTGATACTGGTGGTGATATCACACACTACGCAGACTCCGTGAAGGGC CGATTCACCATCTCCAGAGACAACGCCAACAATATGTTGTATCTGCAAAT GAACAGCCTGAAACCTGAGGACACGGCCGTGTATTGGTGTGCAACAGAT GAGGACTACGCGTTGGGACCGAATGAGTATGACTACTACGGCCAGGGGA CCCAGGTCACCGTCTCCTCAGCGGCCGCA
42G5	187	GAGGTGCAATTGGTGGAGTCTGGGGGAGGCTTGGTGCGGCCTGGGGGTT CTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCGGCAACTATGAC ATGAGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCCCGAGTGGGTCTCAT CTATTGATACTGGTGGTGATATCACACACTACGCAGACCCCGTGAAGGG CCGATTCACCATCTCCAGAGACAACGCCGCCAACACGCTGTATCTGCAA ATGAACAGCCTGAAACCTGAGGACACGGCCGTGTATTACTGTGCAACAG ACGAGGACTACGCGTTGGGACCGAATGAGTATGACTACTACGGCCAGGG GACCCAGGTCACCGTCTCCTCAGCGGCCGCA
36G5	188	GAGGTGCAATTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTT CTCTGAGACTCTCCTGTGTAGCCTCTGGATTCACATTCGGAAGCTATGAC ATGAGCTGGGTCCGCCAGGCTCCAGGAAAGAGGCCCGAGTGGGTCTCAG CTATTAATAGTGGTGGTGGTAGCACATACTATGCAGACTCCGTGAAGGG CCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAG ATGAACAGCCTGAAACCTGAGGACACGGCCGTGTATTACTGTGCAACAG ATGAGGACTACGCATTGGGACCGAATGAGTATGACTACTATGGCCAGGG GACCCAGGTCACCGTCTCCTCAGCGGCCGCA

Table A-4: Sequences of sequence optimized IgE binding Nanobodies.

Name	SEQ ID NO:	SEQUENCE
IGE009	120	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAP GKGPEWVSSIDTGGDITHYADSVKGRFTISRDNANNTLYLQM NSLRPEDTAVYWCATDEDYALGPNEYDYYGQGTLVTVSS
IGE010	121	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAP GKGPEWVSSIDTGGDITHYADSVKGRFTISRDNANNLLYLQM NSLRPEDTAVYWCATDEDYALGPNEYDYYGQGTLVTVSS
IGE011	122	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAP GKGPEWVSSIDTGGDITHYADSVKGRFTISRDNANNTLYLQM NSLRPEDTAVYYCATDEDYALGPNEYDYYGQGTLVTVSS
IGE012	123	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAP GKGPEWVSSIDTGGDITHYADSVKGRFTISRDNANNLLYLQM NSLRPEDTAVYYCATDEDYALGPNEYDYYGQGTLVTVSS
IGE009+N75K	124	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAP GKGPEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQM NSLRPEDTAVYWCATDEDYALGPNEYDYYGQGTLVTVSS
IGE009+G44R IGE56F07	125	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAP GKRPEWVSSIDTGGDITHYADSVKGRFTISRDNANNTLYLQM NSLRPEDTAVYWCATDEDYALGPNEYDYYGQGTLVTVSS
IGE009+G44R+ N75K	126	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAP GKRPEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQM NSLRPEDTAVYWCATDEDYALGPNEYDYYGQGTLVTVSS
IGE025	127	EVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMAWVRQAP GKGPEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQM NSLRPEDTAVYWCATDEEYALGPNEFDYYGQGTLVTVSS
IGE026 IGE0045 IgE66G02	128	EVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMAWVRQAP GKRPEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQM NSLRPEDTAVYWCATDEEYALGPNEFDYYGQGTLVTVSS
IgE66G02(E1D)	129	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMAWVRQAP GKRPEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQM NSLRPEDTAVYWCATDEEYALGPNEFDYYGQGTLVTVSS
IGE027	130	EVQLLQSGGGLVQPGGSLRLSCAASGFTFGNYDMAWVRQAP GKRPEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQM NSLRPEDTAVYWCATDEEYALGPNEFDYYGQGTLVTVSS
IGE028	131	EVQLLQSGGGLVQPGGSLRLSCAASGFTFGNYDMGWVRQAP GKRPEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQM NSLRPEDTAVYWCATDEEYALGPNEFDYYGQGTLVTVSS
IGE029	132	EVQLLQSGGGLVQPGGSLRLSCAASGFTFGSYDMAWVRQAP GKRPEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQM NSLRPEDTAVYWCATDEEYALGPNEFDYYGQGTLVTVSS
IGE030	133	EVQLLQSGGGLVQPGGSLRLSCAASGFTYGNYDMAWVRQA PGKRPEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQ MNSLRPEDTAVYWCATDEEYALGPNEFDYYGQGTLVTVSS

Table A-5: CDR and FR sequences of some preferred "39D11 like sequences".

	39D11		IGE026	
	Sequence	**SEQ ID NO:**	**Sequence**	**SEQ ID NO:**
FR1	EVQLVESGGGLVQPGG SLRLSCAASGFTFG	139	EVQLLESGGGLVQPGGS LRLSCAASGFTFG	140
CDR1	SYDMS	134	NYDMA	135
FR2	WVRQAPGKGPEWVS	141	WVRQAPGKRPEWVS	142
CDR2	SIDTGGDITHYADSVKG	136	SIDTGGDITHYADSVKG	136
FR3	RFTISRDNANNMLYLQ MNSLKPEDTAVYWCAT	143	RFTISRDNAKNTLYLQM NSLRPEDTAVYWCAT	144
CDR3	DEDYALGPNEYDY	137	DEEYALGPNEFDY	138
FR4	YGQGTQVTVSS	145	YGQGTLVTVSS	146

Table A-6: Serum albumin binding peptides for use in the polypeptides of the invention.

Name	SEQ ID NO:	SEQUENCE
EXP89D03	1	WWEQDRDWDFDVFGGGTP
EXP89D03VG	2	WWEQDRDWDFDVFGGGTPVG
NEXP001 (= 89D03V1 of PCT/EP2011/051559)	3	WWEQDRDWDFDVFGGGAP
NEXP002 (= 89D03V1VG of PCT/EP2011/051559)	4	WWEQDRDWDFDVFGGGAPVG
NEXP003 (= 89D03V3 of PCT/EP2011/051559)	5	WWEQDRDWDFTVFGGGDP
NEXP004VG (= 89D03V3 of PCT/EP2011/051559)	6	WWEQDRDWDFTVFGGGDPVG
89D03V2	7	WWEQDRDWDFDVFGGGNP
89D03V2-VG	8	WWEQDRDWDFDVFGGGNPVG
EXP89D03-9GS-EXP89D03	9	WWEQDRDWDFDVFGGGTPGGGGSGGG SWWEQDRDWDFDVFGGGTP
EXP89D03-9GS-EXP89D03VG	10	WWEQDRDWDFDVFGGGTPGGGGSGGG SWWEQDRDWDFDVFGGGTPVG
NEXP001-9GS-NEXP001	11	WWEQDRDWDFDVFGGGAPGGGGSGGG SWWEQDRDWDFDVFGGGAP
NEXP001-9GS-NEXP002	12	WWEQDRDWDFDVFGGGAPGGGGSGGG SWWEQDRDWDFDVFGGGAPVG
NEXP003-9GS-NEXP003	13	WWEQDRDWDFTVFGGGDPGGGGSGGG SWWEQDRDWDFTVFGGGDP
NEXP003-9GS-NEXP004	14	WWEQDRDWDFTVFGGGDPGGGGSGGG SWWEQDRDWDFTVFGGGDPVG
NEXP003-9GS-NEXP001	15	WWEQDRDWDFTVFGGGDPGGGGSGGG SWWEQDRDWDFDVFGGGAP
NEXP003-9GS-NEXP002	16	WWEQDRDWDFTVFGGGDPGGGGSGGG SWWEQDRDWDFDVFGGGAPVG
NEXP001-9GS-NEXP003	17	WWEQDRDWDFDVFGGGAPGGGGSGGG SWWEQDRDWDFTVFGGGDP
NEXP001-9GS-NEXP004	18	WWEQDRDWDFDVFGGGAPGGGGSGGG SWWEQDRDWDFTVFGGGDPVG
17D12	19	aasysdydvfgggtdfgp

Table A-7: Sequence motifs present in the serum albumin binding peptides.

Name	SEQ ID NO:	SEQUENCE
sequence motif	20	DYDV
sequence motif	21	YDVF
sequence motif	22	DVFG
sequence motif	23	VFGG
sequence motif	24	FGGG
sequence motif	25	GGGT
sequence motif	26	DYDVF
sequence motif	27	YDVFG
sequence motif	28	DVFGG
sequence motif	29	VFGGG
sequence motif	30	FGGGT
sequence motif	31	DYDVFG
sequence motif	32	YDVFGG
sequence motif	33	DVFGGG
sequence motif	34	VFGGGT
sequence motif	35	DYDVFGG
sequence motif	36	YDVFGGG
sequence motif	37	DVFGGGT
sequence motif	38	DYDVFGGG
sequence motif	39	YDVFGGGT
sequence motif	40	DYDVFGGGT
sequence motif	41	DAFGGG
sequence motif	42	DVFGGGS
sequence motif	43	DAFGGGT
sequence motif	44	DYTVF
sequence motif	45	YTVFG
sequence motif	46	TVFGG
sequence motif	47	DYTVFG
sequence motif	48	YTVFGG
sequence motif	49	TVFGGG
sequence motif	50	DYTVFGG
sequence motif	51	YTVFGGG
sequence motif	52	TVFGGGT
sequence motif	53	DVFGGGA
sequence motif	54	DVFGGGN
sequence motif	55	DVFGGGD
sequence motif	56	TVFGGGA
sequence motif	57	TVFGGGN
sequence motif	58	TVFGGGD

Name	SEQ ID NO:	SEQUENCE
sequence motif	59	DYTVFGGG
sequence motif	60	YTVFGGGT
sequence motif	61	YDVFGGGA
sequence motif	62	YDVFGGGN
sequence motif	63	YDVFGGGD
sequence motif	64	YTVFGGGA
sequence motif	65	YTVFGGGN
sequence motif	66	YTVFGGGD
sequence motif	67	TAFGGG
sequence motif	68	GGGTPVG
sequence motif	69	GGGAPVG
sequence motif	70	GGGNPVG
sequence motif	71	GGGDPVG
sequence motif	72	LWYML
sequence motif	73	LWYLY
sequence motif	74	YWWER
sequence motif	75	AWYDY
sequence motif	76	WWNWR
sequence motif	77	EWWWR
sequence motif	78	VDWFY
sequence motif	79	RDWFL
sequence motif	80	DWWNR
sequence motif	81	YGDWF
sequence motif	82	WWTWG
sequence motif	83	PIDFW
sequence motif	84	WWTSD
sequence motif	85	QKLYW
sequence motif	86	KWWEI
sequence motif	87	WWSTP
sequence motif	88	LFWWE
sequence motif	89	WWLQE
sequence motif	90	WWEQD
sequence motif	91	NQLIV
sequence motif	92	WWELD
sequence motif	93	RXWDXDVFGGG
sequence motif	94	RXWDXDVFGGGT
sequence motif	95	RXWDXDVFGGGTP
sequence motif	96	RXWDXDVFGGGTPG
sequence motif	97	RXWDXDVFGGGTPGG
sequence motif	98	RYWDYDVFGGG

Name	SEQ ID NO:	SEQUENCE
sequence motif	99	RDWDFDVFGGG
sequence motif	100	RSWDFDVFGGG
sequence motif	101	RYWDFDVFGGG
sequence motif	102	RYWDYDVFGGGT
sequence motif	103	RDWDFDVFGGGT
sequence motif	104	RSWDFDVFGGGT
sequence motif	105	RYWDFDVFGGGT
sequence motif	106	RYWDYDVFGGGTP
sequence motif	107	RDWDFDVFGGGTP
sequence motif	108	RSWDFDVFGGGTP
sequence motif	109	RYWDFDVFGGGTP
sequence motif	110	RYWDYDVFGGGTPV
sequence motif	111	RDWDFDVFGGGTPV
sequence motif	112	RSWDFDVFGGGTPV
sequence motif	113	RYWDFDVFGGGTPV

Table A-8: Linker sequences.

Name	SEQ ID NO:	SEQUENCE
5GS	162	GGGGS
6GS	163	SGGSGGS
9GS	164	GGGGSGGGS
10GS	165	GGGGSGGGGS
15GS	166	GGGGSGGGGSGGGGS
18GS	167	GGGGSGGGGSGGGGGGGS
20GS	168	GGGGSGGGGSGGGGSGGGGS
25GS	169	GGGGSGGGGSGGGGSGGGGSGGGGS
30GS	170	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
35GS	171	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS

Table A-9: Sequences of some anti-IgE polypeptides of the invention.

Name	SEQ ID NO:	SEQUENCE
IGE100 (IGE66G02^{EID}-9GS-EXP89D03)	147	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMA WVRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISR DNAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPN EFDYYGQGTLVTVSSGGGGSGGGSWWEQDRDWDF DVFGGGTP
IGE101 (IgE66G02^{EID}-9GS-EXP89D03-9GS-EXP89D03)	148	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMA WVRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISR DNAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPN EFDYYGQGTLVTVSSGGGGSGGGSWWEQDRDWDF DVFGGGTPGGGGSGGGSWWEQDRDWDFDVFGGGT P
IGE102 (EXP89D03-9GS-IgE66G02)	149	WWEQDRDWDFDVFGGGTPGGGGSGGGGSGGGGSG GGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFGNY DMAWVRQAPGKRPEWVSSIDTGGDITHYADSVKGR FTISRDNAKNTLYLQMNSLRPEDTAVYWCATDEEYA LGPNEFDYYGQGTLVTVSS
IGE103 (EXP89D03-20GS-IgE66G02)	150	WWEQDRDWDFDVFGGGTPGGGGSGGGSWWEQDR DWDFDVFGGGTPGGGGSGGGSEVQLLESGGGLVQP GGSLRLSCAASGFTFGNYDMAWVRQAPGKRPEWVS SIDTGGDITHYADSVKGRFTISRDNAKNTLYLQMNSL RPEDTAVYWCATDEEYALGPNEFDYYGQGTLVTVSS
IGE104 (EXP89D03-9GS-EXP89D03-9GS-IgE66G02)	151	WWEQDRDWDFDVFGGGTPGGGGSGGGSWWEQDR DWDFDVFGGGTPGGGGSGGGGSGGGGSGGGGSEVQ LLESGGGLVQPGGSLRLSCAASGFTFGNYDMAWVRQ APGKRPEWVSSIDTGGDITHYADSVKGRFTISRDNAK NTLYLQMNSLRPEDTAVYWCATDEEYALGPNEFDY YGQGTLVTVSS
IGE105 (EXP89D03-9GS-EXP89D03-20GS-IgE66G02)	152	WWEQDRDWDFDVFGGGTPGGGGSGGGSWWEQDR DWDFDVFGGGTPGGGGSGGGGSGGGGSGGGGSEVQ LLESGGGLVQPGGSLRLSCAASGFTFGNYDMAWVRQ APGKRPEWVSSIDTGGDITHYADSVKGRFTISRDNAK NTLYLQMNSLRPEDTAVYWCATDEEYALGPNEFDY YGQGTLVTVSSGGGGSGGGSWWEQDRDWDFDVFG GGTPGGGGSGGGSWWEQDRDWDFDVFGGGTP
IGE107 (IgE66G02^{EID}-20GS-EXP89D03-9GS-EXP89D03)	153	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMA WVRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISR DNAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPN EFDYYGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS WWEQDRDWDFDVFGGGTPGGGGSGGGSWWEQDR DWDFDVFGGGTP
IGE108 (IGE66G02^{EID}-20GS-NEXP001-9GS- NEXP001)	154	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMA WVRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISR DNAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPN EFDYYGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS

Name	SEQ ID NO:	SEQUENCE
		WWEQDRDWDFDVFGGGAPGGGGSGGGSWWEQDR DWDFDVFGGGAP
IGE109 (IGE66G02^{E1D}-20GS-NEXP001-9GS- NEXP002)	155	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMA WVRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISR DNAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPN EFDYYGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS WWEQDRDWDFDVFGGGAPGGGGSGGGSWWEQDR DWDFDVFGGGAPVG
IGE110 (IGE66G02^{E1D}-20GS-NEXP003-9GS- NEXP003)	156	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMA WVRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISR DNAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPN EFDYYGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS WWEQDRDWDFTVFGGGDPGGGGSGGGSWWEQDR DWDFTVFGGGDP
IGE111 (IGE66G02^{E1D}-20GS-NEXP003-9GS- NEXP004)	157	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMA WVRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISR DNAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPN EFDYYGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS WWEQDRDWDFTVFGGGDPGGGGSGGGSWWEQDR DWDFTVFGGGDPVG
IGE112 (IGE66G02^{E1D}-20GS-NEXP003-9GS-NEXP002)	158	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMA WVRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISR DNAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPN EFDYYGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS WWEQDRDWDFTVFGGGDPGGGGSGGGSWWEQDR DWDFDVFGGGAPVG
IGE113 (IGE66G02^{E1D}-20GS-NEXP001-9GS-NEXP004VG)	159	DVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMA WVRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISR DNAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPN EFDYYGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS WWEQDRDWDFDVFGGGAPGGGGSGGGSWWEQDR DWDFTVFGGGDPVG
EXP476 (IgE66G02-9GS-EXP89D03)	160	EVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMAW VRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISRD NAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPNE FDYYGQGTLVTVSSGGGGSGGGSWWEQDRDWDFD VFGGGTP
EXP483 (IgE66G02-9GS-EXP89D03-9GS-EXP89D03)	161	EVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMAW VRQAPGKRPEWVSSIDTGGDITHYADSVKGRFTISRD NAKNTLYLQMNSLRPEDTAVYWCATDEEYALGPNE FDYYGQGTLVTVSSGGGGSGGGSWWEQDRDWDFD VFGGGTPGGGGSGGGSWWEQDRDWDFDVFGGGTP

Table A-10: Sequence of Alb-8 Nanobody and fusion proteins with Alb-8 Nanobody.

Name	SEQ ID NO:	SEQUENCE
Alb-8 (Alb-11)	174	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS
IGE019 IGE009/ALB-8 IGE/ALB-8-019	175	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGP EWVSSIDTGGDITHYADSVKGRFTISRDNANNTLYLQMNSLRPEDT AVYWCATDEDYALGPNEYDYYGQGTLVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVS SISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC TIGGSLSRSSQGTLVTVSS
IGE020 39D11/ALB-8 IGE/ALB-8-020	176	EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGP EWVSSIDTGGDITHYADSVKGRFTISRDNANNMLYLQMNSLKPEDT AVYWCATDEDYALGPNEYDYYGQGTQVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVS SISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC TIGGSLSRSSQGTLVTVSS
IGE025/ALB-8 IGE/ALB-8-025	177	EVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMAWVRQAPGKGP EWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQMNSLRPEDT AVYWCATDEEYALGPNEFDYYGQGTLVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVS SISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC TIGGSLSRSSQGTLVTVSS
IGE026/ALB-8 IGE/ALB-8-026	178	EVQLLESGGGLVQPGGSLRLSCAASGFTFGNYDMAWVRQAPGKRP EWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQMNSLRPEDT AVYWCATDEEYALGPNEFDYYGQGTLVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVS SISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC TIGGSLSRSSQGTLVTVSS
IGE027/ALB-8 IGE/ALB-8-027	179	EVQLLQSGGGLVQPGGSLRLSCAASGFTFGNYDMAWVRQAPGKRP EWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQMNSLRPEDT AVYWCATDEEYALGPNEFDYYGQGTLVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVS SISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC TIGGSLSRSSQGTLVTVSS
IGE028/ALB-8 IGE/ALB-8-028	180	EVQLLQSGGGLVQPGGSLRLSCAASGFTFGNYDMGWVRQAPGKRP EWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQMNSLRPEDT AVYWCATDEEYALGPNEFDYYGQGTLVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVS SISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC TIGGSLSRSSQGTLVTVSS
IGE029/ALB-8 IGE/ALB-8-029	181	EVQLLQSGGGLVQPGGSLRLSCAASGFTFGSYDMAWVRQAPGKRP EWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQMNSLRPEDT AVYWCATDEEYALGPNEFDYYGQGTLVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVS SISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC TIGGSLSRSSQGTLVTVSS
IGE030/ALB-8 IGE/ALB-8-030	182	EVQLLQSGGGLVQPGGSLRLSCAASGFTYGNYDMAWVRQAPGKR PEWVSSIDTGGDITHYADSVKGRFTISRDNAKNTLYLQMNSLRPED TAVYWCATDEEYALGPNEFDYYGQGTLVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVS SISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC TIGGSLSRSSQGTLVTVSS

Table A-11: Primers used for the amplification of the cyno IgE sequence.

Name	SEQ ID NO:	SEQUENCE
O-2.09 IgE-Fc	189	GTCAACAAAACCTTTGGCGTC
O-2.10 IgE-Fc	190	TTTACCGGGATTTACAGACAC
O-2.42 IgE-Fc	191	ACGACAGCACCAAGAAGTGTGC
O-2.43 IgE-Fc	192	CGCCAGAGAAGCTAGAGAGC

CLAIMS

1. Immunoglobulin single variable domain that (i) competes with 39D11 (SEQ ID NO: 119) for binding to (human) IgE; and/or (ii) binds to the same epitope on (human) IgE as 39D11; and/or (iii) cross-blocks the binding of 39D11 to (human) IgE.

2. Immunoglobulin single variable domain according to claim 1, that comprises or essentially consists of:
   a) a CDR1 which comprises or essentially consists of either (i) the amino acid sequence SYDMS (SEQ ID NO: 134) and/or the amino acid sequence NYDMA (SEQ ID NO: 135) or (ii) an amino acid sequence that has only 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence SYDMS (SEQ ID NO: 134) and/or the amino acid sequence NYDMA (SEQ ID NO: 135); and/or
   b) a CDR2 which comprises or essentially consists of either (i) the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90%, at least 94%, or more than 95% sequence identity with the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136); and/or
   c) a CDR3 which comprises or essentially consists of either (i) the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and/or the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90%, at least 92% or more than 95% sequence identity with the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and/or the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and/or the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138).

3. Immunoglobulin single variable domain according to any of claims 1 or 2, wherein CDR1 comprises or essentially consists of the amino acid sequence SYDMS (SEQ ID NO: 134) and preferably the amino acid sequence NYDMA (SEQ ID NO: 135), CDR2 comprises or essentially consists of the amino acid sequence SIDTGGDITHYADSVKG (SEQ ID NO: 136), and CDR3 comprises or essentially consists of the amino acid sequence DEDYALGPNEYDY (SEQ ID NO: 137) and preferably the amino acid sequence DEEYALGPNEFDY (SEQ ID NO: 138).

4. Immunoglobulin single variable domain according to any of claims 1 to 3, that has at least 70%, such at least 80%, for example at least 85%, such as at least 90% or more than 95% sequence identity with the amino acid sequence 39D11 (SEQ ID NO: 119).

5. Immunoglobulin single variable domain according to any of claims 1 to 4, that is humanized and/or sequence-optimized variant of 39D11 containing one or more mutations (compared to SEQ ID NO: 119) selected from: E1D, V5L, E6Q, F29Y, S31N, S35A or S35G, G44R, N75K, M77T or M77L, K83R, W91Y, D97E, Y100eF and/or Q108L, with the numbering according to Kabat.

6. Immunoglobulin single variable domain according to any of claims 1 to 5, that is selected from any of SEQ ID NO's: 120-133 or an immunoglobulin single variable domain that has at least 70%, such at least 80%, for example at least 85%, such as at least 90% or more than 95% sequence identity with any of SEQ ID NO's: 120-133.

7. Immunoglobulin single variable domain according to any of claims 1 to 6, that is selected from SEQ ID NO's: 128 and 129.

8. Protein, polypeptide, construct or compound that comprises or essentially consists of (i) at least one (such as one or two) immunoglobulin single variable domains according to any of claims 1 to 7 and (ii) at least one other amino acid sequence, protein, (poly)peptide, binding domain, binding unit, group, residue or moiety; suitably linked to each other via one or more suitable linkers or spacers.

9. Protein, polypeptide, construct or compound according to claim 8, which has an increased half-life, compared to the corresponding immunoglobulin single variable domain according to any of claims 1 to 7, per se.

10. Protein, polypeptide, construct or compound according to claim 9, in which said one or more other groups, residues, moieties or binding units provide the protein, polypeptide, construct or compound with increased half-life, compared to the corresponding immunoglobulin single variable domain according to any of claims 1 to 7, per se.

11. Protein, polypeptide, construct or compound according to claim 10, in which said one or more other groups, residues, moieties or binding units that provide the protein, polypeptide, construct or compound with increased half-life is chosen from the group consisting of serum proteins or fragments thereof, binding units that can bind to serum proteins, an Fc portion, and small proteins or peptides that can bind to serum proteins.

12. Protein, polypeptide, construct or compound according to claim 11, in which said one or more other binding units that provides the protein, polypeptide, construct or compound with increased half-life are chosen from the group consisting of binding units that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

13. Polypeptide, protein, construct or compound that comprises or essentially consists of:
    a) one or more (such as one or two) immunoglobulin single variable domains that are directed against IgE; and
    b) one or more (such as one or two, including without limitation a tandem repeat of) peptides directed against (human) serum albumin which can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493.

14. Polypeptide, protein, construct or compound according to claim 13, that comprises or essentially consists of:
    a) one or more (such as one or two) immunoglobulin single variable domains according to any of claims 1 to 7; and
    b) one or more (such as one or two, including without limitation a tandem repeat of) peptides directed against (human) serum albumin which can bind (in)to a subpocket in (human) serum albumin that comprises (at least) one or more of the following amino acid residues of human serum albumin: V442, S443, T446, L484, L487, H488, K490, T491 and/or V493.

15. Polypeptide, protein, construct or compound according to any of claims 13 or 14, wherein the one or more peptides directed against (human) serum albumin are selected from any of SEQ ID NO's: 1 to 18.

16. Polypeptide, protein, construct or compound according to claim 15, selected from any of SEQ ID NO's: 147 to 161, or a polypeptide which has at least 80%, such as at least 85%, for example at least 90%, such as at least 95% sequence identity with any of SEQ ID NO's: 147 to 161.

17. Polypeptide, protein, construct or compound according to claims 16 which is SEQ ID NO: 155.

18. Use of an immunoglobulin single variable domain according to any of claims 1 to 7 as a binding domain or binding unit, in preparing a polypeptide, protein, construct or compound according to any of claims 8 to 17.

19. Nucleic acid or nucleotide sequence, that encodes an immunoglobulin single variable domain according to any of claims 1 to 7; or a polypeptide, protein, construct or compound according to any of claims 8 to 17.

20. Nucleic acid or nucleotide sequence according to claim 19, that is in the form of a genetic construct.

21. Use of a nucleic acid or nucleotide sequence according to claim 19, that encodes an immunoglobulin single variable domain according to any of claims 1 to 7 or a polypeptide, protein, construct or compound according to any of claims 8 to 17, or for the preparation of a genetic construct that encodes an immunoglobulin single variable domain according to any of claims 1 to 7 or a polypeptide, protein, construct or compound according to any of claims 8 to 17.

22. Host or host cell that expresses, or that under suitable circumstances is capable of expressing an immunoglobulin single variable domain according to any of claims 1 to 7, a polypeptide, protein, construct or compound according to any of claims 8 to 17; and/or that comprises a nucleic acid or nucleotide sequence according to claim 19 or a genetic construct according to claim 19.

23. Composition comprising at least one immunoglobulin single variable domain according to any of claims 1 to 7, polypeptide, protein, construct or compound according to any of claims 8 to 17, or nucleic acid or nucleotide sequence according to any of claims 19 and 20.

24. Composition according to claim 23, which is a pharmaceutical composition.

25. Composition according to claim 24, which is a pharmaceutical composition, that further comprises at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and that optionally comprises one or more further pharmaceutically active polypeptides and/or compounds.

26. Method for producing an immunoglobulin single variable domain according to any of claims 1 to 7, a polypeptide according to any of claims 8 to 17, said method at least comprising the steps of:

a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid or nucleotide sequence according to claim 19, or a genetic construct according to claim 20;

optionally followed by:

b) isolating and/or purifying the immunoglobulin single variable domain according to any of claims 1 to 7 or polypeptide according to any of claims 8 to 17, thus obtained.

27. Method for producing an immunoglobulin single variable domain according to any of claims 1 to 7 or polypeptide according to any of claims 8 to 17, said method at least comprising the steps of:

a) cultivating and/or maintaining a host or host cell according to claim 22 under conditions that are such that said host or host cell expresses and/or produces at least one immunoglobulin single variable domain according to any of claims 1 to 7 or polypeptide according to any of claims 8 to 17;

optionally followed by:

b) isolating and/or purifying the immunoglobulin single variable domain according to any of claims 1 to 7 or polypeptide according to any of claims 8 to 17, thus obtained.

28. Method for preparing a polypeptide, protein, construct or compound according to any of claims 13 to 17, said method comprising at least the steps of linking at least one immunoglobulin single variable domain according to any of claims 1 to 7 and one or more peptides directed against (human) serum albumin, and optionally one or more linkers.

29. The immunoglobulin single variable domain according to any of claims 1 to 7, or the polypeptide, protein, construct or compound according to any of claims 8 to 17, for use in therapy.

30. The immunoglobulin single variable domain according to any of claims 1 to 7, or the polypeptide, protein, construct or compound according to any of claims 8 to 17, for the prevention and/or treatment of at least one disease or disorder associated with IgE, with

increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE.

31. The immunoglobulin single variable domain according to any of claims 1 to 7, or the polypeptide, protein, construct or compound according to any of claims 8 to 17, for the prevention and/or treatment of at least one disease or disorder that can be treated by modulating IgE, its biological or pharmacological activity, and/or the biological pathways or signalling in which IgE is involved.

32. The immunoglobulin single variable domain according to any of claims 1 to 7, or the polypeptide, protein, construct or compound according to any of claims 8 to 17, for the prevention and/or treatment of asthma, allergic rhinitis, hay fever, conjunctivitis, eczema, utricaria, food allergies and other allergies, including serious and/or life-threatening allergic reactions such as those to insect bites or stings, snake bites etc., as well as to allergic reaction to medication; and more generally any disease or disorder associated with anaphylactic hypersensitivity and/or (atopic) allergy.

33. Method for the prevention and/or treatment of at least one disease or disorder associated with IgE, with increased levels and/or overproduction of IgE or with abnormal sensitivity (such as hypersensitivity) for IgE, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoglobulin single variable domain according to any of claims 1 to 7 of a polypeptide, protein, construct or compound according to any of claims 8 to 17, and/or of a pharmaceutical composition comprising the same.

34. Method for the prevention and/or treatment of at least one disease or disorder that can be treated by modulating IgE, its biological or pharmacological activity, and/or the biological pathways or signalling in which IgE is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoglobulin single variable domain according to any of claims 1 to 7, of a polypeptide, protein, construct or compound according to any of claims 8 to 17, and/or of a pharmaceutical composition comprising the same.

35. Method for the prevention and/or treatment of asthma, allergic rhinitis, hay fever, conjunctivitis, eczema, utricaria, food allergies and other allergies, including serious and/or life-threatening allergic reactions such as those to insect bites or stings, snake bites etc., as well as to allergic reaction to medication; and more generally any disease or disorder associated with anaphylactic hypersensitivity and/or (atopic) allergy, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoglobulin single variable domain according to any of claims 1 to 7, of a polypeptide, protein, construct or compound according to any of claims 8 to 17, and/or of a pharmaceutical composition comprising the same.

Figure 1

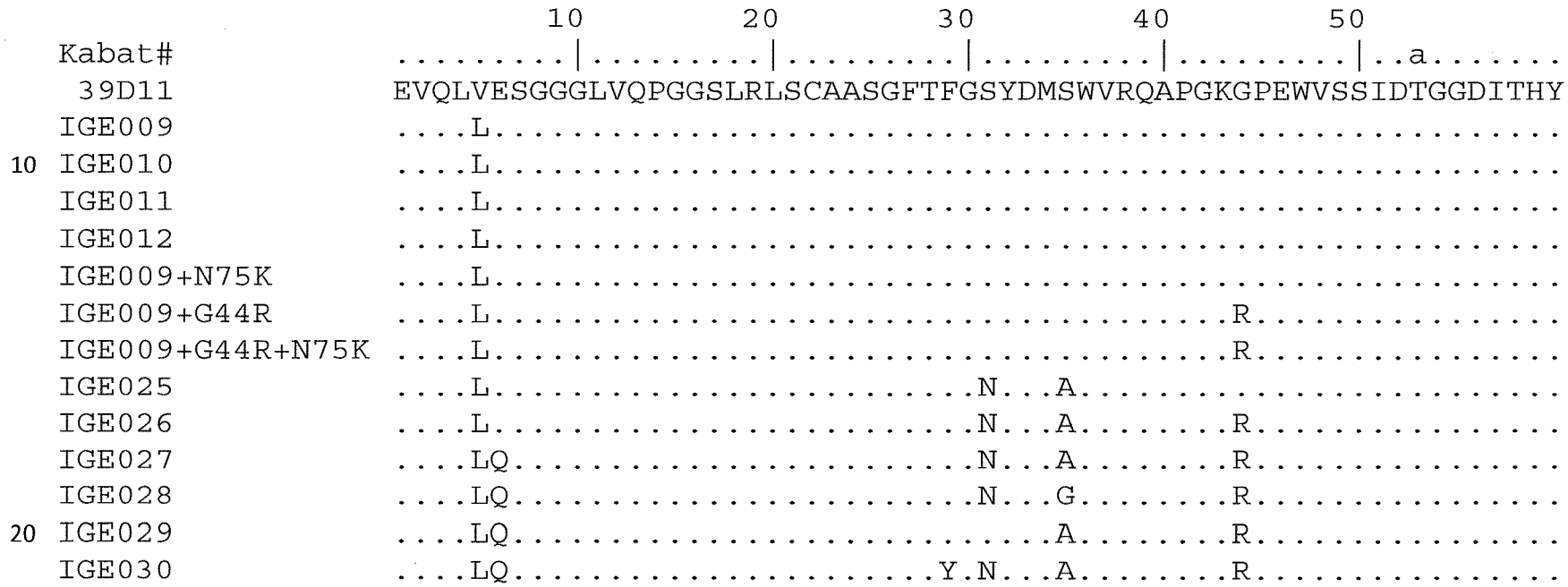
10 20 30 40 50
Kabat# .....|.....|.....|.....|.....|..a.....
39D11 EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSSIDTGGDITHY
IGE009 ....L.....
IGE010 ....L.....
IGE011 ....L.....
IGE012 ....L.....
IGE009+N75K ....L.....
IGE009+G44R ....L.....R.....
IGE009+G44R+N75K ....L.....R.....
IGE025 ....L.....N..A.....
IGE026 ....L.....N..A.....R.....
IGE027 ....LQ.....N..A.....R.....
IGE028 ....LQ.....N..G.....R.....
IGE029 ....LQ.....A.....R.....
IGE030 ....LQ.....Y.N..A.....R.....

Figure 1 continued

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                  60        70        80          90        100           110
Kabat#             |.........|.........|..abc.....|.........|abcde.........|...
 39D11             ADSVKGRFTISRDNANNMLYLQMNSLKPEDTAVYWCATDEDYALGPNEYDYYGQGTQVTVSS
IGE009              ................T........R.............................L.....
IGE010              ................L........R.............................L.....
IGE011              ................T........R.......Y....................L.....
IGE012              ................L........R.......Y....................L.....
IGE009+N75K         ...............K.T........R.............................L.....
IGE009+G44R         ................T........R.............................L.....
IGE009+G44R+N75K    ...............K.T........R.............................L.....
IGE025              ...............K.T........R............E.......F.......L.....
IGE026              ...............K.T........R............E.......F.......L.....
IGE027              ...............K.T........R............E.......F.......L.....
IGE028              ...............K.T........R............E.......F.......L.....
IGE029              ...............K.T........R............E.......F.......L.....
IGE030              ...............K.T........R............E.......F.......L.....
```

Figure 2

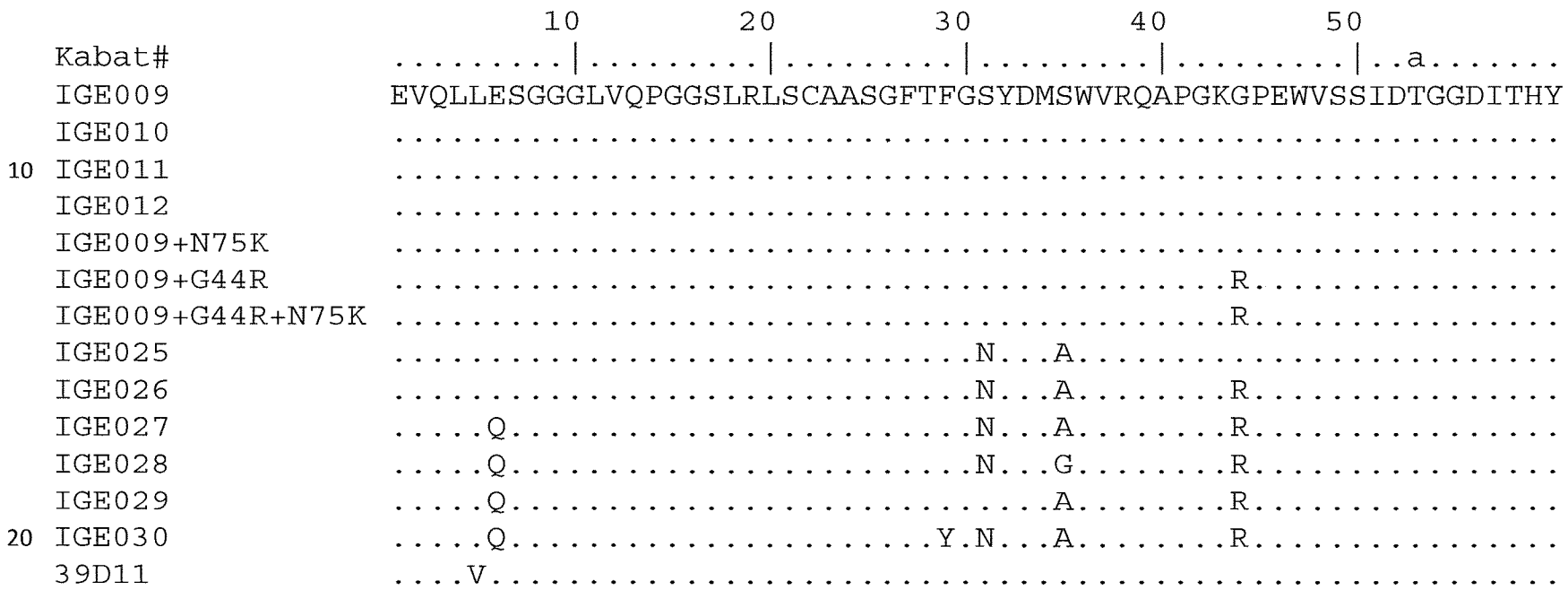

10 20 30 40 50
Kabat# .....|.....|.....|.....|.....|..a.....
IGE009 EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSSIDTGGDITHY
IGE010 ....
IGE011 ....
IGE012 ....
IGE009+N75K ....
IGE009+G44R ....R.....
IGE009+G44R+N75K ....R.....
IGE025 ..............................N...A.....................
IGE026 ..............................N...A........R.....
IGE027 .....Q.....................N...A........R.....
IGE028 .....Q.....................N...G........R.....
IGE029 .....Q.....................A........R.....
IGE030 .....Q.....................Y.N...A........R.....
39D11 ....V..

Figure 2 (continued)

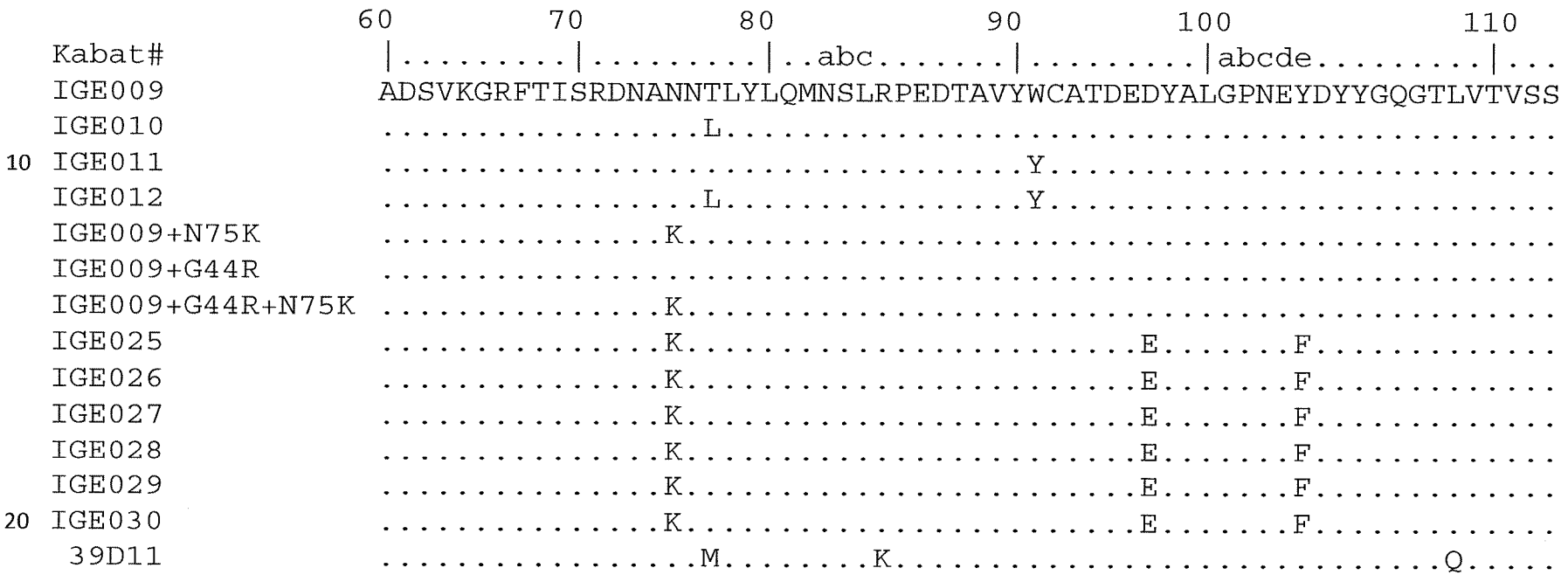
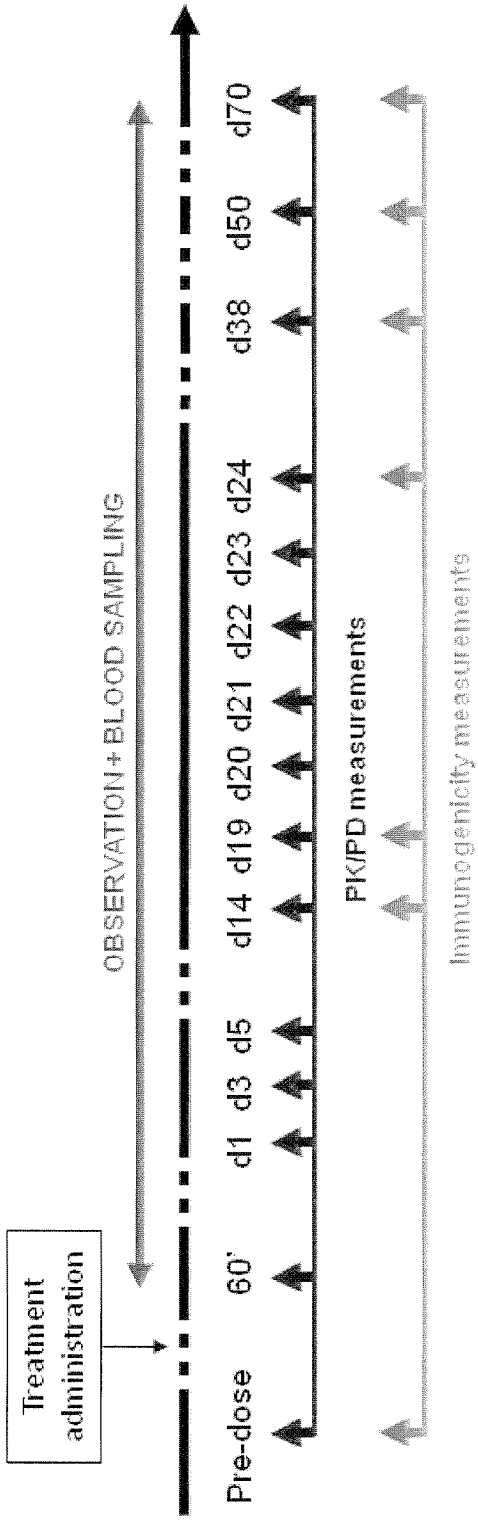


Figure 3:


Treatment administration
OBSERVATION + BLOOD SAMPLING
Pre-dose
60'
d1
d3
d5
d14
d19
d20
d21
d22
d23
d24
d38
d50
d70
PK/PD measurements
Immunogenicity measurements

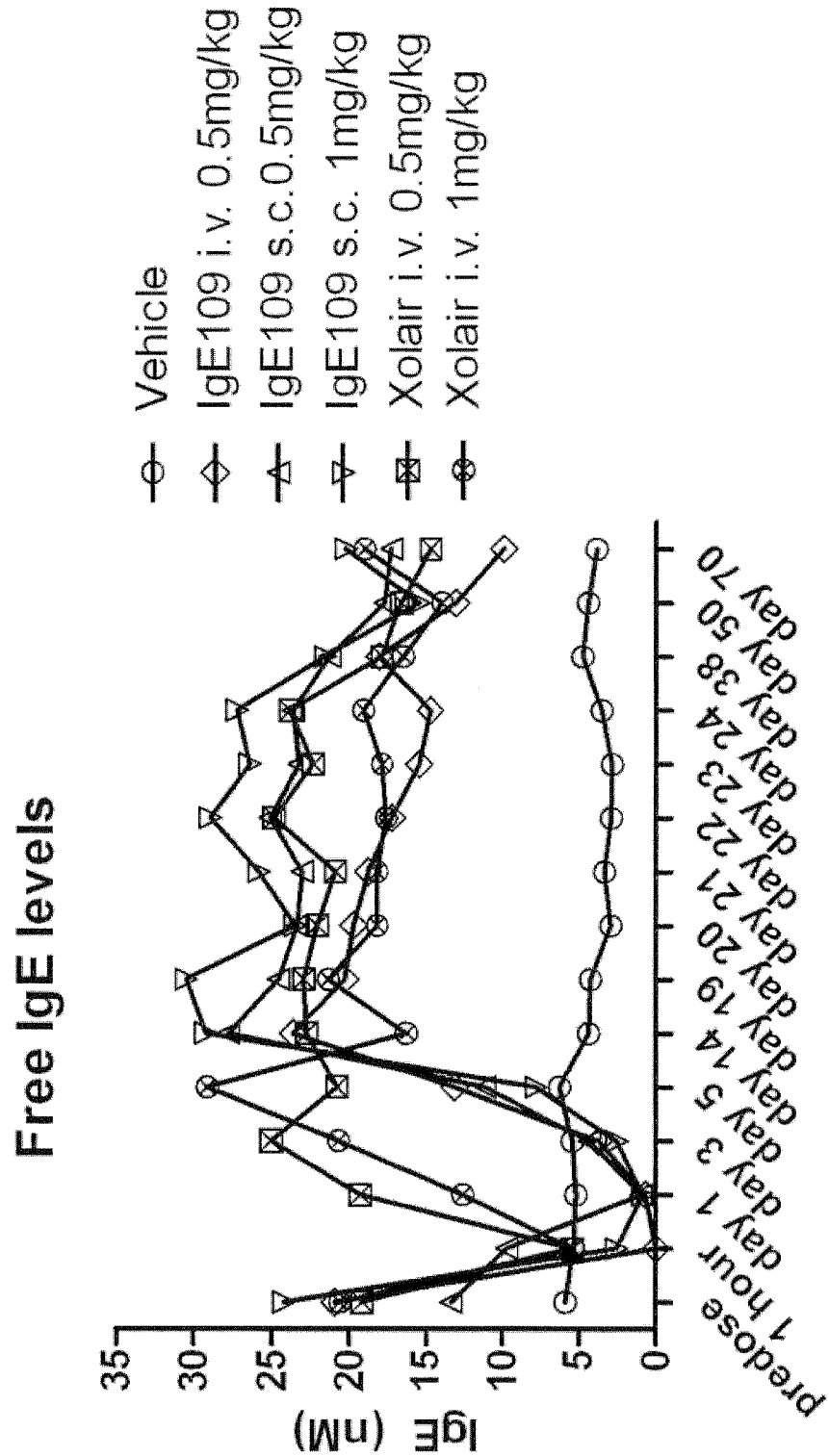

Free IgE levels
IgE (nM)
0
5
10
15
20
25
30
35
predose
1 hour
day 1
day 3
day 5
day 14
day 19
day 20
day 21
day 22
day 23
day 24
day 38
day 50
day 70
Vehicle
IgE109 i.v. 0.5mg/kg
IgE109 s.c.0.5mg/kg
IgE109 s.c. 1mg/kg
Xolair i.v. 0.5mg/kg
Xolair i.v. 1mg/kg

Figure 4:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2012/062250

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [ ] on paper

   [X] in electronic form

   b. (time)

   [X] in the international application as filed

   [ ] together with the international application in electronic form

   [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/062250

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 C07K16/42 A61P37/08
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/041865 A2 (ABLYNX NV [BE]; SILENCE KAREN [BE]; LAUWEREYS MARC [BE]; DREIER TORSTE) 21 May 2004 (2004-05-21)	1
Y	e.g. page 17, paragraph 1; page 46, penultimate paragraph; the whole document	1-12, 18-35
	----- -/--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 September 2012	27/09/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gruber, Andreas

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	"Ablynx", , 1 January 2010 (2010-01-01), XP55037530, Retrieved from the Internet: URL:http://www.ablynx.com/wp-content/uploads/2011/04/IPO_Ablynx-PROSPECTUS-NEDERLANDS-FINAL.pdf [retrieved on 2012-09-07] paragraph bridging page 56 and 57; page A6; the whole document	13-17
X	WO 2008/028977 A2 (ABLYNX NV [BE]; BEIRNAERT ELS [BE]; REVETS HILDE ADI PIERRETTE [BE]; H) 13 March 2008 (2008-03-13) e.g. claim 1,11,14,15; the whole document	13-17
X	WO 2006/122787 A1 (ABLYNX NV [BE]; BEIRNAERT ELS [BE]; REVETS HILDE ADI PIERETTE [BE]; HO) 23 November 2006 (2006-11-23) e.g. claim 1-7; the whole document	13-17

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2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2011/095545 A1 (ABLYNX NV [BE]; REVETS HILDE ADI PIERRETTE [BE]; BOUTTON CARLO [BE]) 11 August 2011 (2011-08-11) page 47, line 11; claim 1-11; the whole document -----	13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/062250

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