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(54) Title: ANTIBODIES TO IL-15

(57) Abstract: The present invention relates to antibodies binding IL-15, in particular humanized antibodies. In particular, the anti-IL-15 antibodies according to the invention are able to neutralize IL-15 activity and are useful in the prevention and/or treatment of an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens.

ANTIBODIES TO IL-15

Field of the Invention

The present invention relates to antibodies that bind interleukin-15 and are, in particular, able to neutralize the activity of said protein, as well as to uses thereof as 5 therapeutics.

Background of the Invention

Interleukin 15 (IL-15), also known as MGC9721, is a 14 to 15 kDa pro-inflammatory cytokine which is expressed in multiple tissues (placenta, skeletal muscle, kidney, lung, heart, monocytes/macrophages) and numerous cell types including monocytes and 10 macrophages, blood derived dendritic cells, epithelial and fibroblast cells, through various stimulatory conditions (*Fenhiger and Caligiuri, 2001, Blood, 97(1):14-32*).

Interleukin-15 regulates T and natural killer (NK) cell activation, survival and proliferation. This cytokine and interleukin 2 (IL-2) share many biological activities, consistent with their shared receptor signaling components (IL-2/15R β and IL-15 15R γ c). However, specificity for IL-15 versus IL-2 is provided by unique private α -chain receptor that completes the IL-15R $\alpha\beta\gamma$ heterotrimeric high-affinity receptor complex and thereby allows differential responsiveness depending on the ligand and high-affinity receptor expressed (*Fenhiger and Caligiuri, 2001, supra*). In addition, while soluble IL-15 is capable of directly stimulating cells expressing either the IL-15R $\alpha\beta\gamma$ high-affinity receptor or the lower affinity IL-15R $\beta\gamma$ receptor, a phenomenon described as IL-15 *cis* presentation, it was suggested that IL-15 bound to IL-15R α for example at the surface of one cell type could associate with and stimulate through the IL-15R $\beta\gamma$ expressed at the surface of another cell, a phenomenon described as IL-15 *trans* presentation (*Stonier et al, 2010, Immunol. Lett., 127:85-92*). Since in the 20 circulation IL-15 may also be preferentially associated with soluble IL-15R α , this *trans* presentation mechanism is unlikely restricted to cell-cell interactions (*Bergamaschi et al, 2012, Blood 120:e1-e8*).

A deleterious role for a dysregulation of IL-15 expression has been suggested in several disorders including autoimmune diseases such as rheumatoid arthritis, psoriasis and 30 celiac disease, as well as in malignancies such as T cell leukemias. In particular, IL-15 triggers an anti-apoptotic pathway in human intraepithelial lymphocytes that is believed

to be a potential new target in celiac disease-associated inflammation and lymphomagenesis (*Malamut et. al.*, 2010, *J. Clin. Invest.*, 120(6):2131-43). Further, it has been found that IL-15 expression is increased in human eosinophilic esophagitis and mediates similar/related pathogenesis in mice (*Zhu et al.*, 2010, *Gastroenterology*, 139(1):182-93). In addition, it has been found that in the observed increased proinflammatory activity in patients with Alzheimer's disease and frontotemporal dementia, IL-15 can be used as a marker since its levels are elevated in cerebrospinal fluid of those patients (*Rentzos et al.*, 2006, *J. Geriatr. Psychiatry Neurol.*, 19(2):114-7).

IL-15 has also been found to play a role of central importance in activating innate immune cells, in particular NK and T cells in transplant rejection response, in particular in the case of allograft transplants (*Ferrari-Lacraz et al.*, 2011, *J Immunol.*, 167(6):3478-3485).

IL-15 is also believed to be a myokine, playing various roles in muscle and fat metabolism (*Raschke and Eckel*, 2013, *Mediators Inflamm.*, 320724). Excess of proinflammatory cytokines including IL-15 has been linked to wasting, hypermetabolic syndromes observed during trauma, injury, and cachexia associated with cancer (*Martínez-Hernández et al.*, 2012, *Oncol Rep.*, 28(4):1443-52).

While it is generally considered to have anti-tumor activities through stimulation of the immune system, IL-15 has also been suggested to play detrimental roles in certain forms of cancers such as acute lymphoid leukemia and large granular lymphocytic leukemia, in addition to its abovementioned role in celiac disease-associated lymphomagenesis (*Cario et al.*, 2007, *J Clin Oncol.* 25(30):4813-20).

Therefore, it would be beneficial to provide potent and specific antibodies that could bind IL-15 and neutralize its biological activities for therapeutic applications, in particular for the treatment of IL-15 related disorders, notably auto-immune and inflammatory disorders.

A fully human monoclonal anti-IL-15 antibody (146B7) has been disclosed (*Villadsen et al.*, 2003, *J. Clin. Invest.*, 112: 1571-1580) as not competing with IL-15 for binding its IL-15R α receptor but potently interfering with the assembly of the IL-15 receptor α , β , γ complex. In a human psoriasis xenograft model, antibody 146B7 reduced the severity of psoriasis. In a phase I-II dose-escalation trial with antibody 146B7 (also

known as AMG 714) in patients with active rheumatoid arthritis, improvements in disease activity have been observed (*Baslund et al, 2005, Arthritis & Rheumatism, 52(9): 2686-2692*). However, this program was discontinued for lack of efficacy (*Fulmer 2009, T. SciBX 2(36)*).

5 A monoclonal mouse anti-IL-15 antibody (B-E29) has been disclosed as preventing IL-15 binding to IL-15R α (*Bernard et al., 2004, J. Biol. Chem., 279(23): 24313-34322*). A fully human anti-IL-15 antibody (DISC0280) has been disclosed as preventing IL-15 binding to IL-15R α even more potently and efficiently than B-E29 when directly compared (*Finch et al., 2011, Brit. J. Pharmacol., 162:480-490*). While DISC0280 was
10 very potent and efficient at neutralizing IL-15 activity *in vitro*, it failed to do so *in vivo*. It was therefore hypothesized that preventing binding of IL-15 to IL-15R α could be detrimental for *in vivo* IL-15 neutralizing activity.

15 Despite the existence of anti-IL-15 antibodies in the prior art, there remains a need for developing alternative anti-IL-15 antibodies which exhibit advantageous properties in comparison to the antibodies of the prior art and/or are more efficiently and/or more easily produced.

20 In view of this need, the present invention desirably provides novel humanized antibodies specific for IL-15 deriving from mouse B-E29 antibody which do not prevent binding of IL-15 to IL-15R α , can neutralize IL-15 *in vivo* and are more potent and efficient at binding and neutralizing IL-15 than the 146B7 antibody.

Summary of the Invention

The present invention is mainly directed towards antibodies which bind interleukin-15, in particular human IL-15, comprising the variable regions described herewith which derive from the humanization and optimization of a mouse anti-IL-15 antibody.

25 A first aspect of the invention provides an isolated antibody binding IL-15 or an antigen-binding fragment thereof comprising:

(1) a heavy chain variable region of SEQ ID NO: 5 or any variant thereof wherein
30 said variant has the amino acid sequence of SEQ ID NO: 5 except that 1, 2, 3 or 4 amino acids are substituted by a different amino acid, wherein substitutions are selected among:

(i) arginine (R) at position H3 (VH RH3) substituted by glutamine (Q),
methionine (M) at position H5 (VH MH5) substituted by valine (V),

alanine (A) at position H6 (VH AH6) substituted by glutamic acid (E), alanine (A) at position H49 (VH AH49) substituted by serine (S), within the heavy chain variable framework region,

(ii) aspartic acid (D) at position H61 (VH DH61) substituted by glutamic acid (E), serine (S) at position H62 (VH SH62) substituted by threonine (T), within the heavy chain CDR2, and

(iii) methionine (M) at position H98 (VH MH98) substituted by leucine (L), phenylalanine (F), isoleucine (I), or alanine (A), tryptophan (W) at position H100C (VH WH100C) substituted by tyrosine (Y), phenylalanine (F) or alanine (A), methionine (M) at position H100E (VH MH100E) substituted by leucine (L), phenylalanine (F) or isoleucine (I), within the heavy chain CDR3;

and

(2) a light chain variable region of SEQ ID NO: 24. 

A second aspect of the invention relates to an isolated nucleic acid molecule encoding said antibody or fragment thereof.

A third and fourth aspects of the invention relate to a recombinant expression vector comprising said nucleic acid molecule, and to a host cell comprising said recombinant vector, respectively.

A fifth aspect of the invention relates to a process for producing antibodies as described herewith comprising culturing a host cell transformed with an expression vector comprising a nucleic acid sequence that encodes said antibodies under conditions sufficient to promote expression of said antibodies or fragments thereof.

10 A sixth aspect of the invention provides a pharmaceutical composition comprising one or more of (i) an isolated antibody binding IL-15 or antigen-binding fragment thereof, (ii) a nucleic acid, (iii) a vector, and/or (iv) a host cell, as described herewith, and at least one pharmaceutically acceptable carrier.

15 A seventh aspect of the invention relates to an imaging composition or a diagnosis composition comprising one or more anti-IL-15 antibody as described herewith.

An eighth aspect of the invention is a kit comprising one or more anti-IL-15 antibody as described herewith.

20 A ninth aspect of the invention relates to the use of an antibody or antigen-binding fragment thereof according to the invention in the manufacture of a medicament for the prevention and/or treatment of IL-15 related disease or disorder, such as an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, a metabolic condition (such as hypermetabolic condition) and/or an infectious disease caused by parasitic, viral or bacterial pathogens.

25 A tenth aspect relates to a method of preventing and/or treating IL-15 related disorders such as an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, a metabolic condition (such as hypermetabolic condition) inherited or related to trauma, injury or cancer and/or an infectious disease caused by parasitic, viral or bacterial pathogens comprising administering in a subject in need thereof a therapeutically effective amount of said antibody or said pharmaceutical composition.

30 In an eleventh aspect, there is provided antibody or antigen-binding fragment thereof according to the invention when used as a medicament.

In a twelfth aspect, there is provided an *ex vivo* method for detecting the presence and/or concentration of IL-15 protein in a biological sample, comprising the steps of:

- (i) Providing a biological sample from a subject,
- 5 (ii) Reacting said biological sample with at least one antibody or antigen-binding fragment thereof according to the invention, under conditions sufficient for binding IL-15 protein present in said biological sample to said at least one antibody or fragment thereof through antigen-antibody interactions; and
- 10 (iii) Detecting a signal proportional to the level of antigen-antibody complex formed in step (ii),

wherein the intensity of the signal correlates with the concentration of IL-15 protein in the biological sample.

In a thirteenth aspect, there is provided method of preventing and/or treating an IL-15 related disease or disorder comprising administering in a subject in need thereof a therapeutically effective amount of an antibody or antigen-binding fragment thereof according to the invention or a pharmaceutical composition thereof.

Other features and advantages of the invention will be apparent from the following detailed description.

Description of the figures

Figure 1. Alignment of sequences of variable regions of humanized and chimeric variants of mouse B-E29, compared to comparative antibody 1, B-E29. (A) Heavy chain variable regions: “cVH1” (SEQ ID NO: 32) represents the heavy chain variable region of mouse B-E29 antibody; “cVH2” (SEQ ID NO: 33), “cVH3” (SEQ ID NO: 34) and “cVH4” (SEQ ID NO: 35) are variants of cVH1; “hVH1” is a humanized form of cVH1. (B) Light chain variable regions: “cVK1” (SEQ ID NO: 36) represents the light chain variable region of mouse B-E29 antibody; “hVK1” (SEQ ID NO: 24), “hVK2” (SEQ ID NO: 37) are two humanized forms of cVK1. CDRs as defined by Kabat are underlined and key residues important for the VH/VL interface and canonical loop structure are asterisked (*).

Figure 2. Dose-response curves of the binding of anti-IL-15 antibodies to human IL-15 as determined by ELISA, expressed as absorbance at 450 nm. Binding of one exemplary anti-IL-15 antibody of the invention (huB-E29-1) and 146B7 antibody to 15 human IL-15 (A), binding of various recombinant anti-IL-15 antibodies to recombinant mouse IL-15 (B) or rat IL-15 (C).

Figure 3. Binding of anti-IL-15 antibodies to recombinant human IL-2 as determined by ELISA. Bars represent the average duplicate values of absorbance at 450 nm for a fixed 5 µg/ml concentration of test anti-IL-15 antibodies or positive control anti-IL-2 antibody. Second step alone is HRP-anti-human immunoglobulin in absence of test antibody.

Figure 4. Dose response-curves of the binding of biotinylated human IL-15 to IL-15Ra-Fc in the presence of various concentrations of antibodies as determined by ELISA, expressed as absorbance at 450 nm. Binding of three exemplary anti-IL-15 antibodies of 25 the invention are shown compared to that of a control antibody known to block IL-15 binding to IL-15Ra, and to the binding of biotinylated IL-15 in absence of antibody or in absence of IL-15Ra-Fc.

Figure 5. Enumeration of NK cells in the spleen of mice injected with vehicle (naïve), or with IL-15/IL-15Ra-Fc complex followed by exemplary anti-IL-15 antibodies or a 30 control human IgG1 isotype. Results are expressed as mean ± standard deviation of 5 animals per group.

Figure 6. Effects of huB-E29-2, 146B7 and control isotype antibodies on IL-15-induced prevention of apoptosis and STA5 phosphorylation in three type II RCD primary cell lines. Results were normalized to levels in control condition (medium, set at 100%) for the percentage of apoptotic cells (A) or the MFI of phosphorylated STAT5 5 intracellular expression (B) induced by IL-15, and expressed as mean plus standard deviation (SD) of results obtained with type II RCD IEL cell lines from three different patients.

Figure 7. Enumeration of CD3⁺CD8⁺ intraepithelial lymphocytes (IEL) in T3b-hIL-15 10 transgenic mice treated for two weeks with two weekly intraperitoneal injections of 100 µg huB-E29-2 (filled circles) or control isotype antibodies (empty circles). Each symbol represents an individual mouse, and group mean plus standard deviation (SD) are plotted. Statistical analysis: unpaired Student's t test.

Detailed Description of the invention

15 Definitions

The terms “interleukin 15”, “interleukin-15”, “IL-15”, designate herewith the interleukin 15 protein, also known as MGC9721, that is a 14 to 15 kDa pro-inflammatory cytokine that, in humans is encoded by the *IL-15* gene whose sequence is disclosed under Hugo Gene Nomenclature Committee ID 5977. The immature form of 20 IL-15 comprises 162 amino acids, where the first 29 amino acids constitute the signal peptide, and the amino acids 30 to 48 constitute the pro-peptide. The immature form of IL-15 is available under UniProtKB accession number P40933. The mature form of the IL-15 protein corresponds to amino acids Asn 49 to Ser 162, where the indicated positions correspond to the amino acid positions on the immature IL-15 amino acid 25 sequence. The amino acid sequence of human mature IL-15 corresponds to SEQ ID NO: 1. The amino acid sequences of immature IL-15 from other species are available in the art and include, for instance, mouse IL-15 (UniProtKB accession number P48346, corresponding to mature IL-15 form of SEQ ID NO: 2), rat IL-15 (UniProtKB accession number P97604, corresponding to mature IL-15 form of SEQ ID NO: 3), Rhesus 30 macaque IL-15 (UniProtKB accession NP_001038196, XP_001091166, XP_001091289 XP_001091416, corresponding to mature IL-15 form of SEQ ID NO: 4) and Cynomolgus monkey IL-15 (predicted sequence from NCBI accession number XP_005556036.1, corresponding to mature IL-15 form of SEQ ID NO: 4). The term

“interleukin 15” also includes any variants or isoforms of interleukin 15 which are naturally expressed by cells. Of note, two alternatively spliced transcript variants of IL-15 have been reported. Although both isoforms produce the same mature protein, they differ in their cellular trafficking.

5 The term “antibody” as referred to herein designates a polypeptide that binds an antigen. This includes whole antibodies and any antigen-binding fragments. The term “antibody” is used in its broadest sense and includes monoclonal antibodies, polyclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, and further engineered antibodies as long as the characteristic properties of the invention are
10 retained, in particular the ability of binding the target antigen (such as IL-15), and optionally to the same epitope of IL-15 as the one recognized by the antibodies of the invention. Examples of antibodies and fragments thereof include a variable domain fragment (“Fv”, consisting of the VH and VL domains of a single arm of an antibody), Fab fragment (monovalent fragment consisting of the VH, VL, CH1 and CL domains),
15 Fab₂ fragment (bivalent), Fab₃ fragment (trivalent), Fab' fragment (Fab with hinge region), F(ab')₂ fragment (bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region), Fd fragment (consisting of the VH and CH1 domains), rIgG (reduced IgG or half-IgG), diabodies, triabodies, tetrabodies, minibodies, domain antibodies (dAb), monovalent antibodies, divalent or multivalent
20 antibodies comprising a fragment of more than one antibody, single chain variable fragment (ScFv), bis-scFv (bispecific), and derivatives of antibodies such as disulfide stabilized Fv fragments, CDR-comprising peptides, as well as epitope-binding fragments of any of the above (*Holliger and Hudson, 2005, Nature Biotechnology, 23(9): 1126-1136*). An antibody refers to a glycoprotein comprising at least two heavy
25 (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding fragment thereof. Each heavy chain comprises a heavy chain variable region (VH) and a heavy chain constant region (CH). Each light chain comprises a light chain variable region (VL) and a light chain constant region (CL). In mammals, the heavy chain can either be alpha (α), delta (δ), epsilon (ε), gamma (γ) or mu (μ), which defines
30 the class of antibody IgA, IgD, IgE, IgG and IgM, respectively. In mammals, the light chain can either be lambda (λ) or kappa (κ). In mammals, depending on the class of antibody, the heavy chain constant region comprises three immunoglobulin

domains, CH1, CH2, and CH3 (for IgA, IgD, IgG) or four immunoglobulin domains, CH1, CH2, CH3, and CH4 (for IgE and IgM). The light chain constant region comprises one immunoglobulin domain, CL. An antibody can have the structure of an IgA, IgG, IgE, IgD and IgM as well as any subtype thereof. Antibodies may be from 5 any source including in particular primate (human and non-human primate) and primatized sources.

The term "variable domain" (variable domain of a light chain (VL), variable domain of a heavy chain (VH)) as used herein refers to each of the pair of light and heavy chain domains which are involved directly in binding the antibody to the antigen. The variable 10 light and heavy chain domains have the same general structure and each domain comprises four framework ("FW") regions whose sequences are widely conserved, connected by three "hypervariable regions" called "complementary determining regions" or "CDRs". The framework regions adopt a β -sheet conformation and the CDRs may form loops connecting the β -sheet structure. The CDRs in each chain are 15 held in their three-dimensional structure by the framework regions and form together with the CDRs from the other chain the antigen binding site. The term "antigen-binding portion of an antibody" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The antigen-binding portion of an antibody comprises amino acid residues from the "complementary determining regions" 20 or "CDRs". "Framework" or "FW" regions are those variable domain regions other than the hypervariable region residues as herein defined. Therefore, the light and heavy chain variable domains of an antibody comprise from N- to C-terminus: the domains FW1, CDR1, FW2, CDR2, FW3, CDR3, and FW4. The residues of the CDR and FW regions are conventionally numbered according to the standard definition of *Kabat et al* 25 (*Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991), Publication No. 91-3242*). This numbering system is used in the present specification except where otherwise indicated. The Kabat residue designations do not always correspond directly to the linear 30 numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain

structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence. The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR- H1), residues 50-65 (CDR-H2) and residues 95-5 102 (CDR-H3) according to the Kabat numbering system. The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system.

In the present application, unless specified otherwise, for all human immunoglobulin heavy and light chain variable domains, numbering is according to the "Kabat 10 numbering system" (*Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991), Publication No. 91-3242*).

In the present application, unless specified otherwise, for all human immunoglobulin heavy chain constant domains, numbering is according to the "EU numbering system" 15 (*Edelman et al, 1969, Proc Natl Acad Sci, 63(1): 78-85*).

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly 20 specific, being directed against a single antigenic site. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

The term "chimeric antibody" generally refers to an antibody comprising a variable 25 region from one source or species and at least a portion of a constant region derived from a different source or species, usually prepared by recombinant DNA techniques. A typical example of chimeric antibodies includes those comprising a mouse variable region and a human constant region. As defined herewith this term also includes an antibody comprising at least one of the CDRs of a first human antibody and at least a 30 portion of a constant region of a second human antibody. It also includes an antibody comprising heavy chain CDR1, CDR2, and CDR3 of a first human antibody and light chain CDR1, CDR2, and CDR3 of a second human antibody.

The term “humanized antibody” designates antibodies from a non-human species having one or more complementarity determining regions (CDRs) from said non-human species and a framework region from a human immunoglobulin molecule. Humanized antibodies may optionally further comprise one or more framework residues derived 5 from the non-human species from which the CDRs were derived.

The term “human antibody” or “fully human antibody” refers to antibodies in which the variable regions and the constant regions of both the heavy and the light chains are all of human origin, or substantially identical to sequences of human origin, but not necessarily from the same antibody.

10 The term “isolated antibody” refers to an antibody that has been separated from a component of its natural environment. For instance, an isolated antibody has been purified to greater than 95% or 99% purity as determined by methods in the art (see e.g. *Flatman et al, 2007, J Chromatogr B Analyt Technol Biomed Life Sci, 848: 79-87*) including electrophoretic (e.g. SDS-PAGE, isoelectric focusing, capillary 15 electrophoresis) or chromatographic (e.g. ion exchange or reverse phase HPLC (high performance liquid chromatography) methods.

The terms “polynucleotide” or “nucleic acid molecule” refers to a polymer comprising nucleotides. Examples of nucleic acid molecules include DNA, RNA, locked nucleic acid (LNA), complementary DNA (cDNA).

20 "Polypeptide" is understood as a peptide, an oligopeptide, an oligomer or a protein comprising at least two amino acids joined to each other by a normal or modified peptide bond, such as in the cases of the isosteric peptides, for example. A polypeptide can be composed of amino acids other than the 20 amino acids defined by the genetic code. A polypeptide can equally be composed of amino acids modified by natural 25 processes, such as post-translational maturation processes or by chemical processes, which are well known to a person skilled in the art. Such modifications are fully detailed in the literature. These modifications can appear anywhere in the polypeptide: in the peptide skeleton, in the lateral chain or even at the carboxy- or amino- terminal ends. For example, polypeptide modifications is understood to include acetylation, 30 acylation, ADP-ribosylation, amidation, covalent fixation of flavine, covalent fixation of heme, covalent fixation of a nucleotide or of a nucleotide derivative, covalent fixation of a lipid or of a lipidic derivative, the covalent fixation of a

phosphatidylinositol, covalent or non-covalent cross-linking, cyclization, disulfide bond formation, demethylation, cysteine formation, pyroglutamate formation, formylation, gamma-carboxylation, glycosylation including pegylation, GPI anchor formation, hydroxylation, iodization, methylation, myristoylation, oxidation, proteolytic processes, 5 phosphorylation, prenylation, racemization, senoylation, sulfatation, amino acid addition such as arginylation or ubiquitination. Such modifications are fully detailed in the literature (*Proteins Structure and Molecular Properties (1993) 2nd Ed., T. E. Creighton, New York; Post-translational Covalent Modifications of Proteins (1983) B. C. Johnson, Ed., Academic Press, New York; Seifter et al. (1990) Analysis for protein 10 modifications and nonprotein cofactors, Meth. Enzymol. 182: 626-646 and Rattan et al., (1992) Protein Synthesis: Post-translational Modifications and Aging, Ann NY Acad Sci, 663: 48-62*).

"Isolated polynucleotide" or "isolated polypeptide" is understood as a polynucleotide or a polypeptide such as previously defined which is isolated from the human body or 15 otherwise produced by a technical process.

The term "variant" can apply to a polynucleotide and/or a polypeptide. For instance, a variant of a peptide or polypeptide, as referred to herein means a peptide or polypeptide substantially homologous to the referenced peptide sequence, but which has an amino acid sequence different from that of the referenced sequence because of one or more 20 amino acid deletions, insertions and/or substitutions. Substantially homologous means a variant amino acid sequence which is identical to the referenced peptide sequence except for the deletion, insertion and/or substitution of a few amino acids, e.g. 1, 2, 3, 4, 5, or 6 amino acids. Substantially homologous means a variant amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, 25 at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the referenced amino acid sequence. A variant nucleic acid sequence can be at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the referenced nucleic acid sequence. The identity of two amino acid sequences or of 30 two nucleic acid sequences can be determined by visual inspection and/or mathematical calculation, or more easily by comparing sequence information using known computer program used for sequence comparison such as Clustal package version 1.83. A variant

may comprise a sequence having at least one conservatively substituted amino acid, meaning that a given amino acid residue is replaced by a residue having similar physiochemical characteristics. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known (Kyte, et al, 1982, *J. Mol. Biol.*, 157: 105- 131). For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a non-native residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Alternatively, substitutions for one or more amino acids present in the original polypeptide are not conservative, which may generate a variant with modified properties compared to the antibody of reference. Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. The term "variant" also includes a peptide or polypeptide substantially homologous to the referenced peptide sequence, but which has an amino acid sequence different from that of the referenced sequence because one or more amino acids have been chemically modified or substituted by amino acid analogs. This term also includes glycosylated polypeptides.

The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinant includes chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody.

As used herewith the term "bind" or "binding" of an antibody to a target antigen means an at least temporary interaction or association of said antibody with, or to, said target antigen (such as IL-15) or with, or to, fragments of said target antigen comprising an epitope recognized by said antibody. As used herewith, an antibody binding IL-15 is also called an anti-IL-15 antibody.

The terms "selectively binds", "specifically binds", "specific for", when applied to an antibody, indicate that the antibody preferentially recognizes and/or binds the target

polypeptide or epitope, i.e. with a higher affinity than to any other antigen or epitope, i.e. the binding to the target polypeptide can be discriminated from non-specific binding to other antigens. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by equilibrium dialysis, equilibrium binding, 5 surface plasmon resonance or spectroscopy (e.g. using a fluorescence assay). Especially, when using the surface plasmon resonance (SPR) technology, biomolecular binding events cause changes in the refractive index at a surface layer where one of the binding partner is immobilized, which are detected as changes in the surface plasmon resonance signal expressed as response units (RU). By measuring the real-time binding 10 kinetics of an antibody to its target antigen, the SPR technology can determine how fast is the association between the antibody and its target (measured as k_a or k_{on} association constant), how strong is its association (measured as k_d or k_{off} dissociation constant). The affinity of an antibody for its target can be quantitatively measured by determining 15 its equilibrium dissociation constant, K_D , defined as $K_D = k_d/k_a$ where k_a is the association rate (k_{on}) and k_d the dissociation rate (k_{off}) (*Murphy, et al, 2006, Curr Protoc Protein Sci, Chapter 19:Unit 19.14*). Comparison of affinity and/or binding properties between two antibodies can be established without actually determining the K_D value for each antibody, but based on a quantitative measurement of binding (e.g. by 20 ELISA or FACS analysis) that is proportional to K_D or a qualitative measurement of affinity or an inference of affinity (e.g. in functional assay or *in vitro* or *in vivo* assay). The term “blocking” or “neutralizing” activity of an antibody refers to its ability to inhibit its target’s activity. The neutralizing activity of an antibody may be determined by *in vitro* assays or *in vivo* assays or functional assays. Applied to an antibody binding 25 IL-15, this term refers to the antibody’s ability to generally neutralize IL-15 activity, which can correspond for instance to the inhibition of the IL-15-induced proliferation and/or survival of activated T cells, natural killer cells, natural killer T cells and B lymphocytes or any other cell expressing the heterotrimeric IL-15Ra β γ or the heterodimeric IL-15R β γ receptor (*Finch, et al, 2011, Br J Pharmacol. 162:480-90*), the IL-15-induced immunoglobulin synthesis by B lymphocytes stimulated by anti-IgM or 30 CD40 ligand (*Litinskiy et al, 2012, Nat Immunol., 3:822-9*), the IL-15 induced activation of human neutrophils (*Rathhé and Girard, 2004, J Leukoc Biol., 76:162-8*), and the IL-15-induced production of proinflammatory cytokines from macrophages,

dendritic cells or epithelial cells (*Nanayakkara, et al, 2013, Am J Clin Nutr., 98:1123-35*). In particular, the neutralizing activity of the anti-IL-15 antibodies can be evaluated by measuring their ability to inhibit IL-15-induced proliferation and/or survival of cell lines such as Kit 225 or M-07e cells as described in the example section. Since IL-15
5 can directly, and alone, act on cells expressing the heterotrimeric IL-15R $\alpha\beta\gamma$ or the heterodimeric IL-15R $\beta\gamma$ receptor (*cis* signalling) or when already bound to IL-15R α receptor (*trans* signalling) (*Stonier, et al, 2010, supra*), an antibody binding to IL-15 could neutralize either, or both, *cis* and *trans* presentation of IL-15. The "potency" of an antibody may be expressed as the concentration of antibody/antigen-binding fragment
10 which produces the half-maximal effect at a given antigen concentration. For example, the "effect" of an antibody may be inhibition or neutralization of its target's activity. In this case, the antibody concentration producing the half-maximal inhibition may be referred to as IC₅₀, which is given in mol/l or M. If binding is the measured "effect" of an antibody, such as in an ELISA assay, the half-maximal binding capacity (BC₅₀) of
15 such an antibody may be expressed as the concentration of antibody which produces the half-maximal signal at a given antigen concentration, which is given in mol/l or M. Potency is usually influenced by affinity until, at a given antigen concentration, an affinity is reached beyond which further improvements in affinity will not enhance binding of the antigen anymore (so-called potency ceiling). Applied to an antibody
20 against IL-15, potency may, for example, be determined by measuring the IC₅₀ value of IL-15 induced proliferation and/or survival of cell lines such as Kit 225 or M-07e cells in presence of the antibody, or the BC₅₀ value for binding to IL-15 from different sources or species.

The term "effector function" as used herein includes a biochemical event that results
25 from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include Fc γ R-mediated effector functions such as ADCC (antibody dependent cell-mediated cytotoxicity) and ADCP (antibody dependent cell-mediated phagocytosis), and complement- mediated effector functions such as CDC (complement dependent cytotoxicity). An effector function of an antibody may be modified by
30 altering, i.e. enhancing or reducing, preferably enhancing, the affinity of the antibody for an effector molecule such as an Fc receptor or a complement component. Binding affinity of an antibody Fc region with an Fc receptor or ligand can be altered by

modifying the effector molecule binding site. It is also possible that an alteration in the binding site on the antibody for the effector molecule alters the geometry of the interaction without significantly altering the overall binding affinity, rendering the effector mechanism ineffective as in non-productive binding. It is also possible to alter an effector function by modifying a site not directly involved in effector molecule binding, but otherwise involved in performance of the effector function. By altering an effector function of an antibody it may be possible to control various aspects of the immune response, e.g. enhancing or suppressing various reactions of the immune system, with possible beneficial effects in diagnosis and therapy.

10 The term “pharmaceutically acceptable” refers to a carrier comprised of a material that is not biologically or otherwise undesirable.

The term “carrier” refers to any components present in a pharmaceutical formulation other than the active agent and thus includes diluents, binders, lubricants, disintegrants, fillers, coloring agents, wetting or emulsifying agents, pH buffering agents, preservatives and the like.

15 As used herein, “treatment” and “treating” and the like generally mean obtaining a desired pharmacological and physiological effect. The effect may be prophylactic in terms of preventing or partially preventing a disease, symptom or condition thereof and/or may be therapeutic in terms of a partial or complete cure of a disease, condition, symptom or adverse effect attributed to the disease. The term “treatment” as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it for example based on familial history; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the 20 disease, i.e., causing regression of the disease and/or its symptoms or conditions such as improvement or remediation of damage. For instance, treatment of celiac disease comprises preventing, decreasing or even eradicating the symptoms of the disease or disorder, for instance partial or total alleviation of abdominal pain, diarrhea, unintended weight loss, malabsorption syndrome, and of abnormalities of the intestinal mucosa 25 such as villous atrophy, erosions, ulcers and infiltration by normal or abnormal intra-epithelial lymphocytes.

The terms “IL-15 related diseases and/or disorders” encompass diseases and disorders characterized by an overexpression of IL-15 and/or increased levels and/or abnormal IL-15 expression by a cell or organ, and/or abnormal expression of a IL-15 variant by a cell or organ. Such diseases and disorders encompass for example autoimmune diseases and/or inflammatory disorders, such as disorders having a pro-inflammatory IL-15 related component and malignancies.

The terms “autoimmune diseases and/or inflammatory disorders” are generally defined herewith as diseases or disorders arising from an abnormal immune response of the subject’s body against substances and tissues normally present in the body and inflammatory abnormalities which may or may not involve the immune system, respectively. Non-limitative examples of autoimmune diseases and inflammatory disorders include mostly rheumatoid arthritis, psoriasis, celiac disease, in particular refractory celiac disease, sarcoidosis, inflammatory bowel disease (e.g. ulcerative colitis, Crohn’s disease), hepatitis C-induced liver diseases, multiple sclerosis, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary atresia, alopecia areata, transplant rejection response, inflammatory diseases of the central nervous system, and eosinophilic esophagitis.

The term “malignancies” mostly cover herewith T-cell leukemia, such as cutaneous T-cell lymphoma (CTCL) (e.g. mycosis fungoides, Sezary syndrome), lymphoproliferative disorder of granular lymphocytes (LDGL), large granular lymphocytic leukemia, and acute lymphocytic leukemia (ALL), but also pre-B cell leukemia, osteosarcoma, Ewing sarcoma, rhabdomyosarcoma, melanoma, small cell lung cancer, renal cell carcinoma, glioblastoma, neuroblastoma, and mesothelioma.

The terms “infectious disease caused by parasitic, viral or bacterial pathogens” mostly cover herewith granulomatous infections (such as tuberculosis, leishmaniasis, schistosomiasis, and cytomegalovirus infections) and hantaviruses infections (such as hantavirus haemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome).

The term “inflammatory diseases of the central nervous system (CNS)” relates to disorders characterized by an inflammation of the CNS, in particular amyloid related disorders. Non-limitative examples of those disorders are Alzheimer’s disease, Parkinson’s disease and Huntington’s disease.

The term “metabolic diseases” mostly cover diabetes, muscular dystrophy and hypermetabolic conditions.

5 The term “hypermetabolic condition” mostly covers inherited conditions such as sickle cell disease or acquired hypermetabolic conditions such as those related to trauma, infection or cancer-associated cachexia.

The term “subject” as used herein refers to mammals. For example, mammals contemplated by the present invention include human, primates, domesticated animals such as cattle, sheep, pigs, horses, laboratory rodents and the like.

10 The term “efficacy” of a treatment or method according to the invention can be measured based on changes in the course of disease or condition in response to a use or a method according to the invention. For example, the efficacy of a treatment or method according to the invention can be measured by its impact on signs or symptoms of illness. A response is achieved when the patient experiences partial or total alleviation, 15 or reduction of unwanted symptoms of illness.

The term “effective amount” as used herein refers to an amount of at least one antibody according to the invention, or a pharmaceutical formulation thereof, that elicits a detectable reduction of the symptoms of the disease in a subject that is being administered said antibody.

20 Throughout the specification and the claims that follow, unless the context requires otherwise, the words “comprise” and “include” and variations such as “comprising” and “including” will be understood to imply the inclusion of a stated integer or group of integers, but not the exclusion of any other integer or group of integers.

25 The reference to any prior art in this specification is not, and should not be taken as, an acknowledgement of any form of suggestion that such prior art forms part of the common general knowledge.

30 It will be appreciated by those skilled in the art that the invention is not restricted in its use to the particular application described. Neither is the present invention restricted in its preferred embodiment with regard to the particular elements and/or features described or depicted herein. It will be appreciated that the invention is not limited to the embodiment or embodiments disclosed, but is capable of numerous rearrangements, modifications and substitutions without departing from the scope of the invention.

Anti-IL-15 antibodies

General characteristics of the antibodies binding IL-15

In a first aspect, the present invention provides antibodies, or antigen-binding fragments

thereof, that bind to IL-15, in particular human IL-15, or a fragment of IL-15, and

5 comprise at least one heavy chain variable region and/or at least one light chain variable
region of an antibody as described herewith.

In one embodiment of the invention are provided isolated antibodies binding IL-15,

more particularly antibodies specific for IL-15, in particular human IL-15, or antigen-
binding fragments thereof, comprising at least one heavy chain variable region and at

10 least one light chain variable region, and optionally at least one fragment of a constant
region, as described herewith.

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Generally, the antigen-binding fragment of the antibody according to the invention comprises CDR1, FW2, CDR2, FW3, CDR3 and FW4 of the heavy chain and/or light chain variable regions of said antibody.

5 In one embodiment, the antigen-binding fragment of the antibody according to the invention comprises amino acids 26 to 111 of SEQ ID NO: 5 or a variant thereof, and/or amino acids 24 to 102 of SEQ ID NO: 24 or a variant thereof.

The protein to which the antibodies according to the invention, or fragments thereof, bind can be the IL-15 protein of any species.

10 The antibodies according to the present invention generally exhibit a high specificity for human IL-15. However, depending on the degree of sequence identity between IL-15 homologs of different species, a given antibody or antigen-binding fragment may show cross-reactivity with IL-15 from at least one other species, e.g. monkey (e.g. Cynomolgus monkey, Rhesus macaque), mouse, rat, marmoset, dog, and/or rabbit. For antibodies directed towards human IL-15, some level of cross-reactivity with other 15 mammalian forms of IL-15 may be desirable in certain circumstances, for example when testing antibodies in animal models of a particular disease or for conducting toxicology, safety and dosage studies.

In a specific embodiment, the antibodies according to the invention or fragments thereof bind preferentially to human IL-15.

20 In another embodiment, the antibodies according to the invention or antigen-binding fragments thereof show cross-reactivity with human IL-15, Cynomolgus monkey IL-15, and Rhesus macaque IL-15.

In a still further embodiment, the antibodies according to the invention or antigen-binding fragments thereof show no cross-reactivity with rat IL-15 and/or mouse IL-15.

25 In some embodiments, the binding affinity (e.g. inversely correlated to the K_D value) of antibodies, and fragments thereof, according to the invention for human IL-15 is at least 2 times, at least 5 times, at least 10 times, at least 50 times, at least 100 times, at least 500 times, or at least 1000 times higher than their binding affinity for a non-human IL-15, such as mouse or rat IL-15.

30 In one embodiment, the antibodies according to the invention or fragments thereof bind preferentially to IL-15 and, optionally, additionally exhibit a weak binding, or virtually

no binding (i.e. negligible or not detectable binding) to other proteins having homology with IL-15 such as IL-2, in particular human IL-2 (SEQ ID NO: 38).

In some embodiments, the quantitative binding of antibodies, and fragments thereof,

according to the invention for IL-15 is at least 2 times, at least 5 times, at least 10 times,

5 at least 50 times, at least 100 times, at least 500 times, or at least 1000 times higher than their quantitative binding for IL-2.

Binding affinity and/or quantitative binding can be measured by any method known in

the art including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance or spectroscopy (e.g. using a fluorescence assay) (*Jiang et*

10 *al. BMC Pharmacology 2010, 10:10*) and can be expressed as, for instance, on-rate, off-

rate, equilibrium dissociation constant (K_D), equilibrium constant (K_{eq}) or any other

term used in the art.

In some embodiments, the antibodies, and antigen-binding fragments thereof, according

to the invention specifically bind to human IL-15 with an equilibrium dissociation

15 constant (K_D) equal to or lower than 100 nM, in particular lower than 10 nM, more

particularly lower than 1 nM, or lower than 0.5 nM, or lower than 0.1 nM, or lower than

0.01 nM, or lower than 0.005 nM.

In a particular embodiment, the antibodies according to the invention or antigen-binding

fragments thereof inhibit IL-15 activity and, optionally, additionally exhibit a weak

20 inhibitory activity, or virtually no inhibitory activity (i.e. negligible or not detectable

activity) towards other proteins having homology with IL-15, such as IL-2.

The ability of an antibody to block or neutralize the activity of its target protein can be

evaluated by its potency as defined herewith, which is itself reflected, for instance, by

the IC_{50} value. Typically, the neutralizing activity of an antibody may be determined by

25 *in vitro* assays, such as an assay for measuring the level of inhibition of IL-15-induced

proliferation and/or survival of cell lines such as Kit 225 or M-07e cells, in the presence

of said antibody, as described in the example section.

In some embodiments, the antibodies, and antigen-binding fragments thereof, according

to the invention have a IC_{50} equal to or lower than 200 nM, in particular lower than 100

30 nM, in particular lower than 50 nM, lower than 30 nM, lower than 20 nM, more

particularly lower than 10 nM, lower than 8 nM, lower than 7 nM, lower than 5 nM,

lower than 4 nM, lower than 3 nM, lower than 2 nM, lower than 1 nM, lower than 0.5

nM, lower than 0.3 nM, lower than 0.2 nM, lower than 0.1 nM, lower than 0.05 nM, or lower than 0.03 nM, for inhibiting IL-15 activity such as IL-15 induced proliferation and/or survival of cell lines such as Kit 225 or M-07e cells as described in the example section.

5 It is understood that any variant of an antibody according to the invention, or fragment thereof, that is described herewith is able to bind IL-15 and optionally neutralize IL-15 activity. In a particular embodiment, such variant can show the same or even higher binding affinity for IL-15 and/or the same or even higher potency and/or the same or greater species-selectivity and/or the same or greater selectivity for IL-15, and/or the
10 same or greater neutralizing efficacy, in comparison to the parental antibody or fragment from which said variant derives.

In another particular embodiment, the antibodies according to the invention or antigen-binding fragments thereof do not substantially prevent the binding of IL-15 to IL-15Ra, i.e. the inhibition of binding of IL-15 to IL-15Ra in presence of the antibodies
15 according to the invention is negligible or not detectable.

The antibodies according to the invention can be monoclonal antibodies, polyclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, and further engineered antibodies as long as the characteristic properties of the antibodies of the invention are retained, in particular the ability of binding to the target antigen, more
20 specifically to the same epitope of IL-15 as the one recognized by the antibodies of the invention, and optionally the ability of neutralizing IL-15 activity.

In a particular embodiment of the invention, the antibodies to IL-15 according to the invention, or antigen-binding fragments thereof which bind to IL-15, are monoclonal antibodies.

25 In a further particular embodiment of the invention, the antibodies to IL-15 according to the invention, or antigen-binding fragments thereof which bind to IL-15, are humanized antibodies.

In a further particular embodiment of the invention, the antibodies to IL-15 according to the invention, or antigen-binding fragments thereof which bind to IL-15, are
30 recombinant antibodies.

The antibodies to IL-15 according to the invention, or antigen-binding fragments thereof which bind to IL-15, can be characterized by their portion interacting with the

target's protein, in particular by their variable region, which typically comprises a heavy chain variable region and a light chain variable region.

Characteristics of the anti-IL-15 antibodies in relation to their variable regions

In one embodiment, the invention relates to an isolated antibody binding IL-15 comprising:

- (1) a heavy chain variable region of SEQ ID NO: 5 or any variant thereof wherein 1, 2, 3, 4, 5, 6, 7, 8, or 9 amino acids of said sequence are substituted by a different amino acid, and
- (2) a light chain variable region of SEQ ID NO: 24 or any variant thereof wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids of said sequence are substituted by a different amino acid,
or an antigen-binding fragment thereof.

In a particular embodiment, the invention relates to an isolated antibody binding IL-15 comprising:

- (1) a heavy chain variable region of SEQ ID NO: 5 or any variant thereof having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 5, and
- (2) a light chain variable region of SEQ ID NO: 24 or any variant thereof having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 24,
or an antigen-binding fragment thereof.

In a more particular embodiment, the antibody according to the invention comprises:

- (1) a heavy chain variable region having at least 96% identity with SEQ ID NO: 5, and
- (2) a light chain variable region having at least 98% identity with SEQ ID NO: 24, or an antigen-binding fragment thereof.

In a still more particular embodiment, the antibody according to the invention comprises:

- (1) a heavy chain variable region having at least 97% identity with SEQ ID NO: 5, and
- (2) a light chain variable region having at least 99% identity with SEQ ID NO: 24 or an antigen-binding fragment thereof.

In one embodiment according to the invention, said variant of SEQ ID NO: 5 has the amino acid sequence of SEQ ID NO: 5 except that at least one, in particular 1, 2, 3, 4, or 5, of the amino acids:

- (i) arginine (R) at position H3 (VH RH3), methionine (M) at position H5 (VH MH5), alanine (A) at position H6 (VH AH6), alanine (A) at position H49 (VH AH49), within the heavy chain variable framework region, and/or
- 5 (ii) aspartic acid (D) at position H61 (VH DH61), serine (S) at position H62 (VH SH62), within the heavy chain CDR2, and/or
- (iii) methionine (M) at position H98 (VH MH98), tryptophan (W) at position 10 H100C (VH WH100C), methionine (M) at position H100E (VH MH100E), within the heavy chain CDR3,

are substituted by a different amino acid.

In a still further embodiment, said variant of SEQ ID NO: 5 has the amino acid sequence of SEQ ID NO: 5 except that:

- 15 (i) VH RH3 is substituted by glutamine (Q), and/or VH MH5 is substituted by valine (V), and/or VH AH6 is substituted by glutamic acid (E), and/or VH AH49 is substituted by serine (S), and/or
- (ii) VH DH61 is substituted by glutamic acid (E), and/or VH SH62 is substituted by threonine (T), and/or
- 20 (iii) VH MH98 is substituted by leucine (L), phenylalanine (F), isoleucine (I), or alanine (A), and/or VH WH100C is substituted by tyrosine (Y), phenylalanine (F), or alanine (A), and/or VH MH100E is substituted by leucine (L), phenylalanine (F), or isoleucine (I).

In one embodiment of the invention, said variant of SEQ ID NO: 24 has the amino acid sequence of SEQ ID NO: 24 except that at least one, in particular 1, 2, 3, or 4, of the amino acids:

- 25 (i) tyrosine (Y) at position L36 (VL YL36), leucine (L) at position L46 (VL LL46), within the light chain variable framework region, and/or
- (ii) aspartic acid (D) at position L91 (VL DL91), serine (S) at position L92 (VL 30 SL92), within the light chain CDR3,

are substituted by a different amino acid.

In a still further embodiment, said variant of SEQ ID NO: 24 has the amino acid sequence of SEQ ID NO: 24 except that:

- (i) VL YL36 is substituted by phenylalanine (F), and/or VL LL46 is substituted by arginine (R), and/or
- 5 (ii) VL DL91 is substituted by glutamic acid (E), and/or VL SL92 is substituted by threonine (T).

In another embodiment, the antibody according to the invention, or antigen-binding fragment thereof, comprises:

- (1) a heavy chain variable region of amino acid sequence of SEQ ID NO: 5 except that:
 - (i) VH RH3 is substituted by glutamine (Q), and/or VH MH5 is substituted by valine (V), and/or VH AH6 is substituted by glutamic acid (E), and/or
 - (ii) VH SH62 is substituted by threonine (T), and/or
 - (iii) VH WH100C is substituted by tyrosine (Y), and
- 15 (2) a light chain variable region of amino acid sequence of SEQ ID NO: 24.

In a more particular embodiment, the antibody according to the invention, or antigen-binding fragment thereof, comprises:

- (i) a heavy chain variable region selected from: SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 12, SEQ ID NO: 21, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 22 and SEQ ID NO: 23, and
- (ii) a light chain variable region selected from: SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29.

25 In a more particular embodiment, the antibody according to the invention, or antigen-binding fragment thereof, comprises a heavy chain variable region of SEQ ID NO: 6. In a more particular embodiment, the antibody according to the invention, or antigen-binding fragment thereof, comprises a light chain variable region of SEQ ID NO: 24. Specific examples of the antibodies according to the invention include those listed in

30 Table 1.

Table 1

Antibody name	VH region	SEQ ID NO:	VL region	SEQ ID NO:
huB-E29-1	huVH1	NO: 5	huVL1	NO: 24
huB-E29-2	huVH2	NO: 6	huVL1	NO: 24
huB-E29-3	huVH3	NO: 7	huVL1	NO: 24
huB-E29-4	huVH1	NO: 5	huVL2	NO: 25
huB-E29-5	huVH1	NO: 5	huVL3	NO: 26
huB-E29-6	huVH4	NO: 8	huVL1	NO: 24
huB-E29-7	huVH5	NO: 9	huVL1	NO: 24
huB-E29-8	huVH6	NO: 10	huVL1	NO: 24
huB-E29-9	huVH7	NO: 11	huVL1	NO: 24
huB-E29-10	huVH8	NO: 12	huVL1	NO: 24
huB-E29-11	huVH9	NO: 13	huVL1	NO: 24
huB-E29-12	huVH10	NO: 14	huVL1	NO: 24
huB-E29-13	huVH11	NO: 15	huVL1	NO: 24
huB-E29-14	huVH12	NO: 16	huVL1	NO: 24
huB-E29-15	huVH13	NO: 17	huVL1	NO: 24
huB-E29-16	huVH14	NO: 18	huVL1	NO: 24
huB-E29-17	huVH15	NO: 19	huVL1	NO: 24
huB-E29-18	huVH1	NO: 5	huVL4	NO: 27
huB-E29-19	huVH1	NO: 5	huVL5	NO: 28
huB-E29-22	huVH16	NO: 20	huVL6	NO: 29
huB-E29-24	huVH18	NO: 21	huVL1	NO: 24
huB-E29-30	huVH20	NO: 22	huVL1	NO: 24
huB-E29-31	huVH20	NO: 22	huVL6	NO: 29
huB-E29-34	huVH21	NO: 23	huVL6	NO: 29

In a more particular embodiment, the antibody according to the invention, or antigen-binding fragment thereof, comprises:

- (1) a heavy chain variable region selected from: SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 12, and SEQ ID NO: 21, and
- (2) a light chain variable region of SEQ ID NO: 24.

Still more particularly, the antibody according to the invention, or antigen-binding fragment thereof, comprises a heavy chain variable region of SEQ ID NO: 5 and a light chain variable region of SEQ ID NO: 24.

Still more particularly, the antibody according to the invention, or antigen-binding fragment thereof, comprises a heavy chain variable region of SEQ ID NO: 6 and a light chain variable region of SEQ ID NO: 24.

Still more particularly, the antibody according to the invention, or antigen-binding fragment thereof, comprises a heavy chain variable region of SEQ ID NO: 12 and a light chain variable region of SEQ ID NO: 24.

Still more particularly, the antibody according to the invention, or antigen-binding fragment thereof, comprises a heavy chain variable region of SEQ ID NO: 21 and a light chain variable region of SEQ ID NO: 24.

Characteristics of the anti-IL-15 antibodies in relation to their constant region

A portion corresponding to a constant region of an antibody is optionally comprised in the isolated antibodies binding IL-15, or antigen-binding fragments thereof, according 10 to the invention.

Depending on the proposed function of the antibodies and, in particular the effector functions which may be required, a constant region of an antibody may or may not be present within the antibodies according to the invention.

Typically, when present within the antibodies or antigen-binding fragments thereof according to the invention, the heavy chain constant region or portion thereof can be from any antibody isotype. For instance, the heavy chain constant region or portion thereof can be that of an antibody selected from IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE, IgM (e.g. IgM1, IgM2). It can be, in particular, the constant region or portion thereof of an IgG, more particularly IgG1.

20 In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simply blocking IL-15 activity.

25 When present within the antibodies or antigen-binding fragments thereof according to the invention, the light chain constant region or portion thereof can be from any light chain's constant region. For instance, the light chain constant region or portion thereof can be from the kappa or lambda light chain.

In a particular aspect of the invention, the antibodies for IL-15, or antigen-binding 30 fragments thereof, comprise (i) at least one heavy chain comprising a variable region as described herewith and a constant region or portion thereof from an IgG antibody (in particular IgG1, more particularly allotype G1m3, and (ii) at least one light chain

comprising a variable region as described herewith and a constant region or portion thereof from a kappa (in particular allotype Km3) light chain. The amino acid sequence of the constant region of allotype G1m3 is SEQ ID NO: 30. The amino acid sequence of the constant region of allotype Km3 is SEQ ID NO: 31.

5 The antibodies, or antigen-binding fragments thereof, of the invention have at least one antigen binding site, e.g. one or two antigen binding sites.

In some embodiments, the isolated antibodies and antigen-binding fragments thereof according to the invention are glycosylated. Typically, monosaccharides such as N-acetylglucosamine, mannose, glucose, galactose, fucose, sialic acid, etc, are assembled 10 to oligosaccharides at individual glycosylation sites on the antibody.

Conjugates comprising an auxiliary molecule

In another aspect of the invention, the isolated antibodies or antigen-binding fragments thereof according to the invention are optionally conjugated to an accessory molecule, and are then also referred to herein as “conjugated antibodies or “conjugated antibody 15 fragments”.

The accessory molecule may be conjugated to the antibody or antibody fragment directly or via a spacer of suitable length for instance as described in *Kellogg et al. (2011, Bioconjug Chem, 22: 717–27)*.

In one embodiment, particularly adapted for therapeutic purposes, the accessory 20 molecule can be a therapeutic effector group such as a cytotoxic (e.g. an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragment thereof), cytostatic, or immunomodulatory agent, including radioactive groups (i.e groups comprising a radionucleide or radioisotope), or small molecules.

In another embodiment, the accessory molecule comprises an antigen-binding fragment 25 of an antibody, which, when conjugated to the antibody or antibody fragment according to the invention, form a bispecific antibody. In particular, said bispecific antibody may be directed to two different epitopes of IL-15 (hence defining a biparatopic antibody).

The conjugated antibodies and conjugated antibody fragments according to the invention can target the drug *in vivo* to a site of disease (e.g. a site of inflammation or a 30 tumor) such that the conjugated auxiliary molecule can have a therapeutic effect on the site of disease.

In an alternative embodiment, particularly adapted for diagnostic purposes, the accessory molecule can be, for example, a labeling group including radioisotopes (e.g. 3H, 14C, 32P, 35S, 125I), chromogenic labels, e.g. enzymes which can be used to convert a substrate to a detectable colored (e.g. horseradish peroxidase, alkaline phosphatase, β -galactosidase) or fluorescent compound (e.g. Green Fluorescent Protein, Red Fluorescent Protein), spectroscopic labels (e.g. fluorescent labels such as fluorescein and its derivatives like FITC, Texas red, cyanine dyes, photocyan, rhodamine, or labels presenting a visible color), luminescent labels including luciferins, affinity labels which may be developed by a further compound specific for the label and allowing easy detection and quantification, or any other label used in standard ELISA.

Nucleic acids encoding the polypeptides of the invention

According to another embodiment, it is provided an isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof according to the invention.

The isolated nucleic acid according to the invention may be, for instance, natural DNA or RNA or a recombinant or synthetic DNA, RNA or LNA or a recombinant nucleic acid molecule comprising any of the nucleic acid molecules according to the invention either alone or in combination. In a particular embodiment, the nucleic acid molecules according to the invention are cDNA.

In a particular embodiment, it is provided an isolated nucleic acid comprising one or more of:

- (1) a nucleic acid sequence encoding a heavy chain variable region of SEQ ID NO: 5 or any variant thereof wherein 1, 2, 3, 4, 5, 6, 7, 8, or 9 amino acids of said sequence are substituted by a different amino acid, or an antigen-binding fragment thereof, and
- 25 (2) a nucleic acid sequence encoding a light chain variable region of SEQ ID NO: 24 or any variant thereof wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids of said sequence are substituted by a different amino acid, or an antigen-binding fragment thereof.

In a particular embodiment, it is provided an isolated nucleic acid comprising one or more of:

- (1) a nucleic acid sequence encoding a heavy chain variable region of SEQ ID NO: 5 or any variant thereof having at least 95%, at least 96%, at least 97%, at least

98%, or at least 99% identity with SEQ ID NO: 5, or an antigen-binding fragment thereof, and

5 (2) a nucleic acid sequence encoding a light chain variable region of SEQ ID NO: 24 or any variant thereof having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO: 24, or an antigen-binding fragment thereof.

In a particular embodiment, it is provided an isolated nucleic acid comprising one or more of:

10 (1) a nucleic acid sequence encoding a heavy chain variable region selected from: SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 12, SEQ ID NO: 21, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 22 and SEQ ID NO: 23, or an antigen-binding fragment thereof, and

15 (2) a nucleic acid sequence encoding a light chain variable region selected from: SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, or an antigen-binding fragment thereof.

In a more particular embodiment, the invention provides an isolated nucleic acid comprising one or more of:

20 (1) a nucleic acid sequence encoding a heavy chain variable region selected from: SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 12, and SEQ ID NO: 21, and

(2) a nucleic acid sequence encoding a light chain variable region of SEQ ID NO: 24.

Vectors and Host cells for production and purification of the polypeptides of the invention

25 In one embodiment, the invention provides a recombinant expression vector comprising a nucleic acid molecule according to the invention, wherein the vector optionally comprises an expression control sequence, allowing expression in prokaryotic or eukaryotic host cells of the encoded polypeptide, operably linked to said nucleic acid molecule.

30 Numerous expression systems can be used, including without limitation chromosomes, episomes, and derived viruses. More particularly, the recombinant vectors used can be derived from bacterial plasmids, transposons, yeast episomes, insertion elements, yeast

chromosome elements, viruses such as baculovirus, papilloma viruses such as SV40, vaccinia viruses, adenoviruses, fox pox viruses, pseudorabies viruses, retroviruses. These recombinant vectors can equally be cosmid or phagemid derivatives.

5 The nucleic acid sequence can be inserted in the recombinant vector by methods well known to a person skilled in the art such as, for example, those that are described in *MOLECULAR CLONING: A LABORATORY MANUAL*, Sambrook et al., 4th Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001.

10 The recombinant vector can include nucleotide sequences that control the regulation of the polynucleotide expression as well as nucleotide sequences permitting the expression and the transcription of a polynucleotide of the invention and the translation of a polypeptide of the invention, these sequences being selected according to the host cells that are used.

15 Thus, for example, an appropriate secretion signal can be integrated in the recombinant vector so that the polypeptide, encoded by the nucleic acid molecule of the invention, will be directed towards the lumen of the endoplasmic reticulum, towards the periplasmic space, on the membrane or towards the extracellular environment. The choice of an appropriate secretion signal may facilitate subsequent protein purification.

In a further embodiment, it is provided a host cell comprising a recombinant vector according to the invention.

20 The introduction of the recombinant vector in a host cell can be carried out according to methods that are well known to a person skilled in the art, such as those described in *BASIC METHODS IN MOLECULAR BIOLOGY*, Davis et al., 2nd ed., McGraw-Hill Professional Publishing, 1995, and *MOLECULAR CLONING: A LABORATORY MANUAL*, supra, such as transfection by calcium phosphate, transfection by DEAE 25 dextran, transfection, microinjection, transfection by cationic lipids, electroporation, transduction or infection.

The host cell can be, for example, bacterial cells such as *Escherichia coli* or *Streptomyces*, cells of fungi such as *Aspergillus* and yeasts such as *Saccharomyces*, insect cells, Chinese Hamster Ovary (CHO) cells, C127 mouse cell line, BHK cell line 30 of Syrian hamster cells, Human Embryonic Kidney 293 (HEK 293) cells. In a particular embodiment, the host cell is a CHO cell or a HEK 293 cell.

The host cells can be used, for example, to express a polypeptide of the invention. After purification by standard methods, the polypeptide of the invention can be used in a method described hereinafter.

For instance, when expression systems that secrete the recombinant protein are 5 employed, the culture medium may first be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration matrix. Alternatively, an anion exchange and/or an affinity resin can be employed. The matrices can be acrylamide, agarose, dextran, 10 cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media can be employed to further purify the antibodies or fragments thereof. Some or all of the foregoing purification steps, in various combinations, are well known 15 and can be employed to provide a substantially homogeneous recombinant protein.

Recombinant protein produced in bacterial culture can be isolated by initial disruption of the host cells, centrifugation, extraction from cell pellets if an insoluble polypeptide, or from the supernatant fluid if a soluble polypeptide, followed by one or more concentration, salting-out, ion exchange, affinity purification or size exclusion 20 chromatography steps. Microbial cells can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

In another embodiment, the invention provides a process for producing cells capable of expressing a polypeptide according to the invention, comprising genetically engineering 25 cells with a recombinant expression vector or a nucleic acid according to the invention.

In another embodiment, the invention provides a process for producing antibodies or fragments thereof according to the invention comprises culturing a host cell transformed with an expression vector comprising a nucleic sequence that encodes said antibodies or fragments thereof under conditions sufficient to promote expression of said 30 polypeptides. The antibody or fragment thereof according to the invention is then recovered from culture medium or cell extracts, depending upon the expression system employed. As known to the skilled artisan, procedures for purifying a recombinant

protein will vary according to such factors as the type of host cells employed and whether or not the recombinant protein is secreted into the culture medium as described above.

Compositions

5 The invention provides pharmaceutical or therapeutic agents as compositions and methods for treating a patient, preferably a mammalian patient, and most preferably a human patient who is suffering from a medical disorder, and in particular an IL-15 related disease or disorder such as an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or an infectious 10 disease caused by parasitic, viral or bacterial pathogens. Alternatively, the invention provides methods for preventing a medical disorder, and in particular an IL-15 related disease or disorder such as an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens.

15 In one embodiment, is provided a pharmaceutical composition comprising one or more of: (i) an antibody binding to IL-15 or antigen-binding fragment thereof according to the invention, (ii) a nucleic acid according to the invention, (iii) a vector according to the invention, and/or (iv) a host cell according to the invention, and at least one pharmaceutically acceptable carrier.

20 Pharmaceutical compositions of the invention can contain one or more antibodies binding IL-15 or antigen-binding fragments thereof in any form described herein. Compositions of this invention may further comprise one or more pharmaceutically acceptable additional ingredient(s) such as alum, stabilizers, antimicrobial agents, buffers, coloring agents, flavoring agents, adjuvants, and the like.

25 The compounds of the invention, together with a conventionally employed adjuvant, carrier, diluent or excipient may be placed into the form of pharmaceutical compositions and unit dosages thereof, and in such form may be employed as solids, such as tablets or filled capsules, freeze-dried forms, or liquids such as solutions, suspensions, emulsions, elixirs, or capsules filled with the same, all for oral use, or in 30 the form of sterile injectable solutions for parenteral (including subcutaneous) use. Such pharmaceutical compositions and unit dosage forms thereof may comprise ingredients in conventional proportions, with or without additional active compounds or principles,

and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed.

Compositions of this invention may be liquid formulations including, but not limited to, aqueous or oily suspensions, solutions, emulsions, syrups, and elixirs. Liquid forms

5 suitable for oral administration may include a suitable aqueous or non-aqueous vehicle with buffers, suspending and dispensing agents, colorants, flavors and the like. The compositions may also be formulated as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain additives including, but not limited to, suspending agents, emulsifying agents, non-aqueous 10 vehicles and preservatives. Suspending agent include, but are not limited to, sorbitol syrup, methylcellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents include, but are not limited to, lecithin, sorbitan monooleate, and acacia. Nonaqueous vehicles include, but are not limited to, edible oils, almond oil, 15 fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol. Preservatives include, but are not limited to, methyl or propyl p-hydroxybenzoate and sorbic acid. Further materials as well as processing techniques and the like are set out in Part 5 of 20 *Remington's The Science and Practice of Pharmacy, 22nd Edition, 2012, Pharmaceutical Press and the University of the Sciences, Philadelphia College of Pharmacy*, which is incorporated herein by reference.

Solid compositions of this invention may be in the form of tablets or lozenges formulated in a conventional manner. For example, tablets and capsules for oral administration may contain conventional excipients including, but not limited to,

25 binding agents, fillers, lubricants, disintegrants and wetting agents. Binding agents include, but are not limited to, syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and polyvinylpyrrolidone. Fillers include, but are not limited to, lactose, sugar,

microcrystalline cellulose, maizestarch, calcium phosphate, and sorbitol. Lubricants include, but are not limited to, magnesium stearate, stearic acid, talc, polyethylene 30 glycol, and silica. Disintegrants include, but are not limited to, potato starch and sodium starch glycollate. Wetting agents include, but are not limited to, sodium lauryl sulfate.

Tablets may be coated according to methods well known in the art.

Injectable compositions are typically based upon injectable sterile saline or phosphate-buffered saline or other injectable carriers known in the art.

Compositions of this invention may also be formulated as transdermal formulations comprising aqueous or non-aqueous vehicles including, but not limited to, creams, 5 ointments, lotions, pastes, medicated plaster, patch, or membrane.

Compositions of this invention may also be formulated for parenteral administration including, but not limited to, by injection or continuous infusion. Formulations for injection may be in the form of suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulation agents including, but not limited to, suspending,

10 stabilizing, and dispersing agents. The composition may also be provided in a powder form for reconstitution with a suitable vehicle including, but not limited to, sterile, pyrogen-free water.

Compositions of this invention may also be formulated as a depot preparation, which may be administered by implantation or by intramuscular injection. The compositions

15 may be formulated with suitable polymeric or hydrophobic materials (as an emulsion in an acceptable oil, for example), ion exchange resins, or as sparingly soluble derivatives (as a sparingly soluble salt, for example).

The compounds of this invention can also be administered in sustained release forms or from sustained release drug delivery systems. A description of representative sustained

20 release materials can also be found in the incorporated materials in *Remington's Pharmaceutical Sciences*.

Injectable formulations are particularly appropriate for administering the compositions according to the invention.

In another embodiment, the invention provides an imaging composition or diagnostic 25 composition comprising an antibody binding IL-15 or an antigen-binding fragment thereof as described herewith.

The imaging composition or diagnostic composition according to the invention is useful for detecting elevated levels of IL-15 associated with an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or

30 an infectious disease caused by parasitic, viral or bacterial pathogens.

Combination

According to the invention, an antibody binding IL-15 or an antigen-binding fragment thereof according to the invention can be administered alone or in combination with a co-agent useful in the prevention and/or treatment of an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens, for example immune modulatory drugs including biologics, small molecules, and vaccines.

Alternatively, an antibody for IL-15 or an antigen-binding fragment thereof according to the invention can be administered or in combination with a co-agent useful in the treatment of cancer, for example an anti-cancerous drug such as cytotoxic drugs, tyrosine kinase inhibitors imatinib (Gleevec/Glivec) or gefitinib (Iressa), and therapeutic antibodies such as trastuzumab (Herceptin) or anti-CD20 antibody rituximab (Rituxan).

The invention encompasses the administration of an antibody for IL-15 or an antigen-binding fragment thereof wherein the antibody or fragment thereof is administered to an individual prior to, simultaneously or sequentially with other therapeutic regimens or co-agents useful in the prevention and /or treatment of an IL-15 related disease or disorder such as an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens, in a therapeutically effective amount. The antibody for IL-15 or an antigen-binding fragment thereof according to the invention that are administered simultaneously with said co-agents can be administered in the same or different compositions and in the same or different routes of administration.

In a particular embodiment, an antibody for IL-15 or an antigen-binding fragment thereof according to the invention can be administered in combination with a compound lowering intestinal inflammation, and/or protecting intestinal mucosa, and/or lowering the immune reactivity of gluten peptides, and/ or modifying the gut microbiota for the treatment of subjects suffering from celiac disease.

In a particular embodiment, an antibody for IL-15 or an antigen-binding fragment thereof according to the invention can be administered in combination with a compound lowering intestinal inflammation, and/or protecting intestinal mucosa, and/or lowering

the immune reactivity of gluten peptides, and/ or modifying the gut microbiota for the treatment of subjects suffering from refractory celiac disease.

Mode of administration

Compositions of this invention may be administered in any manner including, but not limited to, orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal or intranasal administration or intra bladder, or combinations thereof.

Parenteral administration includes, but is not limited to, intravenous, intra-arterial, intra-peritoneal, subcutaneous, intramuscular, intra-thecal, and intra-articular. The compositions of this invention may also be administered in the form of an implant, which allows slow release of the compositions as well as a slow controlled i.v. infusion. In a particular embodiment, an antibody for IL-15 or antigen-binding fragment thereof according to the invention is administered systemically or locally.

In a particular embodiment, an antibody for IL-15 or antigen-binding fragment thereof according to the invention is administered by subcutaneous or intravenous route.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinetic properties, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

Typically, therapeutically effective amounts of a pharmaceutically active antibody range from 0.5 mg/kg up to 50 mg/kg body weight dose. If the regimen is a continuous infusion, it may be in the range of 0.250 mg/kg up to 13 mg/kg of body weight.

Patients

In an embodiment, patients according to the invention are patients suffering from an IL-15 related disease or disorder such as autoimmune disease and/or inflammatory disorder including rheumatoid arthritis, psoriasis, celiac disease such as refractory celiac disease, sarcoidosis, inflammatory bowel disease (e.g. ulcerative colitis, Crohn's disease), hepatitis C-induced liver diseases, multiple sclerosis, auto-immune hepatitis, primary

biliary cirrhosis, sclerosing cholangitis, biliary atresia, alopecia areata, transplant rejection response, inflammatory diseases of the central nervous system, and eosinophilic esophagitis and metabolic conditions such as hypermetabolic conditions.

In a particular embodiment, patients according to the invention are patients suffering from celiac disease.

In a particular embodiment, patients according to the invention are patients suffering from refractory celiac disease.

5 In a particular embodiment, patients according to the invention are patients suffering from eosinophilic esophagitis.

In a particular embodiment, patients according to the invention are patients suffering from auto-immune hepatitis.

In another embodiment, patients according to the invention are patients suffering from a
10 malignancy including T-cell leukemia, such as cutaneous T-cell lymphoma (CTCL)
(e.g. mycosis fungoides, Sezary syndrome), lymphoproliferative disorder of granular
lymphocytes (LDGL), large granular lymphocytic leukemia, and acute lymphocytic
leukemia (ALL), pre-B cell leukemia, osteosarcoma, Ewing sarcoma,
rhabdomyosarcoma, melanoma, small cell lung cancer, renal cell carcinoma,
15 glioblastoma, neuroblastoma, and mesothelioma.

In a particular embodiment, patients according to the invention are patients suffering from large granular lymphocytic leukemia.

In a particular embodiment, patients according to the invention are patients suffering from acute lymphocytic leukemia.

20 In another embodiment, patients according to the invention are patients suffering from a transplant rejection.

In a particular embodiment, patients according to the invention are patients suffering from an infectious disease caused by parasitic, viral or bacterial pathogens.

25 In a particular embodiment, patients according to the invention are patients suffering from an infectious disease caused by Hantavirus (Hantaan virus) such as hantavirus hemorrhagic fever with renal syndrome and/or hantavirus pulmonary syndrome.

In a still other embodiment, patients according to the invention are patients suffering from a hypermetabolic condition including sickle cell disease and cancer-associated cachexia.

30 In a still other embodiment, patients according to the invention are patients suffering from inflammatory diseases of the central nervous system.

In a still other embodiment, patients according to the invention are patients suffering from amyloid related disorders such as Alzheimer's disease.

Uses and methods according to the invention

The antibody binding IL-15 or antigen-binding fragment thereof, the nucleic acids, the vectors, the host cells, the compositions according to the invention are for use in the diagnosis, prevention or treatment of disorders associated with, caused by, or accompanied by elevated levels of IL-15 and/or elevated IL-15 activity.

In one embodiment is provided an antibody to IL-15 or antigen-binding fragment thereof according to the invention for use as a medicament.

10 Another embodiment provides an antibody or fragment thereof according to the invention for use in the prevention and/or treatment of an IL-15 related disease or disorder such as an autoimmune disease and/or inflammatory disorder, in particular rheumatoid arthritis, psoriasis, celiac disease, such as refractory celiac disease, sarcoidosis, inflammatory bowel disease (e.g. ulcerative colitis, Crohn's disease),
15 hepatitis C-induced liver diseases, multiple sclerosis, auto-immune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary atresia, alopecia areata, transplant rejection response, inflammatory diseases of the central nervous system, and eosinophilic esophagitis.

Another embodiment provides an antibody or fragment thereof according to the invention for use in the prevention and/or treatment of a malignancy, in particular T-cell leukemia, such as cutaneous T-cell lymphoma (CTCL) (e.g. mycosis fungoides, Sezary syndrome), lymphoproliferative disorder of granular lymphocytes (LDGL), large granular lymphocytic leukemia, and acute lymphocytic leukemia (ALL), pre-B cell leukemia, osteosarcoma, Ewing sarcoma, rhabdomyosarcoma, melanoma, small cell 25 lung cancer, renal cell carcinoma, glioblastoma, neuroblastoma, and mesothelioma.

A still other embodiment provides an antibody or fragment thereof according to the invention for use in the prevention and/or treatment of transplant rejection, a metabolic condition (such as a hypermetabolic condition) and/or an infectious disease caused by parasitic, viral or bacterial pathogens.

30 In one embodiment is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating an IL-15 related disease or disorder such as

an autoimmune disease and/or inflammatory disorder, in particular rheumatoid arthritis, psoriasis, celiac disease, such as refractory celiac disease, sarcoidosis, inflammatory bowel disease (e.g. ulcerative colitis, Crohn's disease), hepatitis C-induced liver diseases, multiple sclerosis, auto-immune hepatitis, primary biliary cirrhosis, sclerosing 5 cholangitis, biliary atresia, alopecia areata, inflammatory diseases of the central nervous system, and eosinophilic esophagitis.

In a specific embodiment is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating celiac disease, in particular refractory celiac 10 disease.

In a particular embodiment, is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating eosinophilic esophagitis.

In a particular embodiment, is provided a use of an antibody for IL-15 or antigen-15 binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating auto-immune hepatitis.

In one embodiment is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating a malignancy, in particular T-cell leukemia 20 such as acute lymphocytic leukemia, large granular lymphocytic leukemia, cutaneous T-cell lymphoma (CTCL) (e.g. mycosis fungoides, Sezary syndrome), and lymphoproliferative disorder of granular lymphocytes (LDGL), pre-B cell leukemia, osteosarcoma, Ewing sarcoma, rhabdomyosarcoma, melanoma, small cell lung cancer, renal cell carcinoma, glioblastoma, neuroblastoma, and/or mesothelioma.

25 In an alternative embodiment is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating large granular lymphocytic leukemia.

30 In an alternative embodiment is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating acute lymphocytic leukemia.

In a specific embodiment is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating transplant rejection, a metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens.

5 In a specific embodiment is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating hantaviruses infections.

In a specific embodiment is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical 10 composition for preventing and/or treating an inflammatory disease of the central nervous system.

In a specific embodiment is provided a use of an antibody for IL-15 or antigen-binding fragment thereof according to the invention for the preparation of a pharmaceutical composition for preventing and/or treating Alzheimer's disease.

15 In another embodiment is provided a method for preventing and/or treating an IL-15 related disease or disorder such as an autoimmune disease and/or inflammatory disorder, in particular rheumatoid arthritis, psoriasis, celiac disease, in particular refractory celiac disease, sarcoidosis, inflammatory bowel disease (e.g. ulcerative colitis, Crohn's disease), hepatitis C-induced liver diseases, multiple sclerosis, auto-20 immune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary atresia, alopecia areata, inflammatory diseases of the central nervous system, and eosinophilic esophagitis, comprising administering a therapeutically effective amount of an antibody to IL-15 or antigen-binding fragment thereof according to the invention, to a subject in need thereof.

25 In a particular embodiment is provided a method of preventing and/or treating celiac disease, comprising administering a therapeutically effective amount of an antibody for IL-15 or antigen-binding fragment thereof according to the invention, to a subject in need thereof.

In an alternative embodiment is provided a method of preventing and/or treating a 30 malignancy, in particular T-cell leukemia, such as acute lymphocytic leukemia, large granular lymphocytic leukemia, cutaneous T-cell lymphoma (CTCL) (e.g. mycosis fungoides, Sezary syndrome), and lymphoproliferative disorder of granular

lymphocytes (LDGL), pre-B cell leukemia, osteosarcoma, Ewing sarcoma, rhabdomyosarcoma, melanoma, small cell lung cancer, renal cell carcinoma, glioblastoma, neuroblastoma, and/or mesothelioma, comprising administering a therapeutically effective amount of an antibody to IL-15 or antigen-binding fragment thereof according to the invention, to a subject in need thereof.

In a particular embodiment is provided a method of preventing and/or treating large granular lymphocytic leukemia, comprising administering a therapeutically effective amount of an antibody to IL-15 or antigen-binding fragment thereof according to the invention, to a subject in need thereof.

- 5 10 In another embodiment is provided a method for preventing and/or treating transplant rejection, a metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens, comprising administering a therapeutically effective amount of an antibody to IL-15 or antigen-binding fragment thereof according to the invention, to a subject in need thereof.
- 15 In an alternative embodiment is provided a method of detecting IL-15 in a biological sample comprising contacting a biological sample from a subject with an antibody to IL-15 or antigen-binding fragment thereof according to the invention.

As used herewith “biological sample” refers to cells, tissue samples or cell components (such as cellular membranes or cellular components) obtained from a subject, in 20 particular from a subject suspected of, or suffering from, an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens, or at high risk of developing such a disorder.

Examples of biological sample include blood, serum, plasma, cerebrospinal fluid, 25 synovial fluid, and tissue samples including cells isolated from said tissue. Tissue samples include formalin-fixed or frozen tissue sections.

Any suitable method for detection and analysis of IL-15 can be employed, including diagnostic assay techniques known in the art such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either 30 heterogeneous or homogeneous phases.

In a particular embodiment, the invention provides an *ex vivo* method for detecting the presence and/or concentration of IL-15 protein in a biological sample, comprising the steps of:

- (i) Providing a biological sample from a subject,
- 5 (ii) Reacting said biological sample with at least one antibody or antigen-binding fragment thereof according to the invention, under conditions sufficient for binding IL-15 protein present in said biological sample to said at least one antibody or fragment thereof through antigen-antibody interactions; and
- 10 (iii) Detecting a signal proportional to the level of antigen-antibody complex formed in step (ii),

wherein the intensity of the signal correlates with the concentration of IL-15 protein in the biological sample.

More particularly, it is provided an *ex-vivo* method of prognosis or diagnosis of an 15 autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens, associated with an elevated level of IL-15 from a biological sample of a subject comprising the steps of:

- (a) Providing a biological sample from a subject;
- 20 (b) Bringing said biological sample into contact with a solid matrix where at least one antibody or fragment thereof according to the invention is bound to, wherein the contacting is under conditions sufficient for binding IL-15 protein present in said biological fluid sample to said at least one antibody or fragment thereof through antigen-antibody interactions;
- (c) Removing any unbound IL-15 protein from the surface of said solid matrix;
- 25 (d) Detecting a signal proportional to the level of antigen-antibody complex bound to said solid matrix,
- (e) Comparing the level of signal detected in step (d) with the level of signal detected in the same conditions with a negative control,

30 wherein a level of signal detected in the subject's sample that is higher than the level of signal detected in the negative control is indicative of an elevated level of IL-15 associated with an autoimmune disease and/or inflammatory disorder, a

malignancy, transplant rejection, a metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens.

Kit

One aspect of the invention relates to a kit comprising at least one antibody or antigen-binding fragment thereof according to the invention, and/or at least one nucleic acid encoding said antibody or fragment thereof, and/or at least one recombinant vector comprising said nucleic acid, and/or at least one host cell according to the invention, and optionally instructional material.

In a particular embodiment, the kit according to the invention comprises at least one antibody or antigen-binding fragment thereof according to the invention, to be coupled or already coupled to a solid matrix.

Examples of solid matrix suitable for the invention include any solid phase support suitable for carrying out an immunoassay or a method according to the invention, such as beads, microparticles, nanoparticles, tubes, fabrics or plates, films, slides, wells, formed from or coated with glass, polystyrene, polypropylene, nitrocellulose, quartz, ceramic, dextran or other materials. For example, the solid matrix is in a form of microtiter wells, such as a 96-well microtiter plate.

The fixation of the antibodies or fragments thereof according to the invention to the solid matrix in a kit according to the invention may be carried out by adsorption or chemical coupling to a solid phase support. Any mean known in the art for immobilizing a protein or peptide to a solid support can be used. The antibodies or fragments thereof according to the invention can be either covalently or non-covalently bound to the solid matrix by techniques such as covalent binding via an amide or ester linkage or adsorption. Peptides can be bound using binding pairs such as biotin and avidin or antibody and antigen. After the peptides are affixed to the solid matrix, the solid matrix can be incubated with a blocking solution (containing a blocking protein such as bovine serum albumin) to reduce non-specific adsorption of antibodies in a test sample to the support surface. According to one aspect, the antibodies or fragments thereof according to the invention can be synthesized directly on the solid matrix of the kit of the invention.

According to one embodiment, when the kit comprises at least one antibody or fragment thereof according to the invention or a combination thereof for coupling to a solid

matrix as solid phase support, the kit further optionally comprises coupling reagents and/or a solid matrix for performing an immunoassay.

According to another further embodiment, the kit according to the invention further comprises at least one rinsing reagent for washing unbound material before detection in order to avoid background noise detection. Typically rinsing reagents comprise standard buffers known in the art.

References cited herein are hereby incorporated by reference in their entirety. The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustration of individual aspects of the invention,

and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

The invention having been described, the following examples are presented by way of illustration, and not limitation.

EXAMPLES

Example 1. Generation and isolation of the anti-IL-15 antibodies according to the invention

1. Production and sequencing of mouse B-E29

The antibodies according to the invention derive from the commercially available mouse B-E29 antibody, also described in *Bernard et al, 2004, supra*, herein referred to comparative antibody 1. Briefly, mouse monoclonal antibodies, specific for human IL-15, are originally generated by immunizing BALB/c mice with recombinant human IL-15 made in *E. coli* (Peprotech 200-15). Spleens from immunized mice are fused with X6.3.AG.8653 mouse myeloma cell line and hybridoma generated using conventional techniques. Hybridoma supernatants were screened for the presence of IL-15-binding antibodies using an ELISA technique, followed by cloning dilution and isotype determination. The mouse anti-IL-15 monoclonal antibody B-E29, of IgG1 (heavy chain) κ (light chain) isotypes, was selected using this methodology, as well as other anti-IL-15 antibodies. However, when assayed for the inhibition of the proliferation of Kit 225 T cells stimulated with recombinant IL-15, only the B-E29 antibody was able to

block this activity, thus B-E29 neutralizes the biological activity of IL-15 whereas other anti-IL-15 antibodies do not (data not shown).

The variable domain of the heavy chain and the variable domain of the light chain of mouse B-E29 antibody were sequenced using standard protocols. Briefly, messenger RNA (mRNA) was extracted from B-E29 hybridoma cell pellets using conventional RNA extraction and purification protocols. cDNA was created from the RNA by reverse-transcription with an oligo(dT) primer. The VH and VL regions of the monoclonal antibody DNA was amplified by PCR reactions. The VH and VL products were cloned into the Invitrogen sequencing vector pCR2.1 and transformed into TOP10 10 cells and screened for positive transformants by PCR. Selected colonies were picked and analyzed through sequencing. The amino acid sequence of the variable domain of the heavy chain (VH) of mouse B-E29 antibody is provided as SEQ ID NO: 32. The amino acid sequence of the variable domain of the light kappa chain (VL) of mouse B-E29 antibody is provided as SEQ ID NO: 36.

15 2. *Humanization of anti-IL-15 antibodies derived from mouse B-E29*

Using antibody-numbering systems from IMGT (international ImMunoGeneTics information system for immunoglobulins or antibodies) and Kabat, the frameworks and CDRs of the B-E29 mouse antibody were identified. Online databases of Human IgG sequences were searched for comparison to the mouse domains using BLAST search 20 algorithms, and human variable domain frameworks selected from the top 100 BLAST results. These were reduced based on a combination of framework homology, maintaining key framework residues and canonical loop structure. Several humanized, chimeric and combinatorial antibodies were constructed, among which some had mutations on unusual residues located in the N-terminal portion of the mouse VH 25 region, with the aim of selecting the “best” human VH and VL chains for forming antibodies retaining or increasing their binding capacity to IL-15 as well as retaining or increasing the efficiency of their production, compared to mouse B-E29 antibody (comparative antibody 1).

An alignment of the amino acid sequences of the different humanized and chimeric 30 variants of the heavy chain variable domain of mouse B-E29 is shown in Figure 1 (A).

An alignment of the amino acid sequences of the different humanized and chimeric variants of the light chain variable domain of mouse B-E29 is shown in Figure 1 (B).

The VH domain of each of the above-mentioned variants was synthesized in-frame with a human IgG1 isotype constant domain (allotype G1m3) sequence of SEQ ID NO: 30.

The VL domain of each of the above-mentioned variants was synthesized in-frame with a human IgK isotype constant domain (allotype Km3) sequence of SEQ ID NO: 31.

5 Both the heavy chain and light chain coding sequences were cloned into the pVITRO-DHFR3 vector backbone for antibody production. Plasmids were co-transfected into suspension-adapted CHO (Chinese Hamster Ovary) cells (CHO-S cells, Invitrogen, UK). After 7 days, the culture supernatant was harvested and antibodies purified using an Amersham Biosciences AKTA Chromatography system and HiTrap Protein A
10 column.

The combinations cVH3:cVK1 and cVH4:cVK1 did not result in sufficient antibody production to be further tested. In an ELISA assay, the combinations cVH1:cVK1, hVH1:cVK1, cVH1:hVK1 showed similar binding to human IL-15, better than that observed with hVH1:hVK2 and cVH1:hVK2 (data not shown).

15 These results demonstrate that among the variants tested, hVH1 and hVK1 are good candidates to form antibodies which can be efficiently produced and which bind IL-15. Therefore, they were used as starting material for further optimization.

3. Optimization of humanized anti-IL-15 antibodies

Optimization consisted in changing some amino acids or amino acid motifs within the
20 CDRs and/or frameworks of hVH1 (also called “huVH1” herewith) and hVK1 (also called “huVL1” herewith), which might lead to chemical instability or aggregation of antibodies, as well as retaining or increasing the efficiency of their production compared to mouse B-E29 antibody (comparative antibody 1). This strategy allowed the generation of variants of huVH1/huVL1 antibodies comprising the VH/VL regions
25 indicated in above Table 1.

The VH domain of each of the variants indicated in Table 1 was synthesized in-frame with a human IgG1 isotype constant domain (allotype G1m3) sequence of SEQ ID NO: 30.

30 The VL domain of each of the variants indicated in Table 1 was synthesized in-frame with a human IgK isotype constant domain (allotype Km3) sequence of SEQ ID NO: 31.

Recombinant antibodies were produced in transient CHO system and further tested as culture supernatants or after purification through a protein-A FPLC column.

Example 2. Binding potency of various anti-IL-15 antibodies according to the invention for IL-15

5 Some of the antibodies according to the present invention were tested for their ability to bind plate-bound recombinant human or monkey IL-15 in an ELISA assay. Fully human antibody of the prior art (146B7 described in WO 03/017935, herein referred to comparative antibody 2) was produced in the same expression system, purified and used as control.

10 Maxisorp plates were coated with recombinant human IL-15 (Prospec cat no. CYT230) or Rhesus macaque IL-15 (MyBiosource cat. no. MBS948894) at 100 ng/well in carbonate coating buffer for 2 hours at 37°C. Since published sequences of Rhesus macaque and Cynomolgus monkey IL-15 are identical, such recombinant IL-15 protein was referred to as Macaque Monkey IL-15. Tested human IL-15 recombinant protein has a sequence identical to that provided in SEQ ID NO: 1 but with an additional methionine at the N-terminal position as usually done for expressing recombinant proteins in *E. Coli*. Tested recombinant Macaque Monkey IL-15 was produced in yeast and has a sequence identical to that provided in SEQ ID NO: 4 but with an additional N-terminal His tag.

15 The plate was then blocked with 2% normal goat serum for 30 min at 37°C and washed 6 times with PBS-Tween (0.05% Tween-20 in PBS, v/v). Test antibodies were added to the plate in triplicate at various dilutions in PBS-Tween and the plate was incubated with gentle rocking at room temperature for 2 hours. The plate was then washed 6 times with PBS-Tween and an appropriate dilution of goat anti-human IgG antibody (Millipore cat. no. AP309P) conjugated to horseradish peroxidase (HRP) was added to the plate and left rocking at room temperature for 1 hour. The plate was then washed 6 times with PBS-Tween and incubated with TMB substrate. The reaction was stopped by the addition of 1M H₂SO₄ and read at 450 nm. Dose response curves of absorbance versus concentration were plotted and analyzed to determine half-maximal binding

20 concentrations (BC₅₀) using the Graphpad Prism software. BC₅₀ values expressed as molarity were extrapolated from concentration in ng/ml using a 150 kDa molecular weight value for all antibodies.

25

30

Representative BC₅₀ values obtained for several humanized variants of B-E29, as well as fully human 146B7 antibody used as control, are indicated in Table 2.

Table 2

Antibody	Human IL-15 BC ₅₀ (nM)	Macaque Monkey IL-15 BC ₅₀ (nM)
146B7 (comparative antibody 2)	8.407	0.733
huB-E29-1	1.333	0.487
huB-E29-2	1.467	0.340
huB-E29-3	2.620	NT
huB-E29-4	1.013	0.500
huB-E29-5	1.473	0.453
huB-E29-6	1.840	NT
huB-E29-7	0.840	0.433
huB-E29-8	1.787	NT
huB-E29-9	1.987	NT
huB-E29-10	0.507	0.353
huB-E29-11	0.980	NT
huB-E29-12	0.593	NT
huB-E29-13	3.173	NT
huB-E29-14	0.813	0.347
huB-E29-15	2.533	NT
huB-E29-16	2.007	NT
huB-E29-17	2.613	NT
huB-E29-18	0.693	0.367
huB-E29-19	2.207	NT
huB-E29-22	3.867	NT
huB-E29-24	0.647	0.113
huB-E29-30	5.867	NT
huB-E29-31	3.514	NT
huB-E29-34	3.887	NT

NT: not tested

5 As shown in Table 2, while some antibody variants according to the invention had a binding activity comparable to each other, others showed improved binding to human IL-15 (e.g. huB-E29-10 and huB-E29-24). Figure 2A shows that a representative humanized B-E29 variant (huB-E29-1) displays not only better potency in binding to human IL-15 than the 146B7 antibody, but also a higher maximal signal suggesting that

10 the efficacy at binding IL-15 is improved. This improved capacity to bind human IL-15 is further confirmed in example 3, Table 3.

In the cases where binding to Macaque Monkey IL-15 was tested, all antibody variants according to the invention which bind to human IL-15 also bind to Macaque Monkey IL-15, with slightly higher potencies.

**Example 3. Binding kinetics and affinity for human IL-15 with some of the anti-
5 IL-15 antibodies according to the invention**

The association rate (k_a), dissociation rate (k_d) and equilibrium dissociation constant (K_D) of three humanized B-E29 antibody variants according to the invention towards recombinant human IL-15 were determined by surface plasmon resonance (SPR).

Human IL-15 (Prospec cat no. CYT230) was immobilized onto a CM5 chip (GE Healthcare) using standard amine coupling, and antibodies injected at various concentrations in 120 μ l phosphate buffer saline with a flow rate of 40 μ l /ml. Binding kinetics were analyzed on a Biacore 3000 apparatus (GE Healthcare) with a 300 seconds dissociation period. Reference signal and 0 nM antibody concentration signal were substracted and data fitted using a 1:1 (Langmuir) binding model.

15

Table 3

Antibody	k_a ($M^{-1}.s^{-1}$)	k_d (s^{-1})	K_D (pM)	Rmax (RU)
huB-E29-2	1.15×10^6	1.07×10^{-5}	9.4	214
huB-E29-10	1.43×10^6	4.23×10^{-4}	295	212
huB-E29-24	1.75×10^6	5.74×10^{-4}	329	167
146B7 (comparative antibody 2)	1.44×10^6	5.26×10^{-5}	36.7	57.3

RU: Relative units

While the on-rate (k_a , association rate) of all compared humanized B-E29 antibodies was similar, the off-rate (k_d , dissociation rate) of huB-E29-2 was slower, resulting in a lower equilibrium dissociation constant K_D (Table 3). The 146B7 antibody also showed a low K_D , similar to what reported (*Villadsen, et al, 2003, J. Clin. Invest. 112:1571–1580*). However, the maximal (Rmax) signal given by the association of 146B7 on human IL-15 coated chip was much lower than that observed with the three humanized B-E29 antibody variants. This result is similar to what was observed by ELISA in example 2 and suggests that these three humanized B-E29 antibody variants have improved binding capacity to human IL-15, compared to the 146B7 antibody.

25

Example 4. Species-specificity of some anti-IL-15 antibodies according to the invention

Antibodies according to the present invention were tested for their ability to bind plate-bound recombinant mouse or rat IL-15 in an ELISA assay.

5 Maxisorp plates were coated with recombinant mouse IL-15 (R&D Systems cat. no. 447-ML) or rat IL-15 (Sigma cat. no. SRP4172) at 100 ng/well in carbonate coating buffer for 2 hours at 37°C. Tested IL-15 recombinant proteins have a sequence identical to that provided in SEQ ID NO: 2 (mouse IL-15) and SEQ ID NO: 3 (rat IL-15) but with an additional methionine at the N-terminal position as usually done for expressing
10 recombinant proteins in *E. Coli*.

The plate was then blocked with 2% normal goat serum for 30 min at 37°C and washed 6 times with PBS-Tween (0.05% Tween-20 in PBS, v/v). Test antibodies were added to the plate in triplicate at various dilutions in PBS-Tween and the plate was incubated with gentle rocking at room temperature for 2 hours. As positive controls, rabbit-anti-
15 mouse IL-15 (Acris cat. no. AP01124PU-S) or rabbit anti-rat IL-15 (Biovision cat. no. 5172) antibodies were tested at the same concentrations. The plate was then washed 6 times with PBS-Tween and an appropriate dilution of HRP-conjugated goat anti-human IgG antibody (Millipore cat. no. AP309P) for humanized antibodies, or goat-anti-rabbit IgG antibody (Sigma cat. no. A 4416) for the positive controls was added and the plate
20 was left rocking at room temperature for 1 hour. The plate was then washed 6 times with PBS-Tween and incubated with TMB substrate. The reaction was stopped by the addition of 1M H₂SO₄ and read at 450 nm. Dose response curves of absorbance versus concentration were plotted using the Graphpad Prism software.

As shown in Figure 2B and Figure 2C, none of the tested humanized variants of B-E29
25 showed significant binding, at the concentrations tested, to mouse or rat IL-15, while good binding was observed with the positive controls.

Example 5. Selectivity of some anti-IL-15 antibodies according to the invention

Since IL-2 is the cytokine closest to IL-15 by sequence homology, and IL-2 and IL-15 share two common receptor chains (the IL-2/IL-15R β chain and the common γ c chain),
30 the binding of anti-IL-15 antibodies of the present invention to IL-2 was assessed by ELISA.

Maxisorp plates were coated with recombinant human IL-2 (R&D Systems cat. no. 202-IL-010) of SEQ ID NO: 38 at 100 ng/well in carbonate coating buffer for 2 hours at 37°C. The plate was then blocked with 2% normal goat serum for 30 min at 37°C and washed 4 times with PBS-Tween (0.05% Tween-20 in PBS, v/v). Test antibodies or 5 positive control biotinylated antibody (anti-IL-2 Novus Bio cat. no. NBP1-43491) were added to the plate in duplicate at 5 µg/ml in PBS-Tween and the plate was incubated with gentle rocking at room temperature for 2 hours. The plate was then washed 4 times with PBS-Tween and an appropriate dilution of goat anti-human IgG antibody 10 (Millipore cat. no. AP309P) conjugated to horseradish peroxidase (HRP) for test antibodies, or streptavidin-HRP for positive control antibody, was added to the plate and left rocking at room temperature for 1 hour. The plate was then washed 4 times with PBS-Tween and incubated with TMB (3,3',5,5'-tetramethylbenzidine) substrate. The reaction was stopped by the addition of 1 M H₂SO₄ and read at 450 nm.

Figure 3 shows that none of the tested anti-IL-15 antibodies according to the invention 15 binds human IL-2 at the 5 µg/ml concentration tested, whereas a positive control antibody showed strong binding.

Example 6. Inhibition of soluble IL-15-induced proliferation/survival in cell lines by some anti-IL-15 antibodies according to the invention

Antibodies according to the present invention were tested for their ability to inhibit IL-20 15-induced Kit 225 or M-07e cell proliferation/survival (*Finch et al, 2011, supra*).

The Kit 225 cell line was established from a patient with T cell chronic lymphocytic leukemia (*Hori et al., 1987, Blood, 70:1069-1072*). Kit 225 cells express the 3 chains of the IL-15 receptor (IL-15R α , IL-15R β and IL-15R γ) (*Mortier et al., 2006, J. Biol. Chem., 281:1612-1619*). The M-07e cell line was established from the peripheral blood 25 of a 6-month-old girl with acute megakaryoblastic leukemia (*Brizzi et al., 1990, Br J Haematol., 76:203-239*). M-07e cells express only the IL-15R β and IL-15R γ chains of the IL-15 receptor (*Meazza et al., 1998, Int. J. Cancer, 78:189-95*).

Kit 225 cells (*Hori et al., 1987, Blood, 70(4):1069-1072*) and M-07e cells (Leibniz-Institute DSMZ) were grown and maintained in RPMI 1640 medium supplemented with 30 10 mM Hepes, 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 mM L-Glutamine, 1 mM sodium pyruvate, 10% heat-inactivated Fetal Bovine Serum (FBS, PAA) and 200 U/ml human IL-2 (R&D Systems cat. no. 202-IL) of SEQ ID NO: 38. One day prior the

experiments, cells were starved in the same culture medium without IL-2 for 24 hours. After that, 5×10^4 cells were added per well in 96-well plates in triplicate, in the presence of various dilutions of test antibodies as wells as recombinant human IL-15 (R&D systems cat. no. 247-IL) or recombinant monkey IL-15 (My Biosource cat. no.

5 MBS948894) at 1 ng/ml final concentration and a total volume of 100 μ l culture medium per well. As mentioned above, because published sequences of Rhesus macaque and cynomolgus macaque IL-15 are identical, such recombinant IL-15 protein was referred as to Macaque Monkey IL-15.

Cell cultures were maintained for 48h at 37°C, under 5% CO₂ and then 100 μ l of 10 Titerglol solution (which measures ATP consumption, Promega) were dispensed to each well and contents mixed by vigorous pipetting. Plates were incubated for 15-20 min at room temperature before reading. Luminescence signal was read in a plate reader as a measure of cell survival. Using Graphpad Prism software, corrected values against antibody concentrations were plotted and half-maximum inhibitory concentrations 15 (IC₅₀) determined using log inhibitor versus response (three parameters). IC₅₀ values expressed as molarity were extrapolated from concentration in ng/ml using a 150 kDa molecular weight value for all antibodies.

Table 4

Antibody	IC ₅₀ in M-07e cells (nM)		IC ₅₀ in Kit 225 cells (nM)	
	Human IL-15	Macaque Monkey IL-15	Human IL-15	Macaque Monkey IL-15
huB-E29-2	0.017	0.23	0.083	0.112
hub-E29-10	4.2	41.3	17.0	17.5
hub-E29-24	19.1	10.4	29.3	26.7
146B7 comparative antibody 2	3.2	NT	3.7	NT

NT: not tested

20 As shown in Table 4, the three humanized B-E29 antibody variants tested as wells as 146B7 were able to inhibit IL-15-induced proliferation/survival of M-07e and Kit 225 cells. The humanized huB-E29-2 was superior in potency to all the other tested antibodies in M-07e cells, as well as in Kit 225 cells.

Example 7. Inhibition of *trans* IL-15-induced proliferation/survival in cell line by

25 **some anti-IL-15 antibodies according to the invention**

Some antibodies according to the present invention were tested for their ability to inhibit human IL-15-induced M-07e cell (expressing only IL-15R $\beta\gamma$) proliferation/survival, with IL-15 being presented after binding to a IL-15R α -Fc construct immobilized on plastic. This experimental set-up mimics the *trans* presentation of IL-15 described in the literature and seemingly important for the biology of IL-15 (Stonier, et al, 2010, *supra*).

Wells from 96-well plates were coated with 1 μ g recombinant human IL-15R α -Fc chimera (R&D Systems cat. No 7194-IR-050) in 100 μ l PBS for 2 hours at 37°C. Contents of wells were aspirated, then 150 μ l of RPMI 1640 medium supplemented with 10 mM Hepes, 100 IU/ml penicillin, 100 μ g/ml streptomycin, 2mM L-Glutamine, 1 mM sodium pyruvate, 10% heat-inactivated Fetal Bovine Serum (FBS, PAA), referred as culture medium, were added per well and plates were incubated for 30 min at 37°C. Contents of wells were aspirated, then 100 μ l of a 300 ng/ml solution of human IL-15 (R&D Systems) was added and plates were incubated for 1 hour at 37°C. Contents of wells were aspirated, then 200 μ l of culture medium was used twice to wash unbound IL-15. Various concentrations of test antibodies were added to each well in triplicate, then 5×10^4 M-07e cells (left overnight in medium without cytokine) were added. Control wells were adequately designed to make sure that IL-15 did not induce proliferation/survival in this system in absence of IL-15R α -Fc, and that IL-15R α -Fc did not induce proliferation/survival in absence of IL-15.

Cell cultures were maintained for 48h at 37°C, under 5% CO₂ and then 100 μ l of Titerglow solution (which measures ATP consumption, Promega) are dispensed to each well and contents mixed by vigorous pipetting. Plates were incubated for 15-20 min at room temperature before reading. Luminescence signal was read in a plate reader as a measure of cell survival. Using Graphpad Prism software, corrected values against antibody concentrations were plotted and half-maximum inhibitory concentrations (IC₅₀) were determined using log inhibitor versus response (three parameters). IC₅₀ values expressed as molarity were extrapolated from concentration in ng/ml using a 150 kDa molecular weight value for all antibodies.

Table 5

Antibody	IC ₅₀ in M07e cells for IL-15 <i>trans</i> presentation (nM)
huB-E29-2	0.37

Antibody	IC ₅₀ in M07e cells for IL-15 <i>trans</i> presentation (nM)
huB-E29-10	0.53
huB-E29-24	0.65
146B7	0.63
comparative antibody 2	

As shown in Table 5, all tested humanized B-E29 antibody variants inhibited *trans* presentation of human IL-15 to M-07e cells with similar potency.

Example 8: Absence of interference with the binding of human IL-15 to IL-15Ra of some anti-IL-15 antibodies according to the invention

Antibodies according to the present invention were tested for their ability to inhibit binding of biotinylated human IL-15 to a plate-bound IL-15-Ra-Fc recombinant construct (R&D Systems) according to the method described by Finch et al. (*Finch et al, 2011, supra*).

Interleukin-15Ra-Fc in phosphate-buffered saline (PBS) at a concentration of 600 pM was coated onto MaxiSorp 96-well plates by incubation at 4°C for 16 h. The wells were washed with PBS and blocked with PBS containing 3% (w/v) bovine serum albumin (BSA) for 2 h and washed again with PBS. Antibodies and controls (including an antibody known to block IL-15 binding to IL-15Ra, R&D Systems cat. no. NF150) were diluted in PBS with 0.1% (w/v) BSA and added to the IL-15Ra-Fc coated assay wells. Biotinylated human IL-15 (R&D Systems cat. no. NF150) at a final concentration of 100 pM was added and the assay plates incubated for 1 h. The plates were then washed three times with PBS containing 0.1% (v/v) Tween 20 followed by addition of an optimal dilution of streptavidin-peroxidase. After 30 min incubation the plates were washed seven times with PBS containing 0.1% (v/v) Tween 20 and peroxidase substrate added. Once the reaction completed and stopped with H₂SO₄, absorbance was measured at 450 nm emission wavelength.

Figure 4 shows that a positive control antibody was able to inhibit in a dose-dependent manner binding of biotinylated human IL-15 to IL-15-Ra-Fc while the anti-IL-15 antibodies huB-E29-2, huB-E29-10 and huB-E29-24 were not, up to the highest tested dose of 150 µg/ml. In contrast, based on the observations by *Bernard et al., 2004 supra*, monoclonal mouse anti-IL-15 antibody B-E29 (comparative antibody 1) prevents IL-15 binding to IL-15Ra.

Example 9: *In vivo* neutralization of human IL-15 of some anti-IL-15 antibodies according to the invention

Mouse cells are responsive to human IL-15, which allows testing *in vivo* functional inhibition of IL-15 of anti-IL-15 antibodies according to the invention even though

5 those antibodies do not recognize mouse IL-15 as shown in Example 4. Interleukin-15 injected into mice can induce proliferation and accumulation of various lymphocyte subsets such as Natural Killer (NK) cells in the spleen. However, IL-15 by itself is poorly active, may be due to its very short half-life, and stabilized complexes of IL-15 bound to IL-15R α are more efficient *in vivo*.

10 Exemplary antibodies of the present invention were tested for their capacity to inhibit IL-15/IL-15R α -Fc complex-induced NK cell accumulation in the spleen of C57BL/6 male mice (*Finch et al, 2011, supra*). Groups of 5 mice were injected during three consecutive days with a mixture of 1 μ g human IL-15 (Prospec) and 3.6 μ g human IL-15R α -Fc (R&D Systems) and on the first and second days of the experiment with 100
15 μ g and 62 μ g, respectively, of test antibodies and controls. One day after the last injection, spleens were harvested, splenocytes counted and NK cells analysed by flow cytometry and defined as CD45 $^{+}$ NK1.1 $^{+}$ CD3 $^{-}$ cells.

As shown in Figure 5, injection of the IL-15/IL-15R α -Fc complex induced robust NK cell accumulation in mouse spleen that could be fully inhibited by treatment with the
20 exemplary huB-E29-2, huB-E29-10 and huB-E29-24 antibodies of the invention but not a control human IgG1 isotype antibody.

Example 10: Epitope mapping of anti-IL-15 antibodies according to the invention

Epitope mapping of anti-IL-15 antibodies of the invention was performed by analysing the binding of said antibodies to libraries of structured peptides designed to represent

25 linear but also discontinuous epitopes of IL-15 by using the Chemically Linked Peptides on Scaffolds (CLIPS) technology (Pepscan Presto Bv, Lelystad, The Netherlands).

CLIPS technology (*Timmermann et al., 2007, J. Mol. Recognit., 20, 283-99*) allows to structure peptides into single loops, double loops, triple loops, sheet-like folds, helix-like folds, and combinations thereof. Structure peptides are immobilized on arrays. The
30 binding of antibodies to each of the synthesized peptides was tested in a PEPSCAN-based array ELISA. Signal for each peptide is measured using a charge coupled device camera, quantified and results from the whole array processed to define which peptides

are best recognized. Amino-acid substitutions within peptides can allow determining more critical binding residues.

It was found using this technique that the antibodies of the invention huB-E29-2, huB-E29-10 and huB-E29-24 all preferentially bind to linear and constrained peptides containing the peptide stretch of sequence ⁶¹DTVENLII₁₀LANN⁷² (SEQ ID NO: 43) This observation is similar to what reported for comparative antibody 1, B-E29 (*Bernard et al., 2004, supra*). Using single amino-acid substitution, residues found to be most essential for the binding of the antibodies of the invention huB-E29-2, huB-E29-10 and huB-E29-24 antibodies to IL-15 were D61, E64, I68 and N71.

Comparative antibody 1, is characterised by VL and VH as shown in SEQ ID NO: 39 SEQ ID NO: 40 respectively. According to *Bernard et al., 2004, supra*, B-E29 affects binding of IL-15 to IL-15R α through binding to L66 and I67 residues, which are not found as critical for the binding of the antibodies of the invention huB-E29-2, huB-E29-10 and huB-E29-24. On the other hand, still according to *Bernard et al.*, residues E64, N65 and I68 are important for prevention of binding of IL-15 to IL-15R β by B-E29, and two of these three residues, E64 and I68, were found critical for the huB-E29-2, huB-E29-10 and huB-E29-24 antibody binding to IL-15. Prevention of binding of IL-15 to the IL-15R β chain is important to block IL-15 signalling. Therefore, although the huB-E29-2, huB-E29-10 and huB-E29-24 antibodies share an overlapping epitope with the original B-E29 antibody, structural differences may explain a different mode of action, which is the loss of ability to prevent IL-15 binding to IL-15R α , as shown in the present application (Example 8), while preserving the ability to block IL-15 mediated signalling.

It was also observed that the antibodies of the invention huB-E29-10 and huB-E29-24 showed less tolerance for substitutions within the peptide stretch of SEQ ID NO: 43 than the huB-E29-2 antibody, meaning that loss of signal was more frequent, which could be related to their lower affinity for IL-15 as shown in the present application (Example 3).

Finally, comparative antibody 2, 146B7, did not show any reliable binding over background on any of the peptide arrays tested and therefore probably recognizes a complex/discontinuous epitope on IL-15 (*Villadsen et al., 2003, J. Clin. Invest., 112: 1571-1580*). Amino acid mutagenesis, reported that residues D8 and Q108 of IL-15

were essential for the binding of 146B7, without further description of the epitope recognized by this antibody. Data presented in the current example are in accordance with this finding, suggesting a non-linear epitope for 146B7, clearly distinct from the ones recognized by the antibodies of the invention huB-E29-2, huB-E29-10 and huB-E29-24.

The epitope recognized by the DISC0280 anti-IL-15 antibody (comparative antibody 3) was determined using crystallography (*Lowe et al, 2010, J. Mol. Biol., 406, p.160-175*). DISC0280 antibody is characterised by VL CDR3 and VH CDR3 as shown in SEQ ID NO: 41 and SEQ ID NO: 42 respectively. As expected from the fact that comparative antibodies B-E29 and DISC0280 competed for IL-15 binding (*Finch et al, 2010, J Mol Biol., 406(1), p.160-175*), similar residues were found between the DISC0280 epitope and the epitopes recognized by the huB-E29-2, huB-E29-10 and huB-E29-24 antibodies of the present invention, namely E64 and N71. However, other hydrogen bonds were described for DISC0280 with IL-15 residues: K41, E46, Q48, L52, E53, and E89. Hence, DISC0280 and the antibodies of the invention huB-E29-2, huB-E29-10 and huB-E29-24 have an overlapping but distinct epitope. In addition, two residues found most important for the binding of huB-E29-2, huB-E29-10 and huB-E29-24 to IL-15 are not part of DISC0280 epitope: D61 and I68.

Example 11: Effect of anti-IL-15 antibodies according to the invention in cell lines from refractory celiac disease patients

The effects of the antibody of the invention huB-E29-2 and comparative antibody 146B7 on the prevention from apoptosis and activation of type II refractory celiac disease (RCD) patient primary intra epithelial lymphocytes (IEL) cell lines induced by recombinant human IL-15, were assessed *in vitro*. The percentage of apoptotic cells was analysed by staining cells with Annexin V and propidium iodide (PI) and measured by flow cytometry. The expression of phosphorylated STAT5 protein (pSTAT5), also by a flow cytometry technique, was used to measure activation of the primary IEL cell lines by IL-15 and its inhibition by anti-IL-15 antibodies.

The humanized anti-IL-15 antibody of the invention huB-E29-2 potently inhibited IL-15-induced prevention of apoptosis in type II RCD primary IEL cell lines from three different patients *in vitro* (Figure 6), with a half-maximal inhibitory concentration (IC₅₀) of 2.36 nM calculated for one of the three cell lines tested (HAM_RAC). This antibody

also inhibited IL-15-induced STAT5 phosphorylation in all tested type II RCD patient primary IEL cell lines, when used at the concentration calculated to give 80% apoptosis inhibition in the HAM_RAC cell line. Finally, at this same concentration, huB-E29-2 was much more efficient than the fully human anti-IL-15 antibody 146B7 for the inhibition of IL-15-induced prevention of apoptosis and STAT5 phosphorylation in type II RCD patient primary IEL cell lines (Figure 6).

Example 12: Effects of anti-IL-15 antibodies according to the invention in mice transgenic for human IL-15, a model for refractory celiac disease.

10 Transgenic mice overexpressing human IL-15 under the control of T3b, an enterocyte-specific promoter (IL-15TgE mice; *Ohta et al., 2002, J. Immunol. 169(1), 460-468*) show an abnormal and massive accumulation of intra-epithelial lymphocytes (IEL). This model has been postulated to recapitulate some of the features of human refractory celiac disease (*Malamut et al., 2010, supra*). An antibody against IL-15, AMG 714, 15 with an identical sequence as the 146B7 antibody described herein and formerly identified as HuMaxIL-15 (*Villadsen et al., 2003, J Clin Invest, 112(10): 1571-80; Lebrec et al, 2013, J Immunol., 191(11):5551-8*), is able to reverse the accumulation of IELs when administered to these mice, by promoting IEL apoptosis (*Malamut et al., 2010, supra*).

20 The anti-IL-15 antibody of the invention huB-E29-2, or a control isotype antibody IgG1, was administered intraperitoneally twice a week at a dose of 100 µg to groups of T3b-hIL-15 transgenic mice. After the two-week treatment, CD3⁺CD8⁺ IEL were numerated and analysed using standard flow cytometry techniques (*Malamut et al., 2010, supra*). It was observed that the huB-E29-2 treatment resulted in a statistically 25 significant decrease of CD3⁺CD8⁺ IEL when compared to the control antibody (Figure 7).

Example 13: Effects of anti-IL-15 antibodies in an allergen-induced model of eosinophilic esophagitis.

30 Mice genetically deficient for the IL-15R α chain of the IL-15 receptor were resistant to induction of eosinophilic esophagitis following nostril instillation of *Aspergillus fumigatus* (*Zhu et al, 2010, supra*). Antibodies that bind and neutralize mouse IL-15 such as clone AIO.3 (eBiosciences) are administered to mice challenged with

Aspergillus fumigatus to test the activity of anti-IL-15 antibodies for the treatment of eosinophilic esophagitis. Groups of mice are administered intra-nasally with a preparation of *Aspergillus fumigatus* on study days 0, 2, 4, 7, 9, 11, 14, 16, and 18. Animals are treated at different time points with appropriate doses of a neutralizing 5 anti-mouse IL-15 antibody or control isotype antibody. As positive control, mice are treated with dexamethasone. On study Day 19, the esophagus of each mouse is processed and stained and eosinophilic infiltrate is measured via a microscope. In addition, bronchoalveolar lavage is performed to measure the infiltration of inflammatory cells in the airways.

10 **Example 14: Effect of anti-IL-15 antibodies according to the invention on circulating NK cell numbers in non-human primates.**

Administration of anti-IL-15 antibodies to cynomolgus monkeys was shown to induce a decrease of circulating NK cell numbers within a two-week period (Lebrec *et al.*, 2013, *J Immunol*, 191(11), 5551-5558). Different antibodies showed different effect: the 15 minimal dose of anti-IL-15 Hu714MuXHu antibody able to induce NK cell reduction *in vivo* was 0.1 mg/kg, whereas the minimal dose of anti-IL-15 AMG 714 antibody, with an identical sequence as the 146B7 antibody described herein (Villadsen *et al.*, 2003, *J Clin Invest*, 112(10): 1571-80; Lebrec *et al.*, 2013, *J Immunol*, 191(11):5551-8), able to induce NK cell reduction *in vivo* was 150 mg/kg.

20 The capacity of anti-IL-15 antibodies of the invention to modulate circulating NK cell numbers is tested *in vivo* in cynomolgus monkeys. Various doses of said antibodies, ranging from 0.1 mg/kg to 10 mg/kg, are administered to cynomolgus monkeys via intravenous route, and circulating numbers of NK cells are evaluated using a flow cytometry technique applied to blood samples pre-dose and at study days 1, 3, 5, 8, 14, 25 and 21. The modulation of circulating NK cell numbers in non-human primates could define a marker of the pharmacological activities of anti-IL-15 antibodies *in vivo*, and therefore could be advantageously used to define optimal dosing in patients

LIST OF SEQUENCES

Human mature IL-15

30 SEQ ID NO: 1

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDAS
IHDTVENLILANNSSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

Mouse mature IL-15

SEQ ID NO: 2

NWIDVRYDLEKIESLIQSIHIDTLYTDSDFHPSCKVTAMNCFLLELQVILHEYSNMT

5 LNETVRNVLYLANSTLSSNKNVAESGCKECEELEEKTFEFLQSFIRIVQMFINTS

Rat mature IL-15

SEQ ID NO: 3

NWIDVRYDLEKIESLIQFIHIDTLYTDSDFHPSCKVTAMNCFLLELQVILHEYSNMT

10 LNETVRNVLYLANSTLSSNKNVIESGCKECEELEERNFTEFLQSFIHIVQMFINTS

Rhesus macaque/Cynomolgus monkey mature IL-15

SEQ ID NO: 4

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISHESGDTD

15 IHDTVENLILANNILSSNGNITESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

huVH1

SEQ ID NO: 5

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT20 YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGWAMDYWGQGT
LTVSS**huVH2**

SEQ ID NO: 6

25 EVQLVESGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGWAMDYWGQGT
LTVSS**huVH3**

30 SEQ ID NO: 7

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVSTISRGGDYT
YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGWAMDYWGQGT
LTVSS35 **huVH4**

SEQ ID NO: 8

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSLITGGWAMDYWGQGT
LTVSS

40

huVH5

SEQ ID NO: 9

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSFITGGWAMDYWGQGT
45 LTVSS**huVH6**

SEQ ID NO: 10

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDTVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSITGGWAMDYWGQGT
LTVSS

5 **huVH7**

SEQ ID NO: 11

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDTVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSITGGWAMDYWGQGT
LTVSS

10

huVH8

SEQ ID NO: 12

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDTVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGYAMDYWGQGT
15 LTVSS

huVH9

SEQ ID NO: 13

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
20 YYPDTVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGFAMDYWGQGT
LTVSS

huVH10

SEQ ID NO: 14

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
25 YYPDTVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGAAMDYWGQGT
LTVSS

huVH11

30 SEQ ID NO: 15

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGWALDYWGQGT
LTVSS

35 **huVH12**

SEQ ID NO: 16

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGWAFDYWGQGT
LTVSS

40

huVH13

SEQ ID NO: 17

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGWAIDYWGQGT
45 LTVSS

huVH14

SEQ ID NO: 18

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGWAMDYWGQGT
LTVSS

5 **huVH15**

SEQ ID NO: 19

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDTVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGWAMDYWGQGT
LTVSS

10

huVH16

SEQ ID NO: 20

EVQLVESGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSFITGGYAFDYWGQGT
15 LTVSS

huVH18

SEQ ID NO: 21

EVQLVESGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
20 YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSMITGGYAMDYWGQGT
LTVSS

huVH20

SEQ ID NO: 22

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
25 YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSFITGGYAMDYWGQGT
LTVSS

huVH21

30 SEQ ID NO: 23

EVRLMASGGGLVQPGGSLRLSCAASEFTFSNYAMSWVRQAPGKGLEWVATISRGGDYT
YYPESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRVSFITGGYAMDYWGQGT
LTVSS

35 **huVL1**

SEQ ID NO: 24

DVVMTQSPLSLPVTLGQPASISCRSSQSIVDITGNTYLEWYQQRPGQSPRLLIYKVFN
RFSGVPDRFSGSGSGTDFTLKISRVEAEDGVYYCFQDSFVPYTFGQGTKLEIK

40 **huVL2**

SEQ ID NO: 25

DVVMTQSPLSLPVTLGQPASISCRSSQSIVDITGNTYLEWFQQRPGQSPRLLIYKVFN
RFSGVPDRFSGSGSGTDFTLKISRVEAEDGVYYCFQDSFVPYTFGQGTKLEIK

45 **huVL3**

SEQ ID NO: 26

DVVMTQSPLSLPVTLGQPASISCRSSQSIVDITGNTYLEWYQQRPGQSPRLLIYKVFN
RFSGVPDRFSGSGSGTDFTLKISRVEAEDGVYYCFQDSFVPYTFGQGTKLEIK

huVL4

SEQ ID NO: 27

DVVMTQSPLSLPVTLGQPASISCRSSQSIVDITGNTYLEWYQQRPGQSPRLLIYKVFN
RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQESFVPYTFGQGTKLEIK

5

huVL5

SEQ ID NO: 28

DVVMTQSPLSLPVTLGQPASISCRSSQSIVDITGNTYLEWYQQRPGQSPRLLIYKVFN
RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQDTFVPYTFGQGTKLEIK

10

huVL6

SEQ ID NO: 29

DVVMTQSPLSLPVTLGQPASISCRSSQSIVDITGNTYLEWFQQRPGQSPRLLIYKVFN
RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQESFVPYTFGQGTKLEIK

15

IgG1m3 constant region of heavy chain

SEQ ID NO: 30

ASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVWNSGALTSGVHTFPALQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHPNTKVDKRVPEKSCDKHTCPCPAPE

20

LLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTP
REQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

25

IgKm3 constant region of light chain

SEQ ID NO: 31

RTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSTYSLSSTLTLISKADYEKHKVYACEVTHQGLSPVTKSFNRC

30

cVH1 (variable region of heavy chain from mouse B-E29)

SEQ ID NO: 32

EVRLMASGGGLVKPGGSLKLSCAASEFTFSNYAMSWVRQTPEKRLEWVATISRGGDYT
YYPDSVKGRFTISRDNAKNTLYLQMSSLRSEDTALYYCARRVSMITGGWAMDYWGQGT
SVTVSS

35

cVH2

SEQ ID NO: 33

EVRLLASGGGLVKPGGSLKLSCAASEFTFSNYAMSWVRQTPEKRLEWVATISRGGDYT
YYPDSVKGRFTISRDNAKNTLYLQMSSLRSEDTALYYCARRVSMITGGWAMDYWGQGT
SVTVSS**cVH3**

SEQ ID NO: 34

EVQLLASGGGLVKPGGSLKLSCAASEFTFSNYAMSWVRQTPEKRLEWVATISRGGDYT
YYPDSVKGRFTISRDNAKNTLYLQMSSLRSEDTALYYCARRVSMITGGWAMDYWGQGT
SVTVSS**cVH4**

SEQ ID NO: 35

EVRLME SGGLVKPGGSLKLS
CAASEFTFSNYAMS
WVRQTPEKLEWVATISRGGDYT
YYPD
SVKGRFTISRDNAKNTLYLQMSSLRSEDTALYYCARRVSMITGGWAMDYWGQGT
SVTVSS

5 **cVK1**

SEQ ID NO: 36
DVLMTQTPLSLPVSLGDQASISCRSSQSIVDITGNTYLEWYLQKPGQSPKLLIYKVFN
RFSGVPDRFSGSGSGTDFTLKISRVEAEDLGLYYCFQDSFVPYTFGGGTKLEIK

10 **hVK2**

SEQ ID NO: 37
EVVMTQSPATLSLSPGERATLSCRSSQSIVDITGNTYLEWYQQKPGQAPRLLIYKVFN
RFSGIPARFSGSGSGTDFTLTISSL
EPEDFAVYYCFQDSFVPYTFQGKLEIK

15 **Human IL-2**

SEQ ID NO: 38
MYRMQLLSCIALSLALVTNSAP
TSSSTKKTQLQLEHLLLQMI
LNGINNYKNPKLTR
MLTFKFYMPKKATELKHLQCLEEELK
PLEEVLNLAQSKNFHLRPRDLISNINV
VILEL
KGSETTFMCEYADETATIVEFLNRWITFCQSII
STLT

20

VL-146B7

SEQ ID NO: 39
EIVLTQSPGTLSLSPGERATLSCRASQS
VSSSYLAWYQQKPGQAPRLLIYGA
SRRATG
IPDRFSGSGSGTDFLT
LISRLE
PEDFAVYYCQRYGSSHTFGQGKLEIS

25 **VH-146B7**

SEQ ID NO: 40
EVQLVQSGAEVKKPGESLKISCKVSGYFFTYWIGWVRQMPGK
GLEYMGIIY
PGDSDT
RYSPSFQGQVTISADKS
ISTAYLQWSSLKASDTAMYYCARGGN
WNCFDYWGQGTLVTV
SS

30

VL CDR3 DISC0280

Kabat positions: 89, 90, 91, 92, 93, 94, 95, 95A, 95B, 96, 97

SEQ ID NO: 41

35 AWYDRELSEWV

VH CDR3 DISC0280

Kabat positions: 95, 96, 97, 98, 99, 100, 100a, 100b, 100c, 100d, 100e, 100f, 100g, 101, 102

SEQ ID NO: 42

40 DPAAWPLQQSLAWFDP

IL-15 peptide fragment stretch

SEQ ID NO: 43

DTVENLII
LANN

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated antibody binding IL-15 or an antigen-binding fragment thereof comprising:
 - (1) a heavy chain variable region of SEQ ID NO: 5 or any variant thereof wherein said variant has the amino acid sequence of SEQ ID NO: 5 except that 1, 2, 3 or 4 amino acids are substituted by a different amino acid, wherein substitutions are selected among:
 - (i) arginine (R) at position H3 (VH RH3) substituted by glutamine (Q), methionine (M) at position H5 (VH MH5) substituted by valine (V), alanine (A) at position H6 (VH AH6) substituted by glutamic acid (E), alanine (A) at position H49 (VH AH49) substituted by serine (S), within the heavy chain variable framework region,
 - (ii) aspartic acid (D) at position H61 (VH DH61) substituted by glutamic acid (E), serine (S) at position H62 (VH SH62) substituted by threonine (T), within the heavy chain CDR2, and
 - (iii) methionine (M) at position H98 (VH MH98) substituted by leucine (L), phenylalanine (F), isoleucine (I), or alanine (A), tryptophan (W) at position H100C (VH WH100C) substituted by tyrosine (Y), phenylalanine (F) or alanine (A), methionine (M) at position H100E (VH MH100E) substituted by leucine (L), phenylalanine (F) or isoleucine (I), within the heavy chain CDR3;
- and
- (2) a light chain variable region of SEQ ID NO: 24.

2. The isolated antibody or antigen-binding fragment thereof according to claim 1, comprising:
 - (1) a heavy chain variable region of amino acid sequence of SEQ ID NO: 5 having substitutions in said sequence of SEQ ID NO: 5 selected among:
 - (i) VH RH3 is substituted by glutamine (Q), VH MH5 is substituted by valine (V), VH AH6 is substituted by glutamic acid (E),
 - (ii) VH SH62 is substituted by threonine (T), and

(iii) VH WH100C is substituted by tyrosine (Y);
and
(2) a light chain variable region of amino acid sequence of SEQ ID NO: 24.

3. The isolated antibody according to claim 1 or 2, which is a humanized antibody.

4. The isolated antibody or antigen-binding fragment thereof according to any one of claims 1 to 3, comprising:
(1) a heavy chain variable region selected from: SEQ ID NO: 6, SEQ ID NO: 12, SEQ ID NO: 21, and SEQ ID NO: 5, and
(2) a light chain variable region of SEQ ID NO: 24.

5. The isolated antibody or antigen-binding fragment thereof according to any one of claims 1 to 4, comprising a heavy chain variable region of SEQ ID NO: 6 and a light chain variable region of SEQ ID NO: 24.

6. The isolated antibody or antigen-binding fragment thereof according to any one of claims 1 to 5, which inhibits IL-15 activity such as IL-15-induced proliferation and/or survival of Kit 225 or M-07e cell lines.

7. An isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof according to any one of claims 1 to 6.

8. A host cell comprising a recombinant expression vector comprising a nucleic acid molecule according to claim 7.

9. A process for producing an antibody or antigen-binding fragment thereof according to any one of claims 1 to 6 comprising: culturing a host cell transformed with an expression vector comprising a nucleic sequence that encodes said antibody or fragment thereof under conditions sufficient to promote expression of said antibody or fragment thereof.

10. A pharmaceutical composition comprising one or more of: (i) an antibody binding to IL-15 or antigen-binding fragment thereof according to any one of claims 1 to 6, (ii) a nucleic acid according to claim 7, (iii) a recombinant expression vector

comprising said nucleic acid under (ii), and/or (iv) a host cell according to claim 8, and at least one pharmaceutically acceptable carrier.

11. A pharmaceutical composition according to claim 10, further comprising a co-agent useful in the prevention and/or treatment of an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens, or a compound lowering intestinal inflammation, and/or protecting intestinal mucosa, and/or lowering the immune reactivity of gluten peptides, and/ or modifying the gut microbiota.
12. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 6 when used as a medicament.
13. Use of the antibody or antigen-binding fragment thereof according to any one of claims 1 to 6 in the manufacture of a medicament for the prevention and/or treatment of an IL-15 related disease or disorder.
14. The use according to claim 13, wherein the IL-15 related disease or disorder is selected from an autoimmune disease, an inflammatory disorder, a malignancy, a metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens.
15. The use according to claim 13 or 14, wherein the IL-15 related disease or disorder is selected from rheumatoid arthritis, psoriasis, celiac disease such as refractory celiac disease, sarcoidosis, inflammatory bowel disease, hepatitis C-induced liver diseases, multiple sclerosis, auto-immune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary atresia, alopecia areata, transplant rejection, inflammatory diseases of the central nervous system, and eosinophilic esophagitis.
16. The use according to claim 13, wherein the IL-15 related disease or disorder is selected from celiac disease such as refractory celiac disease, eosinophilic esophagitis, auto-immune hepatitis, Alzheimer's disease, viral infections and Hantaan viral infections.

17. The use according to any one of claims 13 to 16, wherein said antibody or antigen-binding fragment thereof is to be administered in combination with a co-agent useful in the prevention and/or treatment of an autoimmune disease and/or inflammatory disorder, a malignancy, transplant rejection, metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens, or a compound lowering intestinal inflammation, and/or protecting intestinal mucosa, and/or lowering the immune reactivity of gluten peptides, and/ or modifying the gut microbiota.
18. An *ex vivo* method for detecting the presence and/or concentration of IL-15 protein in a biological sample, comprising the steps of:
 - (i) Providing a biological sample from a subject,
 - (ii) Reacting said biological sample with at least one antibody or antigen-binding fragment thereof according to any one of claims 1 to 6, under conditions sufficient for binding IL-15 protein present in said biological sample to said at least one antibody or fragment thereof through antigen-antibody interactions; and
 - (iii) Detecting a signal proportional to the level of antigen-antibody complex formed in step (ii),
wherein the intensity of the signal correlates with the concentration of IL-15 protein in the biological sample.
19. A method of preventing and/or treating an IL-15 related disease or disorder comprising administering in a subject in need thereof a therapeutically effective amount of an antibody or antigen-binding fragment thereof according to any one of claims 1 to 6 or a pharmaceutical composition thereof.
20. The method according to claim 19, wherein the IL-15 related disease or disorder is selected from an autoimmune disease, an inflammatory disorder, a malignancy, a metabolic condition and/or an infectious disease caused by parasitic, viral or bacterial pathogens.

Figure 1

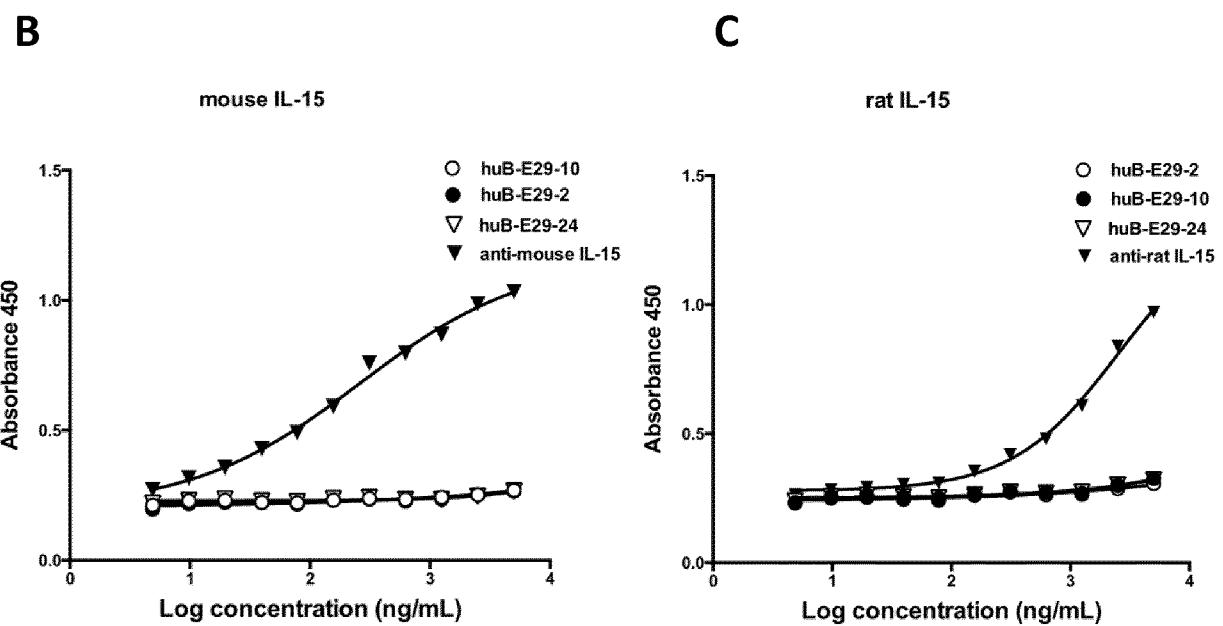
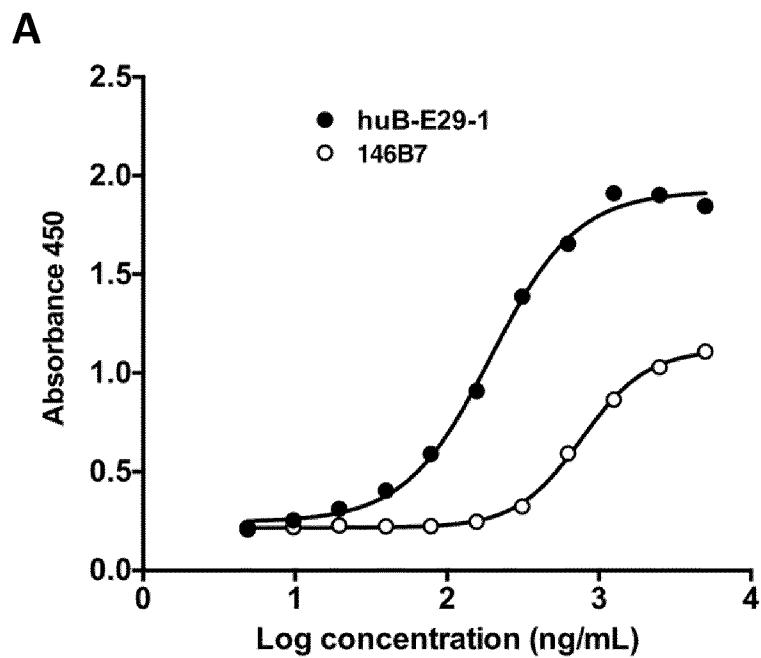


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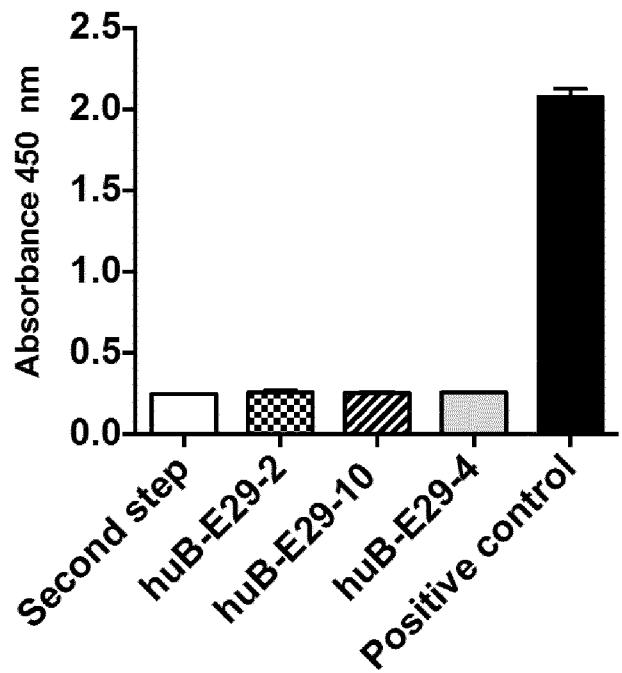


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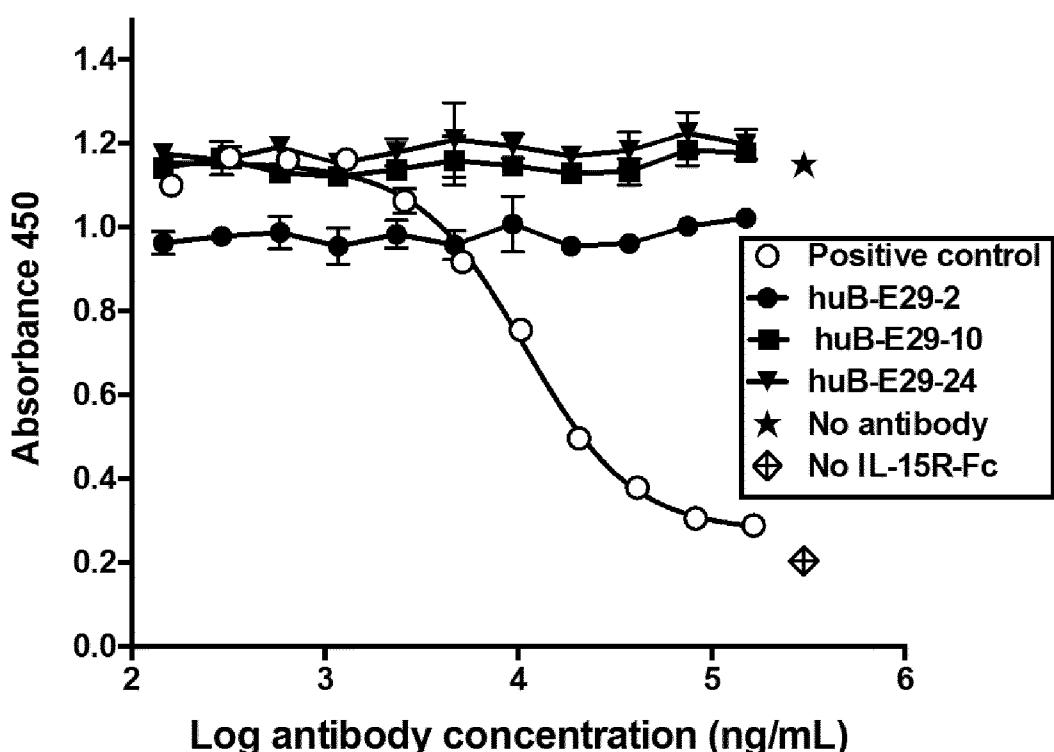
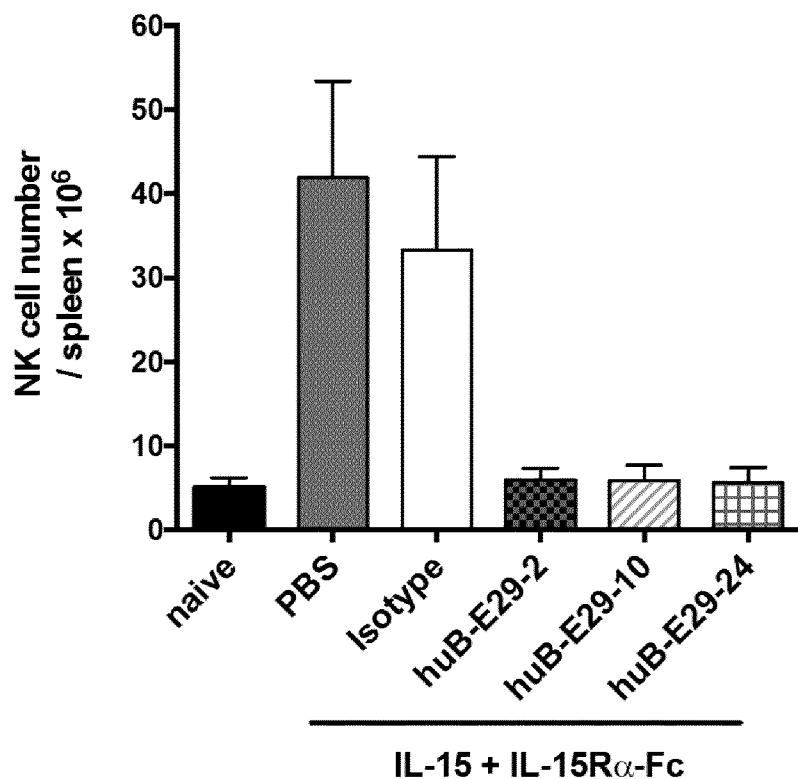
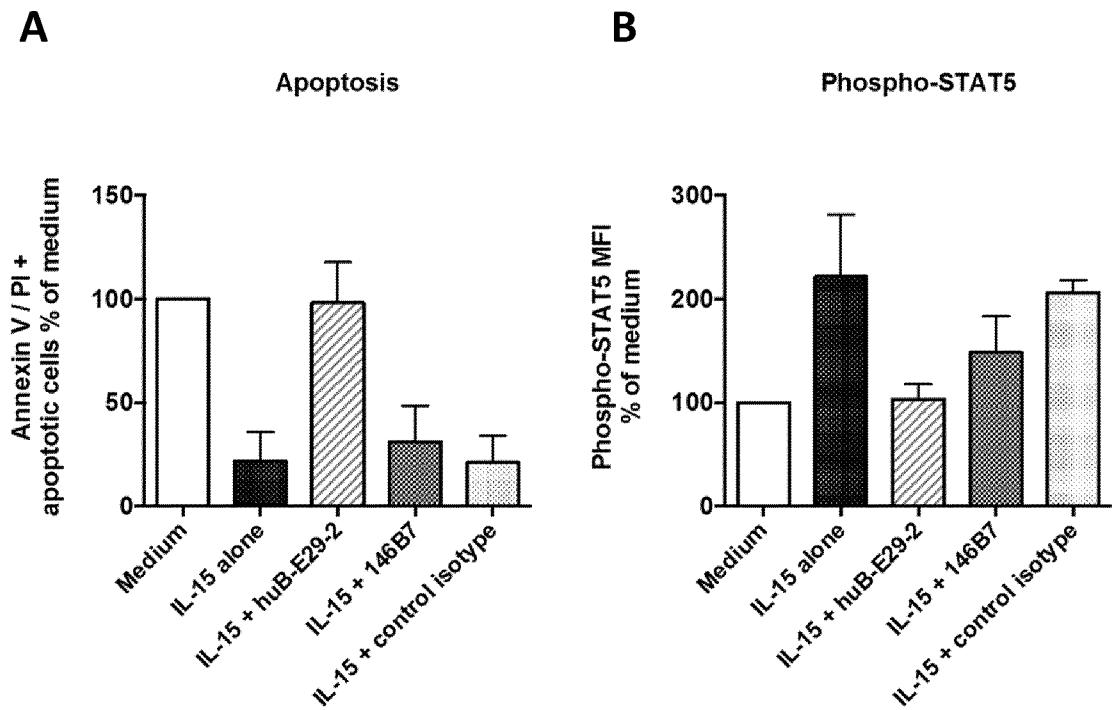


Figure 4

**Figure 5****Figure 6**

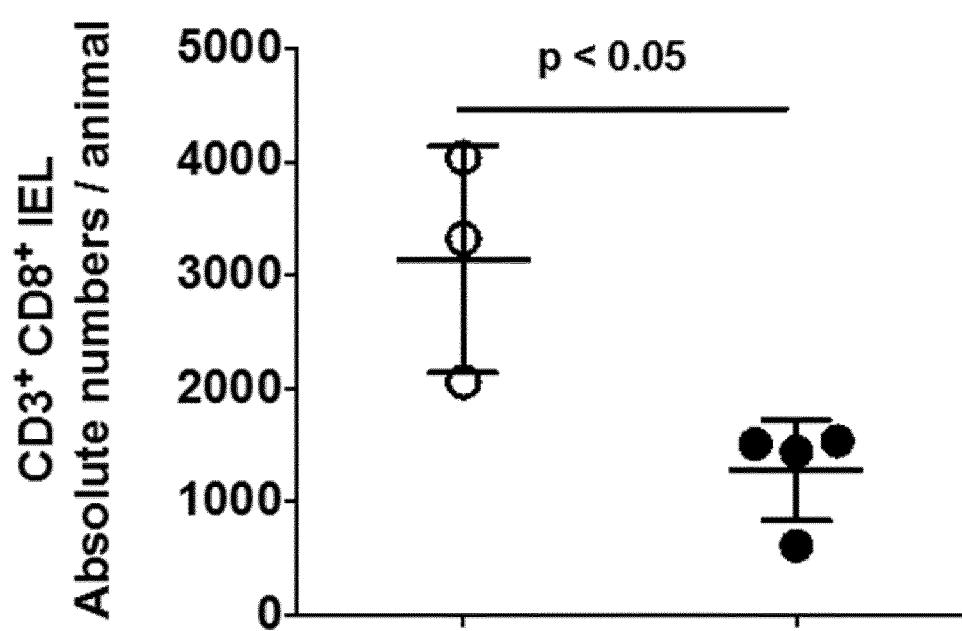


Figure 7

SEQUENCE LISTING
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<130> P1792PC00

<150> EP 14175361.6

<151> 2014-07-02

<160> 43

<170> PatentIn version 3.5

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<212> PRT

<213> Homo sapiens

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Gl n Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
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Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gl n
35 40 45

Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
50 55 60

Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val
65 70 75 80

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
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100 105 110

Thr Ser

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<212> PRT

<213> Mus musculus

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20 25 30

eol f-seql

Pro Ser Cys Lys Val Thr Ala Met Asn Cys Phe Leu Leu Glu Leu Glu
35 40 45

Val Ile Leu His Glu Tyr Ser Asn Met Thr Leu Asn Glu Thr Val Arg
50 55 60

Asn Val Leu Tyr Leu Ala Asn Ser Thr Leu Ser Ser Asn Lys Asn Val
65 70 75 80

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<213> rat

<400> 3

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Pro Ser Cys Lys Val Thr Ala Met Asn Cys Phe Leu Leu Glu Leu Glu
35 40 45

Val Ile Leu His Glu Tyr Ser Asn Met Thr Leu Asn Glu Thr Val Arg
50 55 60

Asn Val Leu Tyr Leu Ala Asn Ser Thr Leu Ser Ser Asn Lys Asn Val
65 70 75 80

Ile Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Arg Asn Phe
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20 25 30

Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gl n
35 40 45

Val Ile Ser His Glu Ser Gly Asp Thr Asp Ile His Asp Thr Val Glu
50 55 60

Asn Leu Ile Ile Leu Ala Asn Asn Ile Leu Ser Ser Asn Gly Asn Ile
65 70 75 80

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
85 90 95

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35 40 45

Al a Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Asn Ser Leu Arg Ala Gl u Asp Thr Ala Val Tyr Tyr Cys
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eol f-seql

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20 25 30

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

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

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

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

Ala Arg Arg Val Ser Met Ile Thr Gly Gly Trp Ala Met Asp Tyr Trp
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Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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20 25 30

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

Ser Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

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

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

Ala Arg Arg Val Ser Met Ile Thr Gly Gly Trp Ala Met Asp Tyr Trp
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Glu Phe Thr Phe Ser Asn Tyr
20 25 30

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

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

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

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

Ala Arg Arg Val Ser Leu Ile Thr Gly Gly Trp Ala Met Asp Tyr Trp
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Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Al a Pro Gl y Lys Gl y Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Gl u Ser Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Asn Ser Leu Arg Al a Gl u Asp Thr Al a Val Tyr Tyr Cys
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Al a Arg Arg Val Ser Phe Ile Thr Gl y Gl y Trp Al a Met Asp Tyr Trp
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Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Al a Pro Gl y Lys Gl y Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Asp Thr Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
Page 6

65 70 eol f-seq1 75 80

Leu Glu Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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Ala Arg Arg Val Ser Ile Ile Thr Gly Gly Trp Ala Met Asp Tyr Trp
100 105 110

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20 25 30

Ala Met Ser Trp Val Arg Glu Ala Pro Glu Lys Glu Leu Glu Trp Val
35 40 45

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Thr Val
50 55 60

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

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

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

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Page 7

1 5 10 15

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20 25 30

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

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Thr Val
50 55 60

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

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

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

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Glu Phe Thr Phe Ser Asn Tyr
20 25 30

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

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Thr Val
50 55 60

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

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

Ala Arg Arg Val Ser Met Ile Thr Gly Gly Phe Ala Met Asp Tyr Trp
100 105 110

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Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Al a Pro Gl y Lys Gl y Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Asp Thr Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Asn Ser Leu Arg Al a Gl u Asp Thr Al a Val Tyr Tyr Cys
85 90 95

Al a Arg Arg Val Ser Met Ile Thr Gl y Gl y Al a Al a Met Asp Tyr Trp
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Gl y Gl n Gl y Thr Leu Val Thr Val Ser Ser
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Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Al a Pro Gl y Lys Gl y Leu Gl u Trp Val
35 40 45

eol f-seql

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

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

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

Ala Arg Arg Val Ser Met Ile Thr Gly Gly Trp Ala Leu Asp Tyr Trp
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Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Ala Met Ser Trp Val Arg Gl n Ala Pro Gl y Lys Gl y Leu Gl u Trp Val
35 40 45

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

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

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

Ala Arg Arg Val Ser Met Ile Thr Gly Gly Trp Ala Phe Asp Tyr Trp
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Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Al a Pro Gl y Lys Gl y Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Asn Ser Leu Arg Al a Gl u Asp Thr Al a Val Tyr Tyr Cys
85 90 95

Al a Arg Arg Val Ser Met Ile Thr Gl y Gl y Trp Al a Ile Asp Tyr Trp
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Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Al a Pro Gl y Lys Gl y Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Gl u Ser Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Asn Ser Leu Arg Al a Gl u Asp Thr Al a Val Tyr Tyr Cys
Page 11

85 eol f-seql
90
95

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

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Glu Phe Thr Phe Ser Asn Tyr
20 25 30

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

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Thr Val
50 55 60

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

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

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

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Glu Phe Thr Phe Ser Asn Tyr
Page 12

20 25 eol f-seql 30

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

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Glu Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

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

Ala Arg Arg Val Ser Phe Ile Thr Glu Gly Tyr Ala Phe Asp Tyr Trp
100 105 110

Gly Glu Gly Thr Leu Val Thr Val Ser Ser
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1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Glu Phe Thr Phe Ser Asn Tyr
20 25 30

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

Ala Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Glu Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

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

Ala Arg Arg Val Ser Met Ile Thr Glu Gly Tyr Ala Met Asp Tyr Trp
100 105 110

Gly Glu Gly Thr Leu Val Thr Val Ser Ser
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Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Al a Pro Gl y Lys Gl y Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Asn Ser Leu Arg Al a Gl u Asp Thr Al a Val Tyr Tyr Cys
85 90 95

Al a Arg Arg Val Ser Phe Ile Thr Gl y Gl y Tyr Al a Met Asp Tyr Trp
100 105 110

Gl y Gl n Gl y Thr Leu Val Thr Val Ser Ser
115 120

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Gl u Val Arg Leu Met Al a Ser Gl y Gl y Gl y Leu Val Gl n Pro Gl y Gl y
1 5 10 15

Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Al a Pro Gl y Lys Gl y Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Gl u Ser Val
50 55 60

eol f-seql

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Asn Ser Leu Arg Al a Gl u Asp Thr Al a Val Tyr Tyr Cys
85 90 95

Al a Arg Arg Val Ser Phe Ile Thr Gl y Gl y Tyr Al a Met Asp Tyr Trp
100 105 110

Gl y Gl n Gl y Thr Leu Val Thr Val Ser Ser
115 120

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Gl n Pro Al a Ser Ile Ser Cys Arg Ser Ser Gl n Ser Ile Val Asp Ile
20 25 30

Thr Gl y Asn Thr Tyr Leu Gl u Trp Tyr Gl n Gl n Arg Pro Gl y Gl n Ser
35 40 45

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

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

Ser Arg Val Gl u Al a Gl u Asp Val Gl y Val Tyr Tyr Cys Phe Gl n Asp
85 90 95

Ser Phe Val Pro Tyr Thr Phe Gl y Gl n Gl y Thr Lys Leu Gl u Ile Lys
100 105 110

<210> 25

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Asp Val Val Met Thr Gl n Ser Pro Leu Ser Leu Pro Val Thr Leu Gl y
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eol f-seql

Gl n Pro Al a Ser Ile Ser Cys Arg Ser Ser Gl n Ser Ile Val Asp Ile
20 25 30

Thr Gl y Asn Thr Tyr Leu Gl u Trp Phe Gl n Gl n Arg Pro Gl y Gl n Ser
35 40 45

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

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

Ser Arg Val Gl u Al a Gl u Asp Val Gl y Val Tyr Tyr Cys Phe Gl n Asp
85 90 95

Ser Phe Val Pro Tyr Thr Phe Gl y Gl n Gl y Thr Lys Leu Gl u Ile Lys
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Gl n Pro Al a Ser Ile Ser Cys Arg Ser Ser Gl n Ser Ile Val Asp Ile
20 25 30

Thr Gl y Asn Thr Tyr Leu Gl u Trp Tyr Gl n Gl n Arg Pro Gl y Gl n Ser
35 40 45

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

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

Ser Arg Val Gl u Al a Gl u Asp Val Gl y Val Tyr Tyr Cys Phe Gl n Asp
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Ser Phe Val Pro Tyr Thr Phe Gl y Gl n Gl y Thr Lys Leu Gl u Ile Lys
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eol f-seqI

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

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

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

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

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

Ser Phe Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
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<210> 28
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1 5 10 15

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

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

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

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

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

Thr Phe Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
Page 17

100 105 eol f-seql 110

<210> 29
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> huVL6

<400> 29

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

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

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

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

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

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

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

<210> 30
<211> 330
<212> PRT
<213> Homo sapiens

<400> 30

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

eol f-seql

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95

Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
 100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
 115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
 145 150 155 160

Tyr Val Asp Glu Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
 180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Glu
 210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
 225 230 235 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Glu Phe Tyr
 245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Glu Gln Pro Glu Asn
 260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Glu Ser Phe Phe
 275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Glu Asn
 290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Glu Lys
 325 330

<210> 31
 <211> 107
 <212> PRT
 <213> Homo sapiens

eol f-seql

<400> 31

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

Gl n Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gl n Trp Lys Val Asp Asn Ala Leu Gl n
35 40 45

Ser Gly Asn Ser Gl n Gl u Ser Val Thr Gl u Gl n Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Gl u
65 70 75 80

Lys His Lys Val Tyr Ala Cys Gl u Val Thr His Gl n Gly Leu Ser Ser
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Gl u Cys
100 105

<210> 32

<211> 122

<212> PRT

<213> Mus musculus

<400> 32

Gl u Val Arg Leu Met Ala Ser Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Thr Pro Gl u Lys Arg Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gly Gly Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Ser Ser Leu Arg Ser Gl u Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Al a Arg Arg Val Ser Met Ile Thr Gly Gly Trp Ala Met Asp Tyr Trp
100 105 110

Gly Gl n Gl y Thr Ser Val Thr Val Ser Ser
115 120

eol f-seql

<210> 33
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> cVH2

<400> 33

Gl u Val Arg Leu Leu Al a Ser Gl y Gl y Gl y Leu Val Lys Pro Gl y Gl y
1 5 10 15

Ser Leu Lys Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Thr Pro Gl u Lys Arg Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Al a Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Ser Ser Leu Arg Ser Gl u Asp Thr Al a Leu Tyr Tyr Cys
85 90 95

Al a Arg Arg Val Ser Met Ile Thr Gl y Gl y Trp Al a Met Asp Tyr Trp
100 105 110

Gl y Gl n Gl y Thr Ser Val Thr Val Ser Ser
115 120

<210> 34
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> cVH3

<400> 34

Gl u Val Gl n Leu Leu Al a Ser Gl y Gl y Gl y Leu Val Lys Pro Gl y Gl y
1 5 10 15

Ser Leu Lys Leu Ser Cys Al a Al a Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Thr Pro Gl u Lys Arg Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

eol f-seql

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Ser Ser Leu Arg Ser Gl u Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Al a Arg Arg Val Ser Met Ile Thr Gl y Gl y Trp Ala Met Asp Tyr Trp
100 105 110

Gl y Gl n Gl y Thr Ser Val Thr Val Ser Ser
115 120

<210> 35
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> cVH4

<400> 35

Gl u Val Arg Leu Met Gl u Ser Gl y Gl y Leu Val Lys Pro Gl y Gl y
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gl u Phe Thr Phe Ser Asn Tyr
20 25 30

Al a Met Ser Trp Val Arg Gl n Thr Pro Gl u Lys Arg Leu Gl u Trp Val
35 40 45

Al a Thr Ile Ser Arg Gl y Gl y Asp Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gl n Met Ser Ser Leu Arg Ser Gl u Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Al a Arg Arg Val Ser Met Ile Thr Gl y Gl y Trp Ala Met Asp Tyr Trp
100 105 110

Gl y Gl n Gl y Thr Ser Val Thr Val Ser Ser
115 120

<210> 36
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> cVK1

eol f-seql

<400> 36

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

Asp Glu Ala Ser Ile Ser Cys Arg Ser Ser Glu Ser Ile Val Asp Ile
20 25 30

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

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

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

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

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

<210> 37

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> hVK2

<400> 37

Glu Val Val Met Thr Glu Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ser Ser Glu Ser Ile Val Asp Ile
20 25 30

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

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

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

Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Phe Glu Asp
85 90 95

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

eol f-seql

<210> 38
<211> 153
<212> PRT
<213> Homo sapiens

<400> 38

Met Tyr Arg Met Glu Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Glu Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Gl u Gl u Leu Lys Pro Leu Gl u Gl u Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Gl u Leu Lys Gl y Ser Gl u Thr Thr Phe Met Cys Gl u Tyr Ala
115 120 125

Asp Gl u Thr Ala Thr Ile Val Gl u Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Thr
145 150

<210> 39
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> VL-146B7

<400> 39

Gl u Ile Val Leu Thr Gl n Ser Pro Gl y Thr Leu Ser Leu Ser Pro Gl y
1 5 10 15

Gl u Arg Ala Thr Leu Ser Cys Arg Ala Ser Gl n Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gl n Gl n Lys Pro Gl y Gl n Ala Pro Arg Leu Leu
35 40 45

eol f-seql

Ile Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

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

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

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Ser
100 105

<210> 40

<211> 118

<212> PRT

<213> Artificial Sequence

<220>

<223> VH-146B7

<400> 40

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

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

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

Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50 55 60

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

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Asn Trp Asn Cys Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 41

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> VL CDR3 DISC0280

<400> 41

eol f-seql

Ala Trp Tyr Asp Arg Glu Leu Ser Glu Trp Val
1 5 10

<210> 42
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> VH CDR3 DISC0280

<400> 42

Asp Pro Ala Ala Trp Pro Leu Glu Glu Ser Leu Ala Trp Phe Asp Pro
1 5 10 15

<210> 43
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> IL-15 peptide fragment stretch

<400> 43

Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn
1 5 10