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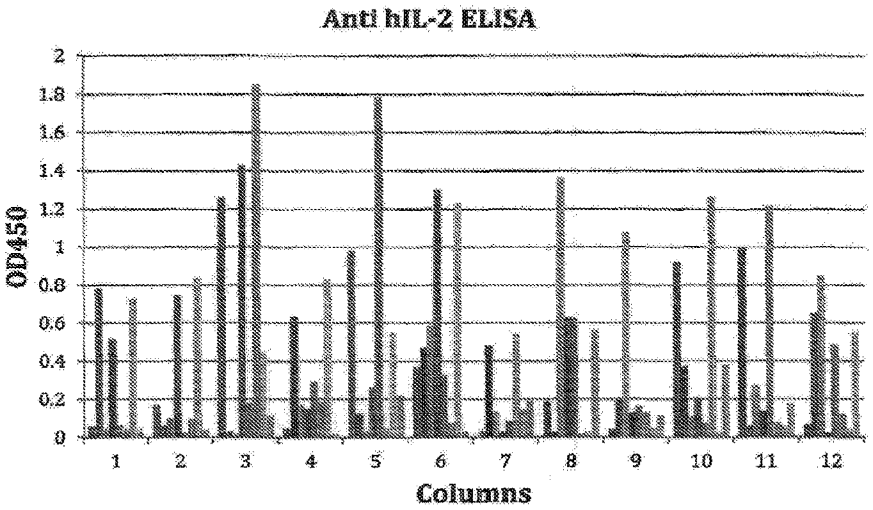
(54) **Titre** : ANTICORPS MONOCLONAUX DE STIMULATION IMMUNITAIRE CONTRE L'INTERLEUKINE-2 HUMAINE
(54) **Title**: IMMUNE-STIMULATING MONOCLONAL ANTIBODIES AGAINST HUMAN INTERLEUKIN-2



(57) **Abrégé/Abstract**:

The invention relates to a human Interieuldn-2 (hIL-2) specific monoclonal antibody (mAb), or antigen binding fragment thereof, the binding of which to hIL-2 inhibits binding of hIL-2 to CD25 and the antibody is characterized by any of the parameters: the variable chain of the mAb comprises the amino acid sequence of SEQ ID NO 005 or SEQ ID NO 006; the binding to hIL-2 is characterized by a dissociation constant (K_D)≤ 7,5 nmol/L; the binding to hIL-2 is characterized by an off-rate (K_{off})≤ $1x10_{-4}$ s^{-1} and/or the antibody displays no measurable cross-reactivity to murine IL-2.

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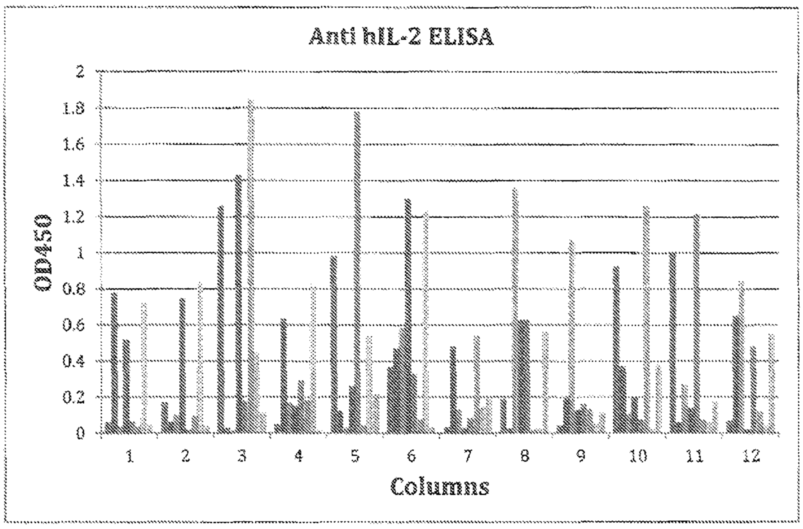

FIG. 1

(57) **Abstract**: The invention relates to a human lnterieuldn-2 (hIL-2) specific monoclonal antibody (mAb), or antigen binding fragment thereof, the binding of which to hIL-2 inhibits binding of hIL-2 to CD25 and the antibody is characterized by any of the parameters: the variable chain of the mAb comprises the amino acid sequence of SEQ ID NO 005 or SEQ ID NO 006; the binding to hIL-2 is characterized by a dissociation constant $(K_D) \leq 7{,}5$ nmol/L; the binding to hIL-2 is characterized by an off-rate $(K_{off}) \leq 1x10^{-4}$ s^{-1} and/or the antibody displays no measurable cross-reactivity to murine IL-2.

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IMMUNE-STIMULATING MONOCLONAL ANTIBODIES AGAINST HUMAN INTERLEUKIN-2

FIELD OF THE INVENTION

The present invention relates to antibodies binding to human interleukin-2 (hIL-2). The invention more specifically relates to antibodies specifically binding a particular epitope of hIL-2 and when bound to this epitope are capable of inhibit binding of hIL-2 to CD25. Furthermore, the invention relates to *in vitro* and *in vivo* therapeutic applications of the antibodies.

BACKGROUND OF THE INVENTION

Malignant melanoma is a frequent cancer type in men and women. Once melanoma becomes metastatic and spreads to distant sites, the 5-year survival rate is quite poor, calculated at about 15%. Currently available treatment strategies for metastatic melanoma barely improve this survival rate.

Interleukin-2 (IL-2) is a cytokine able to potently stimulate cytotoxic lymphocytes against metastatic tumours. However, IL-2 is also able to stimulate so-called $CD25^+$ $CD4^+$ regulatory T cells (Treg cells) that are crucial for prevention of autoimmune disease. Importantly, Treg cells can significantly dampen anti-tumour responses by cytotoxic lymphocytes, thus somewhat antagonizing the beneficial anti-tumour effects of IL-2. Moreover, at doses required to achieve a clinical anti-tumour response, IL-2 can exert toxic adverse effects.

Standard IL-2 immunotherapy has been used since the early 1980's for the immunotherapy of metastatic melanoma and metastatic renal cell carcinoma, leading to the approval by the FDA for these indications in 1996 and 1992, respectively. While IL-2 given at high doses has shown objective response rates in about 17% and complete regression in about 6-9% of patients suffering from these deadly metastatic cancers, IL-2 given at these doses frequently led to toxic adverse effects, such as hypotension, pulmonary edema, liver cell damage, gastrointestinal toxicity, and general edema. Moreover, as mentioned above, IL-2 is able to stimulate Treg cells, which in turn are able to dampen the activity of anti-tumour $CD8^+$ T cells and NK cells.

The combination of IL-2 with a particular anti-IL-2 monoclonal antibody (mAb) has been shown to improve IL-2 therapy in experimental murine models of cancer immunotherapy by

(1) directing IL-2 preferentially to cytotoxic lymphocytes, but not Treg cells, and by

(2) rendering IL-2 more potent but less toxic (Boyman O, Kovar M, Rubinstein MP, Surh CD, and Sprent J. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. *Science* (2006) 311:1924-1927; Krieg C, Letourneau S, Pantaleo G, and Boyman O. Improved IL-2 immunotherapy by selective stimulation of IL-2 receptors on lymphocytes and endothelial cells. *Proceedings of the National Academy of Sciences USA* (2010) 107:11906-11911).

This approach has the advantage that unmutated, natural IL-2 is delivered via anti-IL-2 mAb to $CD8^+$ T cells and NK cells, which subsequently exert potent anti-tumour properties, while IL-2 complexed to this kind of anti-IL-2 mAb barely activates Treg cells. Moreover, IL-2 complexed to this kind of anti-IL-2 mAb is much less toxic than standard IL-2 immuno therapy in mice. However, this therapy has up to date not been available for use in patients due to the lack of appropriate anti-human IL-2 mAbs.

SUMMARY OF THE INVENTION

The problem addressed by the present invention is to provide an anti-human IL-2 monoclonal antibody able to recognize and bind a specific epitope of human IL-2, thereby favoring the stimulation of cytotoxic T cells and NK cells compared to Treg cells, for use in *in vitro* and *in vivo* therapeutic applications. This problem is solved by the subject-matter of the independent claims.

According to a first aspect of the invention a human interleukin-2 (hIL-2) specific monoclonal antibody (mAb), or antigen binding fragment thereof, is provided, wherein the antibody is able to bind to a particular epitope in hIL-2 thereby inhibiting the binding to CD25, thus modulating the immunological effects of hIL-2/IL-2R interaction. The antibody of the invention is further characterized by at least one of the parameters:

a) the variable chain of the mAb comprises an amino acid sequence having an identity of ≥85%, ≥90%, ≥95%, or ≥99% compared to SEQ ID NO 005 or SEQ ID NO 006;

b) the antibody binding to hIL-2 is, i.e. the reaction mAb + hIL-2 $\Leftrightarrow$ mAb*hIL-2, wherein mAb*hIL-2 symbolizes the bound complex of antibody and interleukin, is characterized by a dissociation constant (K_D) ≤7,5 nmol/L, ≤5 nmol/L, ≤3 nmol/L, ≤ 2 nmol/L or ≤ 1,5 nmol/L;

c) the antibody binding to hIL-2 is characterized by an off-rate(K_{off}) ≤ $1x10^{-4}$ s^{-1}, ≤ $8x10^{-5}$ s^{-1}, ≤ $6x10^{-5}$ s^{-1}, ≤ $4x10^{-5}$ s^{-1}, ≤ $3x10^{-5}$ s^{-1} or ≤ $2,1x10^{-5}$ s^{-1};

d) upon mAb binding to hIL-2, the resulting mAb*hIL-2 complex cannot efficiently bind human IL-2 receptor α (also known as CD25) anymore, effectively rendering the binding of human CD25 to mAb*hIL-2 to background levels as compared to the binding of human CD25 to free (non-complexed) hIL-2 when measured by surface plasmon resonance; and/or

e) the antibody displays no measurable cross-reactivity to murine IL-2.

A lack of cross-reactivity with murine IL-2 is advantageous for preclinical studies, which usually involve mouse models, such as the use of mAb*hIL-2 complexes for the treatment of murine tumour models where a cross-reactive anti-IL-2 mAb might bind and seclude endogenous murine IL-2 from endogenous murine Treg cells, thus enhancing the anti-tumour response.

A lack of cross-reactivity with murine IL-2 is also advantageous for preclinical safety and efficacy studies conducted prior to development of a candidate mAb in human patients.

In certain embodiments the hIL-2 mAb comprises at least one V_H and/or V_L sequence having an identity of ≥ 80%, ≥ 85%, ≥ 90%, ≥ 92%, ≥ 93%, ≥ 94%, ≥ 95%, ≥ 96%, ≥ 97% or ≥ 98% compared to SEQ ID NOs 019 or SEQ ID NO 020.

In certain embodiments the variable chain of the hIL-2 mAb comprises an amino acid sequence having an identity of ≥85%, ≥90%, ≥95%, or ≥99% compared to SEQ ID NOs 003, 004, 005 or 006 and the hIL-2 mAb is characterized by a dissociation constant ≤7,5 nmol/L, ≤5 nmol/L, ≤3 nmol/L, ≤ 2 nmol/L or ≤ 1,5 nmol/L.

In certain embodiments the variable chain of the hIL-2 mAb comprises an amino acid sequence having an identity of ≥ 85%, ≥ 90%, ≥ 92%, ≥ 93%, ≥ 94%, ≥ 95%, ≥ 96%, ≥ 97%, ≥ 98% or ≥ 99% compared to SEQ ID NO 005 or 006 and the hIL-2 mAb is characterized by an off-rate $\leq 1x10^{-4}$ s^{-1}, $\leq 8x10^{-5}$ s^{-1}, $\leq 6x10^{-5}$ s^{-1}, $\leq 4x10^{-5}$ s^{-1}, $\leq 3x10^{-5}$ s^{-1} or $\leq 2,1x10^{-5}$ s^{-1}.

In certain embodiments the variable chain of the hIL-2 mAb comprises an amino acid sequence having an identity of ≥ 85%, ≥ 90%, ≥ 92%, ≥ 93%, ≥ 94%, ≥ 95%, ≥ 96%, ≥ 97%, ≥ 98% or ≥ 99% compared to SEQ ID NO 005 or 006 and the hIL-2 mAb displays no measurable cross-reactivity to murine IL-2.

In certain embodiments the sequence of the hIL-2 mAb is humanized for administration to human patients to prevent adverse reactions.

In certain embodiments the hIL-2 mAb is provided as fragment antigen-binding (Fab) or single-chain variable fragment (scFv).

In certain embodiments the hIL-2 mAb comprises at least one complementarity determining (CDR) sequence having an identity of ≥ 80%, ≥ 85%, ≥ 90%, ≥ 92%, ≥ 93%, ≥ 94%, ≥ 95%, ≥ 96%, ≥ 97% or ≥ 98% compared to SEQ ID NOs 007, 008, 009, 010, 011 or 012.

According to a second aspect of the invention, a nucleic acid molecule encoding the monoclonal antibody, or antigen binding fragment thereof, able to bind to human interleukin-2 according to the first aspect of the invention is provided.

In certain embodiments the nucleic acid molecule according to the second aspect of the invention has ≥60%, ≥70%, ≥80%, ≥90%, ≥95%, or ≥99% sequence identity compared to SEQ ID NOs 003 to 004.

According to a third aspect of the invention a vector comprising the nucleic acid molecule according to the invention is provided.

According to a fourth aspect of the invention, a cell is provided, comprising or expressing the nucleic acid molecule according to the invention.

According to a fifth aspect of the invention a cell able to produce the antibodies according to the first aspect of the invention is provided.

According to a sixth aspect of the invention a monoclonal antibody-producing hybridoma cell line is provided, characterized in that the antibodies produced are those of the first aspect of the invention.

According to a seventh aspect of the invention a therapeutic formulation for use in the treatment of cancer or other diseases benefiting from immune stimulatory therapy, such as viral infections, comprising

i. the monoclonal antibody (mAb) according to the first aspect of the invention, and/or

ii. human interleukin-2 or human IL-2 mutants, administered to the subject either contemporaneously or at different time points.

According to an eighth aspect of the invention a fusion protein is provided. The fusion protein comprises:

a. an hIL-2 binding polypeptide fragment, wherein said polypeptide is characterized by any one of the parameters:

i. the hIL-2 binding polypeptide fragment comprises an amino acid sequence having an identity of ≥ 85%, ≥ 90%, ≥ 92%, ≥ 93%, ≥ 94%, ≥ 95%, ≥ 96%, ≥ 97% or ≥ 98% compared to SEQ ID NO 021 or SEQ ID NO 022;

ii. the hIL-2 binding binding of said polypeptide fragment to hIL-2 is characterized by a dissociation constant (K_D) ≤ 7,5 nmol/L, ≤ 5 nmol/L, ≤ 3 nmol/L, ≤ 2 nmol/L or ≤ 1,5 nmol/L;

iii. the binding of said hIL-2 binding polypeptide fragment to hIL-2 is characterized by an off-rate (K_{off}) ≤ $1x10^{-4}$ s^{-1}, ≤ $8x10^{-5}$ s^{-1}, ≤ $6x10^{-5}$ s^{-1}, ≤ $4x10^{-5}$ s^{-1}, ≤ $3x10^{-5}$ s^{-1} or ≤ $2,1x10^{-5}$ s^{-1};

and/or

iv. the hIL-2 binding polypeptide fragment displays no measurable crossreactivity to murine IL-2.

b. a human IL-2 polypeptide fragment having an identity of ≥ 85%, ≥ 90%, ≥ 92%, ≥ 93%, ≥ 94%, ≥ 95%, ≥ 96%, ≥ 97% or ≥ 98% compared to SEQ ID NO 001, and, optionally,

c. an amino acid linker of 1 to 50, particularly of 5 to 40, more particularly of 10 to 30, even more particularly of approx. 15 to 25 amino acids, linking the hIL-2 binding polypeptide fragment to the human IL-2 polypeptide fragment as one single polypeptide chain.

In other words the fusion protein retains the ability of the antibody to bind and direct human interleukin-2 to stimulate selected immune cells, such as CD8$^+$ T cells and NK cells.

The advantage of using such fusion protein is that human IL-2 will not be able to dissociate from the antibody and that the therapy will be composed of one single product instead of two, facilitating various aspects of manufacture, dosing and regulatory compliance.

According to a ninth aspect of the invention, an isolated antibody or antigen binding fragment thereof binding a specific epitope is provided. Said epitope can be the epitope to which an isolated antibody or antigen binding fragment thereof according to other aspects of the invention binds. In an embodiment, the isolated antibody or molecule binds to a human interleukin-2 (hIL-2) epitope which comprises the amino acids K52, P54, K55, T57, R58, T61, F62, K63, Q94, and K96. In another embodiment, the isolated antibody or molecule binds to an epitope further comprising any one or more of the amino acids N50, N53, N91, L92, A93, and N97. An isolated antibody or molecule, which comprises an antigen recognition surface having epitope recognition characteristics equivalent to an antibody or antigen binding fragment thereof according to other aspects is also provided.

Wherever alternatives for single separable features such as, for example, a coding sequence or binding epitope are laid out herein as “embodiments”, it is to be understood that such alternatives may be combined freely to form discrete embodiments of the invention disclosed herein.

The invention is further illustrated by the following examples and figures, from which further embodiments and advantages can be drawn. These examples are meant to illustrate the invention but not to limit its scope.

Definitions

By “human interleukin-2” or “hIL-2” is meant the protein designated UniProt ID P60568 and is reproduced as SEQ ID NO: 1.

Identity in the context of the present specification is a single quantitative parameter representing the result of a sequence comparison position by position. Methods of sequence comparison are known in the art; the BLAST algorithm available publicly is an example. One such example for comparison of nucleic acid sequences is the BLASTN algorithm that uses the default settings: Expect threshold: 10; Word size: 28; Max matches in a query range: 0; Match/Mismatch Scores: 1.-2; Gap costs: Linear. In the absence of other measurement variables, identity shall be measured according to the specification above.

In the context of the present specification, the term *antibody* is used in its meaning known in the art of cell biology and immunology; it refers to whole antibodies, any antigen binding fragment or single chains thereof and related or derived constructs. A whole antibody is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (V_H) and a heavy chain constant region (C_H). The heavy chain constant region is comprised of three domains, C_H1, C_H2 and C_H3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region (C_L). The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions 20 (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs arranged from amino-terminus to carboxyterminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component of the classical complement system.

In the context of the present specification, the term *antigen binding portion* or *antigen binding fragment* is used in its meaning known in the art of cell biology and immunology; it refers to one or more fragments of an intact antibody that retain the ability to specifically bind to a given antigen (e.g., interleukin-2). Antigen binding functions of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term antigen binding portion or antigen binding fragment of an antibody include a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_H domains; a $F(ab)_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; an Fd fragment consisting of the V_H and C_H domains; an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a single domain antibody (dAb) fragment, which consists of a V_H domain or a V_L domain; and an isolated complementarity determining region (CDR). HCDR means a CDR of the heavy chain and LCDR means a CDR of the light chain.

In the context of the present specification, the term *chimeric antibody* is used in its meaning known in the art of cell biology and immunology; it refers to an antibody molecule in which the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, cytokine, toxin, hormone, growth factor, drug, etc. For example, an antibody can be modified by replacing its constant region with a cytokine. Due to the replacement with a cytokine, the chimeric antibody can retain its specificity in recognizing the antigen while having also the function, or part thereof, of the original cytokine molecule.

In the context of the present specification, the term *hybridoma* is used in its meaning known in the art of cell biology and biochemistry; it refers to a hybrid cell created by fusion of a specific antibody-producing B-cell with a myeloma (B-cell cancer) cell. Hybridoma cells can be grown in tissue culture and produce antibodies of a single specificity (monoclonal antibodies).

In the context of the present specification, the term *single-chain variable fragment (scFv)* is used in its meaning known in the art of cell biology and biochemistry; it refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. The scFv retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker.

In the context of the present specification, the term *fragment antigen-binding (Fab)* is used in its meaning known in the art of cell biology and immunology; it refers to a region on an antibody that binds to antigens. It is composed of one constant and one variable domain of each of the heavy (V_H) and light chains (V_L) of immunoglobulins. These domains shape the antigen-binding site at the amino terminal end of the monomer.

In the context of the present specification, the term *dissociation constant (K_D)* is used in its meaning known in the art of chemistry and physics; it refers to an equilibrium constant that measures the propensity of a larger object to dissociate reversibly into smaller components, as when a complex falls apart into its component molecules. K_D is expressed in molar units [M] and corresponds to the concentration of [Ab] at which the binding sites of [Ag] are half occupied. In other words the concentration of unbound [Ab] equals the concentration of the [AbAg] complex. The dissociation constant can be calculated according to the following formula:

$$\mathrm{K_D} = \frac{[\mathrm{Ab}] * [\mathrm{Ag}]}{[\mathrm{AbAg}]}$$

[Ab]: concentration of antibody; [Ag]: concentration of antigen; [AbAg]: concentration of antibodyantigen complex

In the context of the present specification, the terms *off-rate* (Koff;[1/sec]) and *on-rate* (Kon; [1/sec*M]) are used in their meaning known in the art of chemistry and physics; they refer to a rate constant that measures the dissociation (Koff) or association (Kon) of 5 an antibody with its target antigen. Koff and Kon can be experimentally determined using methods well established in the art. A method for determining the Koff and Kon of an antibody employs surface plasmon resonance. This is the principle behind biosensor systems such as the Biacore® or the ProteOn® system. They can also be used to determine the dissociation constant KD by using the following formula:

$$K_D = \frac{[K_{off}]}{[K_{on}]}$$

In the context of the present specification, the term *humanized antibodies* is used in its meaning known in the art of cell biology and biochemistry; it refers to antibodies originally produced by immune cells of a non-human species, whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans.

In the context of the present specification, the term *no measurable cross-reactivity* refers to the lacking capability of an antibody to recognize and bind to orthologous proteins from other species. For example, an antibody directed against human interleukin-2 would have no measurable cross-reactivity to murine interleukin-2 if, under suitable conditions, binding of the antibody to murine interleukin-2 could not be detected with sufficiently sensitive methods such as surface plasmon resonance. One such example of no measurable cross-reactivity is shown in Fig. 9 for the antibody in the lower panel (NARA1).

As used herein, an antibody or a protein that "specifically binds to hIL-2" is intended to refer to an antibody or protein that binds to human IL-2 polypeptide with a K_D of 100nM or less, 10nM or less, 1nM or less, 100pM or less, or 10pM or less. An antibody that "cross-reacts with an antigen other than human IL-2 " is intended to refer to an antibody that binds that antigen with a K_D of 10nM or less, 1 nM or less, or 100 pM or less. An antibody that "does not cross-react with a particular antigen" is intended to refer to an antibody that binds to that antigen, with a K_D of 100 nM or greater, or a K_D of 1 µM or grater, or a K_D of 10 µM or greater. In certain embodiments, such antibodies that do not cross-react with the antigen exhibit essentially undetectable binding against these proteins in standard binding assays.

The term “epitope” means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

The term "epitope binding domain" or "EBD" refers to portions of a binding molecule (e.g., an antibody or epitope-binding fragment or derivative thereof), that specifically interacts with (e.g., by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) a binding site on a target epitope. EBD also refers to one or more fragments of an antibody that retain the ability to specifically interact with (e.g., by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) a IL-2 epitope and inhibit signal transduction. Examples of antibody fragments include, but are not limited to, an scFv, a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)$_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and an isolated complementarity determining region (CDR).

EBDs also include single domain antibodies, maxibodies, unibodies, minibodies, triabodies, tetrabodies, v-NAR and bis-scFv, as is known in the art (see, e.g., Hollinger and Hudson, (2005) Nature Biotechnology 23: 1126-1136), bispecific single chain diabodies, or single chain diabodies designed to bind two distinct epitopes. EBDs also include antibody-like molecules or antibody mimetics, which include, but not limited to minibodies, maxybodies, Fn3 based protein scaffolds, Ankrin repeats (also known as DARpins), VASP polypeptides, Avian pancreatic polypeptide (aPP), Tetranectin, Affililin, Knottins, SH3 domains, PDZ domains, Tendamistat, Neocarzinostatin, Protein A domains, Lipocalins, Transferrin, and Kunitz domains that specifically bind epitopes, which are within the scope of the invention. Antibody fragments can be grafted into scaffolds based on polypeptides such as Fibronectin type III (Fn3) (see U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies).

The present invention also encompasses an antibody to human IL-2, which is an isolated antibody.

The phrase "isolated antibody", as used herein, refers to antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds hIL-2 is substantially free of antibodies that specifically bind antigens other than hIL-2). An isolated antibody that specifically binds hIL-2 may, however, have cross-reactivity to other antigens, such as IL-2 molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The terms “nucleic acid” and “polynucleotide” or “nucleotide coding sequences” are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and can perform any function. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, siRNAs, shRNAs, RNAi agents, and primers. A polynucleotide can be modified or substituted at one or more base, sugar and/or phosphate, with any of various modifications or substitutions described herein or known in the art. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polymer. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

The term “polypeptide” is used interchangeably with the term “protein” and in its broadest sense refers to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits can be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc.

As used herein, the term “treating" or "treatment" of any disease or disorder (e.g. cancer) refers in one embodiment, to ameliorating the disease or disorder (e.g. slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another embodiment "treating" or "treatment" refers to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. In yet another embodiment, "treating" or "treatment" refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. Methods for assessing treatment and/or prevention of disease are generally known in the art, unless specifically described hereinbelow.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows anti-human IL-2 binders. Supernatants of B cell clones obtained after B cell hybridoma fusion were added to a plate previously coated with human IL-2. The anti-human IL-2 mAbs were detected using a biotinylated anti-mouse IgG antibody.

Figure 2 shows screening of anti-human IL-2 mAbs for binding to presumed specific human IL-2 epitope. Plates were coated with 5344 (a hIL-2 mAb without the herein targeted superagonistic behaviour) and blocked, followed by addition of human IL-2 in order to allow the cytokine to bind to 5344, thus covering a specific epitope of the IL-2. Then the supernatants giving a positive signal in the first screening (see Figure 1) were added. After allowing the mAbs in the supernatants to bind to the IL-2-5344 complex, a biotinylated MAB602 antibody was added to the plate in order to assess whether the tested mAbs of the supernatants bound to the same (so-called “competitors”) or to a different region than MAB602. The competitor mAbs led to an absorbance (OD450) that is two-fold lower than the absorbance obtained with MAB602 alone (in this case OD = 1.1, as shown in H11).

Figure 3 shows concentration-dependent competition of B cell hybridomas. The supernatants of 8 competitor B cell hybridoma clones of the first screening (see Figure 2) were expanded and concentrated before use in this assay. The supernatants of these 8 competitor B cell hybridoma clones (labeled 1 to 8) were added in increasing quantities. Competent competitor B cell hybridoma clones reduced the OD450 as much as MAB602 or even more, which is evident for clones 1 and 2. MAB602 at different concentrations (green open circles) served as a control.

Figure 4 shows in vivo proliferation of CD8+ T cells. Carboxyfluorescein succinimidyl ester (CFSE)-labeled CD8+ T cells of CD45.1-congenic IL-7 transgenic mice were transferred to CD45.2-congenic WT recipient mice, followed by daily injections of phosphate-buffered saline (PBS), IL-2, IL-2 plus MAB602 (IL-2/MAB602), IL-2 plus 5344 (IL-2/5344), IL-2 plus hybridoma 1 (IL-2/Hyb#1), or IL-2 plus hybridoma 2 (IL-2/Hyb#2) for 4 days. On day 5, lymph nodes and spleens were analyzed for CFSE profiles of donor CD45.1+ CD8+ T cells. Shown are the results obtained with the lymph nodes, similar results were obtained in the spleens.

Figure 5 shows phenotypic charaterisation of endogenous CD8+ T cells and NK cells following in vivo treatment using IL-2 plus hybridoma 1 and 2. Mice were treated as in Figure 4, followed by assessment by flow cytometry of endogenous CD8+ T cell subsets and NK cells in the lymph nodes and spleen. Shown are (A) CD8 vs. CD3 profiles of total lymph node cells (left graphs) and CD44 (activated or memory T cells) vs. CD122 (IL-2 receptor β-subunit, present on activated or memory T cells) profiles of CD3+ CD8+ lymph node cells, or (B) NK1.1 vs. CD3 profiles of mice receiving the indicated treatment. Activated/memory CD8+ T cells are high for CD44 and intermediate to high for CD122. NK cells are CD3 negative and NK1.1 positive. Similar results were obtained using spleen cells.

Figure 6 shows total cell counts of activated/memory CD8+ T cells and NK cells in lymph nodes and spleens. Animals were treated and analyzed as in Figure 5. Shown are absolute cell counts of CD44high CD8+ T cells (so-called memory phenotype, MP CD8+) and of CD3 negative NK1.1+ NK cells in lymph nodes (top panel) and spleen (lower panel).

Figure 7 shows surface plasmon resonance binding curves of the commercially available monoclonal antibody MAB602 (left graph) and the monoclonal antibody NARA1 (right graph), which is the subject of this invention, to human IL-2. For this experiment an amine coupling GLM chip was used. The activation of the carboxylic acid groups in the chip was done using a mix of 1-ethyl-3-3-dimethylaminopropyl carbodiimide hydrochlorid (EDC at 0.2 M) and sulfo N-hydroxysulfosuccinimids (s_NHS at 0.05M) at 30 ml/min for 420 seconds (s). The antibodies NARA1 and MAB602 were coated in the chip at 100 mg/ml in a sodium acetate buffer (10 mM pH 4.5). Deactivation was followed adding ethanolamine HCl at 30 ml/min for 300 s. Finally human IL-2 was added at different concentrations (starting from 100 nM and followed by three-fold dilutions) at 100 ml/min, 600 s association, and 240 s dissociation.

Figure 8 shows surface plasmon resonance binding curves of human IL-2 bound to the monoclonal antibody NARA1 with the IL-2 receptors subunits CD25 (used here as an Fc fusion of CD25-Fc), CD122, the monoclonal antibody MAB602 or an anti-hIL-2 antibody binding to a different human IL-2 epitope than NARA1 and MAB602. The chip described in Figure 7 coated with NARA1 and MAB602 was re-used. Regeneration of the chip was done using 10 mM glycine, pH 2.5, 30 ml/min, 60 s. Human IL-2 was added at saturating concentration (1 mM), at 100 ml/min, 120 s association, and 0 s dissociation. Immediately after IL-2 association to the antibodies, the second analytes were added at 100 ml/min, 120 s association, and 240s dissociation. The concentration used for the cross-binding were: MAB602: 50 nM; NARA1 : 50 nM; positive control: 50 nM; CD25-Fc: 500 nM; CD122: 138 nM. When hIL-2 is bound to NARA1, an anti-hIL-2 antibody that recognizes a different hIL-2 epitope (here termed 'positive control') binds strongly to the hIL-2/NARA1 complex as expected (green line in Figure 8). Alternatively, IL-2Ra (in the form of CD25-Fc) cannot bind to hIL-2 when hIL-2 is already bound to NARA1 (pink line, Figure 8), however, IL-2Rβ (CD122) still binds to hIL-2 when hIL-2 is already bound to NARA1 (orange line, Figure 8).

Figure 9 shows surface plasmon resonance binding curves of the monoclonal antibodies MAB602 (top graph) and NARA1 (lower graph) to murine IL-2. The same chip used for the generation of the data in Figures 7 and 8 was re-used. Regeneration of the chip was done with 10 mM glycine, pH 2.5, 30 ml/min, 60 s. Mouse IL-2 (mIL-2) or human IL-2 (hIL-2) starting at 10 nM and then doing a three-fold dilution was injected at 100 ml/min, 120 s association, 5 and 240 s dissociation. In the top graph MAB602 shows cross-reactivity by binding to mouse IL-2. Especially, with higher concentrations of murine interleukin-2 (>1 nM) the binding curves differ significantly from background levels with response units 10 (RU) well above 10. Whereas NARA1 (lower graph) displays no measurable cross-reactivity to murine IL-2 at all concentrations tested.

Figure 10 provides the overview of the three-dimension structure of Proleukin®/Fab-NARA1 complex as obtained in Example 1.

Figure 11 provides further analysis of epitope residues. The X-axis lists the amino acid sequence and numbering according to SEQ ID No 1. The upper side of Y-axis shows the total number of atoms of NARA1-Fab that are within 4 Å from corresponding residue from Proleukin® and the lower side of Y-axis shows the reduced solvent-accessible area (Å^2) of corresponding residue from Proleukin® as a consequence of binding to NARA1-Fab.

Figure 12 illustrates the most critical epitope residue recognized by the NARA1-Fab.

Figure 13 shows the overlay of Proleukin®/NARA1-Fab complex with IL-2/CD25/CD122/CD132 quaternary complex.

Figure 14 displays the overlay of C helices from IL-2_C145A (PDB: 3INK), Superkine (PDB: 3QB1), IL-2/CD25/CD122/CD132 (PDB: 2B5I), and Proleukin®/NARA1-Fab.

DETAILED DESCRIPTION OF THE INVENTION

Until now, no monoclonal antibodies suitable for the disclosed invention have been available. The inventors disclose their anti-human IL-2 mAbs that allow the following crucial steps towards the use and commercialization of this technology in clinical applications:

- Further sequencing and fine characterization of the anti-human IL-2 mAbs.

- Humanization of the anti-human IL-2 mAbs, which is essential to avoid (or minimize) immunogenicity in patients.

- Generation of different formats of anti-human IL-2 mAbs, such as IgG, IgG1, IgG4, Fab, and single-chain Fv (scFv).

- Generation of a fusion protein consisting of human IL-2 and an anti-human IL-2 mAb (or a fragment of the anti-human IL-2 mAb): such a construct has the advantage of consisting of one component only, instead of two as in IL-2 bound to an anti-human IL-2 mAb.

The inventors have generated and characterized specific anti-human IL-2 mAbs that are able to bind human IL-2 and, when tested in mice, are able to exert specific and potent stimulation of cytotoxic lymphocytes, including CD8$^+$ T cells and natural killer (NK) cells. Towards these ends several difficulties had to be overcome.

• Human IL-2 shows high similarity with mouse and rat IL-2, thus human IL-2 is able to stimulate mouse lymphocytes in vitro and in vivo. Moreover, IL-2 is present at high concentrations in the primary immune organs (such as the bone marrow), which is the reason why IL-2 is somewhat a “forbidden” antigen, meaning it is very difficult to generate B cell responses leading to neutralizing antibodies against IL-2. Nevertheless, the inventors were able to elicit polyclonal anti-human IL-2 antibody responses, following immunization of C57BL/6 mice using purified recombinant human IL-2 plus adjuvant.

• Of the generated antibody responses, only some mAbs efficiently bound to IL-2 (socalled “binders”) and of those only about 0.35 % interacted with the presumed active site of IL-2.

• Finally, of these anti-human IL-2 mAbs some showed the desired specific and potent in vivo activity as assessed by specialized in vivo assays in mice that are not replaceable by in vitro experiments.

The inventors have developed specific screening assays that allow detection of specific antihuman IL-2 antibodies (so-called “binders”) in the serum of immunized animals and in the supernatant of the B cell clones obtained after B cell hybridoma fusion. In a second step it was discriminated between standard binders and those targeting a presumed specific epitope of the human IL-2 molecule. One example of such an in vitro enzyme-linked immunosorbent assay (ELISA) performed with different B cell clones, is shown in Figures 1 to 3.

After the in vitro screening of the anti-human IL-2 mAbs, these mAbs were characterised in vivo. To this end and in order to obtain sufficient amounts of mAbs, the mAbs were concentrated from the supernatant of the hybridomas, the amount was estimated using an ELISA and finally the anti-human IL-2 mAbs was tested in mice. The results obtained on proliferation and expansion of CD8$^+$ T cells and NK cells is shown in Figures 4 to 6.

In order to characterize the binding properties of the anti-human IL-2 mAbs the binding to human interleukin-2 was tested with surface plasmon resonance binding assays. The commercially available anti-human IL-2 mAb MAB602 was measured as a comparison. In Figure 7 binding curves of MAB602 (left graph) and NARA1 (an antibody according to this invention; right graph) to human interleukin-2 at varying concentrations are shown. The dissociation constant (K_D) as well as the rate constants K_{on} and K_{off} measured for MAB602 and NARA1 are shown in Table 1.

Table 1

	K_{on} (M^*s^{-1})	K_{off} (s^{-1})	K_D (nM)
MAB602	5.8×10^4	4.94×10^{-4}	9.7
NARA1	1.78×10^4	2.08×10^{-5}	1.2

Table 1: Binding properties of anti-human IL-2 mAbs to human IL-2

Examples

Antibodies of the invention include the antibody NARA1, which was derived, isolated and structurally characterized by its full length heavy chain according to SEQ ID NO: 5 and its full length light chain amino acid sequences according to SEQ ID NO: 6.

The corresponding variable regions, V_H and V_L amino acid sequences of NARA1 are. SEQ ID NO: 19 (variable heavy) and SEQ ID NO: 20 (variable light).

Full length light and heavy chains nucleotide coding sequences of NARA1 are SEQ ID NO: 3 (heavy chain coding sequence, including leader sequence) and SEQ ID NO: 4 (light chain coding sequence, including leader sequence).

Variable light and heavy chains nucleotide coding sequences of NARA1 are SEQ ID NO: 21 (variable heavy coding sequence) and SEQ ID NO: 22 (variable light coding sequence).

The CDR regions of NARA1 are delineated using the Kabat system (Kabat, E. A., et al. 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, see also Zhao&Lu 2009, Molecular Immunology 47:694-700).For the ease of reading, when CDR regions are delineated according to Kabat definition, they are called hereafter HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 respectively. The CDR regions of NARA1 are: HCDR1 according to SEQ ID NO: 7, HCDR2 according to SEQ ID NO: 8, HCDR3 according to SEQ ID NO: 9, LCDR1 according to SEQ ID NO: 10, LCDR2 according to SEQ ID NO: 11, LCDR3 according to SEQ ID NO: 12.

Nucleotide coding sequences for the CDR regions of NARA1 are: HCDR1 coding sequence according to SEQ ID NO: 13, HCDR2 coding sequence according to SEQ ID NO: 14, HCDR3 coding sequence according to SEQ ID NO: 15, LCDR1 coding sequence according to SEQ ID NO: 16, LCDR2 coding sequence according to SEQ ID NO: 17, LCDR3 coding sequence according to SEQ ID NO: 18.
Fusion proteins are also provided according to SEQ ID NO: 23 and SEQ ID NO: 24. SEQ ID NO: 23 is a fusion protein comprising the variable heavy chain of NARA1 with its N-terminus fused to the C-terminus of hIL-2 via a GxS linker. SEQ ID NO: 24 is a fusion protein comprising the variable light chain of NARA1 with its N-terminus fused to the C-terminus of hIL-2 via a GxS linker.

(a) Example 1. Crystal structure of NARA1

(i) Material and Methods

The complex structure of a human Interleukin 2 mutant (SEQ ID NO:2), called “Proleukin®”, bound to the Fab fragment of antibody “NARA 1” (SEQ ID NO: 5 and 6) was determined. The resulting numbering of residues on Proleukin® is given according to the numbering of wt IL-2.

As will be discussed in detail below, the differences in sequence between Proleukin® and wt hIL-2 are irrelevant and Proleukin® is a valid model for structural analysis of hIL-2.

To define the epitope, X-ray crystallography was ued to solve the atomic-resolution structure of the complex mentioned above. X-ray crystallography is a technology that has become routinely and widely used to generate structural data for biomolecules including antibodies and their complexes with antigens (Adms et al, (2013) Annual Review Biophysics 42:265-287; Garman, (2014) Science 343:1102-1108; Joachimiak, (2009) Current Opinio Structural Biology 19:573-584.)

The antigen, Proleukin®, is commercially available as lyophilyzed powder together with excipients (every 1 mg Proleukin® is mixed with approximately 50mg mannitol, 0.18mg sodium dodecyl sulfate, 0.173mg sodium dihydrogen phosphate, and 0.89mg disodium hydrogen phosphate). Before used for complex formation, Proleukin® was purified by reverse-phase HPLC to remove the excipients.

The Fab fragment of NARA1 (NARA1-Fab) was generated by papain cleavage of the full-length antibody followed by Protein A chromatography. Briefly, 6.5ml full-length NARA1 (9mg/ml in 50mM citrate buffer with 90mM sodium chloride at pH 7.0) was mixed with 5mM DTT and 590ug Papain (Roche). The cleavage reaction was kept at room temperature for 16h and stopped by addition of 15ul 56mM E64 solution (Roche). The cleavage solution was then diluted 10 times with 25mM Tris, 25mM NaCl, pH 8.0 and loaded onto a 5ml Protein A column (GE Healthcare) equilibrate with 5 column volune of 25mM Tris, 25mM NaCl, pH 8.0 and Fab fragment was in the loading-through fraction and Fc fragment was bound to the Protein A column.

To form complex, Proleukin® powder after HPLC was dissolved in H_2O at the concentration of 5.5 mg/ml. 6.6mg Proleukin®, in excess, was added to 11.5mg NARA1 Fab fragment solution drop by drop. Centrifugation was used to remove the excess Proleukin® that was precipitated under current condition. The complex was then purified by gel filtration with Superdex® 200 10x300 (GE Healthcare) with running buffer of 25mM Tris, 25mM NaCl, pH 7.4.

Proleukin®/NARA1-Fab complex after gel filtration was concentrated to 14mg/ml and was screened by vapour diffusion method as sitting drops. The protein solution was mixed 1:1 with reservoir buffer to a total size of 0.4ul. The experiments were set up with Phoenix robotic system (Art Robbins Instruments), stored in a RockImager hotel (Formulatrix) at 19°C, and imaged automatically. Crystals were harvested 4 days after screening under condition of 20% w/v polyethylene Glycol 3350 and 0.2M sodium nitrate. Crystals were cryo-protected with reservoir buffer containing 10% glycerol and flashed frozen in liquid nitrogen prior to data collection. Diffraction data were collected at the Swiss Light Source (Villigen, Switzerland) at beam-line PX-II with a Pilatus® pixel detector using x-ray radiation wavelength of 0.99998 Å.

The dataset was processed with XDS and XSCALE (version Dec. 6th, 2010) and the structure was resolved with molecular replacement method with the program PHASER®

by using Protein Data Bank entry “3INK” as search model for IL-2 and Protein Data Bank entry “3TTI” as search model for Fab fragment. Iterative model building and refinement were performed with the programs Coot (Crystallographic Object-Oriented Toolkit) and AUTOBUSTER® (Bricogne et al., 2011). All figures were generated with the program PyMOL (Molecular Graphics System; DeLano Scientific: Palo Alto, CA; http://www.pymol.org).

Epitope residues are defined as those residues from Proleukin® that are within 4Å distance from any atom in Fab fragment of NARA1 and are further confirmed by CCP4 program CONTACT and AREAIMOL (Collaborative Computational Project, Number 4, version 6.4.0). Similarly paratope residues are defined as those residues from NARA1-Fab that are within 4Å distance from any atom in Proleukin®.

(ii) Results

The Proleukin®/NARA1-Fab complex was solved to 1.95 Å in space group C 1 2 1 with unit cell dimension a=201.8Å, b= 36.2Å, c= 88.7Å, alpha= 90°, beta= 102.9°, gamma=90 °. Please refer to Table 2 for detailed structure statistics. In each asymmetric unit, there is one complex molecule.

Table 2. Structure statistics for Proleukin®/NARA1-Fab complex

Data collection	
Space group	C1 2 1
Cell dimensions	
a, *b*, *c* (Å)	201.757, 36.233, 88.707
a, b, g (°)	90, 102.93, 90
Resolution (Å)	58.74-1.95
R_{merge}	0.066 (0.472)
I /σ*I*	14.18 (2.59)
Completeness (%)	84.8(96)
Redundancy	3.19
Refinement	
Resolution (Å)	58.74-1.95
No. reflections	34750
R_{work} / R_{free}	0.2052/0.2872
Ramachandran plot	
Outliners	0.0162

Allowed	0.0378
Favored	0.9459
R.m.s. deviations	
Bond lengths (Å)	0.01
Bond angles (°)	1.7

1) *Epitope and paratope analysis*

Figure 10 provides the overview of the three-dimension structure of Proleukin®/Fab-NARA1 complex as obtained in Example 1. Light chain of Fab fragment of NARA1 is designated A, heavy chain of Fab fragment of NARA1 is shown as B, epitope residues recognized by NARA1-Fab are designated D, and Proleukin® is designated C and the mutation, C145S, is highlighted.

Figure 11 provides further analysis of epitope residues. The X-axis lists the amino acid sequence and numbering according to SEQ ID No 1. The upper side of Y-axis demonstrates the total number of atoms of NARA1-Fab that are within 4 Å from corresponding residue from Proleukin® and the lower side of Y-axis demonstrates the reduced solvent-accessible area (Å^2) after binding to NARA1-Fab.

Proleukin used in Example 1 contains mutation of C145S. As shown in Figure 10, C145S is far away from the epitope region. In addition the superposition of Cα atoms between Proleukin® in Example 1 with Cα atoms from wt hIL-2 in complex with CD25, CD122, and CD132 (PDB: 2B5I) shows r.m.s.d of 0.447 Å, which indicates that the mutation does not disturb the over-all structure. Hence Proleukin® with C145S mutation is a valid model for structural analysis for wt hIL-2.

hIL-2 is 4-helix bundle protein and the 4 helices are named from N-terminus to C-terminus as A, B, C, and D, respectively. The epitope recognized by NARA1-Fab as shown in Figure 10 is a conformational epitope and spans two regions as shown in Figure 11: one region (N50-K63) comprises a loop and a short helix and connects helix A and B, and the other region (N91-N97) comprises a loop and connects helix B and C.

The epitope residues together with interacting paratope residues from NARA1-Fab are summarized in Table 3. Among all the epitope residues, Arg58 as shown in Figure 11 is the most critical epitope residue for binding with NARA1-Fab, as this residue alone has 42 interacting atoms from NARA1-Fab and accounts for 17.7% of total reduced solvent-accessible surface area as a consequence of binding to NARA1-Fab. Furthermore Arg58, as shown in Figure 12, forms two strong salt-bridges with Glu35 in HCDR1 and with Asp100 from LCDR3, respectively. Arg58 also makes π-action interaction with the aromatic ring of Try100 from LCDR3. Residues K52, P54, K55, T57, T61, F62, K63, Q94, and K96 are also considered important for the binding to NARA1-Fab, since they all show equal to/more than 5 interacting atoms from NARA1-Fab and larger than 30Å^2 reduced solvent-accessible area as shown in Figure 11.

Table 3. Epitope and paratope summary

Light chain residue	Epitope residue	Heavy chain residue
Y31	N50	
Y31	K52	
Y31	N53	
Y31, Y36, S95, N96	P54	
	K55	W99, G101, G103, Y105
D98	T57	
D98, Y100	R58	L33, E35, W47, W99
	T61	N52, S55, N59
	F62	L33, N52
	K63	S55
	N91	G101, D102, G103
	L92	W99, G101
	A93	G101
	Q94	D102, G103, Y104
D32, D34	K96	Y104
D32	N97	

Figure 12 illustrates Arg58 as the most critical epitope residue recognized the NARA1-Fab. A represents Proleukin®, B represents heavy chain, and C represents light chain. The involved residues are shown as sticks.

2) NARA1-Fab binding properties

Figure 13 shows the overlay of Proleukin®/NARA1-Fab complex with IL-2/CD25/CD122/CD132 quaternary complex. The quaternary complex structure comes from PDB entry “2B5I” with cartoon D in pale cyan representing wt hIL-2, cartoon B in red representing CD122, cartoon C in blue representing CD132, and surface A in green representing CD25. In the Proleukin®/NARA1-Fab complex structure, cyan cartoon D overlayed with wt hIL-2 represents Proleukin®, cartoon E in magenta represents heavy chain, and cartoon F in yellow represents the light chain.

The structure overlay of the two complexes as shown in Figure 13 clearly shows that NARA1-Fab forms direct competition against CD25 but not against CD122/CD132, which is consistent with the observation that IL-2/NARA1 complex demonstrates mainly pro-Teffector cell activity rather than pro-Treg activity.

3) C helix of Proleukin® in complex with NARA1-Fab adopts conformation that is similar to that in quaternary complex

Figure 14 displays the overlay of C helices from IL-2_C145A (PDB: 3INK), Superkine (PDB: 3QB1), IL-2/CD25/CD122/CD132 (PDB: 2B5I), and Proleukin®/NARA1-Fab.

The polar interface between helix C in IL-2 and CD122 plays an important role in binding between the two parts(Wang et al (2005) Science 310:1159-1163). In 2012 Levin, et al have demonstrated that superkine, an IL-2 mutant, alone has a Helix C adopting confirmation similar to that in the quaternary complex and superkine showed ~215 times higher binding affinity towards CD122 than wtIL-2 (Levin et al, (2012) Nature 484:529-533).It was observed that such a conformational change in helix C is associated with conformational stabilization, which then reduces the energetic penalties for binding to CD122. As shown in Figure 14, The conformation of helix C from Proleukin® in complex with NARA1-Fab is also similar to that observed in Superkine as well as in IL-2/CD25/CD122/CD132 quaternary complex, therefore it is possible that Proleukin®/NARA1-Fab complex may demonstrate higher binding affinity towards CD122 than wt hIL-2 does.

(b) Example 2. Linear peptide mapping of NARA1 and MAB602

In order to map the epitope of the NARA1 and MAB602 antibodies, a first library of 15-mer peptides was generated based on the sequence of human IL2. A second library of selected 15-mer peptides was also generated based on the mutation of 3 specific residues F(62), Y(65) and L(92). The latter mutations were done based on the Roche/Glycart IL2 mutein, as disclosed in WO2012/107417A1 which has these 3 mutations. Previous work done in lab Boyman (unpublished) showed that the commercial mouse anti-human IL2 mAb 602 with analogous function as A1 has strongly reduced binding to the F42A mutant of IL2 (one of the IL2 docking sites to CD25).

(i) Material and Methods

Accordingly, each peptide in the first library has 15 amino acids and the sequence is derived by scanning the sequence of interest (see Table 4, reference peptides 1 to 41) with a step of 3 residues, starting from the N-terminus. Therefore a ladder is generated and each peptide contains 12 overlapping residues with the previous peptide and 12 overlapping residues with the following peptide in the ladder. In total, 41 peptides were generated from the expressed human IL2 sequence.

A second library of peptides was generated by mutating F(62), Y(65) and L(92) to alanine in all corresponding peptides in the first library generated as described above (see Table 4, reference peptides no 42 to 60).

For both libraries, the parental cysteines have been replaced by a serine (underlined residues) to avoid unspecific binding.

Table 4. Library of reference peptides

Reference Peptide No.	**Sequence** Residue in bold are the Alanine (A) replacing specific residues. Residue are the serine (S) replacing cysteines (C)	**SEQ ID NOs.**
1	APTSSSTKKTQLQLE	SEQ ID NO. 25
2	SSSTKKTQLQLEHLL	SEQ ID NO. 26
3	TKKTQLQLEHLLLDL	SEQ ID NO. 27
4	TQLQLEHLLLDLQMI	SEQ ID NO. 28
5	QLEHLLLDLQMILNG	SEQ ID NO. 29
6	HLLLDLQMILNGINN	SEQ ID NO. 30
7	LDLQMILNGINNYKN	SEQ ID NO. 31
8	QMILNGINNYKNPKL	SEQ ID NO. 32
9	LNGINNYKNPKLTRM	SEQ ID NO. 33
10	INNYKNPKLTRMLTF	SEQ ID NO. 34
11	YKNPKLTRMLTFKFY	SEQ ID NO. 35
12	PKLTRMLTFKFYMPK	SEQ ID NO. 36
13	TRMLTFKFYMPKKAT	SEQ ID NO. 37
14	LTFKFYMPKKATELK	SEQ ID NO. 38
15	KFYMPKKATELKHLQ	SEQ ID NO. 39
16	MPKKATELKHLQSLE	SEQ ID NO. 40

17	KATELKHLQSLEEEL	SEQ ID NO. 41
18	ELKHLQSLEEELKPL	SEQ ID NO. 42
19	HLQSLEEELKPLEEV	SEQ ID NO. 43
20	SLEEELKPLEEVLNL	SEQ ID NO. 44
21	EELKPLEEVLNLAQS	SEQ ID NO. 45
22	KPLEEVLNLAQSKNF	SEQ ID NO. 46
23	EEVLNLAQSKNFHLR	SEQ ID NO. 47
24	LNLAQSKNFHLRPRD	SEQ ID NO. 48
25	AQSKNFHLRPRDLIS	SEQ ID NO. 49
26	KNFHLRPRDLISNIN	SEQ ID NO. 50
27	HLRPRDLISNINVIV	SEQ ID NO. 51
28	PRDLISNINVIVLEL	SEQ ID NO. 52
29	LISNINVIVLELKGS	SEQ ID NO. 53
30	NINVIVLELKGSETT	SEQ ID NO. 54
31	VIVLELKGSETTFMS	SEQ ID NO. 55
32	LELKGSETTFMSEYA	SEQ ID NO. 56
33	KGSETTFMSEYADET	SEQ ID NO. 57
34	ETTFMSEYADETATI	SEQ ID NO. 58
35	FMSEYADETATIVEF	SEQ ID NO. 59
36	EYADETATIVEFLNR	SEQ ID NO. 60
37	DETATIVEFLNRWIT	SEQ ID NO. 61
38	ATIVEFLNRWITFSQ	SEQ ID NO. 62
39	VEFLNRWITFSQSII	SEQ ID NO. 63
40	LNRWITFSQSIISTL	SEQ ID NO. 64
41	NRWITFSQSIISTLT	SEQ ID NO. 65
42	INNYKNPKLTRMLT**A**	SEQ ID NO. 66
43	YKNPKLTRMLT**A**KFY	SEQ ID NO. 67
47	YKNPKLTRMLTFKF**A**	SEQ ID NO. 68
52	YKNPKLTRMLT**A**KF**A**	SEQ ID NO. 69
44	PKLTRMLT**A**KFYMPK	SEQ ID NO. 70
48	PKLTRMLTFKF**A**MPK	SEQ ID NO. 71
53	PKLTRMLT**A**KF**A**MPK	SEQ ID NO. 72
45	TRMLT**A**KFYMPKKAT	SEQ ID NO. 73
49	TRMLTFKF**A**MPKKAT	SEQ ID NO. 74
54	TRMLT**A**KF**A**MPKKAT	SEQ ID NO. 75
46	LT**A**KFYMPKKATELK	SEQ ID NO. 76
50	LTFKF**A**MPKKATELK	SEQ ID NO. 77
55	LT**A**KF**A**MPKKATELK	SEQ ID NO. 78
51	KF**A**MPKKATELKHLQ	SEQ ID NO. 79
56	SLEEELKPLEEVLN**A**	SEQ ID NO. 80
57	EELKPLEEVLN**A**AQS	SEQ ID NO. 81
58	KPLEEVLN**A**AQSKNF	SEQ ID NO. 82
59	EEVLN**A**AQSKNFHLR	SEQ ID NO. 83
60	**A**NLAQSKNFHLRPRD	SEQ ID NO. 84

Both set of peptides were printed on microarray slides in triplicate, incubated with the antibodies of interest (MAb602 and NARA1) and control antibodies. Additional incubations are with unrelated antibodies from the same isotype (mouse control IgG2a/lambda and mouse control IgG2a/kappa), and secondary antibodies (anti-mouse IgG (Thermo 84545, label DL650) or anti-mouse IgG (JIR 115-175-072, Label Cy5)) to assess unspecific binding due to the detection antibody. The experiments are performed essentially as described in Maksimov P, et al. 2012, PLoS One 7:e34212. doi:10.1371/journal.pone. 0034212.

The determination of peptide-antibody binding was performed by RepliTope-analysis where the peptide microarray (triplicate) was incubated with the primary antibody followed by a fluorescently labelled secondary antibody directed against the Fc-part of the primary one. All steps were performed on a TECAN® microarray processing station enabling highly reliable and reproducible washing and incubation steps. After performing the incubation steps and subsequent to the final washing steps (to remove the unbound secondary antibodies) the microarrays were dried using a nitrogen stream and scanned in a high resolution microarray scanning system with appropriate wavelength settings. Control incubations were performed with an unrelated antibody having the same isotype to exclude false positive signals.
The resulting images were analyzed und quantified using spot-recognition software GenePix (Molecular Devices). For each spot, the mean signal intensity was extracted (between 0 and 65535 arbitrary units). For further data evaluation, the MMC2 values were determined. The MMC2 equals the mean value of all three instances on the microarray. Except the coefficient of variation (CV) – standard-deviation divided by the mean value – is larger 0.5, in this case the mean of the two closest values (MC2) is assigned to MMC2.

(ii) Results

The data are summarized in Table 5.
The anti-IL2 (NARA1) antibody did not show any significant reactivity towards the immobilized peptides. Only peptide 10 exhibited a weak response, however, this peptide was also weakly recognized by the mouse control antibodies.

The commercial antibody MAB602 (mIgG2a) provided some weak signals on peptide 22 to 26 and some strong for peptides 10 to 13.

Table 5. Result of Linear Epitope Mapping

Reference peptide no.	Sequence	Signal intensity for MAB602 after subtraction of control signal (AU)	Signal intensity for NARA1 after subtraction of control signal (AU)
10	INNYKNPKLTRMLTF	45954	20883
11	YKNPKLTRMLTFKFY	49726	1189
12	PKLTRMLTFKFYMPK	28849	1127
13	TRMLTFKFYMPKKAT	5250	224
22	KPLEEVLNLAQSKNF	4998	0
23	EEVLNLAQSKNFHLR	13287	32
24	LNLAQSKNFHLRPRD	3289	282
25	AQSKNFHLRPRDLIS	5220	0
26	KNFHLRPRDLISNIN	7509	0

The overlapping sequences within both set of peptides are considered as containing the binding amino acid to the target antibody (Table 5). One stretch is a strong binder to MAB602 whereas the other is rather a weak binder to MAB602:

Strong:	(57) TRMLTF (62)
Weaker:	(96) KNF (98)

Ala mutation on specific residues F42(62), Y45(65), L72(92) showed that residue F42(62) is clearly an important residue for the binding to antibody MAB602 (Table 6).

Table 6. Mutagenesis characterization

Reference Peptide No.	Sequence Residue in bold are the Alanine (A) which are replacing specific residues	Signal intensity for MAB602 after subtraction of control signal (AU)	Signal intensity for NARA1 after subtraction of control signal (AU)
10	INNYKNPKLTRMLTF	45954	20883
42	INNYKNPKLTRMLT**A**	246	162
11	YKNPKLTRMLTFKFY	49726	1189
43	YKNPKLTRMLT**A**KFY	42784	507
47	YKNPKLTRMLTFKF**A**	21382	251
52	YKNPKLTRMLT**A**KF**A**	13089	238
12	PKLTRMLTFKFYMPK	28849	1127
44	PKLTRMLT**A**KFYMPK	5027	432
48	PKLTRMLTFKF**A**MPK	13394	6205
53	PKLTRMLT**A**KF**A**MPK	0	24
13	TRMLTFKFYMPKKAT	5250	224
45	TRMLT**A**KFYMPKKAT	0	0
49	TRMLTFKF**A**MPKKAT	3018	1492
54	TRMLT**A**KF**A**MPKKAT	0	0

SEQUENCE LIST

Useful amino acids and nucleotide sequences for practicing the invention are found in Table 7.

Table 7. Sequence list

SEQ ID NUMBER	**Ab region**	**Sequence**
SEQ ID NO: 1	Human IL-2	MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

SEQ ID NO: 2	Proleukin ®	MAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLA QSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADE TATIVEFLNRWITFSQSIISTLT
Antibody 1		
SEQ ID NO: 3	DNA Heavy Chain	ATGGAATGGAGCGGAGTCTTTATCTTTCTCCTGTCAG TAACTGCAGGTGTTCACTCCCAGGTCCAGCTGCAGCA GTCTGGAGCTGAGCTGGTAAGGCCTGGGACTTCAGTG AAGGTGTCCTGCAAGGCTTCTGGATACGCCTTCACTA ATTACTTGATAGAGTGGGTAAAGCAGAGGCCTGGACA GGGCCTTGAGTGGATTGGAGTGATTAATCCTGGAAGT GGTGGTACTAACTACAATGAGAAGTTCAAGGGCAAGG CAACACTGACTGCAGACAAATCCTCCAGCACTGCCTA CATGCAGCTCAGCAGCCTGACATCTGATGACTCTGCG GTCTATTTCTGTGCAAGATGGAGGGGGGATGGTTACT ACGCGTACTTCGATGTCTGGGGCGCAGGGACCACGGT CACCGTCTCCTCAGCCAAAACAACAGCCCCATCGGTC TATCCACTGGCCCCTGTGTGTGGAGATACAACTGGCT CCTCGGTGACTCTAGGATGCCTGGTCAAGGGTTATTT CCCTGAGCCAGTGACCTTGACCTGGAACTCTGGATCC CTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCCTGC AGTCTGACCTCTACACCCTCAGCAGCTCAGTGACTGT AACCTCGAGCACCTGGCCCAGCCAGTCCATCACCTGC AATGTGGCCCACCCGGCAAGCAGCACCAAGGTGGACA AGAAAATTGAGCCCAGAGGGCCCACAATCAAGCCCTG TCCTCCATGCAAATGCCCAGCACCTAACCTCTTGGGT GGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGG ATGTACTCATGATCTCCCTGAGCCCCATAGTCACATG TGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTC CAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACA CAGCTCAGACACAAACCCATAGAGAGGATTACAACAG TACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCAC CAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGG TCAACAACAAAGACCTCCCAGCGCCCATCGAGAGAAC CATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAG GTATATGTCTTGCCTCCACCAGAAGAAGAGATGACTA AGAAACAGGTCACTCTGACCTGCATGGTCACAGACTT CATGCCTGAAGACATTTACGTGGAGTGGACCAACAAC GGGAAAACAGAGCTAAACTACAAGAACACTGAACCAG TCCTGGACTCTGATGGTTCTTACTTCATGTACAGCAA GCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAAT AGCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACA ATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGG TAAATGA
SEQ ID NO: 4	DNA Light Chain	ATGGAGACAGACACAATCCTGCTATGGGTGCTGCTGC TCTGGGTTCCAGGCTCCACTGGTGACATTGTGCTGAC CCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAG AGGGCCACCATCTCCTGCAAGGCCAGCCAAAGTGTTG ATTATGATGGTGATAGTTATATGAACTGGTACCAACA GAAACCAGGACAGCCACCCAAACTCCTCATCTATGCT GCATCCAATCTAGAATCTGGGATCCCAGCCAGGTTTA GTGGCAGTGGGTCTGGGACAGACTTCACCCTCAACAT

		CCATCCTGTGGAGGAGGAGGATGCTGCAACCTATTACTGTCAGCAAAGTAATGAGGATCCGTACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
SEQ ID NO: 5	Heavy Chain	MEWSGVFIFLLSVTAGVHSQVQLQQSGAELVRPGTSVKVSCKASGYAFTNYLIEWVKQRPGQGLEWIGVINPGSGGTNYNEKFKGKATLTADKSSSTAYMQLSSLTSDDSAVYFCARWRGDGYYAYFDVWGAGTTVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
SEQ ID NO: 6	Light Chain	METDTILLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNWYQQKPGQPPKLLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC
SEQ ID NO: 7 (Kabat)	HCDR1	NYLIE
SEQ ID NO: 8 (Kabat)	HCDR2	VINPGSGGTNYNEKFKG
SEQ ID NO: 9 (Kabat)	HCDR3	WRGDGYYAYFDV
SEQ ID NO: 10 (Kabat)	LCDR1	KASQSVDYDGDSYMN
SEQ ID NO: 11 (Kabat)	LCDR2	AASNLES
SEQ ID NO: 12 (Kabat)	LCDR3	QQSNEDPYT
SEQ ID NO: 13	HCDR1 DNA	AATTACTTGATAGAG
SEQ ID NO: 14	HCDR2 DNA	GTGATTAATCCTGGAAGTGGTGGTACTAACTACAATGAGAAGTTCAAGGGC
SEQ ID NO: 15	HCDR3 DNA	TGGAGGGGGGATGGTTACTACGCGTACTTCGATGTC

SEQ ID NO: 16	LCDR1 DNA	AAGGCCAGCCAAAGTGTTGATTATGATGGTGATAGTTATATGAAC
SEQ ID NO: 17	LCDR2 DNA	GCTGCATCCAATCTAGAATCT
SEQ ID NO: 18	LCDR3 DNA	CAGCAAAGTAATGAGGATCCGTACACG
SEQ ID NO: 19	VH	QVQLQQSGAELVRPGTSVKVSCKASGYAFTNYLIEWVKQRPGQGLEWIGVINPGSGGTNYNEKFKGKATLTADKSSSTAYMQLSSLTSDDSAVYFCARWRGDGYYAYFDVWGAGTTVTVSS
SEQ ID NO: 20	VL	DIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNWYQQKPGQPPKLLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPYTFGGGTKLEIK
SEQ ID NO: 21	DNA VH	CAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTAAGGCCTGGGACTTCAGTGAAGGTGTCCTGCAAGGCTTCTGGATACGCCTTCACTAATTACTTGATAGAGTGGGTAAAGCAGAGGCCTGGACAGGGCCTTGAGTGGATTGGAGTGATTAATCCTGGAAGTGGTGGTACTAACTACAATGAGAAGTTCAAGGGCAAGGCAACACTGACTGCAGACAAATCCTCCAGCACTGCCTACATGCAGCTCAGCAGCCTGACATCTGATGACTCTGCGGTCTATTTCTGTGCAAGATGGAGGGGGGATGGTTACTACGCGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCA
SEQ ID NO: 22	DNA VL	GACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACCATCTCCTGCAAGGCCAGCCAAAGTGTTGATTATGATGGTGATAGTTATATGAACTGGTACCAACAGAAACCAGGACAGCCACCCAAACTCCTCATCTATGCTGCATCCAATCTAGAATCTGGGATCCCAGCCAGGTTTAGTGGCAGTGGGTCTGGGACAGACTTCACCCTCAACATCCATCCTGTGGAGGAGGAGGATGCTGCAACCTATTACTGTCAGCAAAGTAATGAGGATCCGTACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA
SEQ ID NO: 23	Heavy chain fusion	MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGQVQLQQSGAELVRPGTSVKVSCKASGYAFTNYLIEWVKQRPGQGLEWIGVINPGSGGTNYNEKFKGKATLTADKSSSTAYMQLSSLTSDDSAVYFCARWRGDGYYAYFDVWGAGTTVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

SEQ ID NO: 24	Light chain fusion	MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNWYQQKPGQPPKLLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

CLAIMS

1. A human interleukin-2 (hIL-2) specific monoclonal antibody (mAb), or a human interleukin-2 (hIL-2) specific antigen binding fragment thereof comprising HCDR1 according to SEQ ID NO: 7, HCDR2 according to SEQ ID NO: 8, HCDR3 according to SEQ ID NO: 9, LCDR1 according to SEQ ID NO: 10, LCDR2 according to SEQ ID NO: 11, LCDR3 according to SEQ ID NO: 12, wherein binding of said antibody, or antigen binding fragment thereof, to hIL-2 inhibits binding of hIL-2 to CD25, and the binding to hIL-2 is characterized by a dissociation constant (K_D) $\leq$ 7,5 nmol/L, $\leq$ 5 nmol/L, $\leq$ 3 nmol/L, $\leq$ 2 nmol/L or $\leq$1,5 nmol/L determined in a surface plasmon resonance binding assay.

2. The human interleukin-2 (hIL-2) specific monoclonal antibody, or antigen binding fragment thereof, according to claim 1, wherein the binding to hIL-2 is characterized by an off-rate (K_{off}) $\leq 1x10^{-4}s^{-1}$, $\leq 8x10^{-5}s^{-1}$, $\leq 6x10^{-5}s^{-1}$, $\leq 4x10^{-5}s^{-1}$, $\leq 3x10^{-5}s^{-1}$or $\leq 2,1x10^{-5}s^{-1}$.

3. The human interleukin-2 (hIL-2) specific monoclonal antibody, or antigen binding fragment thereof, according to claim 1 or 2, wherein the antibody, or antigen binding fragment thereof, displays no measurable cross-reactivity to murine IL-2.

4. The human interleukin-2 (hIL-2) specific monoclonal antibody, or antigen binding fragment thereof, according to any one of claims 1-3, characterized in that it comprises at least one VH and/or one VL sequence having a sequence identity of $\geq$ 90% compared to SEQ ID NOs 019 or 20.

5. The antibody or antigen binding fragment thereof according to any one of claims 1 to 4, which binds to a human interleukin-2 (hIL-2) epitope comprising the amino acids K52, P54, K55, T57, R58, T61, F62, K63, Q94, and K96 according to SEQ ID NO: 1.

6. The antibody or antigen binding fragment thereof according to claim 5, wherein the epitope further comprises any one or more of the amino acids N50, N53, N91, L92, A93, N97 according to SEQ ID NO: 1.

7. A nucleic acid molecule encoding the human interleukin-2 (hll-2) specific monoclonal antibody, or antigen binding fragment thereof, according to any one of claims 1-4.

8. The nucleic acid molecule of claim 7 wherein said nucleic acid molecule has ≥ 90% sequence identity compared to SEQ ID NOs 003 or 004.

9. The nucleic acid molecule according to claim 7, comprising the sequence of SEQ ID NOs 13, 14, 15, 16, 17,18, 21 or 22, or a sequence having a sequence identity of ≥ 90% compared to any one of SEQ ID NOs 13, 14, 15, 16, 17, 18, 21 or 22.

10. A vector comprising the nucleic acid molecule according to claim 7, 8 or 9.

11. A cell comprising the nucleic acid molecule according to claim 7, 8 or 9, or expressing the nucleic acid molecule according to claim 7, 8 or 9.

12. A cellproducing a human interleukin-2 (hIL-2) specific monoclonal antibody (mAb), or antigen binding fragment thereof, according to any one of claims 1 to 4.

13. A monoclonal antibody-producing hybridoma cell line characterized in that said produced antibodies are those of any one of claims 1 to 4.

14. A therapeutic formulation for use in the treatment of cancer or viral infections, comprising:

   a. the hIL-2 monoclonal antibody (mAb), or the antigen binding fragment thereof, according to any one of claims 1 to 4, and
   b. human interleukin-2.

15. The human interleukin-2 (hIL-2) specific monoclonal antibody, or antigen binding fragment thereof, according to any one of claims 1 to 4 for use in the treatment of cancer or viral infections, wherein the antibody, or antigen binding thereof is for administration together with human interleukin-2.

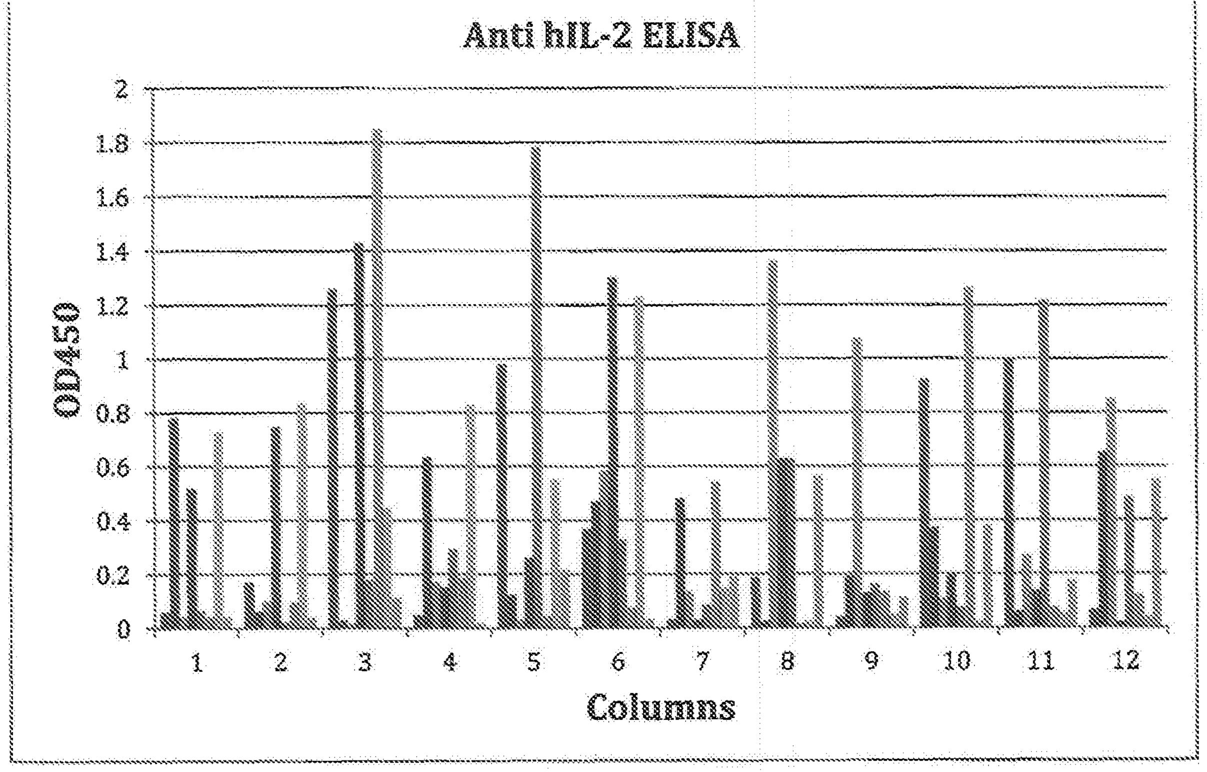
16. A therapeutic formulation for use in the treatment of cancer or viral infections, containing a fusion protein that comprises:

a. an hIL-2 binding polypeptide fragment comprising HCDR1 according to SEQ ID NO: 7, HCDR2 according to SEQ ID NO: 8, HCDR3 according to SEQ ID NO: 9, LCDR1 according to SEQ ID NO: 10, LCDR2 according to SEQ ID NO: 11, LCDR3 according to SEQ ID NO: 12, wherein the binding to hIL-2 is characterized by a dissociation constant (K_D) $\leq$ 7,5 nmol/L, $\leq$ 5 nmol/L, $\leq$ 3 nmol/L, $\leq$ 2 nmol/L or $\leq$1,5 nmol/L determined in a surface plasmon resonance binding assay, and

b. a human IL-2 polypeptide fragment having an identity of $\geq$ 90% compared to SEQ ID NO 001.

17. The therapeutic formulation for use in the treatment of cancer or viral infections according to claim 16, wherein the fusion protein comprises an amino acid linker of 1 to 50 amino acids, linking the hIL-2 binding polypeptide fragment to the human IL-2 polypeptide fragment as one single polypeptide chain.

18. The therapeutic formulation for use in the treatment of cancer or viral infections according to claim 17, wherein the amino acid linker has 15 to 25 amino acids.


Anti hIL-2 ELISA
OD450
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
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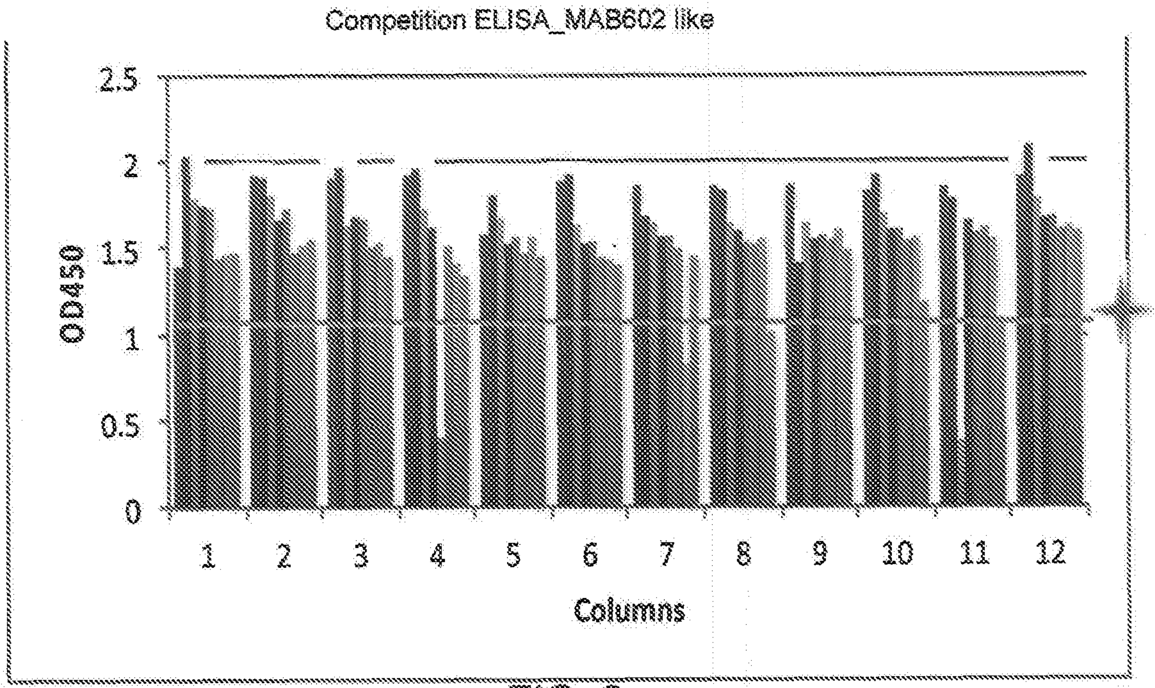
1
2
3
4
5
6
7
8
9
10
11
12
Columns

FIG. 1


Competition ELISA_MAB602 like
OD450
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
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8
9
10
11
12
Columns

FIG. 2

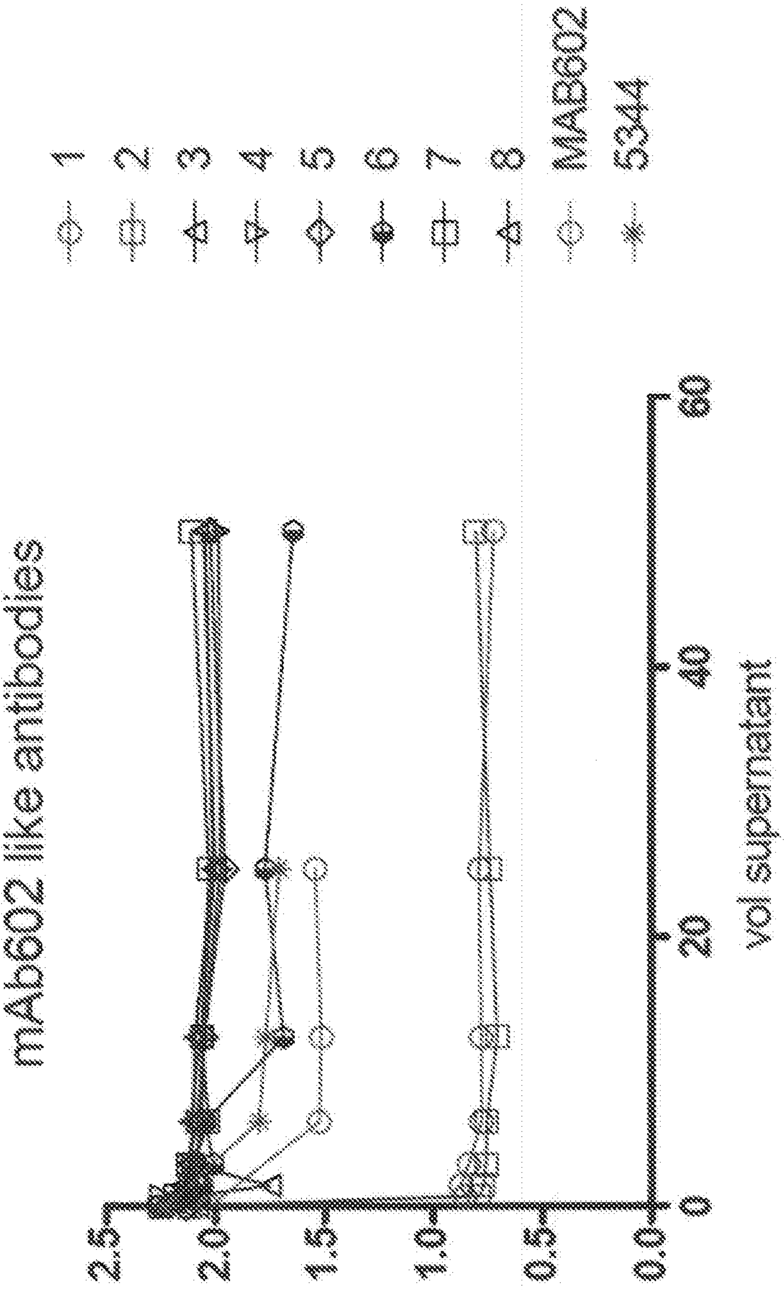

mAb602 like antibodies
OD450
2.5
2.0
1.5
1.0
0.5
0.0
0
20
40
60
vol supernatant
1
2
3
4
5
6
7
8
MAB602
5344

FIG. 3

FIG. 4

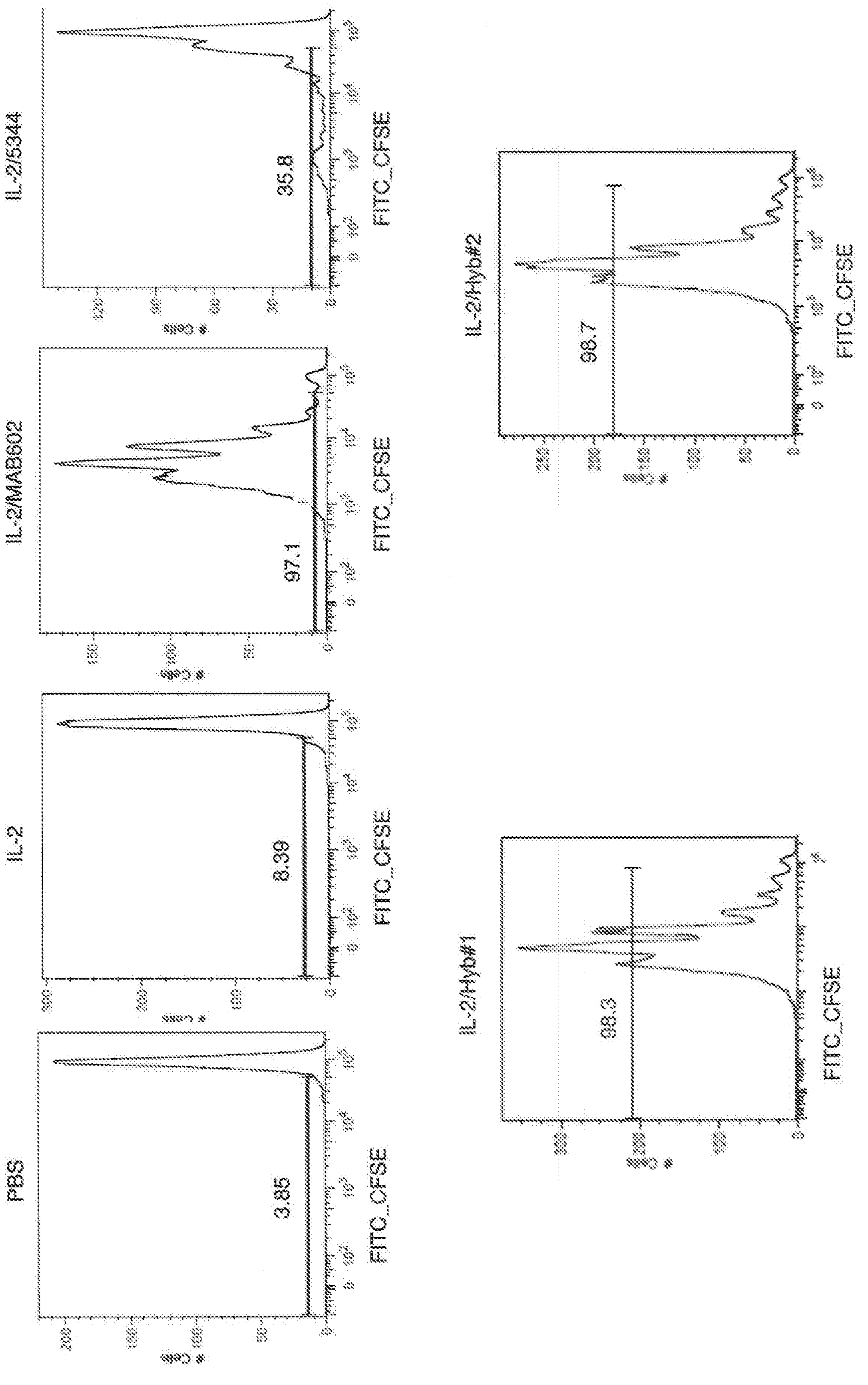

PBS
3.85
FITC_CFSE
Cells
IL-2
8.39
FITC_CFSE
Cells
IL-2/MAB602
97.1
FITC_CFSE
Cells
IL-2/5344
35.8
FITC_CFSE
Cells
IL-2/Hyb#1
98.3
FITC_CFSE
Cells
IL-2/Hyb#2
98.7
FITC_CFSE
Cells

FIG. 5A

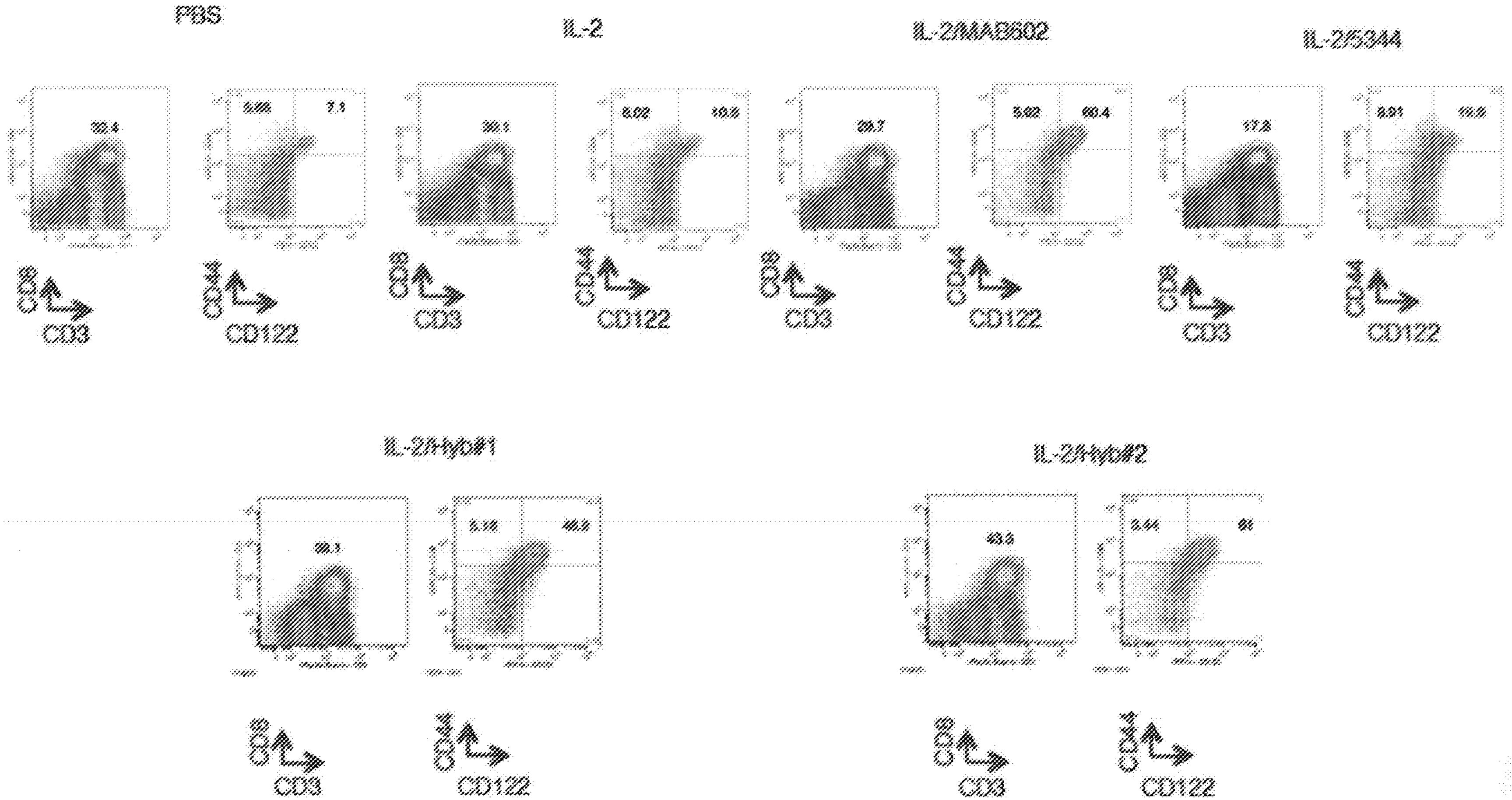
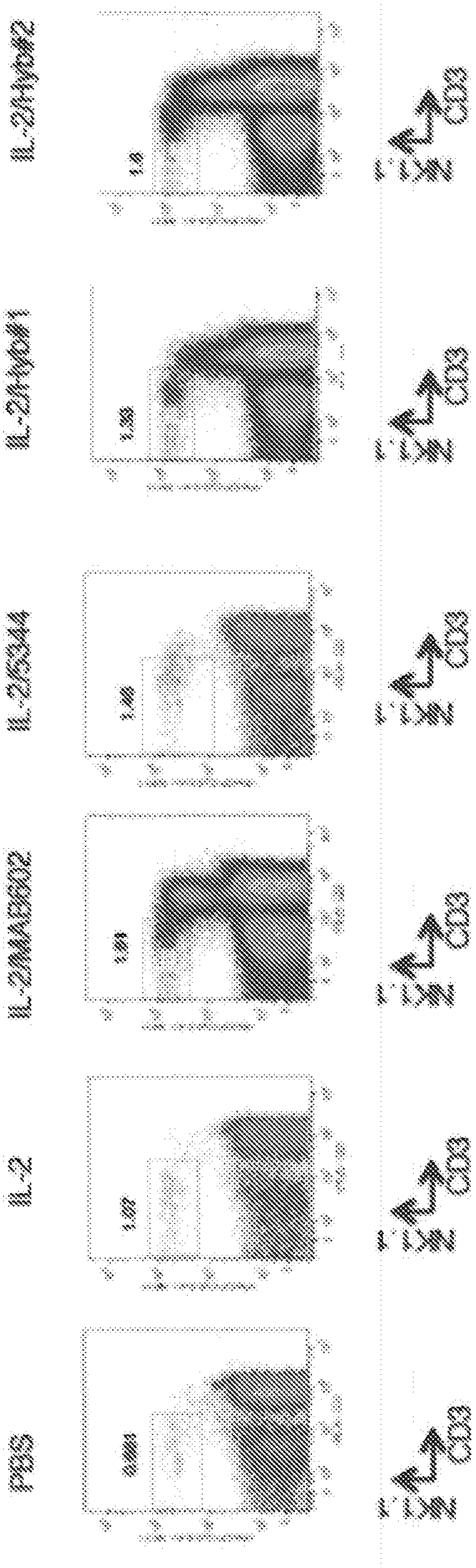

PBS
IL-2
IL-2/MAB602
IL-2/5344
32.4
8.88
7.1
30.1
8.02
10.8
29.7
5.02
60.4
17.8
8.91
IL-2/hyb#1
IL-2/hyb#2
30.1
5.18
40.8
43.3
8.44
60
CD8
CD3
CD44
CD122

FIG. 5B


IL-2/Hyb#2
1.8
NK1.1
CD3
IL-2/Hyb#1
1.38
NK1.1
CD3
IL-2/S4B6
NK1.1
CD3
IL-2/MAB602
NK1.1
CD3
IL-2
NK1.1
CD3
PBS
NK1.1
CD3

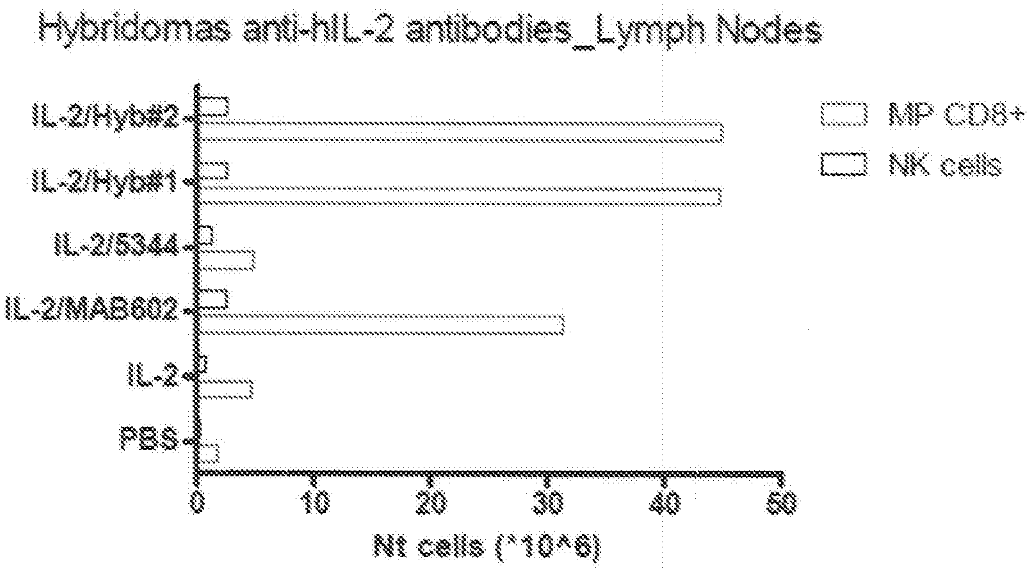
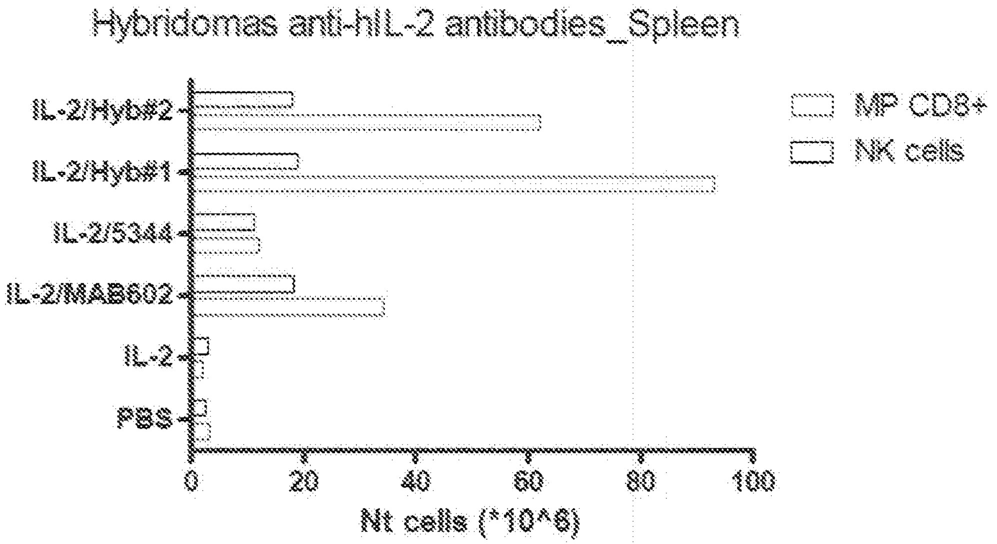

Hybridomas anti-hIL-2 antibodies_Lymph Nodes
IL-2/Hyb#2
IL-2/Hyb#1
IL-2/5344
IL-2/MAB602
IL-2
PBS
0
10
20
30
40
50
Nt cells (*10^6)
MP CD8+
NK cells

FIG. 6A


Hybridomas anti-hIL-2 antibodies_Spleen
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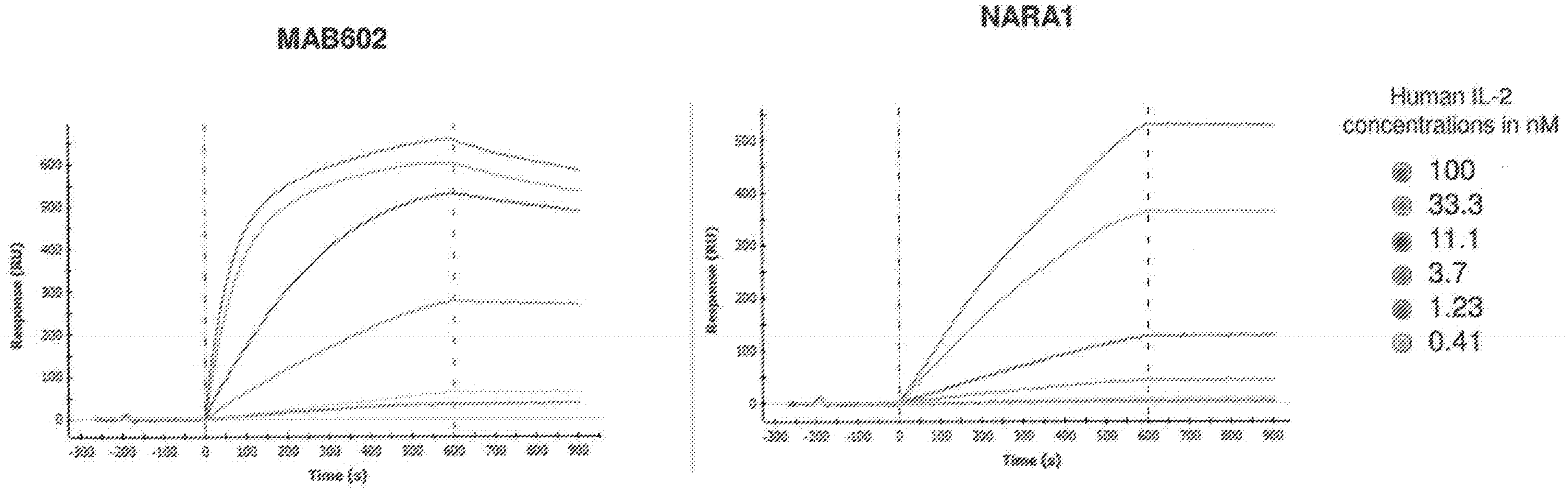
IL-2/Hyb#1
IL-2/5344
IL-2/MAB602
IL-2
PBS
0
20
40
60
80
100
Nt cells (*10^6)
MP CD8+
NK cells

FIG. 6B

FIG. 7


MAB602
NARA1
Response (RU)
0
100
200
300
400
500
600
-300
-200
-100
0
100
200
300
400
500
600
700
800
900
Time (s)
Human IL-2
concentrations in nM
100
33.3
11.1
3.7
1.23
0.41

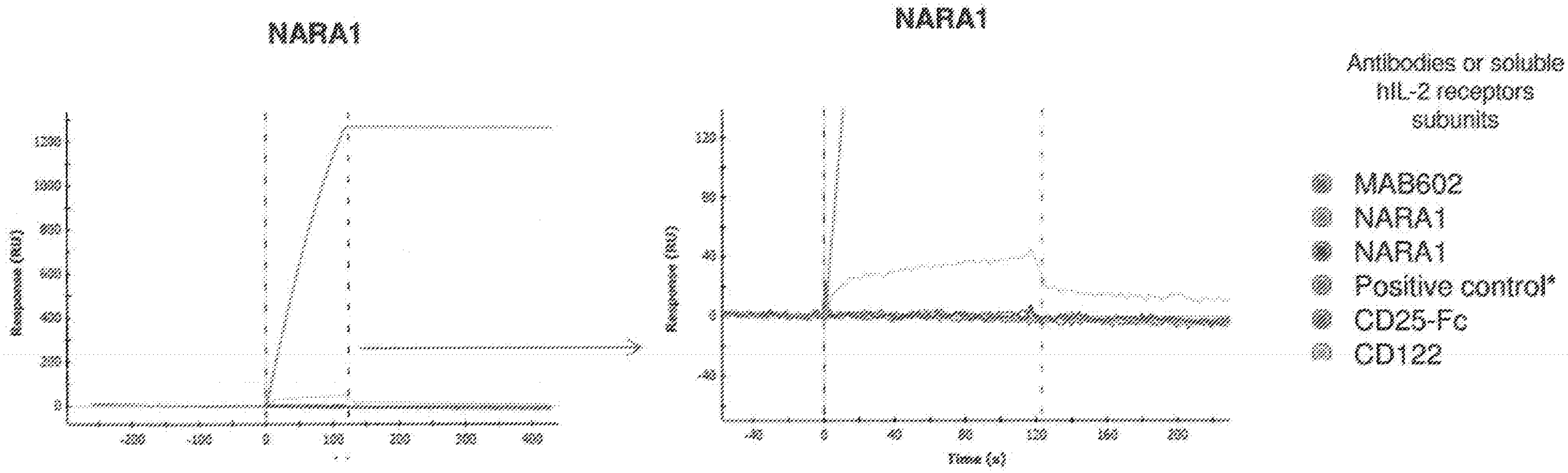
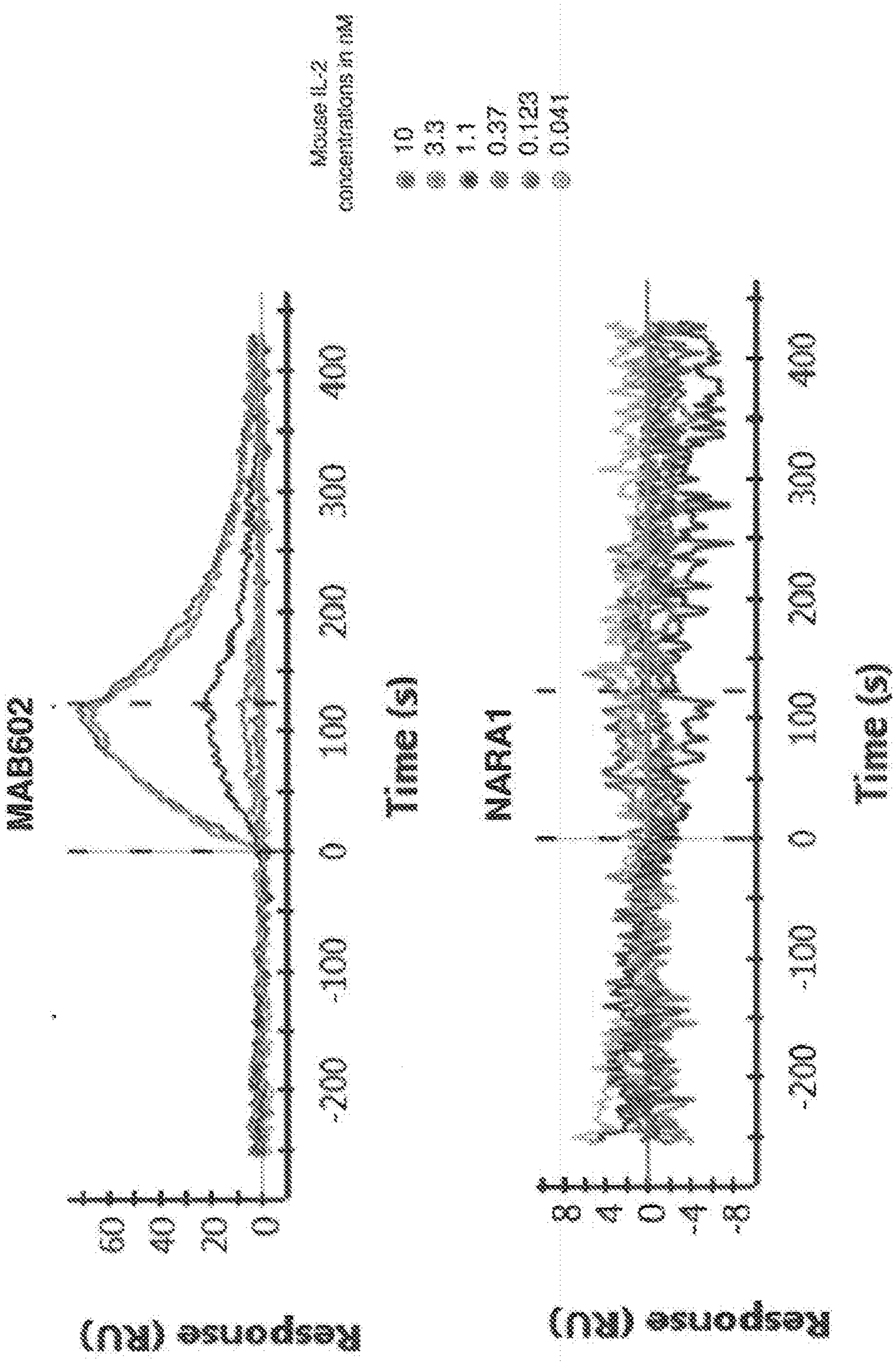

NARA1
NARA1
Response (RU)
1200
1000
800
600
400
200
0
-200
-100
0
100
200
300
400
Response (RU)
120
80
40
0
-40
-40
0
40
80
120
160
200
Time (s)
Antibodies or soluble hIL-2 receptors subunits
MAB602
NARA1
NARA1
Positive control*
CD25-Fc
CD122

FIG. 8

FIG. 9


MAB602
Response (RU)
60
40
20
0
-200
-100
0
100
200
300
400
Time (s)
NARA1
Response (RU)
8
4
0
-4
-8
-200
-100
0
100
200
300
400
Time (s)
Mouse IL-2 concentrations in nM
● 10
● 3.3
● 1.1
● 0.37
● 0.123
● 0.041

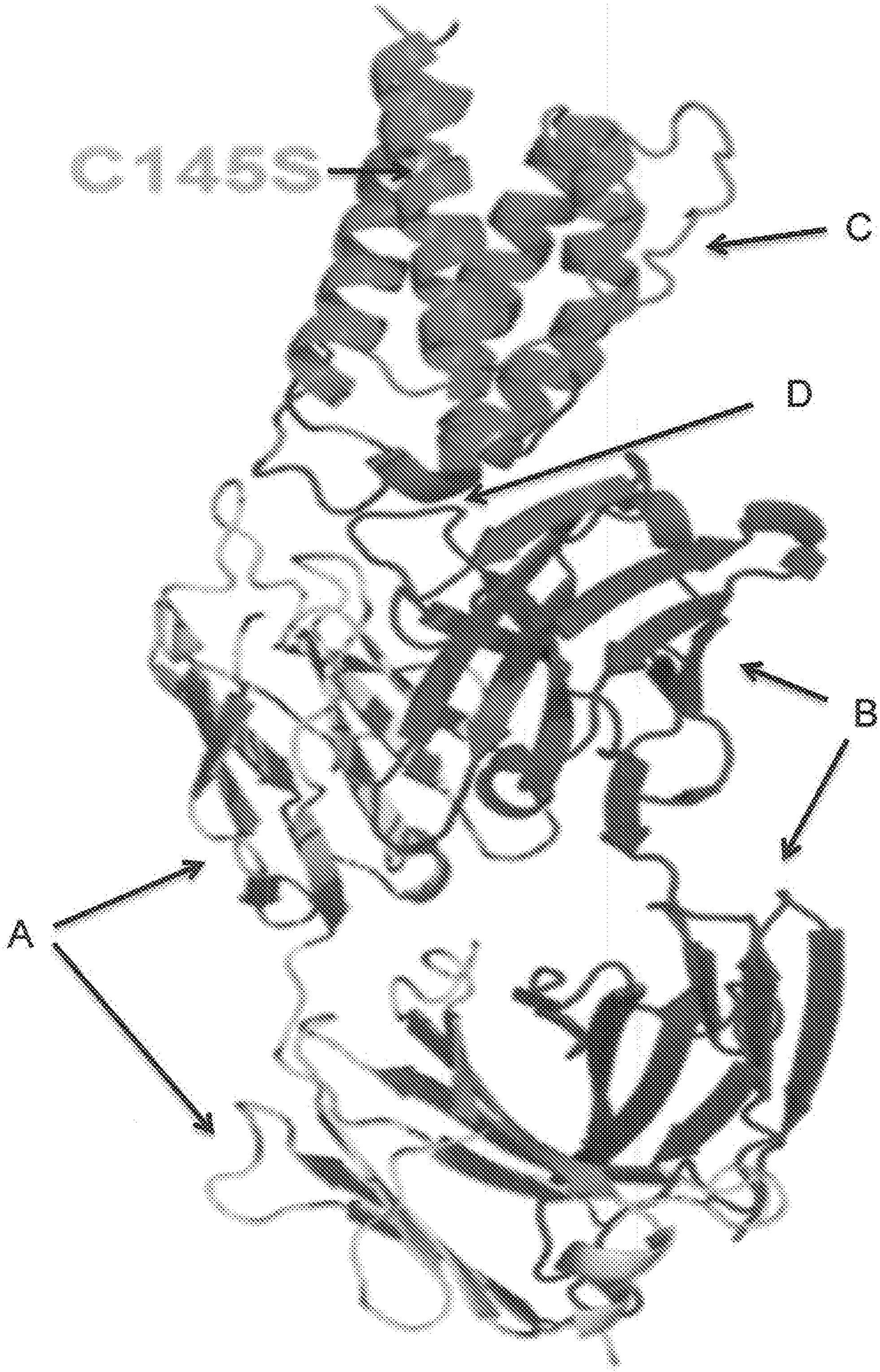


FIG. 10

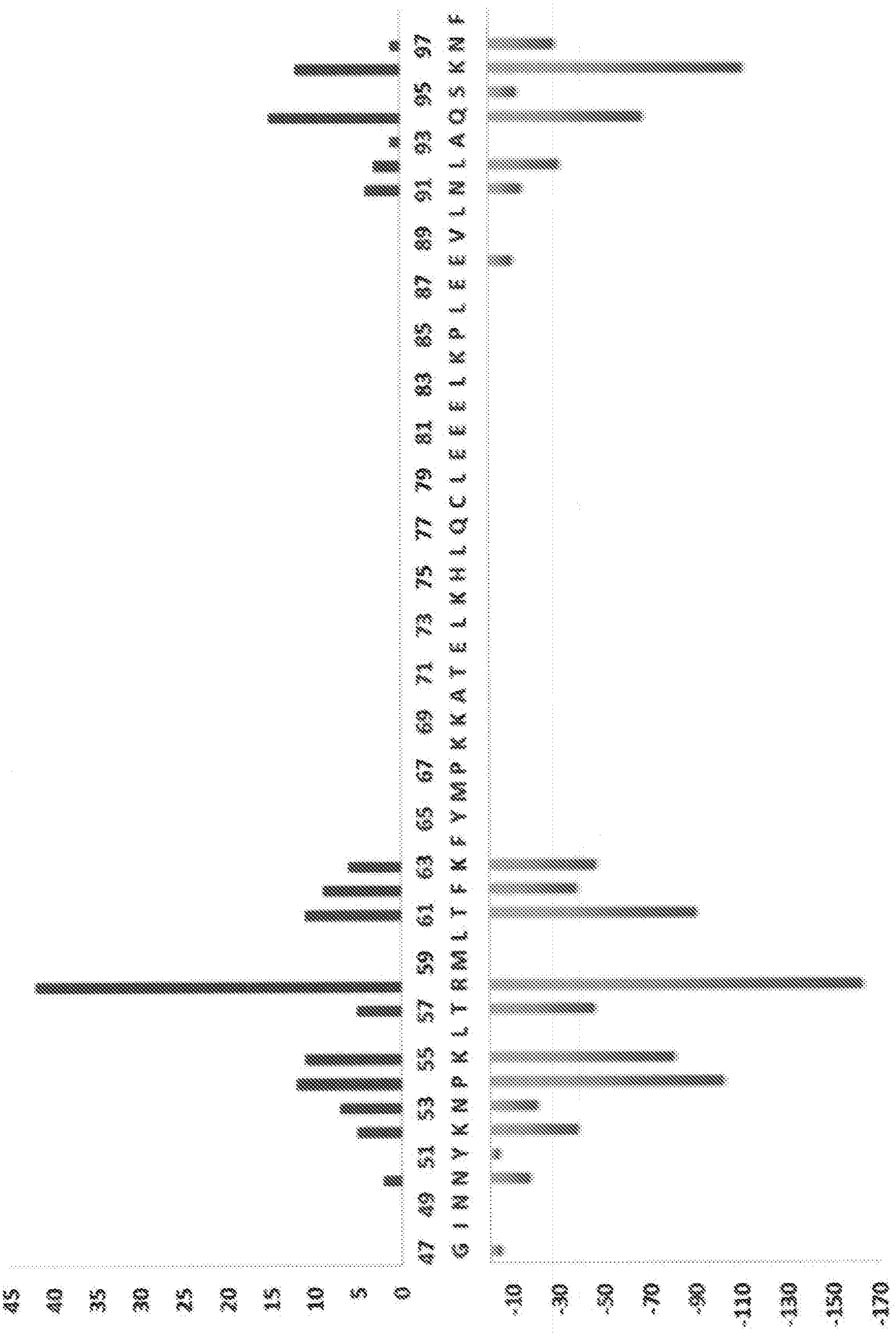

47 49 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81 83 85 87 89 91 93 95 97
G I N N Y K N P K L T R M L T F K F Y M P K K A T E L K H L Q C L E E E L K P L E E V L N L A Q S K N F
45
40
35
30
25
20
15
10
5
0
-10
-30
-50
-70
-90
-110
-130
-150
-170

FIG. 11

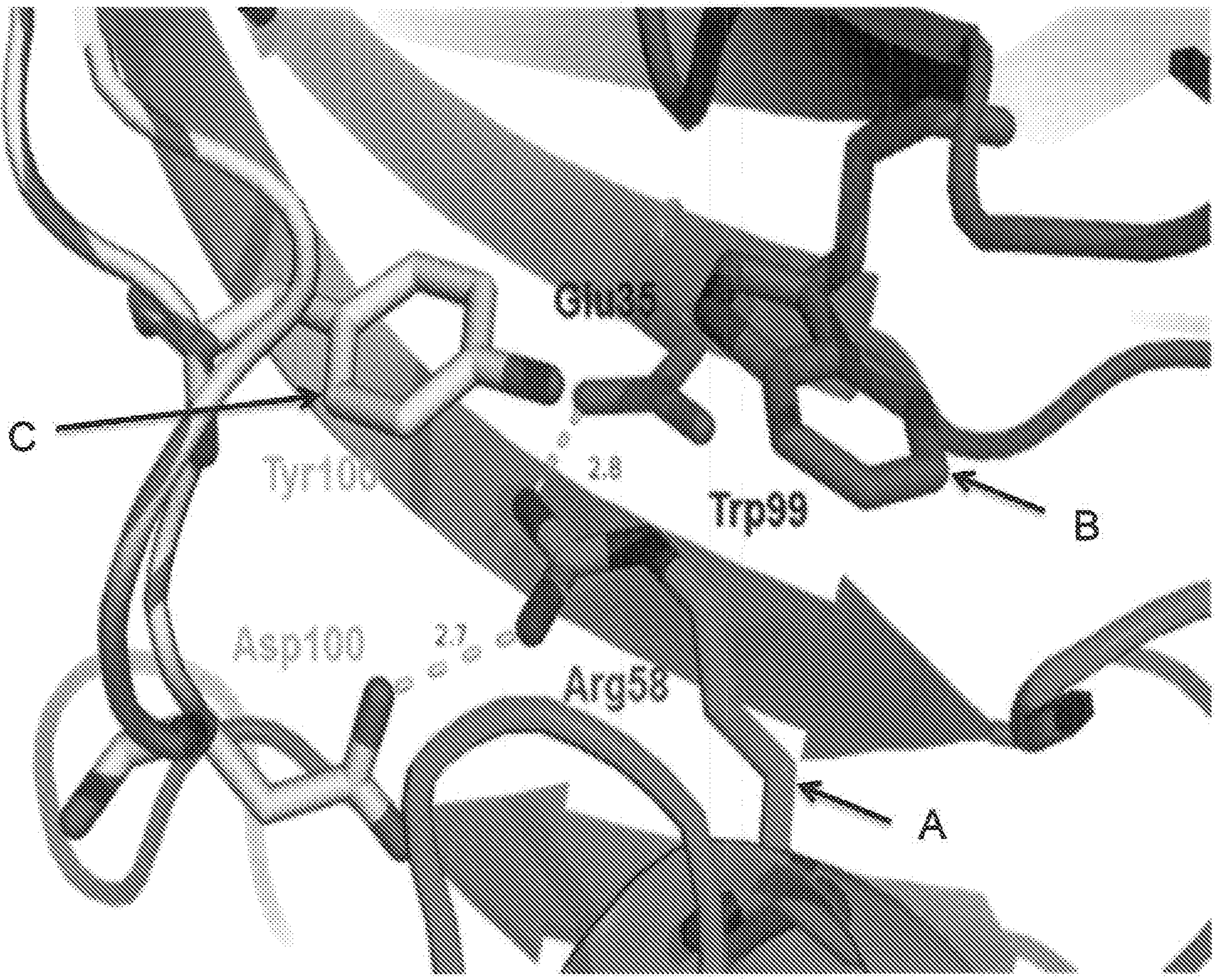


FIG. 12

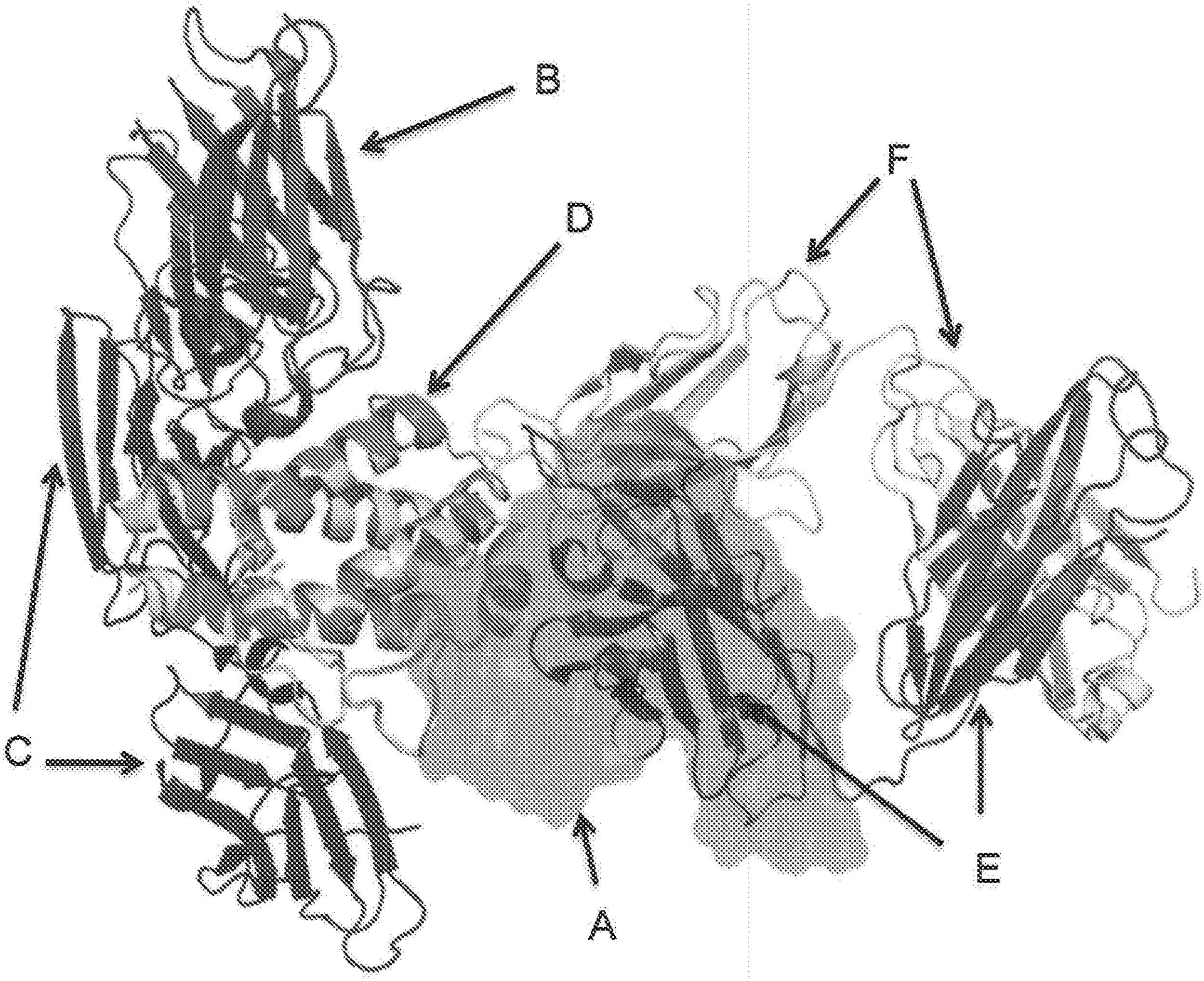


FIG. 13

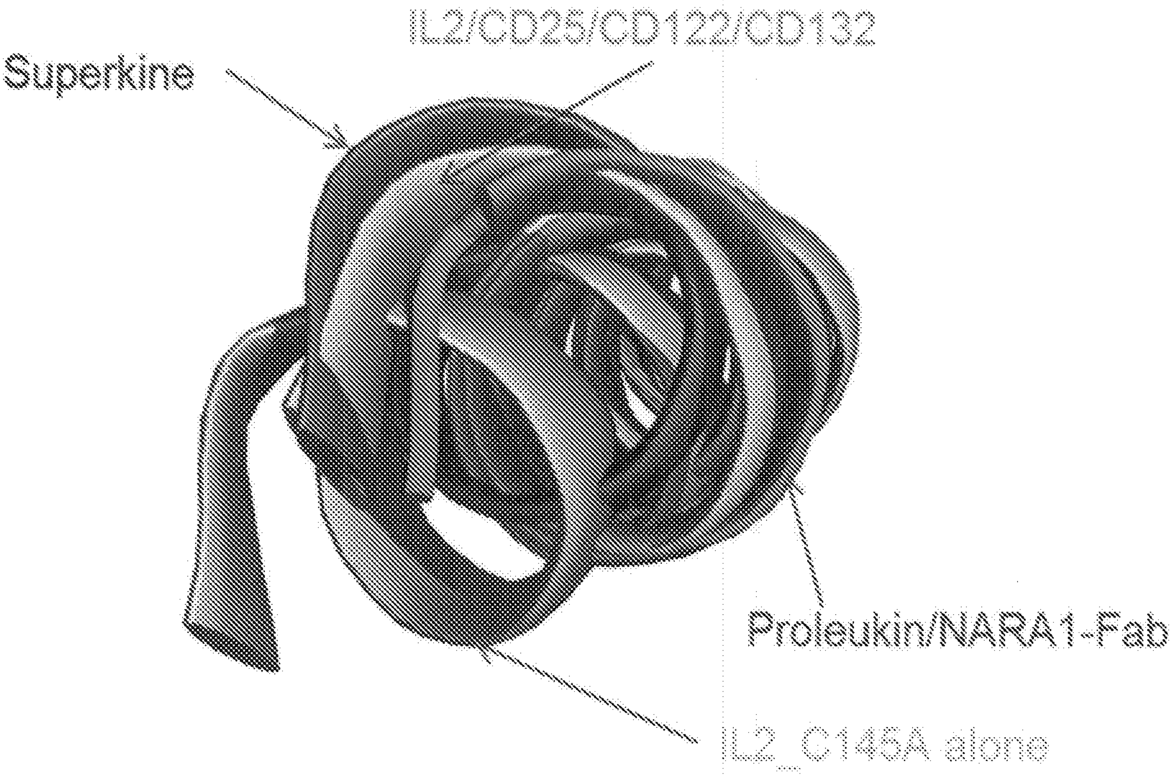
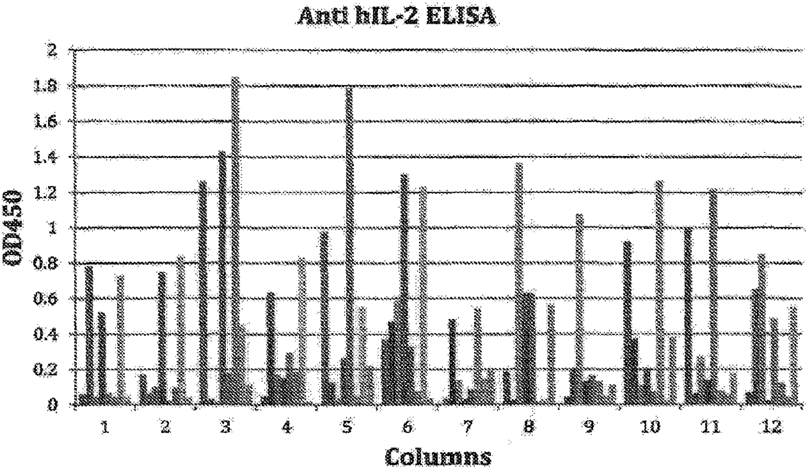

IL2/CD25/CD122/CD132
Superkine
Proleukin/NARA1-Fab
IL2_C145A alone

FIG. 14


Anti hIL-2 ELISA
OD450
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
8
9
10
11
12
Columns