(12) STANDARD PATENT (11) Application No. **AU 2006254333 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Anti-IL2 antibodies

(51) International Patent Classification(s)
C07K 16/24 (2006.01) ***A61K 39/395*** (2006.01)

(21) Application No: **2006254333** (22) Date of Filing: **2006.05.31**

(87) WIPO No: **WO06/128690**

(30) Priority Data

(31) Number	(32) Date	(33) Country
05011845.4	**2005.06.01**	**EP**

(43) Publication Date: **2006.12.07**
(44) Accepted Journal Date: **2011.02.17**

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(56) Related Art
WO 2001/090193 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 December 2006 (07.12.2006)

PCT

(10) International Publication Number
WO 2006/128690 A1

(51) International Patent Classification:
C07K 16/24 (2006.01) *A61K 39/395* (2006.01)

(21) International Application Number:
PCT/EP2006/005194

(22) International Filing Date: 31 May 2006 (31.05.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
05011845.4 1 June 2005 (01.06.2005) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/128690 A1

(54) Title: ANTI-IL2 ANTIBODIES

(57) Abstract: The invention relates to a humanized monoclonal antibody or fragment thereof which specifically binds to human interleukin-2 (IL2), wherein said humanized monoclonal antibody neutralizes the activity of human IL2 by binding to said human IL2 prior to, during, and/or subsequent to the binding of said human IL2 to the human IL2-receptor, and wherein the light chain variable region of said humanized monoclonal antibody comprises in its second framework region the contiguous amino acid sequence KAPKA at amino acid positions 42-46.

Anti-IL2 antibodies

The invention relates to antibodies and fragments thereof which specifically bind the human cytokine IL2. The invention further relates to polynucleotides encoding, pharmaceutical compositions comprising and medical uses involving such antibodies and fragments thereof.

Human IL2 is a protein of 133 amino acids (15.4 kDa) which does not bear significant sequence homology to any other factors. IL2 is synthesized as a precursor protein of 153 amino acids with the first 20 amino-terminal amino acids functioning as a hydrophobic secretory signal sequence. The protein contains a single disulfide bond (joining positions Cys58/105) essential for biological activity.

The biological activities of IL2 are mediated by a membrane receptor that is expressed almost exclusively on activated, but not on resting, T-cells. The complete IL2 receptor consists of three type I trans-membrane protein subunits: alpha, beta and gamma; a lower affinity functional receptor can be constituted by the beta and gamma receptor proteins only. Resting B-cells and resting mononuclear leukocytes rarely express this receptor. The expression of the IL2 receptor, in particular of the alpha subunit, is modulated by multiple factors, for example IL5, IL6 and L2R/p55 inducing factor.

Mouse and human IL2 both cause proliferation of T-cells of the homologous species at high efficiency. Human IL2 is functional also on mouse cells, but not vice versa. IL2 is a growth factor for all subpopulations of T-lymphocytes. It is an antigen-unspecific proliferation factor for T-cells that induces cell cycle progression in resting cells and thus allows clonal expansion of activated T-lymphocytes. IL2 also promotes the proliferation and differentiation of activated B-cells. As with the proliferation of T-cells, this activity also requires the presence of additional factors, for example IL4.

Due to its effects on T-cells and B-cells IL2 is a central regulator of immune responses. The central importance of IL2 in the initiation and amplification of the adaptive immune responses is well illustrated by the clinical efficacy of drugs that are most commonly used to suppress undesirable immune responses such as transplant rejection. The immunosuppressive drugs cyclosporin A and FK506 (tacrolimus) inhibit IL2 production by disrupting signaling through the T-cell receptor, whereas rapamycin (sirolimus) inhibits signaling through the IL2 receptor.

Cyclosporin A and rapamycin act synergistically to limit immune responses by preventing the IL2-driven clonal expansion of T cells. However, all these compounds target intracellular signaling pathways which do not exclusively interfere with IL2 but also with other factors. This implies that clinical application of these drugs imposes a considerable risk of undesirable side effects due to their limited target specificity.

Multiple examples of antibody inhibitors of IL2 activity are also known in the art, for example the commercial antibody daclizumab (Zenapax®, Protein Design Lab, Inc.). However, known antibody inhibitors of IL2 activity exert their biological effect by binding to the IL2 receptor rather than to the antigen itself. Given the important clinical applications of inhibitors of IL2 activity, it is an aim of the present invention to provide alternative specific inhibitors of IL2 activity.

Accordingly, one aspect of the invention provides a humanized monoclonal antibody or fragment thereof which specifically binds to human interleukin-2 (IL2), wherein said humanized monoclonal antibody neutralizes the activity of human IL2 by binding to said human IL2 prior to, during, and/or subsequent to the binding of said human IL2 to the human IL2-receptor, and wherein the light chain variable region of said humanized monoclonal antibody comprises in its second framework region the contiguous amino acid sequence KAPKA, preferably at amino acid positions 42-46.

As used herein, the terms "humanized monoclonal antibody," or "humanized antibody," or "humanized immunoglobulin," or grammatically related variants thereof are used interchangeably to refer to a molecule comprising an antigen binding site derived from one or more non-human immunoglobulins, said molecule additionally comprising at least a portion, e.g. at least one of the framework regions of the light or heavy chain variable domain derived from one or more human immunoglobulins or germline sequences thereof. A "humanized antibody" as used herein includes a humanized light chain variable domain immunoglobulin and a humanized heavy chain variable domain immunoglobulin. The humanized antibody may include a constant region partially or wholly derived from (including synthetic analogs) one or more human gene sequence. A humanized antibody is expected to bind to the same target antigen as a donor antibody which supplied the CDRs. Typically, all segments or portions of the humanized antibody or immunoglobulin, with the exception of the CDRs, are substantially identical or substantially homologous to corresponding segments or portions of naturally occurring or consensus human immunoglobulin sequences.

The light chain variable region (VL) of said humanized monoclonal antibody comprises in its second framework region the contiguous amino acid sequence KAPKA, preferably at amino acid positions 42-46. This preferred sequence numbering, i.e. positions 42-46, refers to the numbering as set out in the "VBase" database (© MRC Centre for Protein Engineering) available under in the internet address http://www.mrc-cpe.cam.ac.uk. For clarity, sequence alignments of framework regions of human germline VL (as V-kappa and V-lambda sequences) and VH regions are included in the present application as they appear in Vbase (see Figs. 7, 8 and 9, respectively; in particular Figs. 8a and 9a for the numbering of the second light chain framework regions in human germline V-kappa and V-lambda sequences).

While the preferred numbering (i.e. amino acid positions 42-46) of the amino acid sequence KAPKA (i.e. Lys-Ala-Pro-Lys-Ala) is provided here for ease of correlation with the reference cited above, it should be understood that the identities of the amino acids within this partial sequence rather than the sequence numbering in and of itself is determinative for the activity of the humanized monoclonal antibody of the invention. As one of skill in the art knows, there exist multiple conventions for numbering human germline antibody sequences, the above cited reference (VBase) being only one of these. Therefore, the partial amino acid sequence KAPKA comprised within the second framework region of certain human germline light chain variable regions may be assigned another numbering according to a numbering convention other than that specified in the above citation. In such a case, the partial amino acid sequence KAPKA would bear a numbering other than preferred amino acid positions 42-46, while the sequence corresponding to the preferred amino acid positions 42-46 under this other numbering convention would likely be an amino acid sequence other than KAPKA. In such a case, as one of skill in the art will understand, the partial amino acid sequence with the "correct" sequence (KAPKA) but deviant numbering (something other than the preferred amino acid positions 42-46) should be regarded as an essential feature of the invention rather than another partial amino acid sequence, the numbering of which is "correct" (preferred amino acid positions 42-46), but the identity of which is not KAPKA.

It has been surprisingly observed that antibodies or fragments thereof lacking the consensus sequence KAPKA in the second light chain framework region, in particular lacking the terminal alanine residue in this stretch, are capable of specifically binding IL2, but not capable of neutralizing its activity. This is especially the case when the CDR regions comprised in the light and heavy variable chains of the humanized monoclonal antibody are as set out in SEQ ID Nos. 1-3 (for light chain variable region CDRs 1-3, respectively), and SEQ ID Nos. 4-6 (for heavy

chain variable region CDRs 1-3, respectively). Without being bound by theory, the inventors attribute this loss of neutralizing activity upon omission of the consensus sequence KAPKA, in particular upon substitution of the terminal alanine residue in this stretch with another amino acid other than alanine, to a destabilization and/or to conformational rearrangements having an adverse effect upon neutralization, but not on binding activity.

The term "specifically binds" or grammatically related expressions such as "specific binding", "binding specifically", "specific binder" etc. as used herein refer to the ability of the humanized monoclonal antibody or fragment thereof to discriminate between human IL2 and any number of other potential antigens different from human IL2 to such an extent that, from a pool of a plurality of different antigens as potential binding partners, only human IL2 is bound, or is significantly bound. Within the meaning of the invention, human IL2 is "significantly" bound when, from among a pool of a plurality of equally accessible different antigens as potential binding partners, human IL2 is bound at least 10-fold, preferably 50-fold, most preferably 100-fold or greater more frequently (in a kinetic sense) than other antigens different than human IL2. As one of ordinary skill in the art understands, such kinetic measurements can be performed i.a. on a Biacore apparatus.

The humanized antibody or fragment thereof according to the invention is monoclonal. As used herein, the term "monoclonal" is to be understood as having the meaning typically ascribed to it in the art, namely an antibody which recognizes a single epitope on the antigen bound. This is in contrast to polyclonal antibody, which represents a collection of distinct antibodies binding to the same antigen, albeit at different epitopes on this antigen. For this reason a single molecule of antigen may simultaneously be bound by multiple molecules of polyclonal antibody specific for this antigen, but only by a single molecule of a particular monoclonal antibody specific for this antigen; following binding by a single molecule of monoclonal antibody, the bound epitope is blocked and therefore no longer available for binding by another molecule of identical monoclonal antibody. The monoclonal nature of the antibody makes it particularly well suited for use as a therapeutic agent, since such antibody will exist as a single, homogeneous molecular species which can be well-characterized and reproducibly made and purified. These factors result in a product whose biological activity can be predicted with a high level of precision, a very important consideration if such a molecule is going to gain regulatory approval for therapeutic administration in mammals, in particular humans.

As used herein, "neutralization," "neutralizer," "neutralizing" and grammatically related variants thereof refers to partial or complete attenuation of the biological effect(s) of IL2. Such partial or complete attenuation of the biological effect(s) of IL2 results from modification, interruption and/or abrogation of IL2-mediated signal transduction, as manifested, for example, in intracellular signalling, cellular proliferation, release of soluble substances, up- or down-regulation of intracellular gene activation, for example that resulting in expression of surface receptors for ligands other than IL2. As one of skill in the art understands, there exist multiple modes of determining whether an agent, for example an antibody in question or fragment thereof is to be classified as a neutralizer. Generally, this may be accomplished by a standard *in vitro* test performed generally as follows: In a first proliferation experiment, a cell line, the degree of proliferation of which is known to depend on the activity of IL2, is incubated in a series of samples with varying concentrations of IL2, following which incubation the degree of proliferation of the cell line is measured. From this measurement, the concentration of IL2 which allowed half-maximal proliferation of the cells is determined. A second proliferation experiment is then performed employing in each of a series of samples the same number of cells as used in the first proliferation experiment, the above-determined concentration of IL2 and, this time, varying concentrations of an antibody or fragment thereof suspected of being a neutralizer of IL2. Cell proliferation is again measured to determine the concentration of antibody or fragment thereof sufficient to effect half-maximal growth inhibition. If the resulting graph of growth inhibition vs. antibody (or fragment thereof) concentration is sigmoidal in shape, then some degree of antibody-dependent growth inhibition has been effected, i.e. the activity of IL2 has been neutralized to some extent. In such a case, the antibody or fragment thereof is to be considered a "neutralizer" in the sense of the present invention. One example of a cell line, the degree of proliferation of which is known to depend on the activity of IL2, is the CTLL-2 cell line, commercially available from LGC Promochem. Another example of a suitable cell line is NK92 (DSMZ).

Surprisingly, the humanized monoclonal antibody of the invention neutralizes the activity of human IL2 by binding to said human IL2 prior to, during, and/or subsequent to the binding of said human IL2 to the human IL2-receptor. This mode of neutralization is highly unexpected. Conventionally, antibody-mediated neutralization of the biological activity of a ligand, said biological activity depending on binding of said ligand to a receptor, is effected by preventing such a ligand-receptor complex from forming. According to this classical scenario for neutralization, a neutralizing antibody binds ligand or receptor at a location in the ligand-receptor interface. In this way, the presence of the antibody sterically and/or electrostatically

prevents formation of the ligand-receptor complex: the ligand-receptor complex is not formed, and the biological activity normally attributed to binding by ligand to its receptor is not effected.

The mode of neutralization observed for a humanized monoclonal antibody according to the invention differs sharply from this classical scenario in that abrogation of biological activity normally attributable to IL2 does not depend on preventing complex formation between IL2 and its receptor. This means that the biological activity of IL2 is abrogated regardless of whether or not IL2 has already bound to the IL2 receptor, implying that the epitope recognized by a humanized monoclonal antibody of the invention is not located on the portion of IL2 which interacts with the IL2 receptor. As such, IL2 neutralization may be achieved with a humanized monoclonal antibody of the invention via several modes.

According to a first mode, the antibody binds to IL2 in solution prior to formation of the complex between IL2 and its receptor so that IL2-mediated signal transduction does not take place in the event that IL2 binds to its receptor.

According to a second mode, the antibody binds to IL2 at the same time as IL2 forms a complex with its receptor. Here again, simultaneous formation of a receptor-IL2-antibody ternary complex does not result in any, or at least any significant signal transduction.

According to a third mode, IL2 has already formed a complex with its receptor, and the antibody binds to IL2 while IL2 exists in complex with its receptor on the surface of an IL2 receptor-bearing cell. In this third scenario, any IL2-mediated signal transduction already taking place prior to binding of IL2 by antibody is abrogated once the antibody is bound.

Such non-classical neutralization, i.e. neutralization as effected by a humanized monoclonal antibody of the invention, is quite surprising, and has several therapeutic advantages.

First, since a humanized monoclonal antibody of the invention recognizes an epitope of IL2 which does not directly participate in contact with the IL2 receptor, no competition arises between the therapeutic antibody on the one hand and the IL2 receptor on the other. This has the effect that lower concentrations of therapeutic antibody may be administered to a patient than would otherwise be possible if a binding competition for the same epitope were to exist between antibody and IL2 receptor. This effectively increases the therapeutic efficacy of a humanized monoclonal antibody of the invention, since the administered concentration may be reduced (relative to that needed given a classical mode of neutralization) with no loss of biological effect.

Administration of a lower amount of therapeutic agent is highly desirable not only from the standpoint of patient tolerability, but also from an economic standpoint, as the cost burden of a given therapy is reduced or, conversely, a larger number of patients may benefit from a given amount of therapeutic antibody.

Second, as alluded to above, the ability of a humanized monoclonal antibody of the invention to bind to and neutralize the biological activity of IL2 already in complex with its receptor has the great advantage that an already-running IL2-mediated signal transduction may be shut off without IL2 first having to dissociate from its receptor binding partner. This has the ultimate effect that the desired neutralizing biological activity of a humanized monoclonal antibody of the invention is realized more quickly in vivo than possible for other "classical" antibody neutralizers which first must compete with the IL2 receptor for the binding epitope on IL2 before eliciting any therapeutic effect. This speed of action may be especially advantageous in acute scenarios such as immune rejection of organ transplants, a known field of anti-IL2 therapy.

A third advantage of such an atypical mode of neutralization as described above relates to the fact that IL2 receptors are located on the surface of T cells. T cells themselves produce IL2 and also respond to IL2 by proliferating, thereby potentiating their own proliferation. In certain acute inflammatory responses, such as tissue rejection following a transplant operation, it is desirable not only to reduce the magnitude of the inflammatory response attributable to the existing T cells, but also to reduce the number of T cells generating the immune response. A humanized monoclonal antibody of the invention is especially effective in achieving this aim. As explained above, the biological activity of IL2 already bound to its receptor on the surface of the T cell will be abrogated. However, following such abrogation, a humanized monoclonal antibody of the invention will typically remain bound to IL2 (itself bound to the IL2 receptor) for a certain time, thus targeting the T cell for destruction via **a**ntibody-**d**ependent **c**ellular **c**ytotoxicity ("ADCC"). In ADCC, a target cell which is coated with immunoglobulin is killed by an effector cell with Fc receptors recognizing the Fc portion of the immunoglobulin coating the target cell. In most cases, the effector cells participating in ADCC are **n**atural **k**iller ("NK") cells which bear on their surface i.a. the Fc receptor Fc-gamma-RIII. In this way, only cells coated with immunoglobulin are killed, so the specificity of cell killing correlates directly with the binding specificity of the antibody. In the context of the present invention, then, T cells which have become decorated with a humanized monoclonal antibody of the invention via IL2 in complex with its receptor become target cells in the above sense which are then lysed by i.a. an NK cell. The effect is a

rapid and effective attenuation of the immune response attributable to cells bearing IL2 receptors, such as T cells.

According to one embodiment of the invention, at least one of the first, third and/or fourth light chain framework region(s) comprised in the human monoclonal antibody or fragment thereof correspond(s) to a human germline sequence for that/those region(s).

According to a further embodiment of the invention, the light chain variable region of a humanized monoclonal antibody or fragment thereof of the invention comprises in its CDR1 region an amino acid sequence as set out in SEQ ID NO: 1. According to a further embodiment of the invention, the light chain variable region of a humanized monoclonal antibody or fragment thereof of the invention comprises in its CDR2 region an amino acid sequence as set out in SEQ ID NO: 2. According to a further embodiment of the invention, the light chain variable region of a humanized monoclonal antibody or fragment thereof of the invention comprises in its CDR3 region an amino acid sequence as set out in SEQ ID NO: 3.

According to a further embodiment of the invention, the light chain variable region of a humanized monoclonal antibody or fragment thereof of the invention further comprises in its CDR1 region an amino acid sequence as set out in SEQ ID NO: 1, in its CDR2 region an amino acid sequence as set out in SEQ ID NO: 2 and in its CDR3 region an amino acid sequence as set out in SEQ ID NO: 3.

According to a further embodiment of the invention, the heavy chain variable region comprises in its CDR1 region an amino acid sequence as set out in SEQ ID NO: 4. According to a further embodiment of the invention, the heavy chain variable region comprises in its CDR2 region an amino acid sequence as set out in SEQ ID NO: 5. According to a further embodiment of the invention, the heavy chain variable region comprises in its CDR3 region an amino acid sequence as set out in SEQ ID NO: 6.

According to a further embodiment of the invention, the heavy chain variable region comprises in its CDR1 region an amino acid sequence as set out in SEQ ID NO: 4, in its CDR2 region an amino acid sequence as set out in SEQ ID NO: 5 and in its CDR3 region an amino acid sequence as set out in SEQ ID NO: 6.

According to a further embodiment of the invention, the light chain variable region of a humanized monoclonal antibody or fragment thereof of the invention further comprises in its

CDR1 region an amino acid sequence as set out in SEQ ID NO: 1, in its CDR2 region an amino acid sequence as set out in SEQ ID NO: 2 and in its CDR3 region an amino acid sequence as set out in SEQ ID NO: 3 and the heavy chain variable region comprises in its CDR1 region an amino acid sequence as set out in SEQ ID NO: 4, in its CDR2 region an amino acid sequence as set out in SEQ ID NO: 5 and in its CDR3 region an amino acid sequence as set out in SEQ ID NO: 6. These CDR regions have been found to be especially advantageous in binding to and neutralizing the biological effect of IL2 in the manner described above.

According to a further embodiment of the invention, the amino acid sequence of the first light chain framework region, the remaining amino acid sequences of the second light chain framework region, and the amino acid sequence of the third light chain framework region correspond to any of those of the human germline subgroup VKI at loci O12, O2, O18, O8, A30, L1, L15, L4, L18, L5, L19, L8, L23, L9, L11 or L12; or of the human germline subgroup VL1 at locus 1a; or any of those of the human germline subgroup VL2 at loci 2c, 2e, 2a2 or 2b2. In this embodiment, the "remaining amino acid sequences of the second light chain framework region" refer to those amino acids in the second light chain framework region other than the sequence KAPKA. Using the numbering of the VBase database, then, "remaining amino acid sequences of the second light chain framework region" denotes amino acids at positions 35-41 and 47-49 inclusive of the second light chain framework region, regardless of whether this light chain framework region is a V-kappa or a V-lambda framework region (see Figs. 7a and 8a for numbering of human germline sequences relating to the V-kappa and V-lambda framework region, respectively). Preferred in this embodiment is the further incorporation in the fourth light chain framework region of a sequence corresponding to that found in the human germline sequence JK4, in particular FGGGTKVEIK. Other amino acid sequences suitable for use as the fourth light chain framework region include but are not limited to FGQGTKVEIK, FGQGTKLEIK, FGPGTKVDIK, FGQGTRLEIK, FGTGTKVTVL, FGGGTKLTVL and FGGGTQLTVL.

According to a further embodiment, at least one of the first, second and/or third heavy chain framework region(s) comprised in the human monoclonal antibody or fragment thereof correspond(s) to a human germline sequence for that/those region(s).

According to a further embodiment, the amino acid sequence of the first heavy chain framework region, the amino acid sequence of the second heavy chain framework region and the amino acid sequence of the third heavy chain framework region correspond to any of those of the human

germline subgroup VH3, in particular at locus 3-07, where the amino acid sequence of the first heavy chain framework region is EVQLVESGGGLVQPGGSLRLSCAASGFTFS, the amino acid sequence of the second heavy chain framework region is WVRQAPGKGLEWVA and the amino acid sequence of the third heavy chain framework region is RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR. The amino acid sequence of the fourth heavy chain framework region may advantageously be chosen, e.g. in combination with the three framework sequences cited above within germline locus 3-07, from one of the following sequences: WGQGTLVTVSS, WGRGTLVTVSS, WGQGTMVTVSS, WGQGTLVTVSS, WGQGTLVTVSS and WGQGTTVTVSS.

In a preferred embodiment, the humanized monoclonal antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence as set out in SEQ ID NO. 7 and a heavy chain variable region comprising an amino acid sequence as set out in SEQ ID NO. 8. Especially preferred is a humanized monoclonal antibody which comprises a light chain comprising an amino acid sequence as set out in SEQ ID NO. 9 and a heavy chain comprising an amino acid sequence as set out in SEQ ID NO. 10. In the following, the humanized anti-IL2 IgG1 antibody comprising SEQ ID NOs. 9 and 10 and/or SEQ ID NOs. 7 and 8 will be referred to as "Anti-IL2".

The humanized monoclonal antibody may be in the form of an IgG antibody, in particular an IgG1 or IgG4 antibody. As is well known in the art, an IgG comprises not only the variable antibody regions responsible for the highly discriminative antigen recognition and binding, but also the constant regions of the heavy and light antibody polypeptide chains normally present in endogenously produced antibodies and, in some cases, even decoration at one or more sites with carbohydrates, such glycosylation normally being on the Fc portion of IgGs. These Fc portions are known to elicit various effector functions in vivo such as ADCC and complement-dependent cytotoxicity ("CDC"). The mechanism of ADCC is described hereinabove. In CDC, two identical immunoglobulins bind to two identical antigens (for example, here IL2 on a T cell) on the surface of a target cell such that their respective Fc portions come into close proximity to one another. This scenario attracts complement proteins, among them complement proteins, for example C1q, C3, C4 and C9, the latter of which creates a pore in the target cell. The target cell is killed by this perforation. At the same time, the target cell/s also become/s decorated at other locations on its/their surface/s. This decoration attracts effector cells, which then kill the target cell/s in a manner analogous to that described above in the context of the ADCC mechanism (see for example Gelderman et al. (2004), Trends Immunology 25, 158-64).

Advantageously, the IgG antibody is an IgG1 antibody or an IgG4 antibody, formats which are preferred since their mechanism of action in vivo is particularly well understood and characterized. This is especially the case for IgG1 antibodies.

According to a further embodiment of the invention, the fragment of the humanized monoclonal antibody may be an scFv, a single domain antibody, an Fv, a diabody, a tandem diabody, a Fab, a Fab' or a F(ab)2. These formats may generally be divided into two subclasses, namely those which consist of a single polypeptide chain, and those which comprise at least two polypeptide chains. Members of the former subclass include an scFv (comprising one VH region and one VL region joined into a single polypeptide chain via a polypeptide linker); and a single domain antibody (comprising a single antibody variable domain which specifically binds human IL2). Members of the latter subclass include an Fv (comprising one VH region and one VL region as separate polypeptide chains which are non-covalently associated with one another); a diabody (comprising two non-covalently associated polypeptide chains, each of which comprises two antibody variable regions – normally one VH and one VL per polypeptide chain – and arranged such that, upon non-covalent association of a VH on one polypeptide chain with the VL on the respective other polypeptide chain and vice-versa, a bivalent antibody molecule results); a tandem diabody (bispecific single-chain Fv antibodies comprising four covalently linked immunoglobulin variable - VH and VL - regions of two different specificities, forming a homodimer that is twice as large as the diabody described above); a Fab (comprising as one polypeptide chain an entire antibody light chain, itself comprising a VL region and the entire light chain constant region and, as another polypeptide chain, a part of an antibody heavy chain comprising a complete VH region and part of the heavy chain constant region, said two polypeptide chains being intermolecularly connected via an interchain disulfide bond); a Fab' (as a Fab, above, except with additional reduced disulfide bonds comprised on the antibody heavy chain); and a F(ab)2 (comprising two Fab' molecules, each Fab' molecule being linked to the respective other Fab' molecule via interchain disulfide bonds). In general, antibody fragments of the type described hereinabove allow greater flexibility in tailoring, for example, the pharmacokinetic properties of an antibody desired for therapeutic administration to the particular exigencies at hand. For example, it may be desirable to reduce the size of the antibody administered in order to increase the degree of tissue penetration when treating tissues known to be poorly vascularized (for example, joints). Under certain circumstances, it may also be desirable to increase the rate at which the therapeutic antibody is eliminated from the body, said rate generally being acceleratable by decreasing the size of the antibody administered.

According to a further embodiment of the invention, the humanized monoclonal antibody may be present in monovalent monospecific or multivalent mono- or multispecific, in particular bivalent mono- or bispecific forms. In general, a multivalent monospecific, in particular bivalent monospecific antibody may bring with it the therapeutic advantage that the neutralization effected by such an antibody is potentiated by avidity effects, i.e. binding to multiple molecules of the same antigen, here human IL2, by the same antibody. Several monovalent monospecific forms of the antibody of the invention have been described above (for example, an scFv, an Fv or a single domain antibody). Multivalent multispecific, in particular bivalent bispecific forms of the humanized monoclonal anti-human IL2 antibody of the invention may include a full IgG in which one binding arm binds to human IL2 while the other binding arm of which binds to another antigen different from human IL2. A further multivalent multispecific, in particular bivalent bispecific form may advantageously be a humanized single chain bispecific antibody, i.e. a recombinant humanized antibody construct comprising two scFv entities as described above, connected into one contiguous polypeptide chain by a short polypeptide spacer between said two scFv entities as known in the art. Here, one scFv portion of the bispecific single chain antibody comprised within the bispecific single chain antibody will specifically bind human IL2 as set out above, while the respective other scFv portion of this bispecific single chain antibody will bind another antigen determined to be of therapeutic benefit.

According to a further embodiment the humanized monoclonal antibody or fragment thereof may be derivatized, for example with an organic polymer, for example with one or more molecules of polyethylene glycol ("PEG"). As is known in the art, such derivatization can be advantageous in modulating the pharmacodynamic properties of antibodies or fragments thereof.

An scFv is an especially preferred (monovalent monospecific) antibody fragment, especially an scFv comprising an amino acid sequence as set out in SEQ ID NO. 11 or SEQ ID NO. 12.

A further aspect of the invention provides a human monoclonal antibody or fragment thereof comprising an amino acid sequence having at least 70% homology, preferably at least 80, 90, or even better at least 95% homology, with an amino acid as set out in any of SEQ ID NOs: 1-12. Homology may be determined by standard sequence alignment programs such as Vector NTI (InforMax™, Maryland, USA). Such programs compare aligned sequences on an amino acid-by-amino acid basis, and can be set to various levels of stringency for the comparison (e.g. identical amino acid, conservative amino acid substitution, etc.). As the term is used herein, two amino acids in question are considered as being a "conservative substitution" of one another, if they

belong to the same main group. By way of non-limiting example, two different amino acids belonging to the group of nonpolar amino acids would be considered a "conservative substitution" of one another, even if these two amino acids were not identical, whereas a nonpolar amino acid on the one hand and a basic amino acid on the other hand would not be considered a "conservative substitution" of one another. Panel 3.1 of "Molecular Biology of the Cell", 4th Edition (2002), by Alberts, Johnson, Lewis, Raff, Roberts and Walter groups amino acids into four main groups: acidic, nonpolar, uncharged polar and basic. Such a grouping may be used for the purposes of determining, for the purposes of the present invention, whether a particular amino acid is a "conservative substitution" of another amino acid in question.

Another aspect of the invention provides a polynucleotide molecule. This polynucleotide molecule comprises a nucleotide sequence encoding an amino acid sequence as set out in any of SEQ ID NOs. 1-12 or a nucleotide sequence exhibiting at least 60%, preferably at least 65, 70, 75, 80, 85, 90, or 95% homology with said nucleotide sequence. Here, homology may be determined by comparing a polynucleotide molecule comprising a nucleotide sequence encoding an amino acid sequence of any of SEQ ID NOs: 1-12 with a polynucleotide molecule having a nucleotide sequence in question ("test sequence") by sequence alignment, and wherein a nucleotide in the test sequence is considered homologous if it is either identical to the corresponding nucleotide in the nucleotide sequence encoding a corresponding amino acid sequence of any of SEQ ID NOs: 1-12 or if one or more nucleotide deviation(s) in the test sequence from corresponding nucleotide(s) in the nucleotide sequence encoding an amino acid sequence of any of SEQ ID NOs: 1-12 results in a nucleotide triplet which, when translated, results in an amino acid which is either identical to (due to a degenerate triplet) or a conservative substitution of the corresponding amino acid in the corresponding amino acid sequence of any of SEQ ID NOs: 1-12. Here, the term "conservative substitution" is to be understood as described above.

Another aspect of the invention provides a polynucleotide molecule comprising a nucleotide sequence encoding an amino acid sequence as set out in any of SEQ ID NOs. 7-12 or a nucleotide sequence exhibiting at least 60%, preferably 65, 70, 75, 80, 85, 90, or 95% homology with said nucleotide sequence, wherein homology may be determined by comparing a polynucleotide molecule comprising a nucleotide sequence encoding an amino acid sequence of any of SEQ ID NOs: 7-12 with a polynucleotide molecule having a nucleotide sequence in question by sequence alignment, wherein a nucleotide in the sequence in question is considered homologous if it is either identical to the corresponding nucleotide in the nucleotide sequence encoding a corresponding amino acid sequence of any of SEQ ID NOs: 7-12 or if one or more

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nucleotide deviation(s) in the sequence in question from the corresponding one or more nucleotide(s) in the nucleotide sequence encoding an amino acid sequence of any of SEQ ID NOs: 7-12 results in a nucleotide triplet which, when translated, results in an amino acid which is either identical to (due to a degenerate triplet) or a conservative substitution of the corresponding amino acid in the corresponding amino acid sequence of any of SEQ ID NOs: 7-12.

A further aspect of the invention provides a pharmaceutical composition comprising a humanized monoclonal antibody or fragment thereof or a polynucleotide molecule having a nucleotide sequence encoding an amino acid sequence as set out in any of SEQ ID NOs. 1-12 or encoding an amino acid sequence comprising an amino acid sequence bearing at least 70% homology to any of SEQ ID NOs. 1-12, wherein "homology" is to be understood as explained hereinabove.

In accordance with this invention, the term "pharmaceutical composition" relates to a composition for administration to a mammalian patient, preferably a human patient. In a

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preferred embodiment, the pharmaceutical composition comprises a composition for parenteral injection or infusion. Such parenteral injection or infusion may take advantage of a resorption process in the form of e.g. an intracutaneous, a subcutaneous, an intramuscular and/or an intraperitoneal injection or infusion. Alternatively, such parenteral injection or infusion may circumvent resorption processes and be in the form of e.g. an intracardial, an intraarterial, an intraveneous, an intralumbal and/or an intrathecal injection or infusion. In another preferred embodiment, the pharmaceutical composition comprises a composition for administration via the skin. One example of administration via the skin is an epicutaneous administration, in which the pharmaceutical composition is applied as e.g. a solution, a suspension, an emulsion, a foam, an unguent, an ointment, a paste and/or a patch to the skin. Alternatively, administration of the pharmaceutical composition may be effected via one or more mucous membranes. For example, administration may be buccal, lingual or sublingual, i.e. via the mucous membrane(s) of the mouth and/or tongue, and may be applied as e.g. a tablet, a lozenge, a sugar coated pill (i.e. dragée) and/or as solution for gargling. Alternatively, administration may be enteral, i.e. via the mucous membrane(s) of the stomach and/or intestinal tract, and may be applied as e.g. a tablet, a sugar coated pill (i.e. dragée), a capsule, a solution, a suspension and/or an emulsion. Alternatively, administration may be rectal, and may be applied as e.g. a suppository, a rectal capsule and/or an ointment or unguent. Alternatively, administration may be intranasal, and may be applied as e.g. drops, an ointment or unguent and/or a spray. Alternatively, administration may be pulmonary, i.e. via the mucous membrane(s) of the bronchi and/or the alveolae, and may be applied as e.g. an aerosol and/or an inhalate. Alternatively, administration may be conjunctival, and may be applied as e.g. eye drops, an eye ointment and/or an eye rinse. Alternatively, administration may be effected via the mucous membrane(s) of the urogenital tract, e.g. may be intravaginal or intraurethal, and may be applied as e.g. a suppository, an ointment and/or a stylus. It should be understood that the above administration alternatives are not mutually exclusive, and that a combination of any number of them may constitute an effective therapeutic regimen.

The pharmaceutical composition of the present invention may further comprise a pharmaceutically acceptable carrier. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, etc.. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in

the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Preparations for e.g. parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, emulsions and liposomes. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Vehicles suitable for general parenteral administration include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Vehicles suitable for intravenous or intraarterial administration include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, inert gases and the like. In addition, the pharmaceutical composition of the present invention might comprise proteinaceous carriers, like, e.g., serum albumin or immunoglobulin, preferably of human origin. It is envisaged that the pharmaceutical composition of the invention might comprise, in addition to the humanized monoclonal antibody or fragment thereof (as described in this invention), further biologically active agents, depending on the intended use of the pharmaceutical composition. Such agents might be drugs acting on the gastro-intestinal system, drugs acting as cytostatica, drugs preventing hyperurikemia, drugs inhibiting immunoreactions (e.g. corticosteroids), drugs modulating the inflammatory response, drugs acting on the circulatory system and/or agents such as cytokines known in the art.

A further aspect of the invention provides a use of a humanized monoclonal antibody or fragment thereof as set out hereinabove or of a polynucleotide molecule as set out hereinabove in the manufacture of a medicament, optionally comprising one or more additional anti-inflammatory agents, for the treatment of inflammatory diseases in mammals, preferably humans. Advantageously, such inflammatory diseases are chosen from the group consisting of rheumatoid arthritis (RA), asthma, multiple sclerosis (MS), chronic obstructive pulmonary disease (COPD), Acute Respiratory Distress Syndrome (ARDS), Idiopathic Pulmonary Fibrosis (IPF), Inflammatory Bowel Disease (IBD), uveitis, macular degeneration, colitis, psoriasis, Wallerian Degeneration, antiphospholipid syndrome (APS), acute coronary syndrome, restinosis, atherosclerosis, relapsing polychondritis (RP), acute or chronic hepatitis, failed orthopedic implants, glomerulonephritis, lupus, autoimmune disorders, acute pancreatitis or ankylosing spondylitis (AS).

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A further aspect of the invention provides a use of a humanized monoclonal antibody or fragment thereof as set out hereinabove or of a polynucleotide molecule as set out hereinabove in the manufacture of a medicament, optionally comprising one or more additional anti-cancer agents, for the treatment of a tumorous disease or another condition with delayed cell apoptosis, increased cell survival or proliferation in mammals, preferably humans. Preferably, the tumorous disease is a cancer, said cancer preferably being a leukaemia, multiple myeloma, gastric carcinoma or skin carcinoma.

A further aspect of the invention provides a method of treating an inflammatory disease in which a humanized monoclonal antibody or fragment thereof as set out hereinabove or a polynucleotide molecule as set out hereinabove is administered (optionally together with one or more additional anti-inflammatory agents) to a mammalian, preferably to a human subject in a sufficient amount and for a sufficient time to prevent and/or ameliorate said inflammatory disease. Advantageously, such inflammatory diseases are chosen from the group consisting of rheumatoid arthritis (RA), asthma, multiple sclerosis (MS), chronic obstructive pulmonary disease (COPD), Acute Respiratory Distress Syndrome (ARDS), Idiopathic Pulmonary Fibrosis (IPF), Inflammatory Bowel Disease (IBD), uveitis, macular degeneration, colitis, psoriasis, Wallerian Degeneration, antiphospholipid syndrome (APS), acute coronary syndrome, restinosis, atherosclerosis, relapsing polychondritis (RP), acute or chronic hepatitis, failed orthopedic implants, glomerulonephritis, lupus, autoimmune disorders, acute pancreatitis or ankylosing spondylitis (AS).

A further aspect of the invention provides a method of treating a tumorous disease in which a humanized monoclonal antibody or fragment thereof as set out hereinabove or a polynucleotide molecule as set out hereinabove is administered (optionally together with one or more additional anti-cancer agents) to a mammalian, preferably to a human subject in a sufficient amount and for a sufficient time to prevent and/or ameliorate said tumorous disease or another condition with delayed cell apoptosis, increased cell survival or proliferation. Preferably, the tumorous disease is a cancer, said cancer preferably being a leukaemia, multiple myeloma, gastric carcinoma or skin carcinoma.

Throughout the specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of

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a stated integer or group of integers but not the exclusion of any other integer or group of integers.

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Reference to cited material or information contained in the text should not be understood as a concession that the material or information was part of the common general knowledge or was known in Australia or any other country.

The invention will now be illustrated by way of the following non-limiting figures and examples. An overview of the figures is as follows:

Fig. 1 Retained antigen binding after humanization of VL region

Fig. 2 Loss of neutralizing activity after humanization of VL region

Fig. 3 Binding of IL2 is not affected by human/mouse framework exchanges within the VL region

Fig. 4 Loss of neutralization activity following incorporation of a human second light chain framework

Fig. 5 Amino acid changes at positions 42-46 of the VL region (within second light chain framework) do not affect antigen binding

Fig. 6 Mutation of leucine to alanine at position 46 of the second light chain framework leads to a regaining of neutralization activity

Fig. 7a Human germline amino acid sequences for first and second light chain framework regions (V-kappa). CDR regions have been omitted; the remaining numbering of the remaining framework regions is as published in the online "Vbase" database (see above for weblink).

Fig. 7b Human germline amino acid sequences for the third light chain framework region (V-kappa). CDR regions have been omitted; the remaining numbering of the remaining framework regions is as published in the online "Vbase" database (see above for weblink).

Fig. 8a Human germline amino acid sequences for first and second light chain framework regions (V-lamdba). CDR regions have been omitted; the remaining numbering of the remaining framework regions is as published in the online "Vbase" database (see above for weblink).

Fig. 8b Human germline amino acid sequences for the third light chain framework region (V-kappa). CDR regions have been omitted; the remaining numbering of the remaining framework regions is as published in the online "Vbase" database (see above for weblink).

Fig. 9a Human germline amino acid sequences for first and second heavy chain framework regions. CDR regions have been omitted; the remaining numbering of the remaining framework regions is as published in the online "Vbase" database (see above for weblink).

Fig. 9b Human germline amino acid sequences for the third heavy chain framework region. CDR regions have been omitted; the remaining numbering of the remaining framework regions is as published in the online "Vbase" database (see above for weblink).

Fig. 10 Specificity of binding of humanized anti-IL2 antibody "Anti-IL2" to the natural killer lymphoma cell line NKL

Fig. 11 Anti-IL2 abrogates IL2-dependent IL2-dependent up-regulation of CD124 cell surface expression on CTLL-2 cells

Fig. 12 Anti-IL2 specifically blocks IL2 signal transduction downstream of the IL2 receptor

Fig. 13 The efficacy of Anti-IL2 and Daclizumab is differentially affected by CD25 expression level

Fig. 14 Results showing the impact of Anti-Il2 and Daclizumab on IL2-dependent proliferation of primary human NK cells

Fig. 15 Results showing the impact of Anti-Il2 and Daclizumab on IL2-dependent release of IFN-gamma by NK cells

Example 1: Procurement of human IL2 ("hIL2") antigen

The purpose of the experimental approaches described below in Examples 1a, 1b and 1c was to provide recombinant IL2 antigen material derived from different sources: antigen expressed in prokaryotic cells, in eukaryotic cells and recombinant protein antigen commercially available as a certified therapeutic.

Example 1a: Recombinant expression from E.coli.

The mature hIL2 (i.e., encoding amino acid residues APTSSS...IISTLT) was cloned as a single open reading frame ("ORF") into the prokaryotic expression vector pBAD (Invitrogen) using standard PCR and molecular biology technology. At the 5'-end three nucleotides encoding a methionine were added, on the 3'-end a nucleotide sequence was inserted before the stop codon that fuses a hexahistidine-tag to the C-terminus of the protein.

This construct was used to transform competent E. coli (strain BL21(DE3), Stratagene) using the instructions provided by the manufacturer. Bacteria were grown in standard LB medium to a density of OD(600 nm) = 0.5, then L-arabinose was added to a concentration of 0.2 % w/v to trigger expression for 5 h. Harvest of E. coli was performed by centrifugation at 10,000 g for 15 min. Then the insoluble fraction (inclusion bodies) was prepared using the BugBuster reagent and protocol (Novagen) according to the manufacturer's instructions.

Inclusion bodies were solubilized in 6 M Guanidine-hydrochloride ("GuHCl"), and were then diluted to 0.1 mg/ml with a buffer containing 2 M GuHCl (pH = 8.0) / 1 mM Glutathione-ox / 10 mM Glutathione-red and incubated for 16 h at 20°C. After incubation, pH was adjusted to 6.0 by slow addition of glacial acetic acid while stirring vigorously. Finally, three sequential

chromatography approaches were applied to obtain a highly purified and homogenous protein preparation of hIL2his: immobilized metal affinity chromatography (IMAC), reversed phase HPLC, ion exchange chromatography. The functionality of the purified protein was verified in a cellular proliferation experiment (see below).

Example 1b: Recombinant expression from mammalian cells

The mature hIL2 (i.e., encoding amino acid residues APTSSS...IISTLT) was cloned into the eukaryotic expression vector pEFdhfr (Mack M. et al. (1995) PNAS 92, 7021-5) using standard PCR and molecular biology technology. At the 5'-end a nucleotide sequence encoding a leader peptide of human IgG was added to allow for efficient processing and secretion, on the 3'-end a nucleotide sequence was inserted before the stop-codon that fuses a hexahistidine-tag to the C-terminus of the protein.

293 cells (DSMZ, order code ACC305) were seeded at a density of 25-35% plate surface coverage and cultured for 24h. Then the cells were transfected with the pEFdhfr-hIL2 expression vector using the "Transfast" reagent (Promega) according to the manufacturer's instructions. After an additional 60 h culture period, cellular supernatants were harvested and hIL2-his protein was purified using an IMAC approach, followed by ion-exchange chromatography. Functionality of the purified protein was verified in a cellular proliferation experiment.

Example 1c: Purchase as Proleukin

Proleukin (formulated recombinant hIL2, expressed in E.coli) was purchased from Chiron.

With the above approaches three different supplies of fully functional recombinant hIL2 antigen were made available.

Example 2: Generation of humanized monoclonal anti-hIL2 antibody

It was desired to generate a humanized monoclonal antibody ("mAb") with a particularly favorable mode of action and that specifically targets human hIL2 and neutralizes its bioactivity. In general, neutralizing mAbs targeting a secreted soluble protein, such as the cytokine hIL2, recognize an epitope that is at least partially overlapping with the epitope recognized by a component of the corresponding cytokine receptor. Thus, the mAb directly competes with the receptor for binding to the cytokine. This mechanism of action implies that neutralization can effectively be achieved. The mAb must be applied at a sufficiently high dose in order to out-compete the cytokine receptor.

Example 2a: Starting point --> commercially available monoclonal anti-hIL2 antibody as protein

To gain an understanding of the extent to which different anti-hIL2 mAbs could neutralize as mentioned above, anti-hIL2 mAbs were produced by immunization of mice, followed by harvest of spleen cells and hybridoma fusion, all according to standard protocols. In addition, commercially available anti-hIL2 mAbs were purchased. The pool of available mAbs was used to compare features of the different antibodies in three assays: binding to soluble antigen as tested by ELISA, binding to cell surface-associated antigen as tested by FACS, and capacity for neutralization of hIL2 bioactivity as tested by a cellular proliferation assay.

The ELISA assay was performed as follows:
All incubations were performed at 20°C. Streptavidin-coated 96-well ELISA plates (Nunc) were used to attach PEG-biotinylated Proleukin, 0.1 µg in 100 µl PBS-TB (phosphate buffered saline, pH = 7.4, 0.05% v/v Tween-20, 1 % w/v BSA) per well for 30 minutes. Then the plate was washed 3 times with 200 µl per well PBS-T (phosphate buffered saline, pH = 7.4, 0.05% v/v Tween-20). The different mAb samples were added, 100 µl per well and samples were incubated for 1 hour. Then the plate was washed 3 times with 200 µl per well PBS-T. The detection antibody applied was a goat anti-human IgG HRP-conjugated mAb, (Jackson Immunoresearch), diluted 1:1000 in PBS-TB, 100 µl per well and incubation for 1 hour. Then the plate was washed 3 times with 200 µl per well PBS-T. Antibody binding to antigen finally was quantified by incubation with the HRP substrate: 100 µl 2,2'-azino-di [3-ethyl-benzthiazoline-6-sulphonic acid] ("ABTS") substrate buffer (Roche Diagnostics, ABTS tablets) and the plate was incubated for 5 to 10 minutes until green dye developed. The staining was measured at 405 nm on a 96-well plate reader.

The FACS assay was performed as follows:
For optimal growth under cell culture conditions, the human natural killer lymphoma cell line NKL depends on the presence of about 5 ng/ml hIL2 in the medium (Basal Iscove's medium (Biochrom AG); 10% v/v fetal bovine serum (Biochrom AG); 100 µg/ml Penicillin/Streptomycin (Biochrom AG)). NKL cells, 1 x 10^6 per ml, were deprived of hIL2 for 24 hours in preparation of the experiment by culturing in hIL2-free medium. Immediately prior to the experiment, the cells were washed with hIL2-free medium. All following incubations were done at 4°C for 30 minutes; for washing, the PBS-F buffer (phosphate buffered saline, 3% v/v fetal bovine serum) was used at 4°C, as well. First 2 x 10^5 NKL cells in 200 µl medium were incubated with 1 µg of recombinant human hIL2 or left without hIL2 under the same conditions. Subsequently, cells were washed 3 times, each wash with 2 ml PBS-F. Then 2 x 10^5 cells were

incubated with the different mouse anti-hIL2 mAbs, 1 µg in 200 µl medium, at 4°C for 30 minutes. The cells were washed again three times, as indicated above, and lastly incubated with a FITC-conjugated goat anti-mouse IgG detection mAb (Jackson Immunoresearch), diluted 1:1000 in 200 µl PBS-F. After three additional washes, cellular fluorescence of cells holding hIL2 on their surface versus plain cells was analyzed on a FACS machine.

The proliferation assay was performed as follows:

For optimal growth under cell culture conditions, the murine CTL cell line CTLL-2 (LGCPromochem) depends on the presence of about 5 ng/ml hIL2 in the medium (Basal Iscove's medium (Biochrom AG); 10% v/v fetal bovine serum (Biochrom AG); 100 µg/ml Penicillin/Streptomycin (Biochrom AG); 0.5 mM 2-Mercaptoethanol (Gibco)). Both mouse and human hIL2 work equally well maintaining survival and proliferation of CTLL-2 cells. CTLL-2 cells, 1 x 10^6 per ml, were deprived of hIL2 for 12 hours in preparation of the experiment by culturing in hIL2-free medium.

Immediately prior to the experiment, the cells were washed with hIL2-free medium. A 96-well tissue culture plate was used to perform the proliferation experiment and assess inhibition of hIL2 bioactivity by the different mAbs. A final assay volume of 200 µl was applied per well, this volume including: 5 x 10^4 CTLL-2 cells, 2 ng/ml hIL2 (to allow for approximately half-maximal proliferation) and the different anti-hIL2 mAbs at a concentration of 5000 ng/ml, 1000 ng/ml, 200 ng/ml and 40 ng/ml. All samples were prepared in triplicate. The respective mixtures were incubated 48 hours at 37°C in a humidified chamber in the presence of 5% carbon dioxide. Then viable cells were detected using the AlamarBlue fluorescent dye readout (Biosource International) and a 96-well fluorescence plate reader according to the manufacturer's recommendation.

The mAb202 (commercially available from R&D Systems) was found to (i) bind to soluble antigen, (ii) bind to cell surface-associated antigen, and (iii) efficiently neutralize hIL2 bioactivity. Among the antibodies tested, only mAb 202 scored in all three assays and therefore was considered a promising candidate according to the features defined above and was therefore chosen as a starting point for subsequent experiments.

Example 2b: Determination of primary sequence of anti-hIL2 antibody by sequencing: Identification of sequences from the variable region of the heavy chain ("VH") and the variable region of the light chain ("VL")

Due to lack of availability of the mAb202 hybridoma clone, the mAb was sequenced to identify VH and VL amino acid sequences. To this end, Fab fragments of mAb202 were prepared. These fragments were subjected to proteolytic digestion with online HPLC for peptide separation. Subsequently, the individual peptides were analyzed with respect to amino acid composition and sequence by an MS/MS mass spectrometry. This approach led to identification of VH and VL protein sequences.

Example 2c: Control for retained functionality: fusion of sequenced VH/VL regions with known mouse constant regions

A functional verification of the sequencing results obtained from mAb202 protein sequencing described above was desired. Thus, a gene encoding the sequenced VH was synthesized and cloned into an expression vector providing the constant regions of a mouse IgG1. Likewise, a gene encoding the sequenced VL was synthesized and cloned into an expression vector providing a mouse C kappa domain. These two expression vectors would ideally allow reconstruction of the original mAb202, the functionality of which could then be re-tested as above. After co-expression of both vectors in 293 cells, an anti-IL2 mAb was detected in the cellular supernatants with features comparable to those observed with the original mAb202. The concordance of activity (by ELISA as well as in a a proliferation assay using a CTLL-2 cell line) observed for the reconstructed mAb following protein sequencing with those of the parental mAb202 may be taken as a confirmation that the sequences determined for the VH and VL regions of this antibody were correct.

Example 2d: Humanization of heavy chain

The intention of humanization is to fully retain binding specificity and biological activity of an antibody while minimizing the content of non-human sequence present in a mAb. The latter aim results in an antibody which is less likely to elicit an immune response when administered to a human subject than its parent antibody, of non-human origin. Initially, an expression vector for a chimeric heavy chain comprising the original mouse VH together with C1, C2 and C3 domains of human IgG1 isotype was generated. After expression of the chimeric heavy chain, when combined with the chimeric light chain (see below), the features of the original mouse mAb could be reproduced (see below). The next logical step was to humanize the VH region. In order to avoid changes in specificity, the CDR sequences remained unchanged. Therefore, on the basis of the original mouse VH, the most closely related human VH framework sequence was searched. Of all human VH frameworks, human framework 1-3/3-07/J6 was found to bear the highest degree of homology to the original murine framework. Human framework 1-3/3-07/J6

was found to differ in 16 amino acid residues from the corresponding mouse VH frameworks. The alignment below shows a direct comparison between original mouse and human 1-3/3-07/J6 VH frameworks; original CDR sequences are underlined and amino acid identity between both sequences is indicated by an asterisk.

```
VH_mouse     DVRLVESGGGLVKPGGSLKLSCAAYGFTFSSYTLAWVRQTPEKRLEWVAAIDSSSYTYSPDTVRG
1-3/3-07/J6  EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVAAIDSSSYTYSPDTVRG
              * ********* ***** ***** ************** * * **********************
VH_mouse     RFTISRDNAKNTLYLQMSSLKSEDTAMYYCTRDSNWDALDYWGQGTSVIVSS
1-3/3-07/J6 RFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDSNWDALDYWGQGTTVTVSS
            *********** ***** **  **** *** *************** * ***
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The constructs containing the original mouse VH or the humanized VH will in the following text be respectively referred to as cHC (**c**himeric **h**eavy **c**hain comprising a mouse VH and human C1, C2, C3) and hHC (**h**umanized **h**eavy **c**hain comprising a VH containing mouse CDR regions within a human VH framework and human C1, C2, C3). For purposes of recombinant protein expression of the humanized heavy chain, open reading frames encoding the humanized VH in combination with C1, C2 and C3 domains of human IgG1 isotype were cloned into a suitable vector (Raum T et al. (2001) Cancer Immunol Immunother. 50, 141-50).

Example 2e: Humanization of light chain

Humanization was performed analogously to the approach described above for the heavy chain. In brief, an expression vector for a chimeric light chain comprising the original mouse VL together with a human Ck domain was generated and tested after co-expression with the chimeric heavy chain (see above). Again, as a second step, on the basis of the original mouse VL the most closely related human VL framework sequence was searched. All three CDRs were retained. Human VL framework O12/Jk4 turned out to be the closest relative in sequence. A total of 22 amino acid residues were different in the VL frameworks between the mouse VL and human O12/Jk4. The alignment below shows a direct comparison between original mouse and human O12/Jk4 frameworks; original CDR sequences are underlined, amino acid identity between both sequences is indicated by an asterisk.

```
VL_mouse     DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVGWYQQKPGQSPKALIYSASFRYS
O12/Jk4      DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVGWYQQKPGKAPKLLIYSASFRYS
             ** ****    * ******  ********************  ** **********

VL_mouse     GVPDRFTGSGSGTDFSLTISNVKSEDLAEYFCQQYYTYPYTFGGGTKLEIK
```

```
O12/Jk4     GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYTYPYTFGGGTKVEIK
            *** ** ******** ****    ** * * ********************* ***
```

The constructs containing the original mouse VL or the humanized VL will in the following text be respectively referred to as cLC (**c**himeric **l**ight **c**hain comprising a mouse VL and human Ckappa) and hLC (**h**umanized **l**ight **c**hain comprising a VL containing mouse CDR regions within a human VL framework and human Ckappa). For purposes of recombinant protein expression of the humanized light chain, open reading frames encoding the humanized VL in combination with the human Ck domain were cloned into a suitable vector (Raum T et al. (2001) Cancer Immunol Immunother. 50, 141-50).

Example 2e.1: Permutation of human and mouse sequences as entire framework regions; Evaluation of binding and neutralization by a proliferation assay

After successful humanization of both the heavy and the light chain had been performed, features of the resulting humanized mAbs were tested in comparison to the chimeric mAb, i.e. the antibody molecule containing fully murine variable domains. Since the chimeric mAb showed neutralization of IL2 bioactivity comparable to the original mAb, it was used as a reference for these experiments. Pairs of expression vectors encoding heavy and light chains were used for transient co-transfection of 293 cells (the applied protocol was identical to the transfection procedure described in Example 1b, except that the cells were transfected with two plasmids simultaneously).

After expression in 293 cells comparable quantities of the different mAb versions in the cellular supernatants were verified by an anti-hIgG ELISA, which was carried out as follows:

A 96-well ELISA plate (Nunc) was incubated with a 1:2,000 dilution in PBS of the anti-hIgG mAb (Abcam LTD), 100 µl per well, 12 hours at 4°C. Each well was washed 3 times with 200 µl of PBS-T buffer, then 100 µl of neat supernatants harvested from 293 cells and serial dilution of supernatants in medium were filled into each well and incubated for 1 hour at 20°C. Again, each well was washed 3 times with 200 µl of PBS-T buffer. A 1:1,000 dilution in PBS-TB of the goat anti-human IgG HRP-conjugated mAb (Jackson Immunoresearch) was added to the wells, 100 µl per well and incubated for 1 hour at 20°C. Then the plate was washed 3 times with 200 µl per well PBS-T. Antibody binding to hIgG finally was quantified by incubation with the HRP substrate: 100 µl ABTS substrate buffer (Roche Diagnostics, ABTS tablets) and the plate incubated for 5 to 10 minutes until green dye developed. The staining was measured at 405 nm on a 96-well plate reader. Only supernatants with comparable quantities of mAb were used for

all follow up experiments. Antigen-binding of the various mAbs was tested by ELISA (see above).

The features of the generated mAbs regarding binding of soluble antigen and regarding neutralization of IL2 bioactivity was analysed by ELISA and a CTLL-2 proliferation assay, respectively (see Example 2a above for detailed experimental protocols). In the ELISA experiment, increasing absorbance units will be indicative for increased amounts of mAb binding to hIL2 antigen. In the CTLL-2 proliferation assay, increasing fluorescence units will be indicative for an increased number of metabolically active (= alive) cells. All cellular supernatants ("SN") containing the different mAb versions were controlled for even mAb concentrations by an anti-hIgG ELISA before they were subjected to the following experiments. The results of a representative experiment are shown in Fig. 1. Here, both combinations hLC+hHC (hLC = humanized light chain, hHC = humanized heavy chain) and cLC+hHC (cLC = chimeric light chain) show comparable binding to hIL2 antigen. The results from the CTLL-2 assay (Fig. 2) show that hLH+hHC does not lead to any detectable neutralization of hIL2 bioactivity because detected fluorescence is not different from the control SN. In contrast, cLH+hHC reduces the number of living cells as evident by a mAb concentration-dependent reduction in fluorescence. Application of the two individual chains cLC or hHC in this assay had no impact on hIL2-dependent cell survival (data not shown). Each data point in the representative experiment shown in Fig. 2 represents the mean result of duplicate samples. The results of the assays described are summarized in Table 1:

Table 1

light chain	heavy chain	antigen binding	Neutralization
hLC	HHC	+	-
cLC	HHC	+	+

These results demonstrate that despite binding to soluble antigen appears not to be different neutralization is lost as soon as hLC is used with the humanized heavy chain variant. The conclusion is that humanization of VL entailed some functional impairment for the mAb.

In order to determine where, i.e. within which framework region, this impairment was introduced, humanization of the VL framework of cLC was performed in different segments, changing only a single framework at a time (i.e. framework region 1, framework region 2 or framework region 3) rather than all three frameworks at a time from mouse to human. An

abbreviated nomenclature was developed, and will be used hereinbelow, in designating these humanization variants. According to this nomenclature, three capital letters are used to create triplets designating each of the first three framework regions 1, 2 and 3, wherein the first position of the triplet designates the nature of framework region 1, second position of the triplet designates the nature of framework region 2, and the third position of the triplet designates the nature of framework region 3. For example, "HMM" would indicate a human framework region 1 in a VL that is otherwise of murine origin, whereas "MHM" indicates that only framework region 2 is human, whereas framework regions 1 and 3 are of murine origin.

The features of the different human/mouse hybrid VL domains were analyzed with respect to their effects. Again, binding of hIL2 antigen and neutralization of IL2 bioactivity were analysed by ELISA and a CTLL-2 proliferation assay, respectively (see Example 2a above for detailed experimental protocols). In the ELISA experiment, increasing absorbance units are indicative of increased amounts of mAb binding to hIL2 antigen. In the CTLL-2 proliferation assay, increasing fluorescence units are indicative of an increased number of metabolically active (= alive) cells. All cellular supernatants ("SN") containing the different mAb versions were controlled for uniform mAb concentrations using an anti-hIgG ELISA before they were subjected to the following experiments. Fig. 3 shows the results from a representative comparative experiment. Here, it can be seen that all human/mouse hybrid VL versions show comparable binding to hIL2 antigen when combined with hHC. The results of the CTLL-2 assay (Fig. 4) show that neutralization of hIL2 bioactivity is only observed as long as VL framework 2 is murine; MHM+hHC did not change the number of living cells compared to control SN. Each data point in the representative experiment shown in Fig. 4 represents the mean of duplicate samples. The results of the assays described are summarized in Table 2:

Table 2:

light chain	heavy chain	antigen binding	Neutralization
HMM	HHC	+	+
MHM	HHC	+	-
MMH	HHC	+	+
HMH	HHC	+	+

These experiments show quite clearly that framework 2 of the VL determines whether or not the mAb is capable of neutralizing IL2 bioactivity. A more detailed comparison of mouse and human sequences of framework 2 reveals that these sequences differ in three amino acids.

Specifically, the mouse framework 2 comprises the partial amino acid sequence QSPKA, whereas the corresponding human sequence is KAPKL (amino acids differing between human and mouse species have been underlined for clarity).

Example 2e.2: Permutation of human and any mouse sequences within framework 2; Evaluation of binding and neutralization by a proliferation assay and a target gene induction assay

To determine whether all three amino acid changes or only some are decisive in terms of providing a neutralizing mAb, an additional set of experiments was performed. To this end, the mouse-derived amino acid residues QS or A were re-introduced into the hLC.

The features of the resulting mAbs with respect to the binding of soluble antigen and the neutralization of IL2 bioactivity were analyzed by ELISA and a CTLL-2 proliferation assay, respectively (see Example 2a above for detailed experimental protocols). In the ELISA experiment, increasing absorbance units are indicative of increased amounts of mAb binding to hIL2 antigen. In the CTLL-2 proliferation assay, increasing fluorescence units are indicative of an increased number of metabolically active (= alive) cells. All cellular SN containing the different mAb versions were controlled for uniform mAb concentrations by an anti-hIgG ELISA before they were subjected to the following experiments. Fig. 5 shows results from a representative experiment. As can clearly be seen, both versions QSPKL+hHC and KAPKA+hHC show comparable binding to hIL2 antigen. The CTLL-2 assay (Fig. 6) shows that QSKPL+hHC does not lead to any detectable neutralization of hIL2 bioactivity because detected fluorescence is not different from the control SN. In contrast, KAPKA+hHC reduces the number of living cells as evident by a mAb concentration dependent reduction in fluorescence. Each data point in the representative experiment shown in Fig. 6 represents the means of duplicate samples. The results of the assays described are summarized in Table 3.

Table 3:

light chain	heavy chain	antigen binding	neutralization
hLC	HHC	+	-
hLC_QSPKL	HHC	+	-
hLC_KAPKA	HHC	+	+

This shows that a single amino acid residue located in VL framework 2 defines if the mAb neutralizes IL2 bioactivity: the alanine residue in this position, derived from mouse framework 2, permits neutralization, the leucine residue, derived from human framework 2, does not.

Example 3: Determination of the mode of neutralization

In the following, the term "Anti-IL2" denotes a humanized anti-IL2 antibody comprising a light chain comprising an amino acid sequence as set out in SEQ ID NO: 9 (itself comprising a VL region with an amino acid sequence as set out in SEQ ID NO: 7) and a heavy chain comprising an amino acid sequence as set out in SEQ ID NO: 10 (itself comprising a VL region with an amino acid sequence as set out in SEQ ID NO: 8). The VL of Anti-IL2 comprises the amino acid sequence "KAPKA" as explained above in Example 2e.2.

It was desired to better understand the mode of neutralization of hIL2 by Anti-IL2. To this end, experiments were performed to study the nature of binding of hIL2 to, on the one hand, components of the hIL2 receptor and, on the other hand, to Anti-IL2.

Since NKL cells require IL2 for survival, it can be inferred that these cells do express a functional receptor for IL2. A FACS experiment was performed according to the procedures detailed above. Briefly, the cells were incubated with a mixture of the anti-hIL2 mAb and a species-specific secondary detection antibody ("premix"). The secondary antibody was conjugated to a fluorescent label. Cellular fluorescence was monitored in the presence and absence of hIL2 using FACS. The following experimental scenarios were carried out to address the question of whether a certain order of incubations was required for the effects observed.

In a first scenario the premix was incubated with or without hIL2 for 30 min, then NKL cells were added. Cellular fluorescence was observed in a hIL2-dependent fashion. In a second scenario NKL cells were incubated with or without hIL2 for 30 min, then the premix was added. Again, cellular fluorescence was observed in a hIL2-dependent fashion.
These experiments demonstrate that hIL2, when bound to Anti-IL2, can still bind to its receptor and further, that hIL2 when associated with its receptor can still interact with Anti-IL2.

These results demonstrate that the epitope of hIL2 bound by Anti-IL2generated as described above is distinct – at least partially – from the epitope of hIL2 bound by the hIL2 receptor. This mode of neutralization is noteworthy in that it implies that neutralization of hIL2 may be accomplished by binding this molecule in either its soluble or its receptor-bound form. Seen chronologically, then, this means that the binding event between hIL2 and Anti-IL2may take place either before or following formation of the complex between hIL2 and the hIL2-receptor; in either case, neutralization of the bioactivity of hIL2 is effected. By extrapolation one may

therefore assume that neutralization is also effected in the event that the two relevant binding events – formation of the complex between hIL2 and hIL2-receptor and the complex between hIL2 and Anti-IL2 – occur simultaneously.

It should be noted that such a mode of neutralization as observed for Anti-IL2 stands in sharp contrast to other known modes of neutralization in which the epitope bound by a neutralizing anti-ligand antibody and a the ligand receptor are one and the same; In such a conventional scenario, it is not possible for the ternary complex between ligand, ligand-receptor and neutralizing anti-ligand antibody to exist. Expressed differently, in such a conventional mode of neutralization, ligand must be bound by neutralizing anti-ligand antibody while the ligand is still in soluble form, and so that formation of a complex between ligand and ligand-receptor is precluded.

Example 4: IL2-dependent binding of Anti-IL2 to the human natural killer lymphoma cell line NKL

In this example, the specificity of Anti-IL2 binding to cell surface associated hIL2 was studied. The Anti-IL2 parent Ab (mAb202) showed strictly IL2-dependent binding to the cell surface of NKL cells. This particular feature therefore had to be confirmed for Anti-IL2.

NKL cells were deprived of hIL2 for 24 h prior to the experiment. Anti-IL2 or a human IgG1 isotype control antibody were incubated in the absence or presence of a 2-fold molar excess of hIL2 at 20°C for 60 min. The respective mixes were then added to NKL cells (10^5 cells per sample) and further incubated for 30 min on ice. Subsequently, the cells were washed extensively and a fluorescence-labeled goat anti-human IgG detection antibody was added, followed by incubation for 30 min on ice. Again the cells were washed and then subjected to FACS analysis to study cell-associated fluorescence.

As expected, in the absence of hIL2, no significant cell-associated fluorescence was detectable with either Anti-IL2 or the control antibody (Fig. 10, left plot). In the presence of hIL2, cell associated fluorescence with the control antibody was unchanged (Fig. 10, right plot, shaded peak). In contrast, incubation with hIL2 and Anti-IL2 resulted in a substantial increase in fluorescence (Fig. 10, right plot, black-outlined peak), indicative of specific IL2-dependent binding of Anti-IL2 to the cell surface. Thus, the ability to recognize cell surface- associated hIL2 was conserved in Anti-IL2. This experiment provided evidence that Anti-IL2 not only

recognizes hIL2 in solution, but also recognizes hIL2 that is associated with one or several of its receptor components. Consequently, hIL2 can associate with Anti-IL2 and the IL2 receptor component(s) in a non-exclusive fashion.

Example 5: Anti-IL2 abrogates IL2-dependent up-regulation of CD124 cell surface expression on CTLL-2 cells

Following stimulation with hIL2, CTLL-2 cells proliferate and up-regulate cell surface expression of CD124 (IL-4R alpha) (Puri, R. K., et al. (1990). Immunology 70, 492). Consequently, CTLL-2 cells acquire increased sensitivity to concomitant stimulation through IL-4 via the IL2 stimulus. Therefore, Anti-IL2 may not only limit IL2 mediated proliferation but also affect CD124 expression.

To test this hypothesis, CTLL-2 cells were cultured in the absence of hIL2 for 12 h prior to the experiment and then stimulated with 0.5 ng/ml hIL2 for 5 h in the presence or absence of titrated Anti-IL2 concentrations. CD124 expression levels were assessed by FACS analysis using a fluorescence labeled CD124-specific antibody. The mean fluorescence intensities detected were plotted versus the different Anti-IL2 concentrations (Fig. 11, open squares with black line); mean fluorescence values recorded in the absence of Anti-IL2 (Fig. 11, filled diamond) or in the absence of IL2 (Fig. 11, filled triangle) were included as controls. As evident from Fig. 11, Anti-IL2 reduced CD124 expression in a dose-dependent fashion; the IC50 computed from this assay was approximately 3.3×10^{-10} M. These data imply that Anti-IL2 not only affects proliferation of CTLL-2 cells but also other IL2-dependent cellular responses, such as CD124 expression.

Example 6: Anti-IL2 specifically blocks IL2 signal transduction downstream of the IL2R

This experiment was performed to further rule out the possibility that Anti-IL2 mediates its effects on hIL2-dependent cellular responses in part by some cytotoxic mechanism, and to confirm that the mechanism of Anti-IL2 action is highly specific for hIL2- driven signals but does not affect related pathways. Among the most rapid cellular events of IL2-mediated cellular signals is the tyrosine phosphorylation of the transcription factor STAT3 (Leonard, W. J. 2000. *IL2 Family Cytokines and their Receptors*). Other cytokines, such as IL-6, trigger partially overlapping cellular signalling pathways, which also involve STAT3 (Hemmann, U., et al. (1996). J Biol Chem 271, 12999; Stahl, N., et al. (1995). Science 267, 1349).

Therefore, Anti-IL2 was tested regarding its effects on IL2- and IL6-driven tyrosine phosphorylation of STAT3. Peripheral blood lymphocytes were isolated from fresh donor blood, incubated at 2 x 10^6 cells/ml, prestimulated 48 h with lectin and then allowed to rest in medium for 24 h prior to stimulation. Cells were then stimulated with saturating concentrations of IL2 or IL6/sIL6Rα without mAb or in the presence of Anti-IL2 or an isotype control monoclonal antibody for 15 minutes. Following separation of cytoplasmic extracts by SDS-PAGE, the phosphorylation status of STAT3 was investigated by immune blotting using a STAT3 tyrosine phosphorylation-specific antibody (Fig. 12, upper panel). To control for comparable loading, a blot for total STAT3 protein was also performed (Fig. 12, lower panel). The electrophoretic mobility of standard proteins is indicated on the left of each panel in Fig. 12.

Both IL2 and IL-6 stimulation greatly enhanced cellular tyrosine phosphorylation of STAT3 in the absence of Anti-IL2 (Fig. 12, lanes 2 and 3 versus 1, or lanes 6 and 7 versus 1). Anti-IL2 therefore specifically affects STAT3 tyrosine phosphorylation after IL2 stimulation, but not after IL6 stimulation (Fig. 12, lanes 4 versus 5). These data demonstrate that Anti-IL2 is highly specific for interference with hIL2 biology and does not affect pathways regulated by other factors, nor does Anti-IL2 possess evident cytotoxic effects.

Example 7: The efficacy of Anti-IL2 and Daclizumab is differentially affected by CD25 expression levels

The inhibitory activity of Daclizumab, a humanized anti-CD25 mAb, was compared side-by-side to that of Anti-IL2 and an isotype control antibody in a proliferation assay using the IL2-dependent cell line NKL (Fig. 13).

To investigate the effect of CD25 cell surface expression levels on the inhibition of IL2-induced cell proliferation by either Anti-IL2 or Daclizumab, NKL cells were FACS-sorted for low or high level expression of CD25 and both cell populations studied side-by-side in this experiment. The anti-CD25 mAb used for FACS sorting did not interfere with the binding of IL2 or Daclizumab to CD25 (data not shown). Immediately after sorting, a clear distinction of $CD25^{low}$ and $CD25^{high}$ populations was possible by FACS, in the course of the five-day experiment the two populations would converge, leading to CD25 expression levels comparable to the population prior to sorting. This implies that the results obtained in this assay only reflect clearly separated $CD25^{low}$ versus $CD25^{high}$ populations for the initial phase but not for the later phases of this experiment. Therefore, differences to be observed regarding inhibition of proliferation by

Anti-IL2 or Daclizumab comparing $CD25^{low}$ and $CD25^{high}$ populations are limited due to non-stable CD25 expression levels; still a clear trend indicating differential CD25 dependence of Daclizumab and Anti-IL2 efficacy can be deduced from these data. The NKL cells were starved for 16 hours in preparation of the experiment by culturing in hIL2-free medium. Per well a final assay volume of 200 µl was applied which included: 1 x 10^4 NKL cells, 2 ng/ml hIL2 (to allow for half-maximal proliferation), and the different monoclonal antibodies at titrated concentrations. All samples were prepared in duplicate. Incubation of the respective mixtures took place for 120 hours, then viable cells were visualized using a fluorescent dye.

In general Anti-IL2 was more efficient in neutralization of IL2-mediated proliferation compared to Daclizumab in this assay. As anticipated, the efficacy of Anti-IL2 was not affected by CD25 expression levels: in $CD25^{low}$ and $CD25^{high}$ NKL cells, the curves obtained with Anti-IL2 run essentially on top of one another. In contrast, the curves obtained with Daclizumab show a clear difference in $CD25^{low}$ compared to $CD25^{high}$ NKL cells. The isotype control Ab had no effect (Fig. 13). In summary, this experiment provided in vitro evidence that efficacy of Daclizumab but not Anti-IL2 is dependent on CD25 levels.

Example 8: Impact of Anti-IL2 or Daclizumab on IL2-dependent proliferation of primary human NK cells

Not only primary T cells, but also primary NK cells can proliferate in response to IL2 stimulation. Thus, in a further experiment, inhibition of IL2-induced proliferation of freshly isolated human NK cells was studied.

The cells were obtained by negative isolation from donor blood and incubated with hIL2 (5.5 ng/ml) in the presence or absence of titrated Anti-IL2 or Daclizumab. A control antibody was only applied at the highest concentration; another control was performed with cells in the absence of IL2 and antibody. Viable cells were quantified using a fluorescent dye at the end of a one-week incubation period. Anti-IL2 substantially reduced IL2-driven proliferation of primary human NK cells in this experiment. With high Anti-IL2 concentrations, proliferation is essentially limited to the levels observed in the absence of IL2 indicative of Anti-IL2 affecting all IL2-responsive NK cells present in this assay. In contrast, Daclizumab only showed effect of much reduced amplitude, suggesting that only a fraction of NK cells was affected by the presence of this antibody (Fig. 14). To further investigate this finding, the levels of CD25 expression were monitored during the one-week incubation with IL2 and antibodies: only about

11% of total NK cells from the donor shown acquired CD25 expression with a maximum on day 3, and a drop to 2% on day 7. Consistently, freshly isolated NK cells from all donors were devoid of detectable CD25 expression and similar levels and kinetics of CD25 expression were found with NK cells from all donors analyzed (data not shown). This explained why Daclizumab could inhibit proliferation of only a fraction of NK cells (Fig. 14). Anti-IL2 again showed independence of CD25 expression levels and blocked proliferation of all NK cells with an IC50 value of approximately 3 x 10^{-10} M. These results provide a strong indication that Anti-IL2 but not Daclizumab is capable of interference with IL2-mediated signals through the intermediate affinity IL2 receptor CD122/CD132, independent of CD25.

Ex. 9: Impact of Anti-IL2 or Daclizumab on IL2-dependent release of IFN-gamma by NK cells

Besides proliferation, a typical and rapid response of primary NK cells to cytokine stimulation is the release of IFN-gamma. The release of the latter was measured in a further experiment, as dependent on both Anti-IL2 and Daclizumab.

In this assay, freshly isolated human NK cells were stimulated with a cocktail comprising hIL2 (5.5 ng/ml), hIL12 (5 ng/ml) and hIL18 (5 ng/ml), triggering efficient production and release of IFN-gamma by these cells. The effects of titrated Anti-IL2, Daclizumab and an isotype control antibody on IFN-gamma release within the first 48 h of incubation were compared. Both Anti-IL2 and Daclizumab reduced expression of IFN-gamma in a dose-dependent fashion, whereas the control antibody had no effect (Fig. 15). Anti-IL2 was a more potent inhibitor of IFN-gamma release, scoring an IC50 of approximately 1.3 x 10^{-10} M, as compared to approximately 1.1 x 10^{-9} M for Daclizumab (Fig. 15). In contrast to the experiment described in the previous example, all NK cells in this experimental setup acquired CD25 expression (data not shown), explaining the more profound effect of Daclizumab on IFN-gamma compared to NK cell proliferation.

Table 4 summarizes the equilibrium binding constant (K_D) for Anti-IL2 and Daclizumab. Furthermore, IC50 values obtained in side-by-side comparative experiments with both Abs as described above in Examples 8 and 9.

Table 4:

Characteristic	Unit	Anti-IL2	Daclizumab
Binding Affinity	Equilibrium dissociation constant (K_D)	$6.8 \pm 6.1 \times 10^{-10}$ M (BiaCore) $2.5 \pm 1.6 \times 10^{-9}$ M (Cell Surface)	3.0×10^{-9} M#
Proliferation of Human Primary NK Cells	IC50	$1.0 \pm 0.6 \times 10^{-10}$ M	$1.4 \pm 0.4 \times 10^{-9}$ M*
IFN-gamma production by Human NK Cells	IC50	$1.3 \pm 1.0 \times 10^{-10}$ M	$1.1 \pm 0.8 \times 10^{-9}$ M

according to Junghans, R. P., et al. (1990). Cancer Res 50, 1495.

* based on ~10% of total NK cell population, which expressed CD25

Claims

1. A humanized monoclonal antibody or fragment thereof which specifically binds to human interleukin-2 (IL2),

- wherein said humanized monoclonal antibody neutralizes the activity of human IL2 by binding to said human IL2 prior to, during, and/or subsequent to the binding of said human IL2 to the human IL2-receptor, and
- wherein the light chain variable region of said humanized monoclonal antibody comprises in its second framework region the contiguous amino acid sequence KAPKA.

2. The humanized monoclonal antibody or fragment thereof of claim 1, wherein the contiguous amino acid sequence KAPKA is located at amino acid positions 42-46 of the second framework region.

3. The humanized monoclonal antibody or fragment thereof of claim 1 or 2, wherein at least one of the first, third and/or fourth light chain framework regions correspond(s) to the human germline sequence for that/those region(s).

4. The humanized monoclonal antibody or fragment thereof of any of the preceding claims, wherein the light chain variable region further comprises in its CDR1 region an amino acid sequence as set out in SEQ ID NO: 1, in its CDR2 region an amino acid sequence as set out in SEQ ID NO: 2 and in its CDR3 region an amino acid sequence as set out in SEQ ID NO: 3; and wherein the heavy chain variable region comprises in its CDR1 region an amino acid sequence as set out in SEQ ID NO: 4, in its CDR2 region an amino acid sequence as set out in SEQ ID NO: 5 and in its CDR3 region an amino acid sequence as set out in SEQ ID NO: 6.

5. The humanized monoclonal antibody or fragment thereof of any of the preceding claims, wherein at least one of the first, third and/or fourth light chain framework regions correspond(s) to the human germline sequence for that/those region(s).

6. The humanized monoclonal antibody or fragment thereof of any of the preceding claims, wherein the amino acid sequence of the first light chain framework region, the remaining amino acid sequences of the second light chain framework region (i.e. at amino acid positions 35-41 and 47-49 inclusive), and the amino acid sequence of the third light chain framework region correspond to any of those of the human germline subgroup VKI at loci O12, O2, O18, O8, A30,

L1, L15, L4, L18, L5, L19, L8, L23, L9, L11 or L12; or of the human germline subgroup VL1 at locus 1a; or any of those of the human germline subgroup VL2 at loci 2c, 2e, 2a2 or 2b2.

7. The humanized monoclonal antibody or fragment thereof of any of the preceding claims, wherein the amino acid sequence of the first heavy chain framework region, the amino acid sequence of the second heavy chain framework region, and the amino acid sequence of the third heavy chain framework region independently correspond to any of those of the human germline subgroup VH3.

8. The humanized monoclonal antibody or fragment thereof of claim 7, wherein the amino acid sequence of the first heavy chain framework region, the amino acid sequence of the second heavy chain framework region, and the amino acid sequence of the third heavy chain framework region are as in locus 3-07 of the human germline subgroup VH3.

9. The humanized monoclonal antibody or fragment thereof of any of claims 6 to 8, wherein the amino acid sequence of the fourth light chain framework region corresponds to that of human JK4 (FGGGTKVEIK).

10. The humanized monoclonal antibody or fragment thereof of any of the preceding claims, wherein said humanized monoclonal antibody comprises a light chain variable region comprising an amino acid sequence as set out in SEQ ID NO. 7 and a heavy chain variable region comprising an amino acid sequence as set out in SEQ ID NO. 8.

11. The humanized monoclonal antibody of any of the preceding claims, wherein said humanized monoclonal antibody comprises a light chain comprising an amino acid sequence as set out in SEQ ID NO. 9 and a heavy chain comprising an amino acid sequence as set out in SEQ ID NO. 10.

12. The humanized monoclonal antibody according to any of the preceding claims, wherein said antibody is an IgG.

13. The humanized monoclonal antibody according to claim 12, wherein said IgG is an IgG1 or IgG4.

14. The fragment of said humanized monoclonal antibody according to any of claims 1 to 10, wherein said fragment is an scFv, a single domain antibody, an Fv, a diabody, a tandem diabody, a Fab, a Fab' or a F(ab)2.

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15. The fragment of said humanized monoclonal antibody according to claim 14, wherein said fragment is an scFv, in particular wherein said scFv comprises in its light chain variable region an amino acid sequence as set out in SEQ ID NO. 7 and in its heavy chain variable region an amino acid sequence as set out in SEQ ID NO. 8.

16. The fragment of said humanized monoclonal antibody according to claim 15, wherein said scFv comprises an amino acid sequence as set out in SEQ ID NO. 11 or SEQ ID NO. 12.

17. The humanized monoclonal antibody or fragment thereof of any of the preceding claims, said humanized monoclonal antibody or fragment thereof comprising an amino acid sequence bearing at least 70% homology, preferably at least 80, 90, or even better at least 95% homology, to the respective amino acid sequence as set out in any of SEQ ID NOs: 1-12.

18. A polynucleotide molecule comprising a nucleotide sequence encoding an amino acid sequence as set out in any of SEQ ID NOs. 7-12 or a nucleotide sequence exhibiting at least 60%, preferably 65, 70, 75, 80, 85, 90, or 95% homology with said nucleotide sequence,

wherein homology may be determined by comparing a polynucleotide molecule comprising a nucleotide sequence encoding an amino acid sequence of any of SEQ ID NOs: 7-12 with a polynucleotide molecule having a nucleotide sequence in question by sequence alignment,

wherein a nucleotide in the sequence in question is considered homologous if it is either identical to the corresponding nucleotide in the nucleotide sequence encoding a corresponding amino acid sequence of any of SEQ ID NOs: 7-12 or if one or more nucleotide deviation(s) in the sequence in question from the corresponding one or more nucleotide(s) in the nucleotide sequence encoding an amino acid sequence of any of SEQ ID NOs: 7-12 results in a nucleotide triplet which, when translated, results in an amino acid which is either identical to (due to a degenerate triplet) or a conservative substitution of the corresponding amino acid in the corresponding amino acid sequence of any of SEQ ID NOs: 7-12.

19. A pharmaceutical composition comprising a humanized monoclonal antibody or fragment thereof according to any of the claims 1-17 or a polynucleotide molecule according to claim 18.

20. The pharmaceutical composition of claim 19, wherein said pharmaceutical composition further comprises one or more anti-inflammatory or anti-cancer medicaments.

21. Use of a humanized monoclonal antibody or fragment thereof according to any of claims 1 to 17 or of a polynucleotide molecule according to claim 18 in the manufacture of a medicament,

COMS ID No: ARCS-306750 Received by IP Australia: Time (H:m) 18:54 Date (Y-M-d) 2011-01-19

optionally comprising one or more additional anti-inflammatory agents, for the treatment of inflammatory diseases in mammals, preferably humans.

22. The use of claim 21, wherein said inflammatory diseases are chosen from the group consisting of rheumatoid arthritis (RA), asthma, multiple sclerosis (MS), chronic obstructive pulmonary disease (COPD), Acute Respiratory Distress Syndrome (ARDS), Idiopathic Pulmonary Fibrosis (IPF), Inflammatory Bowel Disease (IBD), uveitis, macular degeneration, colitis, psoriasis, Wallerian Degeneration, antiphospholipid syndrome (APS), acute coronary syndrome, restinosis, atherosclerosis, relapsing polychondritis (RP), acute or chronic hepatitis, failed orthopedic implants, glomerulonephritis, lupus, autoimmune disorders, acute pancreatitis or ankylosing spondylitis (AS).

23. Use of a humanized monoclonal antibody or fragment thereof according to any of claims 1-17 or of a polynucleotide molecule according to claim 18 in the manufacture of a medicament, optionally comprising one or more additional anti-cancer agents, for the treatment of a tumorous disease or another condition with delayed cell apoptosis, increased cell survival or proliferation in mammals, preferably humans.

24. The use of claim 23, wherein said tumorous disease is a cancer.

25. The use of claim 24, wherein said cancer is leukaemia, multiple myeloma, gastric carcinoma or skin carcinoma.

26. A method of treating an inflammatory disease in which a humanized monoclonal antibody or fragment thereof according to any of claims 1-17 or a polynucleotide molecule of claim 18 is administered (optionally together with one or more additional anti-inflammatory agents) to a mammalian, preferably to a human subject in a sufficient amount and for a sufficient time to prevent and/or ameliorate said inflammatory disease.

27. The method of claim 26, wherein said inflammatory diseases are chosen from the group consisting of rheumatoid arthritis (RA), asthma, multiple sclerosis (MS), chronic obstructive pulmonary disease (COPD), Acute Respiratory Distress Syndrome (ARDS), Idiopathic Pulmonary Fibrosis (IPF), Inflammatory Bowel Disease (IBD), uveitis, macular degeneration, colitis, psoriasis, Wallerian Degeneration, antiphospholipid syndrome (APS), acute coronary syndrome, restinosis, atherosclerosis, relapsing polychondritis (RP), acute or chronic hepatitis,

failed orthopedic implants, glomerulonephritis, lupus, autoimmune disorders, acute pancreatitis or ankylosing spondylitis (AS).

28. A method of treating a tumorous disease in which a humanized monoclonal antibody or fragment thereof as set out hereinabove or a polynucleotide molecule as set out hereinabove is administered (optionally together with one or more additional anti-cancer agents) to a mammalian, preferably to a human subject in a sufficient amount and for a sufficient time to prevent and/or ameliorate said tumorous disease or another condition with delayed cell apoptosis, increased cell survival or proliferation.

29. The method of claim 28, wherein said tumorous disease is a cancer.

30. The method of claim 29, wherein said cancer is leukaemia, multiple myeloma, gastric carcinoma or skin carcinoma.

31. A humanized monoclonal antibody or fragment thereof accordingly to anyone of Claims 1 – 17 as hereinbefore described with reference to the Examples.

Fig. 1

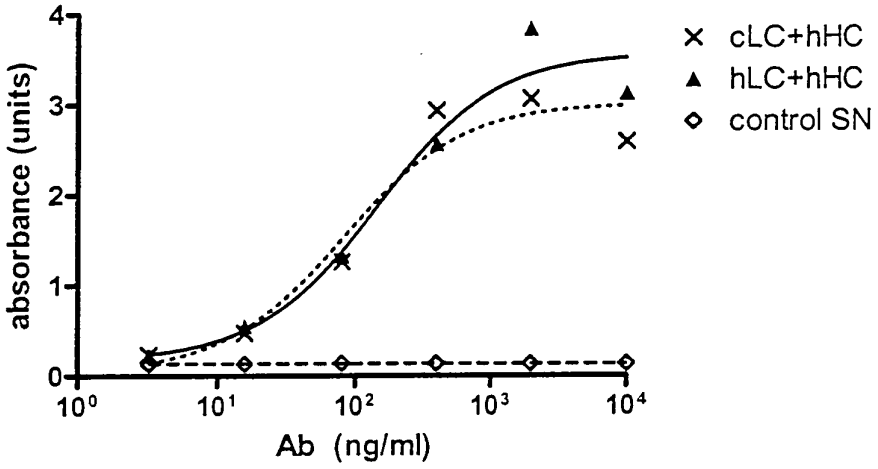

absorbance (units)
4
3
2
1
0
10^0
10^1
10^2
10^3
10^4
Ab (ng/ml)
cLC+hHC
hLC+hHC
control SN

Fig. 2

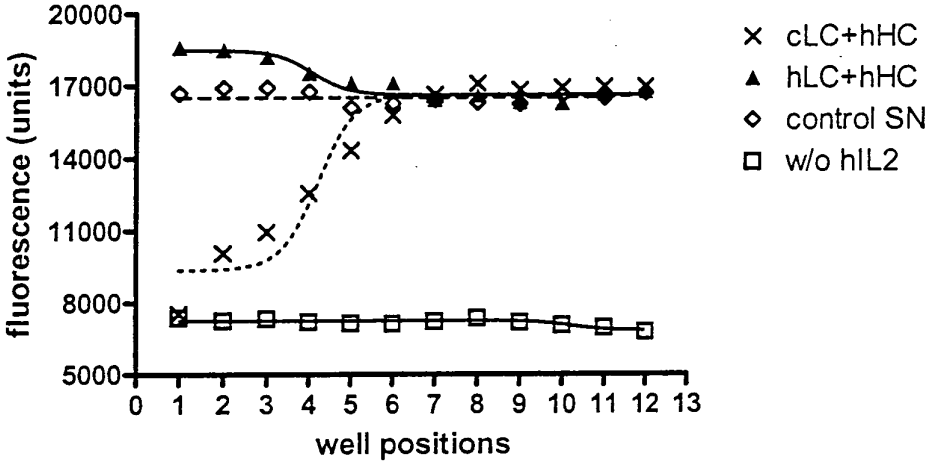

20000
17000
14000
11000
8000
5000
fluorescence (units)
0 1 2 3 4 5 6 7 8 9 10 11 12 13
well positions
cLC+hHC
hLC+hHC
control SN
w/o hIL2

Fig. 3

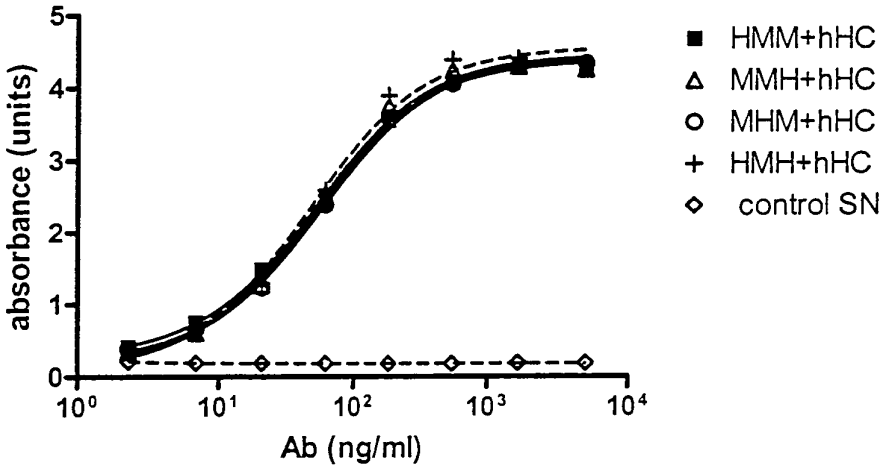

absorbance (units)
5
4
3
2
1
0
10^0
10^1
10^2
10^3
10^4
Ab (ng/ml)
HMM+hHC
MMH+hHC
MHM+hHC
HMH+hHC
control SN

Fig. 4

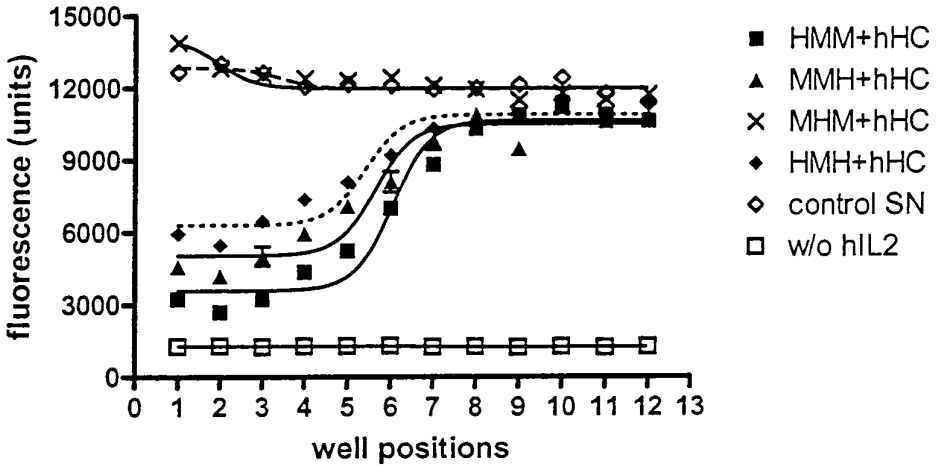

15000
12000
9000
6000
3000
0
fluorescence (units)
0 1 2 3 4 5 6 7 8 9 10 11 12 13
well positions
HMM+hHC
MMH+hHC
MHM+hHC
HMH+hHC
control SN
w/o hIL2

Fig. 5

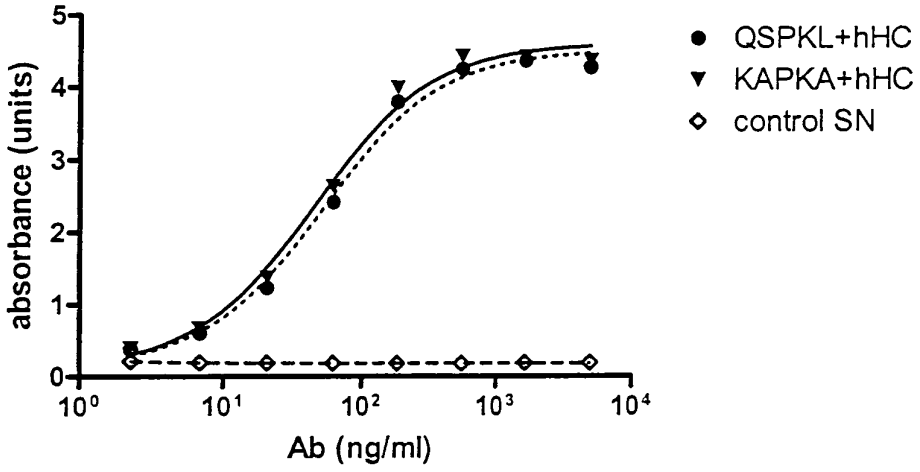

absorbance (units)
5
4
3
2
1
0
10^0
10^1
10^2
10^3
10^4
Ab (ng/ml)
QSPKL+hHC
KAPKA+hHC
control SN

Fig. 6

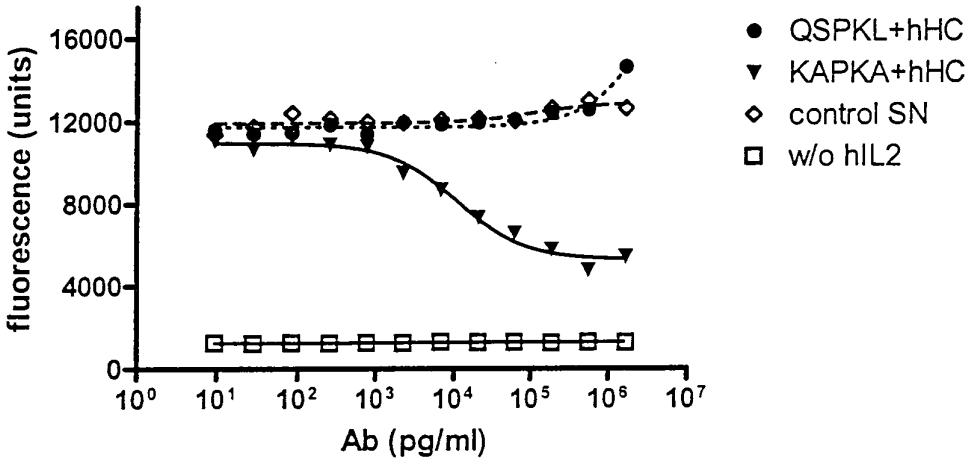

fluorescence (units)
16000
12000
8000
4000
0
10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7
Ab (pg/ml)
QSPKL+hHC
KAPKA+hHC
control SN
w/o hIL2

Fig. 7a

		FR1	FR2
		1 2	3 4
	Locus	12345678901234567890123	567890123456789
VKI	O12	DIQMTQSPSSLSASVGDRVTITC	WYQQKPGKAPKLLIY
	O2	DIQMTQSPSSLSASVGDRVTITC	WYQQKPGKAPKLLIY
	O18	DIQMTQSPSSLSASVGDRVTITC	WYQQKPGKAPKLLIY
	O8	DIQMTQSPSSLSASVGDRVTITC	WYQQKPGKAPKLLIY
	A20	DIQMTQSPSSLSASVGDRVTITC	WYQQKPGKVPKLLIY
	A30	DIQMTQSPSSLSASVGDRVTITC	WYQQKPGKAPKRLIY
	L14	NIQMTQSPSAMSASVGDRVTITC	WFQQKPGKVPKHLIY
	L1	DIQMTQSPSSLSASVGDRVTITC	WFQQKPGKAPKSLIY
	L15	DIQMTQSPSSLSASVGDRVTITC	WYQQKPEKAPKSLIY
	L4	AIQLTQSPSSLSASVGDRVTITC	WYQQKPGKAPKLLIY
	L18	AIQLTQSPSSLSASVGDRVTITC	WYQQKPGKAPKLLIY
	L5	DIQMTQSPSSVSASVGDRVTITC	WYQQKPGKAPKLLIY
	L19	DIQMTQSPSSVSASVGDRVTITC	WYQQKPGKAPKLLIY
	L8	DIQLTQSPSFLSASVGDRVTITC	WYQQKPGKAPKLLIY
	L23	AIRMTQSPFSLSASVGDRVTITC	WYQQKPAKAPKLFIY
	L9	AIRMTQSPSSFSASTGDRVTITC	WYQQKPGKAPKLLIY
	L24	VIWMTQSPSLLSASTGDRVTISC	WYQQKPGKAPELLIY
	L11	AIQMTQSPSSLSASVGDRVTITC	WYQQKPGKAPKLLIY
	L12	DIQMTQSPSTLSASVGDRVTITC	WYQQKPGKAPKLLIY
VKII	O11	DIVMTQTPLSLPVTPGEPASISC	WYLQKPGQSPQLLIY
	O1	DIVMTQTPLSLPVTPGEPASISC	WYLQKPGQSPQLLIY
	A17	DVVMTQSPLSLPVTLGQPASISC	WFQQRPGQSPRRLIY
	A1	DVVMTQSPLSLPVTLGQPASISC	WFQQRPGQSPRRLIY
	A18	DIVMTQTPLSLSVTPGQPASISC	WYLQKPGQSPQLLIY
	A2	DIVMTQTPLSLSVTPGQPASISC	WYLQKPGQPPQLLIY
	A19	DIVMTQSPLSLPVTPGEPASISC	WYLQKPGQSPQLLIY
	A3	DIVMTQSPLSLPVTPGEPASISC	WYLQKPGQSPQLLIY
	A23	DIVMTQTPLSSPVTLGQPASISC	WLQQRPGQPPRLLIY
VKIII	A27	EIVLTQSPGTLSLSPGERATLSC	WYQQKPGQAPRLLIY
	A11	EIVLTQSPATLSLSPGERATLSC	WYQQKPGLAPRLLIY
	L2	EIVMTQSPATLSVSPGERATLSC	WYQQKPGQAPRLLIY
	L16	EIVMTQSPATLSVSPGERATLSC	WYQQKPGQAPRLLIY
	L6	EIVLTQSPATLSLSPGERATLSC	WYQQKPGQAPRLLIY
	L20	EIVLTQSPATLSLSPGERATLSC	WYQQKPGQAPRLLIY
	L25	EIVMTQSPATLSLSPGERATLSC	WYQQKPGQAPRLLIY
VKIV	B3	DIVMTQSPDSLAVSLGERATINC	WYQQKPGQPPKLLIY
VKV	B2	ETTLTQSPAFMSATPGDKVNISC	WYQQKPGEAAIFIIQ
VKVI	A26	EIVLTQSPDFQSVTPKEKVTITC	WYQQKPDQSPKLLIK
	A10	EIVLTQSPDFQSVTPKEKVTITC	WYQQKPDQSPKLLIK
	A14	DVVMTQSPAFLSVTPGEKVTITC	WYQQKPDQAPKLLIK

Fig. 7b

		FR3
		5 6 7 8
	Locus	7890123456789012345678901234 5678
VKI	O12	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
	O2	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
	O18	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC
	O8	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC
	A20	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC
	A30	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC
	L14	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC
	L1	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
	L15	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
	L4	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
	L18	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
	L5	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
	L19	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
	L8	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC
	L23	GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC
	L9	GVPSRFSGSGSGTDFTLTISCLQSEDFATYYC
	L24	GVPSRFSGSGSGTDFTLTISCLQSEDFATYYC
	L11	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
	L12	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC
VKII	O11	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
	O1	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
	A17	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
	A1	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
	A18	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
	A2	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
	A19	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
	A3	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
	A23	GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYC
VKIII	A27	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
	A11	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
	L2	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC
	L16	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC
	L6	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC
	L20	GIPARFSGSGPGTDFTLTISSLEPEDFAVYYC
	L25	GIPARFSGSGSGTDFTLTISSLQPEDFAVYYC
VKIV	B3	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC
VKV	B2	GIPPRFSGSGYGTDFTLTINNIESEDAAYYFC
VKVI	A26	GVPSRFSGSGSGTDFTLTINSLEAEDAATYYC
	A10	GVPSRFSGSGSGTDFTLTINSLEAEDAATYYC
	A14	GVPSRFSGSGSGTDFTFTISSLEAEDAATYYC

Fig. 8a

		FR1	FR2
		2	3 4
	Locus	123456789123456789012 3	56789012345678 9
VL1	1a	QSVLTQPPSVSEAPRQRVTISC	WYQQLPGKAPKLLIY
	1e	QSVLTQPPSVSGAPGQRVTISC	WYQQLPGTAPKLLIY
	1c	QSVLTQPPSASGTPGQRVTISC	WYQQLPGTAPKLLIY
	1g	QSVLTQPPSASGTPGQRVTISC	WYQQLPGTAPKLLIY
	1b	QSVLTQPPSVSAAPGQKVTISC	WYQQLPGTAPKLLIY
VL2	2c	QSALTQPPSASGSPGQSVTISC	WYQQHPGKAPKLMIY
	2e	QSALTQPRSVSGSPGQSVTISC	WYQQHPGKAPKLMIY
	2a2	QSALTQPASVSGSPGQSITISC	WYQQHPGKAPKLMIY
	2d	QSALTQPPSVSGSPGQSVTISC	WYQQPPGTAPKLMIY
	2b2	QSALTQPASVSGSPGQSITISC	WYQQHPGKAPKLMIY
VL3	3r	SYELTQPPSVSVSPGQTASITC	WYQQKPGQSPVLVIY
	3j	SYELTQPLSVSVALGQTARITC	WYQQKPGQAPVLVIY
	3p	SYELTQPPSVSVSPGQTARITC	WYQQKSGQAPVLVIY
	3a	SYELTQPPSVSVSLGQMARITC	WYQQKPGQFPVLVIY
	3l	SSELTQDPAVSVALGQTVRITC	WYQQKPGQAPVLVIY
	3h	SYVLTQPPSVSVAPGKTARITC	WYQQKPGQAPVLVIY
	3e	SYELTQLPSVSVSPGQTARITC	WYQQKPGQAPELVIY
	3m	SYELMQPPSVSVSPGQTARITC	WYQQKPGQAPVLVIY
	2-19	SYELTQPSSVSVSPGQTARITC	WFQQKPGQAPVLVIY
VL4	4c	LPVLTQPPSASALLGASIKLTC	WYQQRPGRSPQYIMK
	4a	QPVLTQSSSASASLGSSVKLTC	WHQQQPGKAPRYLMK
	4b	QLVLTQSPSASASLGASVKLTC	WHQQQPEKGPRYLMK
VL5	5e	QPVLTQPPSSSASPGESARLTC	WYQQKPGSPPRYLLY
	5c	QAVLTQPASLSASPGASASLTC	WYQQKPGSPPQYLLR
	5b	QPVLTQPSSHSASSGASVRLTC	WYQQKPGNPPRYLLY
VL6	6a	NFMLTQPHSVSESPGKTVTISC	WYQQRPGSSPTTVIY
VL7	7a	QTVVTQEPSLTVSPGGTVTLTC	WFQQKPGQAPRALIY
	7b	QAVVTQEPSLTVSPGGTVTLTC	WFQQKPGQAPRTLIY
VL8	8a	QTVVTQEPSFSVSPGGTVTLTC	WYQQTPGQAPRTLIY
VL9	9a	QPVLTQPPSASASLGASVTLTC	WYQQRPGKGPRFVMR
VL10	10a	QAGLTQPPSVSKGLRQTATLTC	WLQQHQGHPPKLLSY

Fig. 8b

		FR3
		5 6 7 8
	Locus	789012345678ab901234567890123456 78
VL1	1a	GVSDRFSGSKSG--TSASLAISGLQSEDEADYYC
	1e	GVPDRFSGSKSG--TSASLAITGLQAEDEADYYC
	1c	GVPDRFSGSKSG--TSASLAISGLQSEDEADYYC
	1g	GVPDRFSGSKSG--TSASLAISGLRSEDEADYYC
	1b	GIPDRFSGSKSG--TSATLGITGLQTGDEADYYC
VL2	2c	GVPDRFSGSKSG--NTASLTVSGLQAEDEADYYC
	2e	GVPDRFSGSKSG--NTASLTISGLQAEDEADYYC
	2a2	GVSNRFSGSKSG--NTASLTISGLQAEDEADYYC
	2d	GVPDRFSGSKSG--NTASLTISGLQAEDEADYYC
	2b2	GVSNRFSGSKSG--NTASLTISGLQAEDEADYYC
VL3	3r	GIPERFSGSNSG--NTATLTISGTQAMDEADYYC
	3j	GIPERFSGSNSG--NTATLTISRAQAGDEADYYC
	3p	GIPERFSGSSSG--TMATLTISGAQVEDEADYYC
	3a	GIPERFSGSSSG--TIVTLTISGVQAEDEADYYC
	3l	GIPDRFSGSSSG--NTASLTITGAQAEDEADYYC
	3h	GIPERFSGSNSG--NTATLTISRVEAGDEADYYC
	3e	GIPERFSGSTSG--NTTTLTISRVLTEDEADYYC
	3m	GIPERFSGSSSG--TTVTLTISGVQAEDEADYYC
	2-19	GIPERFSGSSSG--TTVTLTISGAQVEDEADYYC
VL4	4c	GIPDRFMGSSSG--ADRYLTFSNLQSDDEAEYHC
	4a	GVPDRFSGSSSG--ADRYLTISNLQLEDEADYYC
	4b	GIPDRFSGSSSG--AERYLTISSLQSEDEADYYC
VL5	5e	GVPSRFSGSKDASANTGILLISGLQSEDEADYYC
	5c	GVPSRFSGSKDASANAGILLISGLQSEDEADYYC
	5b	GVPSRFSGSNDASANAGILRISGLQPEDEADYYC
VL6	6a	GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYC
VL7	7a	WTPARFSGSLLG--GKAALTLSGVQPEDEAEYYC
	7b	WTPARFSGSLLG--GKAALTLSGAQPEDEAEYYC
VL8	8a	GVPDRFSGSILG--NKAALTITGAQADDESDYYC
VL9	9a	GIPDRFSVLGSG--LNRYLTIKNIQEEDESDYHC
VL10	10a	GISERLSASRSG--NTASLTITGLQPEDEADYYC

Fig. 9a

		FR1			FR2	
		1	2	3	3	4
	Locus	12345678901234567890123456789			0	67890123456789
VH1	1-02	QVQLVQSGAEVKKPGASVKVSCKASGYTFT				WVRQAPGQGLEWMG
	1-03	QVQLVQSGAEVKKPGASVKVSCKASGYTFT				WVRQAPGQRLEWMG
	1-08	QVQLVQSGAEVKKPGASVKVSCKASGYTFT				WVRQATGQGLEWMG
	1-18	QVQLVQSGAEVKKPGASVKVSCKASGYTFT				WVRQAPGQGLEWMG
	1-24	QVQLVQSGAEVKKPGASVKVSCKVSGYTLT				WVRQAPGKGLEWMG
	1-45	QMQLVQSGAEVKKTGSSVKVSCKASGYTFT				WVRQAPGQALEWMG
	1-46	QVQLVQSGAEVKKPGASVKVSCKASGYTFT				WVRQAPGQGLEWMG
	1-58	QMQLVQSGPEVKKPGTSVKVSCKASGFTFT				WVRQARGQRLEWIG
	1-69	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS				WVRQAPGQGLEWMG
	1-e	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS				WVRQAPGQGLEWMG
	1-f	EVQLVQSGAEVKKPGATVKISCKVSGYTFT				WVQQAPGKGLEWMG
VH2	2-05	QITLKESGPTLVKPTQTLTLTCTFSGFSLS				WIRQPPGKALEWLA
	2-26	QVTLKESGPVLVKPTETLTLTCTVSGFSLS				WIRQPPGKALEWLA
	2-70	QVTLKESGPALVKPTQTLTLTCTFSGFSLS				WIRQPPGKALEWLA
VH3	3-07	EVQLVESGGGLVQPGGSLRLSCAASGFTFS				WVRQAPGKGLEWVA
	3-09	EVQLVESGGGLVQPGRSLRLSCAASGFTFD				WVRQAPGKGLEWVS
	3-11	QVQLVESGGGLVKPGGSLRLSCAASGFTFS				WIRQAPGKGLEWVS
	3-13	EVQLVESGGGLVQPGGSLRLSCAASGFTFS				WVRQATGKGLEWVS
	3-15	EVQLVESGGGLVKPGGSLRLSCAASGFTFS				WVRQAPGKGLEWVG
	3-20	EVQLVESGGGVVRPGGSLRLSCAASGFTFD				WVRQAPGKGLEWVS
	3-21	EVQLVESGGGLVKPGGSLRLSCAASGFTFS				WVRQAPGKGLEWVS
	3-23	EVQLLESGGGLVQPGGSLRLSCAASGFTFS				WVRQAPGKGLEWVS
	3-30	QVQLVESGGGVVQPGRSLRLSCAASGFTFS				WVRQAPGKGLEWVA
	3-30.3	QVQLVESGGGVVQPGRSLRLSCAASGFTFS				WVRQAPGKGLEWVA
	3-30.5	QVQLVESGGGVVQPGRSLRLSCAASGFTFS				WVRQAPGKGLEWVA
	3-33	QVQLVESGGGVVQPGRSLRLSCAASGFTFS				WVRQAPGKGLEWVA
	3-43	EVQLVESGGVVVQPGGSLRLSCAASGFTFD				WVRQAPGKGLEWVS
	3-48	EVQLVESGGGLVQPGGSLRLSCAASGFTFS				WVRQAPGKGLEWVS
	3-49	EVQLVESGGGLVQPGRSLRLSCTASGFTFG				WFRQAPGKGLEWVG
	3-53	EVQLVETGGGLIQPGGSLRLSCAASGFTVS				WVRQAPGKGLEWVS
	3-64	EVQLVESGGGLVQPGGSLRLSCAASGFTFS				WVRQAPGKGLEYVS
	3-66	EVQLVESGGGLVQPGGSLRLSCAASGFTVS				WVRQAPGKGLEWVS
	3-72	EVQLVESGGGLVQPGGSLRLSCAASGFTFS				WVRQAPGKGLEWVG
	3-73	EVQLVESGGGLVQPGGSLKLSCAASGFTFS				WVRQASGKGLEWVG
	3-74	EVQLVESGGGLVQPGGSLRLSCAASGFTFS				WVRQAPGKGLVWVS
	3-d	EVQLVESRGVLVQPGGSLRLSCAASGFTVS				WVRQAPGKGLEWVS
VH4	4-04	QVQLQESGPGLVKPSGTLSLTCAVSGGSIS				WVRQPPGKGLEWIG
	4-28	QVQLQESGPGLVKPSDTLSLTCAVSGYSIS				WIRQPPGKGLEWIG
	4-30.1	QVQLQESGPGLVKPSQTLSLTCTVSGGSIS				WIRQHPGKGLEWIG
	4-30.2	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS				WIRQPPGKGLEWIG
	4-30.4	QVQLQESGPGLVKPSQTLSLTCTVSGGSIS				WIRQPPGKGLEWIG
	4-31	QVQLQESGPGLVKPSQTLSLTCTVSGGSIS				WIRQHPGKGLEWIG
	4-34	QVQLQQWGAGLLKPSETLSLTCAVYGGSFS				WIRQPPGKGLEWIG
	4-39	QLQLQESGPGLVKPSETLSLTCTVSGGSIS				WIRQPPGKGLEWIG
	4-59	QVQLQESGPGLVKPSETLSLTCTVSGGSIS				WIRQPPGKGLEWIG
	4-61	QVQLQESGPGLVKPSETLSLTCTVSGGSVS				WIRQPPGKGLEWIG
	4-b	QVQLQESGPGLVKPSETLSLTCAVSGYSIS				WIRQPPGKGLEWIG
VH5	5-51	EVQLVQSGAEVKKPGESLKISCKGSGYSFT				WVRQMPGKGLEWMG
	5-a	EVQLVQSGAEVKKPGESLRISCKGSGYSFT				WVRQMPGKGLEWMG
VH6	6-01	QVQLQQSGPGLVKPSQTLSLTCAISGDSVS				WIRQSPSRGLEWLG
VH7	7-4.1	QVQLVQSGSELKKPGASVKVSCKASGYTFT				WVRQAPGQGLEWMG

Fig. 9b

		FR3
		6 7 8 9
	Locus	67890123456789012abc345678901234
VH1	1-02	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR
	1-03	RVTITRDTSASTAYMELSSLRSEDTAVYYCAR
	1-08	RVTMTRNTSISTAYMELSSLRSEDTAVYYCAR
	1-18	RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR
	1-24	RVTMTEDTSTDTAYMELSSLRSEDTAVYYCAT
	1-45	RVTITRDRSMSTAYMELSSLRSEDTAMYYCAR
	1-46	RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
	1-58	RVTITRDMSTSTAYMELSSLRSEDTAVYYCAA
	1-69	RVTITADESTSTAYMELSSLRSEDTAVYYCAR
	1-e	RVTITADKSTSTAYMELSSLRSEDTAVYYCAR
	1-f	RVTITADTSTDTAYMELSSLRSEDTAVYYCAT
VH2	2-05	RLTITKDTSKNQVVLTMTNMDPVDTATYYCAHR
	2-26	RLTISKDTSKSQVVLTMTNMDPVDTATYYCARI
	2-70	RLTISKDTSKNQVVLTMTNMDPVDTATYYCARI
VH3	3-07	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR
	3-09	RFTISRDNAKNSLYLQMNSLRAEDTALYYCAKD
	3-11	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR
	3-13	RFTISRENAKNSLYLQMNSLRAGDTAVYYCAR
	3-15	RFTISRDDSKNTLYLQMNSLKTEDTAVYYCTT
	3-20	RFTISRDNAKNSLYLQMNSLRAEDTALYHCAR
	3-21	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR
	3-23	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
	3-30	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
	3-30.3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
	3-30.5	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
	3-33	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
	3-43	RFTISRDNSKNSLYLQMNSLRTEDTALYYCAKD
	3-48	RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR
	3-49	RFTISRDGSKSIAYLQMNSLKTEDTAVYYCTR
	3-53	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
	3-64	RFTISRDNSKNTLYLQMGSLRAEDMAVYYCAR
	3-66	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
	3-72	RFTISRDDSKNSLYLQMNSLKTEDTAVYYCAR
	3-73	RFTISRDDSKNTAYLQMNSLKTEDTAVYYCTR
	3-74	RFTISRDNAKNTLYLQMNSLRAEDTAVYYCAR
	3-d	RFTISRDNSKNTLHLQMNSLRAEDTAVYYCKK
VH4	4-04	RVTISVDKSKNQFSLKLSSVTAADTAVYYCAR
	4-28	RVTMSVDTSKNQFSLKLSSVTAVDTAVYYCAR
	4-30.1	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
	4-30.2	RVTISVDRSKNQFSLKLSSVTAADTAVYYCAR
	4-30.4	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
	4-31	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
	4-34	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
	4-39	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
	4-59	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
	4-61	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
	4-b	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
VH5	5-51	QVTISADKSISTAYLQWSSLKASDTAMYYCAR
	5-a	HVTISADKSISTAYLQWSSLKASDTAMYYCAR
VH6	6-01	RITINPDTSKNQFSLQLNSVTPEDTAVYYCAR
VH7	7-4.1	RFVFSLDTSVSTAYLQICSLKAEDTAVYYCAR

Fig. 10

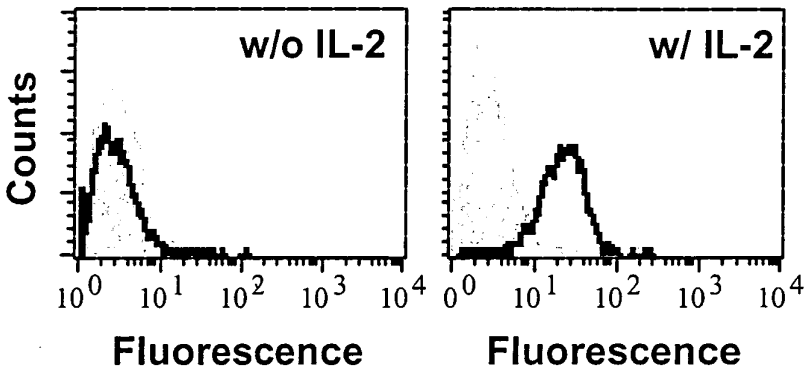

w/o IL-2
w/ IL-2
Counts
10^0 10^1 10^2 10^3 10^4
0^0 10^1 10^2 10^3 10^4
Fluorescence
Fluorescence

Fig. 11

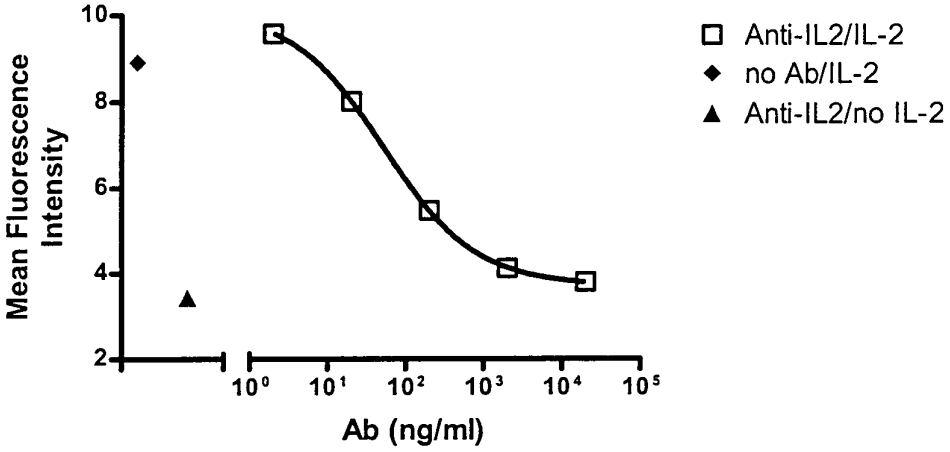

Mean Fluorescence Intensity
10
8
6
4
2
10^0
10^1
10^2
10^3
10^4
10^5
Ab (ng/ml)
Anti-IL2/IL-2
no Ab/IL-2
Anti-IL2/no IL-2

Fig. 12

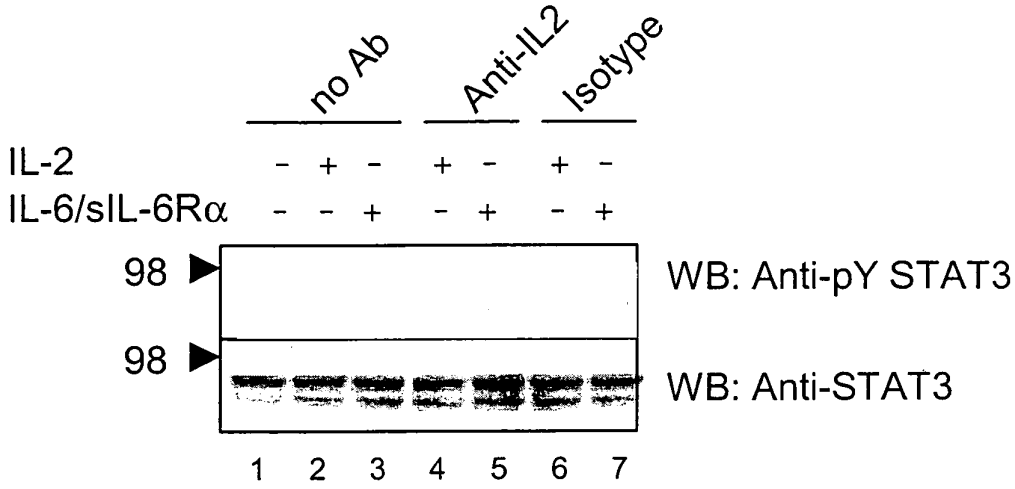

no Ab
Anti-IL2
Isotype
IL-2
- + - + - + -
IL-6/sIL-6Rα
- - + - + - +
98
98
WB: Anti-pY STAT3
WB: Anti-STAT3
1 2 3 4 5 6 7

Fig. 13

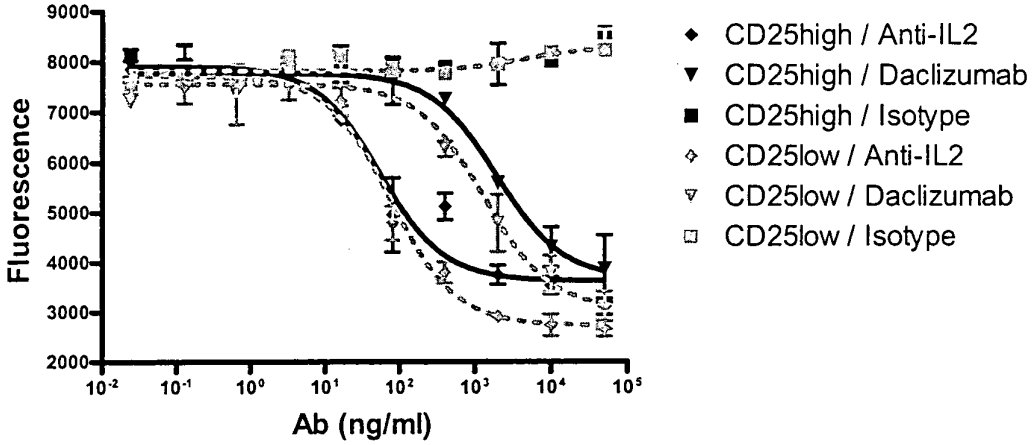

9000
8000
7000
6000
5000
4000
3000
2000
Fluorescence
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
Ab (ng/ml)
CD25high / Anti-IL2
CD25high / Daclizumab
CD25high / Isotype
CD25low / Anti-IL2
CD25low / Daclizumab
CD25low / Isotype

Fig. 14

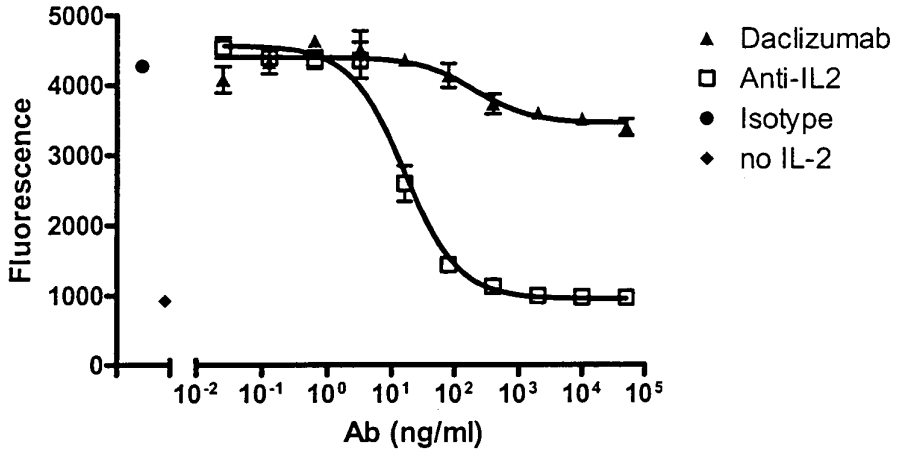
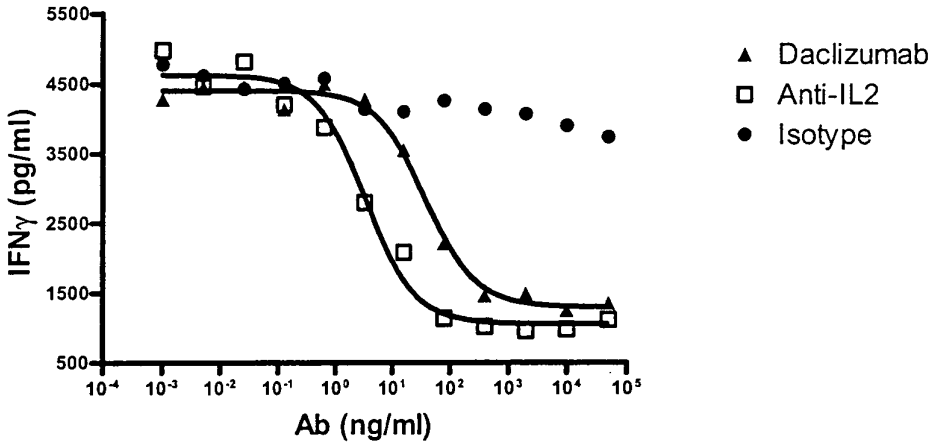

5000
4000
3000
2000
1000
0
Fluorescence
10-2 10-1 100 101 102 103 104 105
Ab (ng/ml)
Daclizumab
Anti-IL2
Isotype
no IL-2

Fig. 15


5500
4500
3500
2500
1500
500
IFNγ (pg/ml)
10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁰ 10¹ 10² 10³ 10⁴ 10⁵
Ab (ng/ml)
Daclizumab
Anti-IL2
Isotype

SEQUENCE LISTING

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<120> Anti-IL2 antibodies

<130> MIC-026 PCT

<140> unknown
<141> 2006-05-31

<150> EP 05 011 845.4
<151> 2005-06-01

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Val Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile
35 40 45
Tyr Ser Ala Ser Phe Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys

100 105

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35 40 45
Ala Ala Ile Asp Ser Ser Ser Tyr Thr Tyr Ser Pro Asp Thr Val Arg
50 55 60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Asp Ser Asn Trp Asp Ala Leu Asp Tyr Trp Gly Gln Gly Thr Thr
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Val Thr Val Ser Ser
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35 40 45
Tyr Ser Ala Ser Phe Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Tyr Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
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145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

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Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190
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Phe Asn Arg Gly Glu Cys
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Thr Leu Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Ala Ile Asp Ser Ser Ser Tyr Thr Tyr Ser Pro Asp Thr Val Arg
    50                  55                  60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65                  70                  75                  80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Arg Asp Ser Asn Trp Asp Ala Leu Asp Tyr Trp Gly Gln Gly Thr Thr
            100                 105                 110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
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Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
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        355                 360                 365
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
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Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
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            20                  25                  30
Val Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile
        35                  40                  45
Tyr Ser Ala Ser Phe Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Tyr Pro Tyr
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Gly Ser
            100                 105                 110
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu
        115                 120                 125
Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys
    130                 135                 140
Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Thr Leu Ala Trp Val Arg
145                 150                 155                 160
Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Ala Ile Asp Ser Ser
                165                 170                 175
Ser Tyr Thr Tyr Ser Pro Asp Thr Val Arg Gly Arg Phe Thr Ile Ser
            180                 185                 190
Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg
        195                 200                 205
Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Ser Asn Trp Asp
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Thr Leu Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Ala Ile Asp Ser Ser Ser Tyr Thr Tyr Ser Pro Asp Thr Val Arg
    50                  55                  60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65                  70                  75                  80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Arg Asp Ser Asn Trp Asp Ala Leu Asp Tyr Trp Gly Gln Gly Thr Thr
            100                 105                 110
Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
        115                 120                 125
Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
    130                 135                 140
Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn
145                 150                 155                 160
Val Gly Thr Asn Val Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
                165                 170                 175
Lys Ala Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Ser Gly Val Pro Ser
            180                 185                 190
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
        195                 200                 205
Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr
    210                 215                 220
Thr Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
225                 230                 235
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