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(54) Title: NEUTRALIZING ANTI-CD95L MONOCLONAL ANTIBODIES

(57) Abstract: Chronic inflammatory diseases are becoming a leading cause of death throughout the world. Although such diseases appear to be clinically different, they share many similarities in terms of genetic background and pathophysiological pathways. There is an interest to develop drugs for inhibiting the CD95 -mediated non-apoptotic signaling pathway that contributes to inflammation. In particular, neutralizing anti-CD95L monoclonal antibodies are highly desirable. The inventors a neutralizing anti-CD95L monoclonal antibody (mAb), designated JQ3 (IgGl K). In particular, the neutralizing effect of JQ3 was confirmed since this home-made monoclonal antibody inhibited the CD95-mediated apoptotic signaling pathway induced in T- cell line Jurkat more efficiently than NOK-1 mAb. Interestingly, JQ3 blocked the CD95- mediated Ca^{2+} response in neutrophils exposed to sera from various inflammatory conditions (COVID19 patients and anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) patients). The present invention thus relates to neutralizing anti-CD95L monoclonal antibodies that revie from JQ3.

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NEUTRALIZING ANTI-CD95L MONOCLONAL ANTIBODIES

FIELD OF THE INVENTION:

The present invention is in the field of immunology and oncology.

BACKGROUND OF THE INVENTION:

Chronic inflammatory diseases are becoming a leading cause of death throughout the world. Although such diseases appear to be clinically different, they share many similarities in terms of genetic background and pathophysiological pathways. Accumulating evidence suggests that trafficking of IL-17-producing CD4+ effector T cells, termed Th17 cells, and their subsequent accumulation in organs triggers inflammation and tissue lesions responsible for the clinical symptoms associated with such diseases.

CD95L (FasL) belongs to the *Tumor Necrosis Factor* (TNF) family and is the ligand of the death receptor CD95 (also known as Fas). While CD95 is ubiquitously expressed on healthy cells, CD95L exhibits a restricted expression pattern, mainly detected at the surface of lymphocytes, where it plays a pivotal role in the elimination of infected and transformed cells (*Strasser, A.; Jost, P. J.; Nagata, S. The many roles of FAS receptor signaling in the immune system. Immunity. 2009, 30, 180-192*). CD95L is a transmembrane glycoprotein that acts locally through cell-to-cell contact and after cleavage by metalloproteases, a soluble CD95L (s-CD95L) is released into the bloodstream. This soluble ligand contributes to aggravate inflammation in chronic inflammatory disorders such as systemic lupus erythematosus (SLE) (*Tauzin, S. et al. PLoS Biol. 2011, 9, e1001090.*) by inducing non-apoptotic signaling pathways such as NF-κB and PI3K and may exert pro-oncogenic functions by promoting the survival of ovarian and liver cancers and chemotherapy resistance of lung cancers. CD95L receptor, designated CD95 or Fas carries an intracellular conserved stretch, the death domain (DD), which serves as a docking platform to trigger cell death. Binding of membrane-bound hexameric CD95L to CD95 leads to the recruitment of the adaptor protein FADD (Fas Associated Death Domain) through homotypic interactions via their respective DD (*Holler, N. et al., Mol Cell Biol. 2003, 23, 1428-1440*). FADD in turn aggregates the initiator caspase-8 and caspase-10. The CD95/FADD/caspase complex is called death-inducing signalling complex (DISC) and leads to the elimination of cancer cells through an apoptotic mechanism *(Kischkel, F. C. et al. Embo J. 1995, 14, 5579-5588*). By contrast, homotrimeric s-CD95L fails

to induce DISC formation, but instead triggers the formation of a non-apoptotic complex termed motility-inducing signaling complex (MISC) implementing a Ca^{2+} response (*Tauzin, S. et al. PLoS Biol. 2011, 9, e1001090.*). Recent data highlighted that s-CD95L induces a calcium response by inducing the direct interaction of CD95 with PLCγ1 (*Poissonnier, A. et al. Immunity. 2016, 45, 209-223*). Indeed, in presence of s-CD95L, the juxtamembrane region of CD95, called calcium-inducing domain (CID), recruits PLCγ1 to induce endothelial transmigration of Th17 cells in SLE (*Poissonnier, A. et al. Immunity. 2016, 45, 209-223*).

There is thus an interest to develop drugs for inhibiting the CD95-mediated non-apoptotic signaling pathway that contributes to inflammation. In particular, neutralizing anti-CD95L monoclonal antibodies are highly desirable.

WO1996029350 discloses several neutralizing anti-CD95L monoclonal antibodies and in particular the NOK1 antibody.

SUMMARY OF THE INVENTION:

The present invention is defined by the claims. In particular, the present invention relates to neutralizing anti-CD95L monoclonal antibodies and their uses for therapeutic purposes.

DETAILED DESCRIPTION OF THE INVENTION:

Main Definitions:

As used herein, the terms "**polypeptide**", "**peptide**", and "**protein**" are used interchangeably herein to refer to polymers of amino acids of any length. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, phosphorylation, or conjugation with a labeling component. Polypeptides when discussed in the context of gene therapy refer to the respective intact polypeptide, or any fragment or genetically engineered derivative thereof, which retains the desired biochemical function of the intact protein.

As used herein, the term "**polynucleotide**" refers to a polymeric form of nucleotides of any length, including deoxyribonucleotides or ribonucleotides, or analogs thereof. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs,

and may be interrupted by non-nucleotide components. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide, as used herein, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

As used herein, the expression "**derived from**" refers to a process whereby a first component (e.g., a first polypeptide), or information from that first component, is used to isolate, derive or make a different second component (e.g., a second polypeptide that is different from the first one).

As used herein, the "**percent identity**" between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described below. The percent identity between two amino acid sequences can be determined using the Needleman and Wunsch algorithm (Needleman, Saul B. & Wunsch, Christian D. (1970). "A general method applicable to the search for similarities in the amino acid sequence of two proteins". Journal of Molecular Biology. 48 (3): 443–53.). The percent identity between two nucleotide or amino acid sequences may also be determined using for example algorithms such as EMBOSS Needle (pair wise alignment; available at www.ebi.ac.uk). For example, EMBOSS Needle may be used with a BLOSUM62 matrix, a "gap open penalty" of 10, a "gap extend penalty" of 0.5, a false "end gap penalty", an "end gap open penalty" of 10 and an "end gap extend penalty" of 0.5. In general, the "percent identity" is a function of the number of matching positions divided by the number of positions compared and multiplied by 100. For instance, if 6 out of 10 sequence positions are identical between the two compared sequences after alignment, then the identity is 60%. The % identity is typically determined over the whole length of the query sequence on which the analysis is performed. Two molecules having the same primary amino acid sequence or nucleic acid sequence are identical irrespective of any chemical and/or biological modification. According to the invention, a first amino acid sequence having at least 70% of

identity with a second amino acid sequence means that the first sequence has 70; 71; 72; 73; 74; 75; 76; 77; 78; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; 99 or 100% of identity with the second amino acid sequence.

As used herein, the term "**encoding**" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as, for example, a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*e.g.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase "nucleotide sequence that encodes a protein or a RNA" may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

As used herein, the term "**CD95L**" has its general meaning in the art and refers to the cognate ligand of CD95 that is a transmembrane protein. The term is also known as APT1LG1, FASL, or TNFSF6. An exemplary amino acid sequence for CD95L is represented by SEQ ID NO:1. The extracellular domain of human CD95L typically consists of the amino acid sequence that ranges from the amino acid residue at position 103 to the amino acid residue at position 281 in SEQ ID NO:1.

SEQ ID NO:1 >sp|P48023|TNFL6_HUMAN Tumor necrosis factor ligand superfamily member 6 OS=Homo sapiens OX=9606 GN=FASLG PE=1 SV=1. The extracellular domain is underlined in the sequence.
MQQPFNYPYPQIYWVDSSASSPWAPPGTVLPCPTSVPRRPGQRRPPPPPPPPPLPPPPPPPPLPPLPLP
PLKKRGNHSTGLCLLVMFFMVLVALVGLGLGMFQLFHLQKELAELRESTSQMHTASSLEKQIGHPSPPP
EKKELRKVAHLTGKSNSRSMPLEWEDTYGIVLLSGVKYKKGGLVINETGLYFVYSKVYFRGQSCNNLPL
SHKVYMRNSKYPQDLVMMEGKMMSYCTTGQMWARSSYLGAVFNLTSADHLYVNVSELSLVNFEESQTFF
GLYKL

As used herein the term "**soluble CD95L**" has its general meaning in the art and refers to the soluble ligand produced by the cleavage of the transmembrane CD95L by a metalloprotease. The term "**serum CD95L**", "**soluble CD95L**", "**metalloprotease-cleaved CD95L**" and "**s-CD95L**" have the same meaning along the specification.

As used herein the term "**antibody**" and "**immunoglobulin**" have the same meaning, and will be used equally in the present invention. The term "**antibody**" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. In natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (1) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain includes three (α, δ, γ) to five (μ, ε) domains, a variable domain (VH) and three to four constant domains (CH1, CH2, CH3 and CH4 collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from nonhypervariable or framework regions (FR) can participate to the antibody binding site or influence the overall domain structure and hence the combining site. CDRs refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated L-CDR1, L-CDR2, L-CDR3 and H-CDR1, H-CDR2, H-CDR3, respectively. An antigen-binding site, therefore, typically includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. Framework

Regions (FRs) refer to amino acid sequences interposed between CDRs. According to the invention, the amino acid residues in the variable domain, complementarity determining regions (CDRs) and framework regions (FR) of the antibody of the present invention are identified using the Immunogenetics (IMGT) database (http://imgt.cines.fr). Lefranc et al. (2003) Dev Comp Immunol. 27(1):55-77. The IMGT database was developed using sequence information for immunoglobulins (IgGs), T-cell receptors (TcR) and Major Histocompatibility Complex (MHC) molecules and unifies numbering across antibody lambda and kappa light chains, heavy chains and T-cell receptor chains and avoids the use of insertion codes for all but uncommonly long insertions. IMGT also takes into account and combines the definition of the framework (FR) and complementarity determining regions (CDR) from Kabat et al., the characterization of the hypervariable loops from Chothia et al., as well as structural data from X-ray diffraction studies.

As used herein, the terms "**monoclonal antibody**", "**monoclonal Ab**", "**monoclonal antibody composition**", "**mAb**", or the like, as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody is obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprised in the population are identical except for possible naturally occurring mutations that may be present in minor amounts.

As used herein, the term "**chimeric antibody**" refers to an antibody which comprises a VH domain and a VL domain of a non-human antibody, and a CH domain and a CL domain of a human antibody.

As used herein, the term "**humanized antibody**" refers to an antibody having variable region, framework and constant regions from a human antibody but retains the CDRs of a previous non-human antibody. In some embodiments, a humanized antibody contains minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies and antibody fragments thereof may be human immunoglobulins (recipient antibody or antibody fragment) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity.

As used herein, the term "**antibody fragment**" refers to at least one portion of an intact antibody, preferably the antigen binding region or variable region of the intact antibody, that retains the ability to specifically interact with (*e.g.*, by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen. "**Fragments**" comprise a portion of the intact antibody, generally the antigen binding site or variable region. Examples of antibody fragments include Fab, Fab', Fab'-SH, F(ab')2, and Fv fragments; diabodies; any antibody fragment that is a polypeptide having a primary structure consisting of one uninterrupted sequence of contiguous amino acid residues (referred to herein as a "single-chain antibody fragment" or "single chain polypeptide"), including without limitation (1) single - chain Fv molecules (2) single chain polypeptides containing only one light chain variable domain, or a fragment thereof that contains the three CDRs of the light chain variable domain, without an associated heavy chain moiety and (3) single chain polypeptides containing only one heavy chain variable region, or a fragment thereof containing the three CDRs of the heavy chain variable region, without an associated light chain moiety; and multispecific antibodies formed from antibody fragments. Fragments of the present antibodies can be obtained using standard methods.

As used herein, the term "**Fc region**" has its general meaning in the art and includes the polypeptides comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus, Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the hinge between Cgamma1 (Cγ1) and Cgamma2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat et al. (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, Va.). The "EU index as set forth in Kabat" refers to the residue numbering of the human IgG1 EU antibody as described in Kabat et al. supra. Fc may refer to this region in isolation, or this region in the context of an antibody, antibody fragment, or Fc fusion protein. An Fc variant protein may be an antibody, Fc fusion, or any protein or protein domain that comprises an Fc region. Particularly preferred are proteins comprising variant Fc regions, which are non-naturally occurring variants of an Fc region. The amino acid sequence of a non-naturally occurring Fc region (also referred to herein as a

"**variant Fc region**") comprises a substitution, insertion and/or deletion of at least one amino acid residue compared to the wild type amino acid sequence. Any new amino acid residue appearing in the sequence of a variant Fc region as a result of an insertion or substitution may be referred to as a non-naturally occurring amino acid residue. Note: Polymorphisms have been observed at a number of Fc positions, including but not limited to Kabat 270, 272, 312, 315, 356, and 358, and thus slight differences between the presented sequence and sequences in the prior art may exist.

As used herein, the terms "**Fc receptor**" or "**FcR**" are used to describe a receptor that binds to the Fc region of an antibody. The primary cells for mediating ADCC express FcγRIII, whereas monocytes express FcγRI, FcγRII, FcγRIII and/or FcγRIV. FcR expression on hematopoietic cells is summarized in Ravetch and Kinet, Annu. Rev. Immunol., 9:457-92 (1991). To assess ADCC activity of a molecule, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecules of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes et al., Proc. Natl. Acad. Sci. (USA), 95:652-656 (1998). As used herein, the term "effector cells" are leukocytes which express one or more FcRs and perform effector functions. The cells express at least FcγRI, FCγRII, FcγRIII and/or FcγRIV and carry out ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils.

As used herein, the term "**specificity**" refers to the ability of an antibody to detectably bind target molecule (e.g. an epitope presented on an antigen) while having relatively little detectable reactivity with other target molecules. Specificity can be relatively determined by binding or competitive binding assays, using, *e.g.*, Biacore instruments, as described elsewhere herein. Specificity can be exhibited by, *e.g.*, an about 10:1, about 20:1, about 50:1, about 100:1, 10.000:1 or greater ratio of affinity/avidity in binding to the specific antigen versus nonspecific binding to other irrelevant molecules.

The term "**affinity**", as used herein, means the strength of the binding of an antibody to a target molecule (e.g. an epitope). The affinity of a binding protein is given by the dissociation constant Kd. For an antibody said Kd is defined as [Ab] x [Ag] / [Ab-Ag], where [Ab-Ag] is the molar

concentration of the antibody-antigen complex, [Ab] is the molar concentration of the unbound antibody and [Ag] is the molar concentration of the unbound antigen. The affinity constant Ka is defined by 1/Kd. Preferred methods for determining the affinity of a binding protein can be found in Harlow, et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Coligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, Meth. Enzymol. 92:589-601 (1983), which references are entirely incorporated herein by reference. One preferred and standard method well known in the art for determining the affinity of binding protein is the use of Biacore instruments.

The term "**binding**" as used herein refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. In particular, as used herein, the term "binding" in the context of the binding of an antibody to a predetermined target molecule (e.g. an antigen or epitope) typically is a binding with an affinity corresponding to a K_D of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less, about 10^{-10} M or less, or about 10^{-11} M or even less.

As used herein, the term "**epitope**" refers to a specific arrangement of amino acids located on a protein or proteins to which an antibody binds. Epitopes often consist of a chemically active surface grouping of molecules such as amino acids or sugar side chains, and have specific three-dimensional structural characteristics as well as specific charge characteristics. Epitopes can be linear or conformational, *i.e.*, involving two or more sequences of amino acids in various regions of the antigen that may not necessarily be contiguous.

As used herein, the term "**neutralizing anti-CD95L monoclonal antibody**" refers to an antibody to a monoclonal antibody having specificity for CD95L and that reduces at least one activity of CD95L. In particular, the neutralizing anti-CD95L monoclonal antibody reduces the CD95-mediated non-apoptotic signaling pathway and/or reduces the CD95-mediated apoptotic signaling pathway. Said activities can be measured by any well-known method in the art and typically as described in EXAMPLE.

As used herein, the term "**JQ3**" refers to the antibody having the VL domain as set forth in SEQ ID NO:1 and the VH domain as set forth in SEQ ID NO:5.

SEQ ID NO: 2: VL domain of the JQ3 antibody having the following structure FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4
QIVFTQSPAIMSAFLGEEITLTCSASSSVSYMHWYQQKSGTSPKLLIYNTFNLASGVPSRFSGSGSGTFYSLTIS
SVEAEDAADYYCHQWSSYPTFGGGTKLEIK

SEQ ID NO: 6: VH domain of the JQ3 antibody having the following structure FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4
EIQLQQTGPELVKPGASVKISCKASGFSFTDYIMVWVKQSHGKILEWIGDISPYYGTATYNLKFKGKATLTVVKS
SSTAYMQLNSLTSEDSAVYYCARAPNRYEVMDYWGQGTSVTVSS

As used herein, the term "**treatment**" or "**treat**" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of patient at risk of contracting the disease or suspected to have contracted the disease as well as patients who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a patient having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a patient beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a patient during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a patient during treatment of an illness, e.g., to keep the patient in remission for long periods of time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at a regular interval, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., pain, disease manifestation, etc.]).

As used herein, the term "**pharmaceutical composition**" refers to a composition described herein, or pharmaceutically acceptable salts thereof, with other agents such as carriers and/or excipients. The pharmaceutical compositions as provided herewith typically include a pharmaceutically acceptable carrier.

As used herein, "**consisting essentially of**", with reference to a composition, means that the at least one antibody of the invention as described hereinabove is the only one therapeutic agent or agent with a biologic activity within said composition.

As used herein, the term "**pharmaceutically acceptable carrier**" includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical-Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof.

Antibodies of the present invention:

The first object of the present invention relates to a neutralizing anti-CD95L monoclonal antibody having

- a VL domain comprising the complementarity determining regions CDR1L, CDR2L and CDR3L, the CDR1L having the amino acid sequence SSVSY (SEQ ID NO:3) the CDR2L having the amino acid sequence NTF (SEQ ID NO:4) and the CDR3L having the amino acid sequence HQWSSYPT (SEQ ID NO:5) and
- a VH domain comprising the complementarity determining regions CDR1H, CDR2H and CDR3H, the CDR1H having the amino acid sequence GFSFTDYI (SEQ ID NO:7), the CDR2H having the amino acid sequence ISPYYGTA (SEQ ID NO:8), and the CDR3H having the amino acid sequence ARAPNRYEVMDY (SEQ ID NO:9).

In some embodiments, the antibody of the present invention is a chimeric antibody.

In some embodiments, the antibody of the present invention is a humanized antibody.

In some embodiments, the antibody of the present invention is an antibody fragment. Fragments can be produced by techniques that are known in the art. For instance, Fab or $F(ab')_2$ fragments may be produced by protease digestion of the isolated antibodies, according to conventional techniques. It will be appreciated that immunoreactive fragments can be modified using known methods, for example to slow clearance *in vivo* and obtain a more desirable pharmacokinetic profile the fragment may be modified with polyethylene glycol (PEG). Methods for coupling and site-specifically conjugating PEG to a Fab' fragment are described in, for example, Leong et al., Cytokines 16 (3): 106-119 (2001) and Delgado et al., Br. J. Cancer 5 73 (2): 175- 182 (1996), the disclosures of which are incorporated herein by reference.

In some embodiments, the antibody of the present invention comprises a VL domain having at least 70 % of identity with SEQ ID NO:1 and/or a VH domain having at least 70 % of identity with SEQ ID NO:5.

In some embodiments, the VH domain and/or the VL domain of the antibody of the invention comprises conservative sequence modifications. The term "**conservative sequence modifications**" refers to amino acid modifications that do not significantly affect or alter the biologic function of the protein containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into a protein by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. A “conservative substitution” is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine. Amino acid substitutions may further be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine

and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. Other families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within an antibody of the invention can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for binding to CD95L.

The antibody of the present invention is produced by any technique known in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination. Typically, knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce said antibodies, by standard techniques for production of polypeptides. For instance, they can be synthesized using well-known solid phase method, preferably using a commercially available peptide synthesis apparatus (such as that made by Applied Biosystems, Foster City, California) and following the manufacturer's instructions. Alternatively, antibodies of the present invention can be synthesized by recombinant DNA techniques well-known in the art. For example, antibodies can be obtained as DNA expression products after incorporation of DNA sequences encoding the antibodies into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired antibodies, from which they can be later isolated using well-known techniques.

Accordingly, a further object of the invention relates to a polynucleotide encoding an antibody according to the invention. More particularly the polynucleotide encodes a VH domain and/or a VL domain of an antibody of the present invention. In some embodiments the polynucleotide encodes a VH domain or a VL domain of an antibody of the present invention. In some embodiments the polynucleotide encodes a VH domain and a VL domain of an antibody of the present invention.

Typically, said polynucleotide is a DNA or RNA molecule, which may be included in any suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector. As used herein, the terms "**vector**", "**cloning vector**" and "**expression vector**" mean the vehicle by which a DNA or RNA sequence (*e.g.*, a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (*e.g.*, transcription and translation) of the introduced sequence. So, a further object of the invention relates to a vector comprising a polynucleotide of the invention. Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said antibody upon administration to a subject. Examples of promoters and enhancers used in the expression vector for animal cell include early promoter and enhancer of SV40, LTR promoter and enhancer of Moloney mouse leukemia virus, promoter and enhancer of immunoglobulin H chain and the like. Any expression vector for animal cell can be used, so long as a gene encoding the human antibody C region can be inserted and expressed. Examples of suitable vectors include pAGE107, pAGE103, pHSG274, pKCR, pSG1 beta d2-4 and the like. Other examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like. Other examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO 95/14785, WO 96/22378, US 5,882,877, US 6,013,516, US 4,861,719, US 5,278,056 and WO 94/19478.

As used herein, the term "**promoter/regulatory sequence**" refers to a nucleic acid sequence (such as, for example, a DNA sequence) recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence, thereby allowing the expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term "**operably linked**" or "**transcriptional control**" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, *e.g.*, where necessary to join two protein coding regions, are in the same reading frame.

A further object of the present invention relates to a host cell which has been transfected, infected or transformed by a polynucleotide and/or a vector according to the invention.

As used herein, the term "**transformation**" means the introduction of a "foreign" (*i.e.*, extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA bas been "transformed".

The polynucleotides of the invention may be used to produce an antibody of the present invention in a suitable expression system.

The term "**expression system**" means a host cell and compatible vector under suitable conditions, *e.g.*, for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell. Common expression systems include E. coli host cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors. Other examples of host cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, etc.). Specific examples include E.coli, Kluyveromyces or Saccharomyces yeasts, mammalian cell lines (*e.g.*, Vero cells, CHO cells, 3T3 cells, COS cells, etc.) as well as primary or established mammalian cell cultures (*e.g.*, produced from lymphoblasts, fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes, etc.). Examples also include mouse SP2/0-Ag14 cell (ATCC CRL1581), mouse P3X63-Ag8.653 cell (ATCC CRL1580), CHO cell in which a dihydrofolate reductase gene (hereinafter referred to as "DHFR gene") is defective (Urlaub G et al; 1980), rat YB2/3HL.P2.G11.16Ag.20 cell (ATCC CRL1662, hereinafter referred to as

"YB2/0 cell"), and the like. The present invention also relates to a method of producing a recombinant host cell expressing an antibody according to the invention, said method comprising the steps of: (i) introducing *in vitro* or *ex vivo* a recombinant polynucleotide or a vector as described above into a competent host cell, (ii) culturing *in vitro* or *ex vivo* the recombinant host cell obtained and (iii), optionally, selecting the cells which express and/or secrete said antibody. Such recombinant host cells can be used for the production of antibodies of the present invention.

Examples of vectors include all those known in the art, including, without limitation, cosmids, plasmids (*e.g.*, naked or contained in liposomes) and viruses (*e.g.*, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

Antibodies of the present invention are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

In some embodiments, the antibody (preferably the monoclonal antibody) of the present invention does not comprise a Fc region that mediates antibody-dependent cell-mediated cytotoxicity and thus does not comprise an Fc portion that induces antibody dependent cellular cytotoxicity (ADCC). In some embodiments, the antibody (preferably the monoclonal antibody) of the present invention does not comprise an Fc region that induces complement dependent cytotoxicity (CDC) or antibody-dependent phagocytosis. In some embodiments, the antibody (preferably the monoclonal antibody) of the present invention does not lead, directly or indirectly, to the depletion of cells expressing CD95L polypeptides (*e.g.*, do not lead to a 10%, 20%, 50%, 60% or greater elimination or decrease in number of $CD95L^+$ Cells). In some embodiments, the antibody (preferably the monoclonal antibody) of the present invention does not comprise an Fc domain capable of substantially binding to a FcγRIIIA (CD16) polypeptide. In some embodiments, the antibody (preferably the monoclonal antibody) of the present invention lacks an Fc domain (*e.g.*, lacks a CH2 and/or CH3 domain) or comprises an Fc domain of IgG2 or IgG4 isotype. In some embodiments, the antibody (preferably the monoclonal antibody) of the present invention comprises an Fc domain (e.g., of IgG1) with an altered glycosylation profile, resulting in the absence of ADCC activity of the antibody. In some embodiments, the antibody (preferably the monoclonal antibody) of the present invention

consists of or comprises a Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, a diabody, single-chain antibody fragment, or a multispecific antibody comprising multiple different antibody fragments. In some embodiments, the antibody (preferably the monoclonal antibody) of the present invention is not linked to a toxic moiety. In some embodiments, one or more amino acids selected from amino acid residues can be replaced with a different amino acid residue such that the antibody has altered C2q binding and/or reduced or abolished CDC. This approach is described in further detail in U.S. Patent Nos. 6,194,551 by Idusogie et al.

Therapeutic uses:

Accordingly, a further object of the present invention relates to the antibody of the present invention for use as a drug. More specifically, the present invention provides a method of therapy in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of an antibody of the present invention.

In some embodiments, the antibody of the present invention is particularly the treatment of cancer in a subject in need thereof.

As used herein, the term "**cancer**" has its general meaning in the art and includes, but is not limited to, solid tumors and blood borne tumors. The term cancer includes diseases of the skin, tissues, organs, bone, cartilage, blood and vessels. The term "cancer" further encompasses both primary and metastatic cancers. Examples of cancers that may be treated by methods and compositions of the present invention include, but are not limited to, cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma;

chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous; adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; and roblastoma, malignant; Sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas;

malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

In some embodiments, the subject suffers from a cancer selected from the group consisting of breast cancer, colon cancer, lung cancer, prostate cancer, testicular cancer, brain cancer, skin cancer, rectal cancer, gastric cancer, esophageal cancer, sarcomas, tracheal cancer, head and neck cancer, pancreatic cancer, liver cancer, ovarian cancer, lymphoid cancer, cervical cancer, vulvar cancer, melanoma, mesothelioma, renal cancer, bladder cancer, thyroid cancer, bone cancers, carcinomas, sarcomas, and soft tissue cancers.

In some embodiments, the antibody of the present invention is particularly for the treatment of triple negative breast cancer. As used herein the expression "**triple negative breast cancer**" has its general meaning in the art and means that said breast cancer lacks or expresses low levels of receptors for the hormones estrogen (ER-negative) and progesterone (PR-negative), and for the protein HER2.

In some embodiments, the antibody of the present invention is particularly for the prevention of metastases (e.g. in a subject suffering from a triple negative breast cancer).

In some embodiments, the antibody of the present invention is particularly for the treatment of an inflammatory disease. In some embodiments, the inflammatory disease is selected from the group consisting of arthritis, rheumatoid arthritis, acute arthritis, chronic rheumatoid arthritis, gouty arthritis, acute gouty arthritis, chronic inflammatory arthritis, degenerative arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, vertebral arthritis, and juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, gutatte psoriasis, pustular psoriasis, and psoriasis of the nails, dermatitis including contact dermatitis, chronic contact dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, and atopic dermatitis, x-linked hyper IgM syndrome, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria,

polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma, systemic scleroderma, sclerosis, systemic sclerosis, multiple sclerosis (MS), spino-optical MS, primary progressive MS (PPMS), relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, and ataxic sclerosis, inflammatory bowel disease (IBD), Crohn's disease, colitis, ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, transmural colitis, autoimmune inflammatory bowel disease, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis, respiratory distress syndrome, adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, rheumatoid spondylitis, sudden hearing loss, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis, Rasmussen's encephalitis, limbic and/or brainstem encephalitis, uveitis, anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, autoimmune uveitis, glomerulonephritis (GN), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), rapidly progressive GN, allergic conditions, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) or systemic lupus erythematodes such as cutaneous SLE, subacute cutaneous lupus erythematosus, neonatal lupus syndrome (NLE), lupus erythematosus disseminatus, lupus (including nephritis, cerebritis, pediatric, non-renal, extra-renal, discoid, alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis, lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitides, including vasculitis, large vessel vasculitis, polymyalgia rheumatica, giant cell (Takayasu's) arteritis, medium vessel vasculitis, Kawasaki's disease, polyarteritis nodosa, microscopic polyarteritis, CNS vasculitis, necrotizing, cutaneous, hypersensitivity vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving

leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus, optionally pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, pemphigus erythematosus, autoimmune polyendocrinopathies, Reiter's disease or syndrome, immune complex nephritis, antibody-mediated nephritis, neuromyelitis optica, polyneuropathies, chronic neuropathy, IgM polyneuropathies, IgM-mediated neuropathy, thrombocytopenia, thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis); subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis, allergic encephalomyelitis, experimental allergic encephalomyelitis (EAE), myasthenia gravis, thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, primary biliary cirrhosis, pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac disease, Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AGED), autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory or relapsed polychondritis, pulmonary alveolar proteinosis, amyloidosis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis, optionally benign monoclonal gammopathy or monoclonal garnmopathy of undetermined significance, MGUS, peripheral neuropathy, paraneoplastic syndrome, channelopathies such as

epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine opthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases, diabetic nephropathy, Dressler's syndrome, alopecia greata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyl), and telangiectasia), male and female autoimmune infertility, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant cell polymyalgia, endocrine ophthamopathy, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, aspermiogenese, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensoneural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica, orchitis granulomatosa, pancreatitis (e.g. chronic pancreatitis), polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyreoiditis,

acquired splenic atrophy, infertility due to antispermatozoan antobodies, non-malignant thymoma, vitiligo, SCID and Epstein-Barr virus-associated diseases, acquired immune deficiency syndrome (AIDS), parasitic diseases such as Lesihmania, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulitis, polyendocrine failure, peripheral neuropathy, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, dilated cardiomyopathy, epidermolisis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Loffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, ischemic re-perfusion disorder, reduction in blood pressure response, vascular dysfunction, antgiectasis, tissue injury, cardiovascular ischemia, hyperalgesia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, reperfusion injury of myocardial or other tissues, dermatoses with acute inflammatory components, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, acute serious inflammation, chronic intractable inflammation, pyelitis, pneumonocirrhosis, diabetic retinopathy, diabetic large-artery disorder, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis.

In some embodiments, the antibody of the present invention is particularly suitable for the treatment of systemic lupus erythematosus or anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV).

In some embodiments, the antibody of the present invention is particularly suitable for the treatment of viral induced inflammation, in particular COVID19.

Pharmaceutical compositions:

A further object of the present invention relates to a composition comprising, consisting of or consisting essentially of an antibody of the present invention.

In some embodiments, the composition of the invention is a pharmaceutical composition and further comprises a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene- block polymers, polyethylene glycol and wool fat. For use in administration to a patient, the composition will be formulated for administration to the patient. The compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Sterile injectable forms of the compositions of this invention may be aqueous or an oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water,

Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono-or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation. The compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include, *e.g.*, lactose. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added. Alternatively, the compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols. The compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs. For topical applications, the compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-

octyldodecanol, benzyl alcohol and water. Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Patches may also be used. The compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents. For example, an antibody present in a pharmaceutical composition of this invention can be supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The product is formulated for IV administration in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection. The pH is adjusted to 6.5. An exemplary suitable dosage range for an antibody in a pharmaceutical composition of this invention may between about 1 mg/m^2 and 500 mg/m^2. However, it will be appreciated that these schedules are exemplary and that an optimal schedule and regimen can be adapted taking into account the affinity and tolerability of the particular antibody in the pharmaceutical composition that must be determined in clinical trials. A pharmaceutical composition of the invention for injection (*e.g.*, intramuscular, i.v.) could be prepared to contain sterile buffered water (*e.g.*, 1 ml for intramuscular), and between about 1 ng to about 100 mg, *e.g.*, about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an antibody of the invention.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. JQ3 recognizes human and simian CD95L and is a stronger neutralizing anti-CD95L mAb as compared to the commercial anti-CD95L antibody NOK-1. A. Kon, Koff and Kd of JQ3 (IgG1κ) and NOK-1 (IgG1κ) hybridoma measured by surface plasmon resonance. IgCD95L was immobilized on a sensor chip S CM5 and antibody binding features was evaluated using a Biacore T200. **B.** The ability of the anti-human CD95L monoclonal antibodies NOK-1 and JQ3 and anti-mouse CD95L monoclonal antibody MFL3 (1μg/ml) to recognize human, simian or mouse CD95L was determined by flow cytometry using HEK/293T

cells transiently transfected with indicated CD95L-encoding pcDNA3.1 vectors. **C.** The binding efficiency of NOK-1 and JQ3 was compared by flow cytometry against CD95L-expressing 1A12 cell line. *Left panel:* Histograms report the intensity of CD95L staining (CD95L mean of fluorescence – isotypic mean of fluorescence) according to the antibody concentration (M). *Right panels:* examples of CD95L staining, in red the JQ3 or NOK-1 staining and in grey the isotypic staining. Table indicates the half maximal effective concentration (EC50) for each antibody. **D.** The neutralizing ability of NOK1 and JQ3 mAbs was compared by incubating CD95-sensitive Jurkat cell line (5.10^5 cells) for 24 hours with 100 ng/mL of Ig-CD95L (soluble and cytotoxic human CD95L) in the presence or absence of the indicated concentrations of antibody.

Figure 2. NOK-1 and JQ3 mAbs and the CD95 inhibitor DB550 inhibit neutrophil activation (calcium response) by sera from AAV or COVID-19 patients. Neutrophils from healthy donors (HD) were loaded with the calcium (Ca^{2+}) probe cal-520. Fluorescence values (F) were normalized to pre-stimulated values (F_0) to yield F/F_0 values (relative $[Ca^{2+}]_{cyt}$). Data represent the mean ± s.d. $[Ca^{2+}]_{cyt}$ (cytoplasmic Ca^{2+} concentration). **A.** Neutrophils were pre-incubated for 30 minutes with DB550 (1µM) or without (control) and then exposed to indicated sera. On the other hand, sera from COVID-19 patients were pre-incubated for 30 min in the presence of NOK-1 or JQ3 (10 µg/mL) and then, neutrophils were stimulated with indicated sera and the intracellular calcium concentration was monitored. **B.** HD Neutrophils were pre-incubated for 30 minutes with DB550 (1µM) or without (control) and then exposed to indicated sera. On the other hand, sera from anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) patients were pre-incubated for 30 min in the presence or absence of NOK-1 or JQ3 (10 µg/mL). Then, HD neutrophils were exposed to indicated sera and the intracellular calcium concentration was monitored. **C.** DMSO treatment induces differentiation of the myeloid HL60 cell line into neutrophils. DMSO-treated HL60 cells were pre-incubated for 30 minutes in the presence or absence (Control) of DB550 (1µM). On the other hand, sera from healthy donor (HD3), AAV (AAV 45) and COVID-19 (Covid 15) patients were pre-incubated for 30 minutes with or without NOK-1 or JQ3 (10 µg/mL). Then, DMSO-treated HL-60 cells were stimulated with indicated sera and the intracellular calcium concentration was monitored.

EXAMPLE:

Methods

Neutralizing anti-CD95L monoclonal antibody JQ3 production

Balb/c mice were immunized and boosted repeatedly with soluble recombinant CD95L (Peprotech) by intra-splenic route. Sera were collected and their immune response efficiency was assessed by ELISA using IgCD95L-coated plates. Splenocytes from the mice with the highest titer were fused with mouse SP2/O-Ag14 Cell Line murine (DSMZ, ACC 146) and selected to generate antibody-producing hybridoma. Hybridomas producing CD95L-targeting IgG were sorted based on their ability to bind IgCD95L-immobilized in microtiter plates and next validated using flow cytometry against CD95L-expressing 1A12 cells. After cloning steps, monoclonal antibody JQ3 was selected and produced in roller bottle and purified on protein A columns (GE).

Measurement of cell death

Cell viability was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as previously described (48). In brief, a medium containing 100 ng/mL of IgCD95L (*i.e.*, concentration of IgCD95L inducing 100% of cell death in Jurkat T-cells), was pre-incubated for 30 min with the indicated concentrations of JQ3, NOK-1 or control antibody and then incubated for 24 h with Jurkat T-cells (4.10^4) in flat-bottom 96-well plates in a final volume of 100 µL. Then, MTT (15 µl at 5 mg/mL in PBS) was added, and after 4 h of incubation at 37°C, the absorbance was measured at 570 nm wavelength using the Infinite200Pro (Tecan, Männedorf, Switzerland).

DMSO-treated HL60 cells

To induce the neutrophil differentiation of HL60 cell line, viable log-phase cells were resuspended in complete RPMI 1640 growth medium containing 1.25% DMSO for 24–96 h.

Imaging of calcium concentration ($[Ca^{2+}]i$)

Single-cell cytosolic calcium imaging was performed using Cal-520, a new fluorogenic calcium-sensitive dye. Cal-520 was shown to be the best performing green-emitting dye because of its low basal fluorescence and a large increase in fluorescence in response to small changes in $[Ca^{2+}]$, resulting in a good signal-to-noise ratio (44). Moreover, this Ca^{2+} probe shows a better intracellular retention than other calcium dyes. Cells were loaded with Cal-520 (2 µM) at room temperature (20–25 °C) in Hank's Balanced Salt Solution (HBSS) for 30 min.

The cells were centrifuged to eliminate the extracellular probe, rinsed with HBSS and incubated for 15 min to complete de-esterification of the dye. The loaded cells are deposited on a glass coverslip placed in a recording chamber positioned on the stage of an inverted epifluorescence microscope (Olympus IX70) equipped with a ×40 UApo/340-1.15W water-immersion objective (Olympus, Tokyo, Japan). To minimize UV light exposure, 4×4-binning function was used. Cal-520 was excited at 485 +/- 22 nm, and images were captured at 530 +/- 30 nm at constant 10-s intervals, at 12-bit resolution, by a fast-scan camera (CoolSNAP fx Monochrome, Photometrics). Regions of interest were drawn on certain recorded cells to restrict data collection to specific regions. Imaging was controlled by Universal Imaging software, including Metafluor and Metamorph. Fluorescence intensity changes were normalized to the initial fluorescence value F_0 (pixel averaged over 20 frames before stimulation) and expressed as F/F_0 (relative $[Ca^{2+}]_{cyt}$). One field was acquired from each coverslip and the data pooled from six independent coverslips on three different days.

Results

We generated a neutralizing anti-CD95L monoclonal antibody (mAb), designated JQ3 (IgG1κ). The features of the antibody are depicted in **Figure 1A, 1B and 1C**. In particular, the neutralizing effect of JQ3 was confirmed since this home-made monoclonal antibody inhibited the CD95-mediated apoptotic signaling pathway induced in T-cell line Jurkat more efficiently than NOK-1 mAb (**Figure 1D**). Interestingly, JQ3 blocked the CD95-mediated Ca^{2+} response (**Figure 2**) in neutrophils isolated from healthy donors and stimulated with sera isolated from patients suffering from various inflammatory disorders including COVID19 and anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV).

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

CLAIMS:

1. A neutralizing anti-CD95L monoclonal antibody having

- a VL domain comprising the complementarity determining regions CDR1L, CDR2L and CDR3L, the CDR1L having the amino acid sequence SSVSY (SEQ ID NO:3) the CDR2L having the amino acid sequence NTF (SEQ ID NO:4) and the CDR3L having the amino acid sequence HQWSSYPT (SEQ ID NO:5) and

- a VH domain comprising the complementarity determining regions CDR1H, CDR2H and CDR3H, the CDR1H having the amino acid sequence GFSFTDYI (SEQ ID NO:7), the CDR2H having the amino acid sequence ISPYYGTA (SEQ ID NO:8), and the CDR3H having the amino acid sequence ARAPNRYEVMDY (SEQ ID NO:9).

2. The neutralizing anti-CD95L monoclonal antibody of claim 1 is a chimeric antibody.

3. The neutralizing anti-CD95L monoclonal antibody of claim 1 is a humanized antibody.

4. The neutralizing anti-CD95L monoclonal antibody of claim 1 that comprises a VL domain having at least 70 % of identity with SEQ ID NO:1 and/or a VH domain having at least 70 % of identity with SEQ ID NO:5

5. The neutralizing anti-CD95L monoclonal antibody of claim 1 wherein the VH domain and/or the VL domain of the antibody of the invention comprises conservative sequence modifications

6. A polynucleotide that encodes the neutralizing anti-CD95L monoclonal antibody of claim 1.

7. The polynucleotide of claim 6 that encodes a VH domain and/or a VL domain of the neutralizing anti-CD95L monoclonal antibody of claim 1.

8. A vector that comprises the polynucleotide of claim 6.

9. A host cell which has been transfected, infected or transformed by the polynucleotide of claim 6 and/or the vector of claim 8.

10. The neutralizing anti-CD95L monoclonal antibody of claim 1 for use as a drug.

11. A method of therapy in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of the neutralizing anti-CD95L monoclonal antibody of claim 1.

12. A method of treating cancer in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of the neutralizing anti-CD95L monoclonal antibody of claim 1.

13. The method of claim 12 wherein the patient suffers from a cancer selected from the group consisting of breast cancer, colon cancer, lung cancer, prostate cancer, testicular cancer, brain cancer, skin cancer, rectal cancer, gastric cancer, esophageal cancer, sarcomas, tracheal cancer, head and neck cancer, pancreatic cancer, liver cancer, ovarian cancer, lymphoid cancer, cervical cancer, vulvar cancer, melanoma, mesothelioma, renal cancer, bladder cancer, thyroid cancer, bone cancers, carcinomas, sarcomas, and soft tissue cancers.

14. The method of claim 12 wherein the patient suffers from a triple negative breast cancer.

15. A method of treating an inflammatory disease in patient in need thereof, comprising administering to the patient a therapeutically effective amount of the neutralizing anti-CD95L monoclonal antibody of claim 1.

16. The method of claim 15 wherein the inflammatory disease is selected from the group consisting of arthritis, rheumatoid arthritis, acute arthritis, chronic rheumatoid arthritis, gouty arthritis, acute gouty arthritis, chronic inflammatory arthritis, degenerative arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, vertebral arthritis, and juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, gutatte psoriasis, pustular psoriasis, and psoriasis of the nails, dermatitis including contact dermatitis, chronic contact dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, and atopic dermatitis, x-linked hyper IgM syndrome, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma, systemic scleroderma, sclerosis, systemic sclerosis, multiple sclerosis

(MS), spino-optical MS, primary progressive MS (PPMS), relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, and ataxic sclerosis, inflammatory bowel disease (IBD), Crohn's disease, colitis, ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, transmural colitis, autoimmune inflammatory bowel disease, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis, respiratory distress syndrome, adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, rheumatoid spondylitis, sudden hearing loss, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis, Rasmussen's encephalitis, limbic and/or brainstem encephalitis, uveitis, anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, autoimmune uveitis, glomerulonephritis (GN), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), rapidly progressive GN, allergic conditions, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) or systemic lupus erythematodes such as cutaneous SLE, subacute cutaneous lupus erythematosus, neonatal lupus syndrome (NLE), lupus erythematosus disseminatus, lupus (including nephritis, cerebritis, pediatric, non-renal, extra-renal, discoid, alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis, lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitides, including vasculitis, large vessel vasculitis, polymyalgia rheumatica, giant cell (Takayasu's) arteritis, medium vessel vasculitis, Kawasaki's disease, polyarteritis nodosa, microscopic polyarteritis, CNS vasculitis, necrotizing, cutaneous, hypersensitivity vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia,

diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus, optionally pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, pemphigus erythematosus, autoimmune polyendocrinopathies, Reiter's disease or syndrome, immune complex nephritis, antibody-mediated nephritis, neuromyelitis optica, polyneuropathies, chronic neuropathy, IgM polyneuropathies, IgM-mediated neuropathy, thrombocytopenia, thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis); subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis, allergic encephalomyelitis, experimental allergic encephalomyelitis (EAE), myasthenia gravis, thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, primary biliary cirrhosis, pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac disease, Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AGED), autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory or relapsed polychondritis, pulmonary alveolar proteinosis, amyloidosis, scleritis, a non-cancerous lymphocytosis, a primary

lymphocytosis, which includes monoclonal B cell lymphocytosis, optionally benign monoclonal gammopathy or monoclonal garnmopathy of undetermined significance, MGUS, peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine opthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases, diabetic nephropathy, Dressler's syndrome, alopecia greata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyl), and telangiectasia), male and female autoimmune infertility, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant cell polymyalgia, endocrine ophthamopathy, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, aspermiogenese, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica,

enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensoneural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica, orchitis granulomatosa, pancreatitis (e.g. chronic pancreatitis), polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyreoiditis, acquired splenic atrophy, infertility due to antispermatozoan antobodies, non-malignant thymoma, vitiligo, SCID and Epstein-Barr virus-associated diseases, acquired immune deficiency syndrome (AIDS), parasitic diseases such as Lesihmania, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulitis, polyendocrine failure, peripheral neuropathy, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, dilated cardiomyopathy, epidermolisis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Loffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, ischemic re-perfusion disorder, reduction in blood pressure response, vascular dysfunction, antgiectasis, tissue injury, cardiovascular ischemia, hyperalgesia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, reperfusion injury of myocardial or other tissues, dermatoses with acute inflammatory components, acute purulent

meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, acute serious inflammation, chronic intractable inflammation, pyelitis, pneumonocirrhosis, diabetic retinopathy, diabetic large-artery disorder, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis.

17. The method of claim 15 wherein the patient suffers from systemic lupus erythematosus or anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV).

18. The method of claim 15 wherein the patient suffers from viral induced inflammation, in particular COVID19.

19. A pharmaceutical composition comprising the antibody of claim 1.

	K_{on} (M-1 s-1)	K_{off} (s-1)	Kd (M)
JQ3	1.35E+05	3.03E-04	2.2E-09
NOK1	4.35E+04	2.33E-04	5.3E-09

Figure 1A

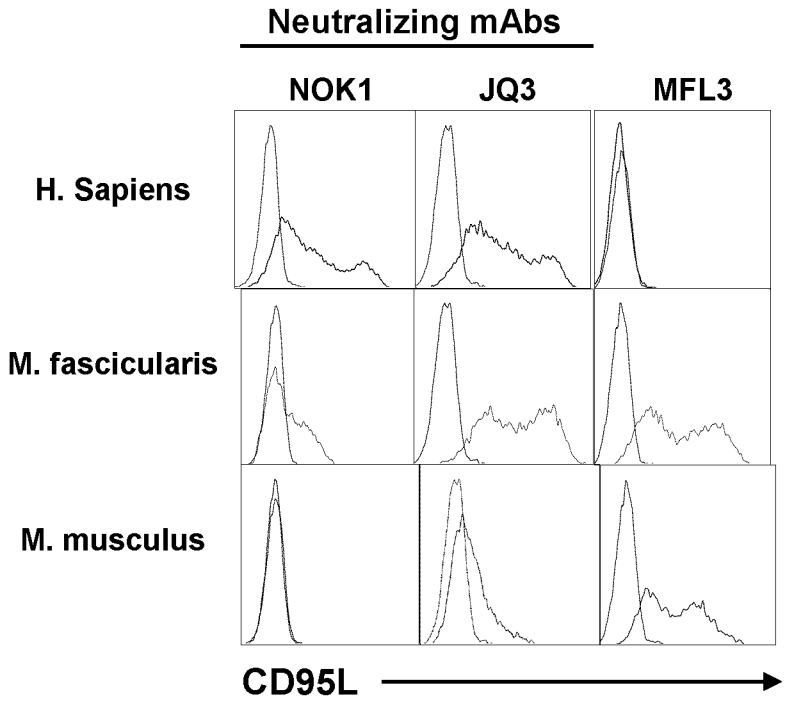
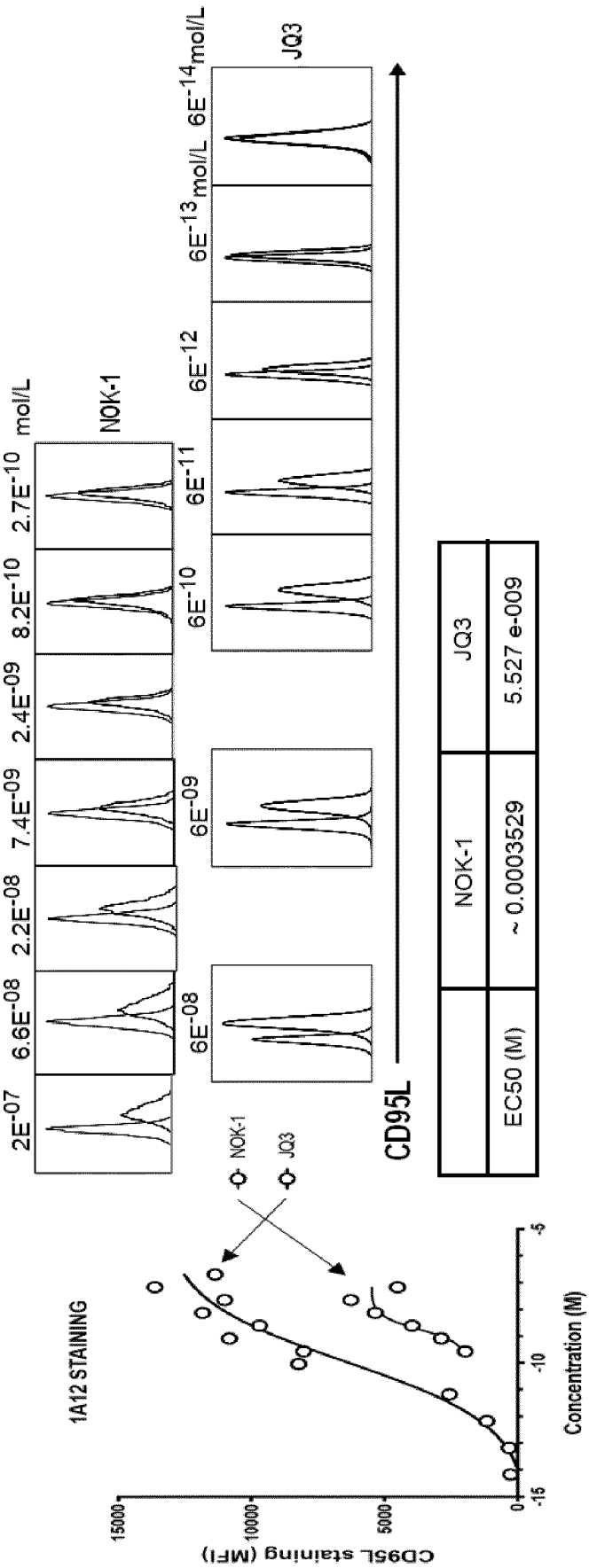


Figure 1B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2.2E-08
7.4E-09
2.4E-09
8.2E-10
2.7E-10 mol/L
NOK-1
6E-08
6E-09
6E-10
6E-11
6E-12
6E-13 mol/L
6E-14 mol/L
JQ3
CD95L
NOK-1
JQ3
EC50 (M)
~ 0.0003529
5.527 e-009
1A12 STAINING
CD95L staining (MFI)
15000
10000
5000
0
-15
-10
-5
Concentration (M)

Figure 1C

	Isotype	NOK-1	JQ3
IC_{50} (µg/mL)	-	0.006	0.000001

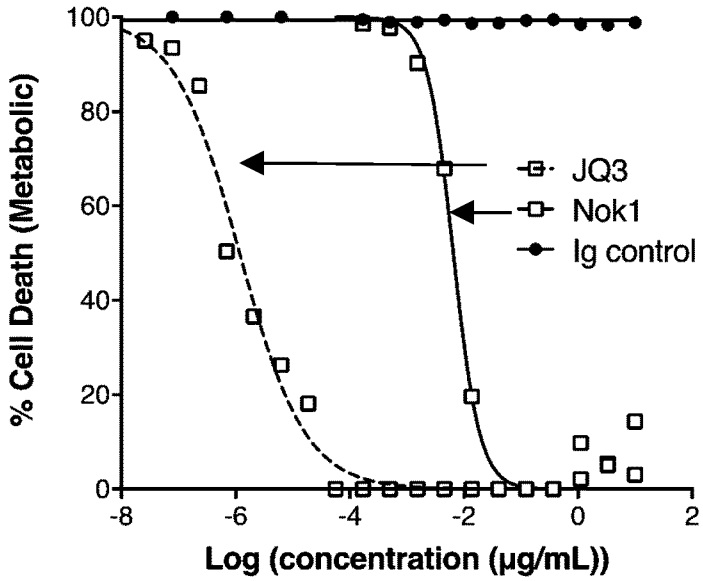
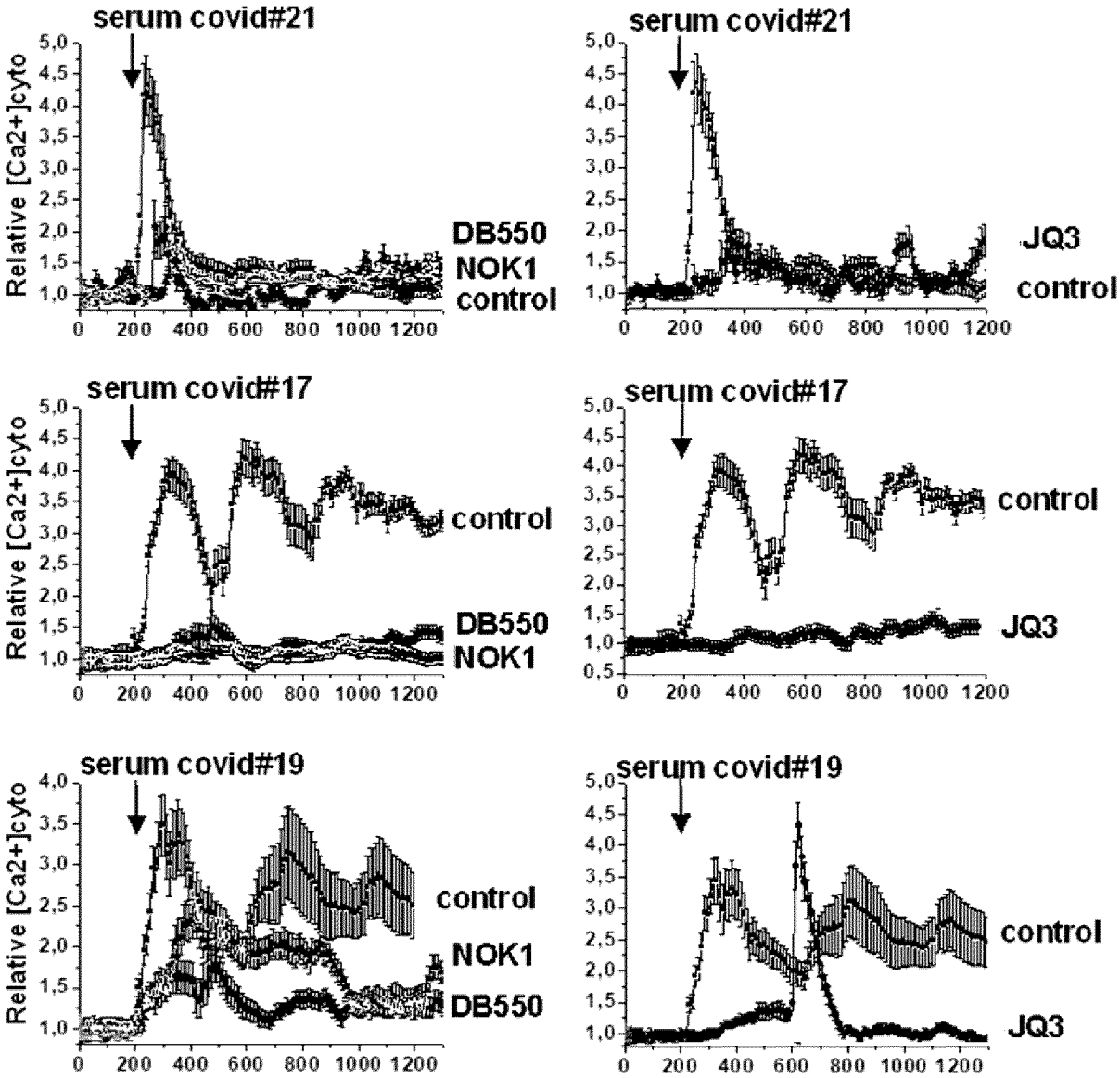


Figure 1D


serum covid#21
Relative [Ca2+]cyto
DB550
NOK1
control
serum covid#21
JQ3
control
serum covid#17
control
DB550
NOK1
serum covid#17
control
JQ3
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control
NOK1
DB550
serum covid#19
control
JQ3

Figure 2A

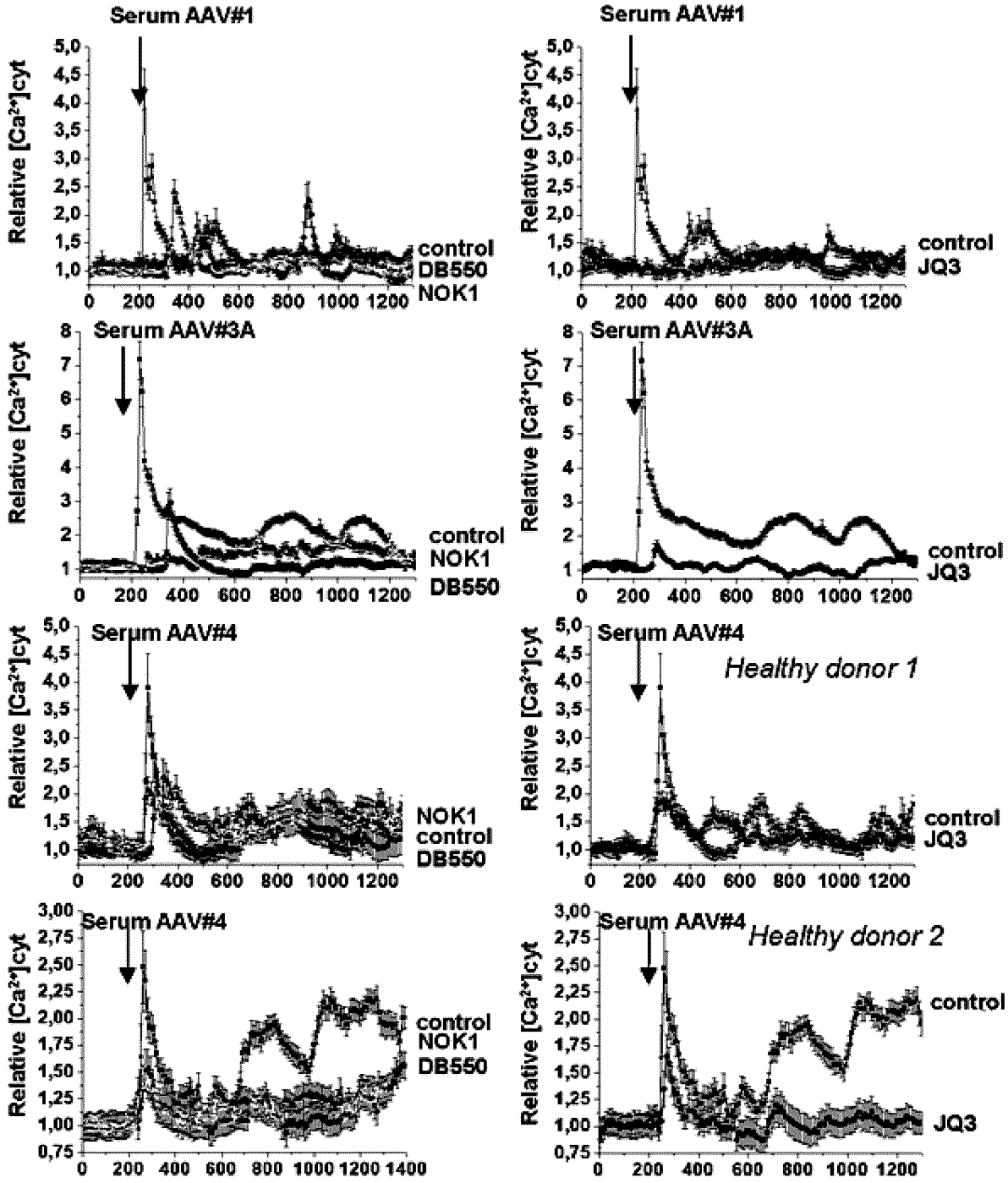
Serum AAV#1
Relative [Ca2+]cyt
control
DB550
NOK1
Serum AAV#1
control
JQ3
Serum AAV#3A
control
NOK1
DB550
Serum AAV#3A
control
JQ3
Serum AAV#4
NOK1
control
DB550
Serum AAV#4
Healthy donor 1
control
JQ3
Serum AAV#4
control
NOK1
DB550
Serum AAV#4
Healthy donor 2
control
JQ3

Figure 2B

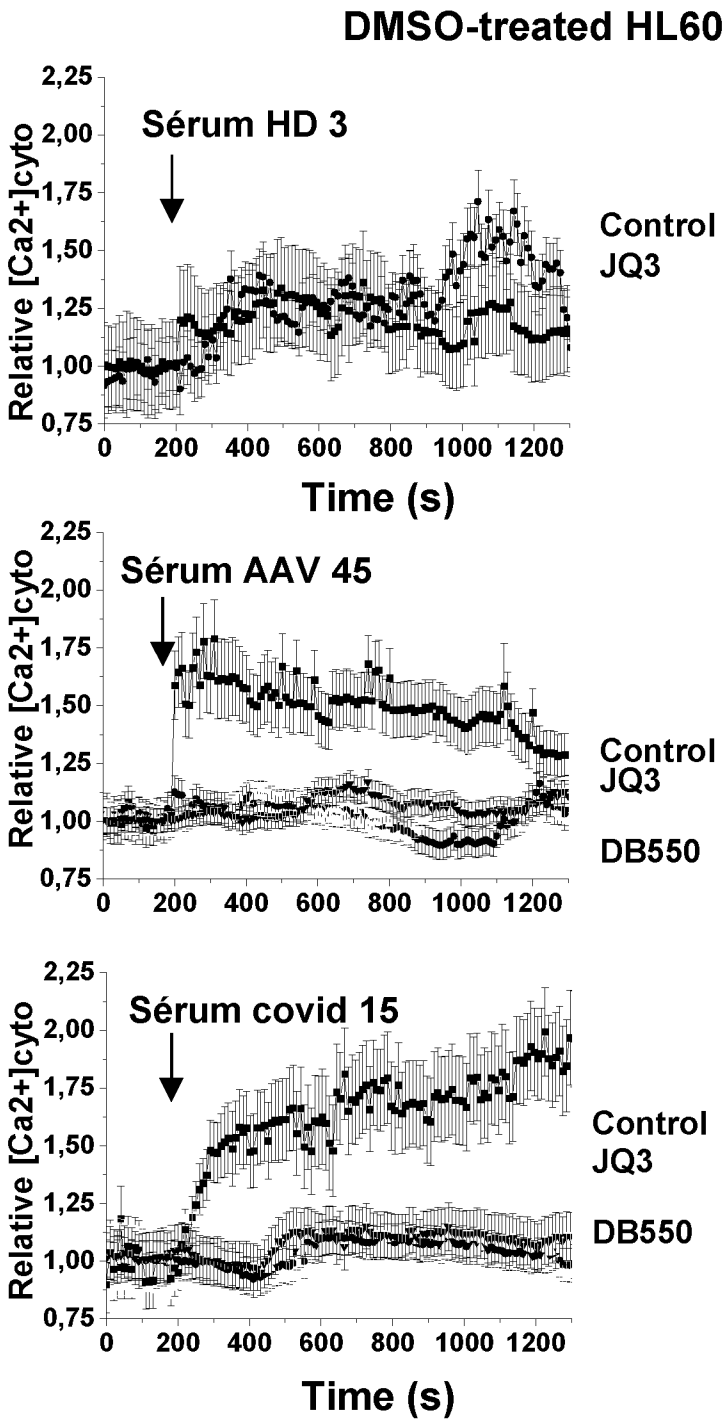
DMSO-treated HL60
Sérum HD 3
Control
JQ3
Relative [Ca2+]cyto
2,25
2,00
1,75
1,50
1,25
1,00
0,75
0
200
400
600
800
1000
1200
Time (s)
Sérum AAV 45
Control
JQ3
DB550
Sérum covid 15
Control
JQ3
DB550

Figure 2C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2022/083881

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

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

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/083881

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P37/00 A61P35/00 A61P37/02
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/051002 A1 (APOGENIX AG [DE]) 30 March 2017 (2017-03-30) claims 1-24; figures 7-12, 16; examples 7-12, 16	1-19
X	US 2011/038867 A1 (PINCELLI CARLO [IT] ET AL) 17 February 2011 (2011-02-17) claims 1-9 paragraph [0075] - paragraph [0082]; figures 1-7 paragraph [0085] - paragraph [0092]; figures 10-14 paragraph [0006] paragraph [0013]	1-19

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 February 2023	06/03/2023

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2022/083881

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