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(54) Title: ANTAGONISTIC ANTI-HTNFSF13B HUMAN ANTIBODIES

(57) Abstract: Human monoclonal antibodies that specifically bind to TNFSF13b polypeptides are disclosed. These antibodies have high affinity for hTNFSF13b (e.g., K_D = 10^{-8} M or less), a slow off rate for TNFSF13b dissociation (e.g., K_{off} = 10^{-3} sec^{-1} or less) and neutralize TNFSF13b activity in vitro and in vivo. The antibodies of the invention are useful in one embodiment for inhibiting TNFSF13b activity in a human subject suffering from a disorder in which hTNFSF13b activity is detrimental. Nucleic acids encoding the antibodies of the present invention, as well as, vectors and host cells for expressing them are also encompassed by the invention.

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Antagonistic Anti-hTNFSF13b Human Antibodies

The TNF family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including proliferation, cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes. The TNF family of cytokines and receptors has undergone a large expansion in the last few years with the advent of massive EST sequencing. TNFSF13b is the official name adopted by the TNF Congress for BLyS, TALL-1, BAFF, THANK, neutrokine-α, and zTNF (for review see Locksley et al. Cell 2001 104:487). Human TNFSF13b (hTNFSF13b) is a 285-amino acid type II membrane-bound protein that possesses a N-terminal cleavage site that allows for the existence of both soluble and membrane bound proteins. Functionally, TNFSF13b appears to regulate B cell and some T cell immune responses.

Studies of septic shock syndrome and other disorders arising from overproduction of inflammatory cytokines have shown that an afflicted host will often counter high cytokine levels by releasing soluble cytokine receptors or by synthesising high-affinity anti-cytokine antibodies. Methods of treatment that mimic such natural responses are considered as viable therapeutic approaches for alleviating cytokine-mediated disease. Thus, there is a well-recognised need for human antibodies that bind cytokines, such as TNFSF13b, that are involved in the regulation of cellular immune processes with high affinity and that have the capacity to antagonise the activity of the targeted cytokine *in vitro* and *in vivo*. Although international patent application WO00/50597 non-descriptively discloses antibodies directed at TNFSF13b, that application does not specifically describe the structural or functional characteristics of such antibodies.

The present invention provides anti-hTNFSF13b human antibodies, or antigen-binding portions thereof. The antibodies of the invention are characterised by high affinity binding to TNFSF13b polypeptides, slow dissociation kinetics, and the capacity to antagonise at least one *in vitro* and/or *in vivo* activity associated with TNFSF13b polypeptides.

According to a first aspect of the invention there is provided an anti-hTNFSF13b human antibody comprising at least three of the polypeptides selected from the group consisting of:

a. SEQ ID NO: 4 located at CDR1 of the light chain variable region (LCVR);

b. SEQ ID NO: 6 located at CDR2 of the LCVR;

c. SEQ ID NO: 8 located at CDR3 of the LCVR;

d. SEQ ID NO: 12 located at CDR1 of the heavy chain variable region (HCVR);

e. SEQ ID NO: 14 located at CDR2 of the HCVR; and

f. SEQ ID NO: 16 located at CDR3 of the HCVR.

According to a second aspect of the present invention there is provided an antibody that neutralizes TNFSF13b activity by binding an epitope of TNFSF13b, wherein the epitope comprises lysine at position 71, threonine at position 72, tyrosine at position 73, and glutamic acid at position 105.

According to a third aspect of the present invention there is provided an antibody or fragment thereof comprising an amino acid sequence of heavy chain 4A5-3.1.1-B4 represented by SEQ ID NO 17 and an amino acid sequence of light chain 4A5-3.1.1-B4 represented SEQ ID NO 19.

The present invention also provides an anti-hTNFSF13b human antibody comprising at least three, four, five or all sequences selected from the group consisting of:

a. SEQ **10** NO: 4 located at COR1 of the light chain variable region (LCVR);

b. SEQ **10** NO: 6 located at COR2 of the LCVR;

c. SEQ **10** NO: 8 located at COR3 of the LCVR;

d. SEQ **10** NO: 12 located at COR1 of the HCVR;

e. SEQ **10** NO: 14 located at COR2 of the heavy chain variable region (HCVR);- and

f. SEQ **10** NO: 16 located at COR3 of the HCVR. The invention also provides an anti-h TNFSF13b human antibody comprising a light chain variable region (LCVR) polypeptide as shown in SEQ 10 NO: 2 or a heavy chain variable region (HCVR) polypeptide as shown in SEQ 10 NO: 10.

In another embodiment, the invention provides an isolated anti-hTNFSF13b human antibody which binds to a hTNFSF13b polypeptide and dissociates from the hTNFSF13b polypeptide with a K_{off} rate constant of 1 X 10^{-4} s^{-1} or less, as determined by surface plasmon resonance, or which inhibits TNFSF13b induced proliferation in an *in vitro* neutralisation assay with an IC_{50} of 1 x 10^{-8} M or less.

In an preferred embodiment, the invention provides an isolated anti-hTNFSF13b human antibody that has the following characteristics:

a) inhibits TNFSF13b induced proliferation in an *in vitro* neutralisation assay with an IC_{50} of 1 x 10^{-8} M or less;

b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:16; and

c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:8.

The invention also provides methods of treating or preventing acute or chronic diseases or conditions associated with B cell and some T cell activity including, but not limited to, autoimmune disorders, such as systemic lupus erythematosus, rheumatoid arthritis, and/or neoplasia comprising the administration of an anti-hTNFSF13b human antibody of the present invention.

In another embodiment, the present invention provides sequences that encode the novel anti-hTNFSF13b human antibodies, vectors comprising the polynucleotide sequences encoding anti-hTNFSF13b human antibodies, host cells transformed with vectors incorporating polynucleotides that encode the anti-hTNFSF13b human antibodies, formulations comprising anti-hTNFSF13b human antibodies and methods of making and using the same.

In another embodiment, the present invention provides the epitope of the antigen to which the novel anti-hTNFSF13b human antibodies bind. Thus, the invention also provides a use of an antibody that binds the epitope of the present invention thereby neutralising the TNFSF13b activity for the treatment or prevention of acute or chronic diseases or conditions associated with B cell and some T cell activity including, but not limited to, autoimmune disorders, such as systemic lupus erythematosus, rheumatoid arthritis, and/or neoplasia.

The invention also encompasses an article of manufacture comprising a packaging material and an antibody contained within said packaging material, wherein the antibody neutralises TNFSF13b activity for treatment or prevention of a human subject suffering from a disorder in which TNFSF13b activity is detrimental, and wherein the packaging material comprises a package insert which indicates that the antibody neutralises by binding an epitope of TNFSF13b, wherein the epitope comprises lysine at position 71, threonine at position 72, tyrosine at position 73, and glutamic acid at position 105; and a package insert that provides for administration of the dosage form that results in neutralising TNFSF13b activity.

Also included within the scope of the invention is an antibody or fragment thereof comprising SEQ ID NO: 17; an antibody or fragment thereof comprising SEQ ID NO: 18; an antibody or fragment thereof comprising SEQ ID NO: 19; an antibody or fragment thereof comprising SEQ ID NO: 17 and SEQ ID NO: 19; an antibody or fragment thereof comprising SEQ ID NO: 18 and SEQ ID NO: 19; and an antibody or fragment thereof that competitively inhibits the specific binding of the 4A5-3.1.1-B4 antibody produced by the hybridoma of ATCC Deposit Number PTA- 3674 to hTNFSF13b.

Fig. 1. Graph illustrating the inhibition of hTNFSF13b and IL-1 induced proliferation of T1165.17 cells by human antibody 4A5-3.1.1-B4.

Fig. 2. Graph illustrating the neutralisation of hTNFSF13b induced proliferation by human antibody 4A5-3.1.1-B4 in primary human B cells stimulated with anti-IgM.

In order that the present invention may be more readily understood, certain terms are first defined.

An antibody is an immunoglobulin molecule comprised of four polypeptide chains, two heavy (H) chains (about 50-70kDa) and two light (L) chains (about 25kDa) inter-connected by disulfide bonds. Light chains are classified as kappa and lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD, and IgE, respectively. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2, and CH3 for IgG, IgD and IgA, and 4 domains, CH1, CH2, CH3, CH4 for IgM and IgE. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The HCVR and LCVR regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each HCVR and LCVR is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The assignment of amino acids to each domain is in accordance with well known conventions. [Kabat, *et al*, *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991); Chothia, *et al.*, *J. Mol. Biol.* 196:901-917 (1987); Chothia, *et al.*, *Nature* 342:878-883 (1989)]. The functional characteristics of the antibody to bind a particular antigen are determined by the CDRs.

In the present disclosure the term "antibody" is intended to refer to a monoclonal antibody *per se*. A monoclonal antibody can be a human antibody, chimeric antibody, humanised antibody, Fab fragment, Fab' fragment, F(ab')2, or single chain FV fragment. Preferably the term "antibody" refers to human antibody.

The term "human antibody" includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences. Human antibodies have several advantages over non-human and chimeric antibodies for use in human therapy. For example, the effector portion of a human antibody may interact better with the other parts of the human immune system (eg., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)). Another advantage is that the human immune system should not recognise the human antibody as foreign, and, therefore the antibody response against such an injected antibody should be less than against a totally foreign non-human antibody or a partially foreign chimeric antibody. In addition, injected non-human antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of human antibodies. Injected human antibodies will have a half-life essentially identical to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

The term "hTNFSF13b" refers to the human form of a member of the tumour necrosis factor family of ligands described in international patent applications WO98/18921 and WO00/50597 (referred to therein as neutrokine-α). The term "TNFSF13b" is intended to encompass hTNFSF13b as well as homologues of hTNFSF13b derived from other species. The terms "hTNFSF13b" and

"TNFSF13b" are intended to include forms thereof, which can be prepared by standard recombinant expression methods or purchased commercially (Research Diagnostics Inc. Catalogue No. RDI-3113, rhuBAFF, Flanders, N.J.) as well as generated synthetically.

The phrase "biological property", "biological characteristic", and the term "activity" in reference to an antibody of the present invention are used interchangeably herein and include, but are not limited to, epitope affinity and specificity (eg., anti-hTNFSF13b human antibody binding to hTNFSF13b), ability to antagonise the activity of the targeted polypeptide (eg., TNFSF13b activity), the *in vivo* stability of the antibody, and the immunogenic properties of the antibody. Other identifiable biological properties or characteristics of an antibody recognised in the art include, for example, cross-reactivity, (ie., with non-human homologues of the targeted polypeptide, or with other proteins or tissues, generally), and ability to preserve high expression levels of protein in mammalian cells. The aforementioned properties or characteristics can be observed or measured using art-recognised techniques including, but not limited to ELISA, competitive ELISA, surface plasmon resonance analysis, *in vitro* and *in vivo* neutralisation assays (eg., Example 2), and immunohistochemistry with tissue sections from different sources including human, primate, or any other source as the need may be. Particular activities and biological properties of anti-hTNFSF13b human antibodies are described in further detail in the Examples below.

The phrase "contact position" includes an amino acid position in the CDR1, CDR2 or CDR3 of the heavy chain variable region or the light chain variable region of an antibody which is occupied by an amino acid that contacts antigen. If a CDR amino acid contacts the antigen, then that amino acid can be considered to occupy a contact position.

"Conservative substitution" or "conservative amino acid substitution" refers to amino acid substitutions, either from natural mutations or human manipulation, wherein the antibodies produced by such substitutions have substantially the same (or improved or reduced, as may be desirable) activity(ies) as the antibodies disclosed herein.

The term "epitope" as used herein refers to a region of a protein molecule to which an antibody can bind. An "immunogenic epitope" is defined as the part of a protein that elicits an antibody response when the whole protein is the immunogen.

The term "binds" as used herein, generally refers to the interaction of the antibody to the epitope of the antigen. More specifically, the term "binds" relates to the affinity of the antibody to the epitope of the antigen. Affinity is measured by K_D.

The term "inhibit" or "inhibiting" includes the generally accepted meaning, which includes neutralising, prohibiting, preventing, restraining, slowing, stopping, or reversing progression or severity of a disease or condition.

The term "neutralising" or "antagonising" in reference to an anti-TNFSF13b antibody or the phrase "antibody that antagonises TNFSF13b activity" is intended to refer to an antibody or antibody fragment whose binding to TNFSF13b results in inhibition of a biological activity induced by TNFSF13b polypeptides. Inhibition of TNFSF13b biological activity can be assessed by measuring one or more *in vitro* or *in vivo* indicators of TNFSF13b biological activity including, but not limited to, TNFSF13b-induced proliferation, TNFSF13b-induced immunoglobulin secretion, TNFSF13b-induced

prevention of B cell apoptosis, or inhibition of receptor binding in a TNFSF13b receptor binding assay. Indicators of TNFSF13b biological activity can be assessed by one or more of the several *in vitro* or *in vivo* assays known in the art. (see, for example, Moore, P.A., et al., Science, 285:260-263 (1999); Schneider, P., *et al., J. Exp. Med.*, 189:1747-1756 (1999); Shu, H., *et al., J. Leuko. Biol.*, 65:680-683 (1999); Mukhopadhyay, A., *et al., J. Biol. Chem.*, 274:15978-15981 (1999); Mackay, F. *et al., J. Exp. Med.*, 190:1697-1710 (1999); Gross, J.A., *et al., Nature*, 404:995-999 (2000); and Example 2). Preferably, the ability of an antibody to neutralise or antagonise TNFSF13b activity is assessed by inhibition of B cell proliferation as shown in Example 2.

The term "K_{off}", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

The term "K_D", as used herein, is intended to refer to the dissociation constant or the "off" rate divided by the "on" rate, of a particular antibody-antigen interaction. For purposes of the present invention K_D was determined as shown in Example 4

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. Ordinarily, an isolated antibody is prepared by at least one purification step. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, and (2) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue, or preferably, silver stain. Preferably, an "isolated antibody" is an antibody that is substantially free of other antibodies having different antigenic specificities (eg., an isolated antibody that specifically binds hTNFSF13b substantially free of antibodies that specifically bind antigens other than hTNFSF13b polypeptide).

The phrase "nucleic acid molecule" includes DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The phrase "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody fragments (eg., HCVR, LCVR, CDR3) that bind hTNFSF13b polypeptide, includes a nucleic acid molecule in which the nucleotide sequences encoding the antibody, or antibody portion, are free of other nucleotide sequences encoding antibodies or antibody fragments that bind antigens other than hTNFSF13b polypeptide, which other sequences may naturally flank the nucleic acid in human genomic DNA. Thus, for example, an isolated nucleic acid of the invention encoding a HCVR region of an anti-hTNFSF13b human antibody contains no other sequences encoding HCVR regions that bind antigens other than hTNFSF13b polypeptide.

The term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (eg., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (eg.,

non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (eg., replication defective retroviruses, adenoviruses, and adeno-associated viruses), that serve equivalent functions.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "recombinant" in reference to an antibody includes antibodies that are prepared, expressed, created or isolated by recombinant means. Representative examples include antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal (eg., a mouse) that is transgenic for human immunoglobulin genes or antibodies prepared, expressed, created or isolated by any means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences.

The phrase "recombinant host cell" (or simply "host cell") includes a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

Recombinant human antibodies may also be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and, thus, the amino acid sequences of the HCVR and LCVR regions of the recombinant antibodies are sequences that, while derived from those related to human germline HCVR and LCVR sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

Transgenic animals (eg., mice) that are capable, upon immunisation, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production can be

employed. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, eg., Jakobovits, *et al., Proc. Natl. Acad. Sci.* USA, 90:2551-2555, (1993); Jakobovits, *et al., Nature*, 362:255-258, (1993; Bruggemann, *et al., Year in Immun.*, 7:33 (1993); *Nature* 148:1547-1553 (1994), *Nature Biotechnology* 14:826 (1996); Gross, J.A., *et al.*, Nature, 404:995-999 (2000); and U.S. patents nos. 5,877,397, 5,874,299, 5,814,318, 5,789,650, 5,770,429, 5,661,016, 5,633,425, 5,625,126, 5,569,825, and 5,545,806 (each of which is incorporated herein by reference in its entirety for all purposes)). Human antibodies can also be produced in phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1992); Marks, *et al.*, J. Mol. Biol., 222:581 (1991)). The techniques of Cole et al. and Boerner, *et al.* are also available for the preparation of human monoclonal antibodies (Cole, *et al., Monoclonal Antibodies and Cancer Therap*, Alan R. Liss, p. 77 (1985) and Boerner, *et al., J. Immunol.*, 147(I):86-95 (1991)).

"Container" means any receptacle and closure suitable for storing, shipping, dispensing, and/or handling a pharmaceutical product.

"Packaging material" means a customer-friendly device allowing convenient administration and/or ancillary devices that aid in delivery, education, and/or administration. The packaging material may improve antibody administration to the patient, reduce or improve educational instruction time for the patient, provide a platform for improved health economic studies, and/or limit distribution channel workload. Also, the packaging material may include but not be limited to a paper-based package, shrink wrapped package, see-through top packaging, trial-use coupons, educational materials, ancillary supplies, and/or delivery device.

"Package insert" means information accompanying the product that provides a description of how to administer the product, along with the safety and efficacy data required to allow the physician, pharmacist, and patient to make an informed decision regarding use of the product, and/or patient education information. The package insert generally is regarded as the "label" for a pharmaceutical product.

A "subject" means a mammal; preferably a human in need of a treatment. In regards to the present invention subjects in need of treatment include mammals that are suffering from, or are prone to suffer from a disorder in which TNFSF13b activity is detrimental, for example immune diseases, including autoimmune diseases, and inflammatory diseases. Preferred disorders include, but are not limited to, systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, Lyme arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, asthma, allergic diseases, psoriasis, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, infectious diseases, parasitic diseases, female infertility, autoimmune thrombocytopenia, autoimmune thyroid disease, Hashimoto's disease, Sjogren's syndrome, and cancers, particularly B or T cell lymphomas or myelomas.

Various aspects of the invention are described in further detail in the following subsections.

The present invention relates to human monoclonal antibodies that are specific for and neutralise bioactive hTNFSF13b polypeptides. Also disclosed are antibody heavy and light chain amino acid sequences which are highly specific for and neutralise TNFSF13b polypeptides when they

are bound to them. This high specificity enables the anti-hTNFSF13b human antibodies, and human monoclonal antibodies with like specificity, to be immunotherapy of TNFSF13b associated diseases.

In one aspect, the invention provides an isolated human antibody comprising at least one of the amino acid sequences shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, or 16 and that binds a TNFSF13b polypeptide epitope with high affinity, dissociates from a bound TNFSF13b polypeptide with a low K_{off} rate constant of 1 x 10^{-4} s^{-1} or less, and has the capacity to antagonise TNFSF13b polypeptide activity. In one embodiment, the anti-hTNFSF13b human antibody comprises a polypeptide selected from the group consisting of: CDR1 polypeptide of the LCVR as shown in SEQ ID NO: 4; CDR2 polypeptide of the LCVR as shown in SEQ ID NO: 6; CDR3 polypeptide of the LCVR as shown in SEQ ID NO: 8; CDR1 polypeptide of the HCVR as shown in SEQ ID NO: 12; CDR2 polypeptide of the HCVR as shown in SEQ ID NO: 14; and CDR3 polypeptide of the HCVR as shown in SEQ ID NO: 16. In another embodiment, the anti-hTNFSF13b human antibody comprises at least two of the polypeptides selected from the group consisting of: CDR1 polypeptide of the LCVR as shown in SEQ ID NO: 4; CDR2 polypeptide of the LCVR as shown in SEQ ID NO: 6; CDR3 polypeptide of the LCVR as shown in SEQ ID NO: 8; CDR1 polypeptide of the HCVR as shown in SEQ ID NO: 12; CDR2 polypeptide of the HCVR as shown in SEQ ID NO: 14; and CDR3 polypeptide of the HCVR as shown in SEQ ID NO: 16. In another embodiment, the anti-hTNFSF13b human antibody comprises at least three of the polypeptides selected from the group consisting of: CDR1 polypeptide of the LCVR as shown in SEQ ID NO: 4; CDR2 polypeptide of the LCVR as shown in SEQ ID NO: 6; CDR3 polypeptide of the LCVR as shown in SEQ ID NO: 8; CDR1 polypeptide of the HCVR as shown in SEQ ID NO: 12; CDR2 polypeptide of the HCVR as shown in SEQ ID NO: 14; and CDR3 polypeptide of the HCVR as shown in SEQ ID NO: 16. In another embodiment, the anti-hTNFSF13b human antibody comprises at least four of the polypeptides selected from the group consisting of: CDR1 polypeptide of the LCVR as shown in SEQ ID NO: 4; CDR2 polypeptide of the LCVR as shown in SEQ ID NO: 6; CDR3 polypeptide of the LCVR as shown in SEQ ID NO: 8; CDR1 polypeptide of the HCVR as shown in SEQ ID NO: 12; CDR2 polypeptide of the HCVR as shown in SEQ ID NO: 14; and CDR3 polypeptide of the HCVR as shown in SEQ ID NO: 16. In another embodiment, the anti-hTNFSF13b human antibody comprises at least five of the polypeptides selected from the group consisting of: CDR1 polypeptide of the LCVR as shown in SEQ ID NO: 4; CDR2 polypeptide of the LCVR as shown in SEQ ID NO: 6; CDR3 polypeptide of the LCVR as shown in SEQ ID NO: 8; CDR1 polypeptide of the HCVR as shown in SEQ ID NO: 12; CDR2 polypeptide of the HCVR as shown in SEQ ID NO: 14; and CDR3 polypeptide of the HCVR as shown in SEQ ID NO: 16. In another embodiment, the anti-hTNFSF13b human antibody comprises the polypeptides of CDR1 polypeptide of the LCVR as shown in SEQ ID NO: 4; CDR2 polypeptide of the LCVR as shown in SEQ ID NO: 6; CDR3 polypeptide of the LCVR as shown in SEQ ID NO: 8; CDR1 polypeptide of the HCVR as shown in SEQ ID NO: 12; CDR2 polypeptide of the HCVR as shown in SEQ ID NO: 14; and CDR3 polypeptide of the HCVR as shown in SEQ ID NO: 16.

More preferred, the anti-hTNFSF13b human antibody comprises a light chain variable region (LCVR) polypeptide as shown in SEQ ID NO: 2 or a heavy chain variable region (HCVR) polypeptide as shown in SEQ ID NO: 10. Even more preferred, the anti-hTNFSF13b human antibody comprises

the LCVR polypeptide as shown in SEQ ID NO: 2 and the HCVR polypeptide as shown in SEQ ID NO: 10.

In preferred embodiments, the isolated human antibody dissociates from a bound TNFSF13b polypeptide with a K_{off} rate constant of 5 x 10^{-5} s^{-1} or less, and inhibits TNFSF13b induced proliferation in an *in vitro* neutralisation assay with an IC_{50} of 1 x 10^{-7}M or less. In more preferred embodiments, the isolated human antibody dissociates from a bound TNFSF13b polypeptide epitope with a K_{off} rate constant of 1 x 10^{-5} s^{-1} or less and inhibits TNFSF13b induced proliferation in an *in vitro* neutralisation assay with an IC_{50} of 1 x 10^{-8}M or less. In an even more preferred embodiment, the isolated anti-TNFSF13b human antibody dissociates from a bound hTNFSF13b polypeptide with a K_{off} rate constant of 5 x 10^{-6} s^{-1} or less and inhibits TNFSF13b induced proliferation in an *in vitro* assay with an IC_{50} of 1 x 10^{-9}M or less. Examples of anti-hTNFSF13b human antibodies that meet, the aforementioned kinetic and neutralisation criteria include 4A5-3.1.1-B4 antibodies.

The most preferred anti-hTNFSF13b human antibody of the present invention is referred to herein as 4A5-3.1.1-B4. 4A5-3.1.1-B4 has LCVR and HCVR polypeptide sequences as shown in SEQ ID NO:2 and SEQ ID NO:10, respectively. The poly-nucleotide sequence encoding the LCVR and HCVR of 4A5-3.1.1-B4 is shown in SEQ ID NO:1 and SEQ ID NO:9, respectively. The properties of the anti-hTNFSF13b human antibodies of the present invention are specifically disclosed in the Examples. Particularly notable is the high affinity for TNFSF13b polypeptide, slow dissociation kinetics, and high capacity to antagonise TNFSF13b polypeptide activity demonstrated by 4A5-3.1.1-B4.

The K_{off} of an anti-hTNFSF13b human antibody can be determined by surface plasmon resonance as generally described in Example 4. Generally, surface plasmon resonance analysis measures real-time binding interactions between ligand (recombinant TNFSF13b polypeptide immobilised on a biosensor matrix) and analyte (antibodies in solution) by surface plasmon resonance (SPR) using the BIAcore system (Pharmacia Biosensor, Piscataway, NJ). SPR analysis can also be performed by immobilising the analyte (antibodies on a biosensor matrix) and presenting the ligand (recombinant TNFSF13b in solution).

In one aspect, the present invention is also directed to the cell lines which produce the anti-hTNFSF13b human antibodies of the present invention. The isolation of cell lines producing monoclonal antibodies of the invention can be accomplished using routine screening techniques known in the art. A hybridoma which produces an anti-hTNFSF13b human antibody of the present invention has been deposited with ATCC, (ATCC PTA-3674) as disclosed herein.

A wide variety of host expression systems can be used to express the antibodies of the present invention including bacterial, yeast, baculoviral, plant, and mammalian expression systems (as well as phage display expression systems). An example of a suitable bacterial expression vector is pUC119 (Sfi). Other antibody expression systems are also known in the art and are contemplated herein.

An antibody of the invention can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are

expressed in the host cell. Preferably, the recombinant antibodies are secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors, and introduce the vectors into host cells.

The isolated DNA encoding the HCVR region can be converted to a full-length heavy chain gene by operatively linking the HCVR-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2, and CH3). The DNA sequences of human heavy chain constant region genes are known in the art and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region and any allotypic variant therein as described in Kabat, (Kabat, *et al, Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)), but most preferably is an IgG1, or IgG4 constant region. Alternatively, the antibody portion can be an Fab fragment, a Fab' fragment, F(ab')2, or a single chain FV fragment. For a Fab fragment heavy chain gene, the HCVR-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the LCVR region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the LCVR-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The DNA sequences of human light chain constant region genes are known in the art and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region.

To create a scFV gene, the HCVR- and LCVR-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, eg., encoding the amino acid sequence Gly-Gly-Gly-Gly-Ser- Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (Gly4-Ser)3, such that the HCVR and LCVR sequences can be expressed as a contiguous single-chain protein, with the LCVR and HCVR regions joined by the flexible linker (see eg., Bird *et al. Science* 242:423-426 (1988); Huston, *et al., Proc. Natl. Acad Sci.* USA 85:5879-5883 (1988); McCafferty, *et al., Nature* 348:552-554 (1990)).

To express the antibodies of the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. The antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (eg., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Additionally, or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the anti-hTNFSF13b human antibody chain from a host cell. The

anti-hTNFSF13b human antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (ie., a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. Regulatory sequences comprise promoters, enhancers and other expression control elements (eg., polyadenylation signals) that control the transcription or translation of the antibody chain genes. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*eg.*, the adenovirus major late promoter (AdMLP)) and polyoma.

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (eg., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced. For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin, or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, eg., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216-4220 (1980), used with a DHFR selectable marker, eg., as described in R.J. Kaufman and P.A. Sharp, *Mol. Biol.*, 159:601-621 (1982)), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the

antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce portions of intact antibodies, such as Fab fragments of scFV molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody of this invention. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to hTNFSF13b. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In one system for recombinant expression of an antibody of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (eg., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are culture to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Antibodies or antigen-binding portions thereof of the invention can be expressed in an animal (*eg.*, a mouse) that is transgenic for human immunoglobulin genes (see *eg.*, Taylor, L.D., *et al.* Nucl. Acids Res., 20:6287-6295(1992)). Plant cells can also be modified to create transgenic plants that express the antibody or antigen binding portion thereof, of the invention.

In view of the foregoing, another aspect of the invention pertains to nucleic acids, vectors, and host cell compositions that can be used for recombinant expression of the antibodies and antibody portions of the invention. Preferably, the invention features isolated nucleic acids that encode CDRs of 4A5-3.1.1-B4, or the full heavy and/or light chain variable region of 4A5-3.1.1-B4. Accordingly, in one embodiment, the invention features an isolated nucleic acid encoding an antibody heavy chain variable region that encodes the 4A5-3.1.1-B4 heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:16. Preferably, the nucleic acid encoding the antibody heavy chain variable region further encodes a 4A5-3.1.1-B4 heavy chain CDR2 which comprises the amino acid sequence of SEQ ID NO:14. More preferably, the nucleic acid encoding the antibody heavy chain variable region further encodes a 4A5-3.1.1-B4 heavy chain CDR1 which comprises the amino acid sequence of SEQ ID NO:12. Even more preferably, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO:10 (the full HCVR region of 4A5-3.1.1-B4).

In other embodiments, the invention features an isolated nucleic acid encoding an antibody light chain variable region that encodes the 4A5-3.1.1-B4 light chain CDR3 comprising the amino acid

sequence of SEQ ID NO:8 Preferably, the nucleic acid encoding the antibody light chain variable region further encodes a 4A5-3.1.1-B4 light chain CDR1 which comprises the amino acid sequence of SEQ ID NO:4. Even more preferably, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO:2 (the full LCVR region of 4A5-3.1.1-B4).

In other embodiments, the invention features an isolated nucleic acid encoding an antibody light chain variable region that encodes the 4A5-3.1.1-B4 light chain CDR3 comprising the amino acid sequence of SEQ ID NO:8 Preferably, the nucleic acid encoding the antibody light chain variable region further encodes a 4A5-3.1.1-B4 light chain CDR1 which comprises the amino acid sequence of SEQ ID NO:4. Even more preferably, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO:2 (the full LCVR region of 4A5-3.1.1-B4).

In another embodiment, the invention provides an isolated nucleic acid encoding a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO:16 (ie., the 4A5-3.1.1-B4 HCVR CDR3). This nucleic acid can encode only the CDR3 region or, more preferably, encodes an entire antibody heavy chain variable region (HCVR). For example, the nucleic acid can encode a HCVR having a CDR2 domain comprising the amino acid sequence of SEQ ID NO:14 (ie., the 4A5-3.1.1-B4 HCVR CDR2) and a CDR1 domain comprising the amino acid sequence of SEQ ID NO:12 (ie., 4A5-3.1.1-B4 HCVR CDR1).

In still another embodiment, the invention provides an isolated nucleic acid encoding an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO:2 (ie., the 4A5-3.1.1-B4 LCVR). Preferably this nucleic acid comprises the nucleotide sequence of SEQ ID NO:1, although the skilled artisan will appreciate that due to the degeneracy of the genetic code, other nucleotide sequences can encode the amino acid sequence of SEQ ID NO:2. The nucleic acid can encode only the LCVR or can also encode an antibody light chain constant region, operatively linked to the LCVR. In one embodiment, this nucleic acid is in a recombinant expression vector.

In still another embodiment, the invention provides an isolated nucleic acid encoding an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO:10 (ie., the 4A5-3.1.1-B4 HCVR). Preferably this nucleic acid comprises the nucleotide sequence of SEQ ID NO:9, although the skilled artisan will appreciate that due to the degeneracy of the genetic code, other nucleotide sequences can encode the amino acid sequence of SEQ ID NO:10. The nucleic acid can encode only the HCVR or can also encode a heavy chain constant region, operatively linked to the HCVR. For example, the nucleic acid can comprise an IgG1 or IgG4 constant region. In one embodiment, this nucleic acid is in a recombinant expression vector.

Those of ordinary skill in the art are aware that modifications in the amino acid sequence of the antibody can result in an antibody that display equivalent or superior functional characteristics when compared to the original antibody. Alterations in the antibodies of the present invention can include one or more amino acid insertions, deletions, substitutions, truncations, fusions, and the like, either from natural mutations or human manipulation. The present invention encompasses antibodies disclosed herein further comprising one or more amino acid substitutions provided that the substituted

antibodies have substantially the same (or improved or reduced, as may be desirable) activity(ies) as the antibodies disclosed herein. Preferably, a CDR of the present invention has 3 or less conservative substitutions. Preferably, a CDR of the present invention has 2 or less conservative substitutions. Preferably, a CDR of the present invention has one conservative substitution. The skilled artisan will recognise that antibodies having conservative amino acid substitutions can be prepared by a variety of techniques known in the art. For example, a number of mutagenesis methods can be used, including PCR assembly, Kunkel (dut-ung-) and thiophosphate (Amersham Sculptor kit) oligonucleotide-directed mutagenesis. Conservative substitutions of interest are shown in Table 1 along with preferred substitutions.

Table 1. Conservative Substitutions

Residue	Substitutions	Preferred Substitution
Ala (A)	Gly, Val, Leu, Ile, Ser, Met, Thr	Val
Arg (R)	Lys, Gln, Asn, His	Lys
Asn (N)	Gln	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Ala, Ile, Leu, Pro, Ser, Met, Val Val	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala Phe	Ile
Lys (K)	Arg, Gln, Asn, His	Arg
Met (M)	Ala, Gly, Ile, Leu, Phe, Ser, Val	Leu
Phe (F)	Leu, Val, Ile, Ala, Trp, Tyr	Tyr
Pro (P)		
Ser (S)	Ala, Gly, Ile, Leu, Met, Thr, Val	Thr
Thr (T)	Ala, Gly, Ile, Leu, Met, Ser, Val	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ala, Ile, Leu, Met, Ser, Met, Norleu	Leu

The invention also provides recombinant expression vectors encoding an antibody comprising a polypeptide selected from the group consisting of a polypeptide as shown in SEQ ID NO: 2, a polypeptide as shown in SEQ ID NO: 4, a polypeptide as shown in SEQ ID NO: 6, a polypeptide as shown in SEQ ID NO: 8, a polypeptide as shown in SEQ ID NO: 10, a polypeptide as shown in SEQ ID NO: 12, a polypeptide as shown in SEQ ID NO: 14; and a polypeptide as shown in SEQ ID NO: 16.

The invention also provides recombinant expression vectors encoding both an antibody heavy chain and an antibody light chain. For example, in one embodiment, the invention provides a recombinant expression vector encoding:

a) an antibody heavy chain having a variable region comprising the amino acid sequence of SEQ ID NO:10; and

b) an antibody light chain having a variable region comprising the amino acid sequence of SEQ ID NO:2.

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of

the art, including ammonium sulfate precipitation, ion exchange, affinity, reverse phase, hydrophobic interaction column chromatography, gel electrophoresis and the like. Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically or prophylactically, as directed herein.

The antibodies of the present invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody or antibody portion of the invention and a pharmaceutically acceptable diluent, carrier, and/or excipient. The pharmaceutical compositions for administration are designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable diluents, carrier, and/or excipients such as dispersing agents, buffers, surfactants, preservatives, solubilising agents, isotonicity agents, stabilising agents and the like are used as appropriate.

A pharmaceutical composition comprising an anti-hTNFSF13b human antibody of the present invention can be administered to a mammal at risk for or exhibiting autoimmunity related symptoms or pathology such as systemic lupus erythematosus using standard administration techniques by intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration.

The antibodies of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. Peripheral systemic delivery by intravenous or intraperitoneal or subcutaneous injection is preferred. Suitable vehicles for such injections are straightforward. In addition, however, administration may also be effected through the mucosal membranes by means of nasal aerosols or suppositories. Suitable formulations for such modes of administration are well known and typically include surfactants that facilitate cross-membrane transfer.

The pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. Therefore, pharmaceutical compositions may be sterile filtered after making the formulation, or otherwise made microbiologically acceptable. A typical composition for intravenous infusion could have a volume as much as 250mL of fluid, such as sterile Ringer's solution, and 1-100mg per mL, or more in antibody concentration. Therapeutic agents of the invention can all be frozen or lyophilised for storage and reconstituted in a suitable sterile carrier prior to use. Lyophilisation and reconstitution can lead to varying degrees of antibody activity loss (eg. with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies). Dosages may have to be adjusted to compensate. The pH of the formulation will be selected to balance antibody stability (chemical and physical) and comfort to the patient when administered. Generally, pH between 6 and 8 is tolerated.

TNFSF13b plays a critical role in the pathology associated with a variety of diseases involving immune and inflammatory factors. Therefore, a pharmaceutical composition comprising an anti-hTNFSF13b human antibody of the invention can be used to treat disorders in which TNFSF13b activity is detrimental, for example immune diseases including autoimmune diseases and inflammatory diseases. Preferred disorders include, but are not limited to, systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, Lyme arthritis, Crohn's disease,

ulcerative colitis, inflammatory bowel disease, asthma, allergic diseases, psoriasis, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, infectious diseases, parasitic diseases, female infertility, autoimmune thrombocytopenia, autoimmune thyroid disease, Hashimoto's disease, Sjogren's syndrome, and cancers, particularly B or T cell lymphomas or myelomas.

More preferably, a pharmaceutical composition comprising an anti-hTNFSF13b human antibody and/or antibody fragment of the invention is used to treat systemic lupus erythematosus.

The use of the antibody of an anti-hTNFSF13b human antibody of the present invention in the manufacture of a medicament for the treatment of at least one of the aforementioned disorders in which TNFSF13b activity is detrimental is also contemplated herein.

In certain situations, an antibody of the invention will be co-formulated with and/or co-administered with one or more additional therapeutic agents that are used in the treatment of autoimmune and/or inflammatory diseases. Such combination therapies may advantageously utilise lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies. It will be appreciated by the skilled practitioner that when the antibodies of the invention are used as part of a combination therapy, a lower dosage of antibody may be desirable than when the antibody alone is administered to a subject (eg., a synergistic therapeutic effect may be achieved through the use of combination therapy which, in turn, permits use of a lower dose of the antibody to achieve the desired therapeutic effect).

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (eg., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

Given their ability to bind to hTNFSF13b, antibodies, of the invention can be used to detect TNFSF13b polypeptides (eg, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The invention provides a method for detecting TNFSF13b in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of

the invention and detecting either the antibody (or antibody portion) bound to hTNFSF13b or unbound antibody (or antibody portion), to thereby detect hTNFSF13b in the biological sample. The antibody is directly or indirectly labelled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinyl-amine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H.

Alternative to labelling the antibody, TNFSF13b can be assayed in biological fluids by a competition immunoassay utilising TNFSF13b standards labelled with a detectable substance and an unlabelled anti-hTNFSF13b human antibody. In this assay, the biological sample, the labelled TNFSF13b standards and the anti-hTNFSF13b human antibody are combined and the amount of labelled TNFSF13b standard bound to the unlabelled antibody is determined. The amount of TNFSF13b in the biological sample is inversely proportional to the amount of labelled rTNFSF13b standard bound to the anti-hTNFSF13b human antibody.

In another embodiment, the present invention provides a use of an antibody that neutralises TNFSF13b activity by binding an epitope of TNFSF13b. The epitope was identified as described in Example 10. For reference, the soluble portion of hTNFSF13b is represented as follows:

```
Human TNFSF13b      1 AVQGPEETVT QDCLQLIADS ETPTIQKGSY TFVPWLLSFK  40
                   41 RGSALEEKEN KILVKETGYF FIYGQVLYTD KTYAMGHLIQ  80
                   81 RKKVHVFGDE LSLVTLFRCI QNMPETLPNN SCYSAGIAKL 120
                  121 EEGDELQLAI PRENAQISLD GDVTFFGALK LL         152
```

The hTNFSF13b amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise at least one of the amino acids selected from the group consisting of: threonine at position 69, lysine at position 71, threonine at position 72, tyrosine at position 73, glutamic acid at position 105, threonine at position 106, leucine at position 107, and asparagine at position 109. In another embodiment, the amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise at least two of the amino acids selected from the group consisting of: threonine at position 69, lysine at position 71, threonine at position 72, tyrosine at position 73, glutamic acid at position 105, threonine at position 106, leucine at position 107, and asparagine at position 109. In another embodiment, the amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise at least three of the amino acids selected from the group consisting of: threonine at position 69, lysine at position 71, threonine at position 72, tyrosine at position 73, glutamic acid at position 105, threonine at position 106, leucine at position 107, and asparagine at position 109. In another embodiment, the amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise at least four of the amino acids selected from the group consisting of: threonine at position 69, lysine at position 71, threonine at position 72, tyrosine at position 73, glutamic acid at position 105, threonine at position 106, leucine at position 107, and asparagine at position 109.

In another embodiment, the amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise lysine at position 71, threonine at position 72, tyrosine at position 73, and glutamic acid at position 105.

In another embodiment, the amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise glutamic acid at position 105 and at least one of the amino acids selected from the group consisting of: threonine at position 69, lysine at position 71, threonine at position 72, and tyrosine at position 73. In another embodiment, the amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise threonine at position 106 and at least one of the amino acids selected from the group consisting of: threonine at position 69, lysine at position 71, threonine at position 72, and tyrosine at position 73. In another embodiment, the amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise leucine at position 107 and at least one of the amino acids selected from the group consisting of: threonine at position 69, lysine at position 71, threonine at position 72, and tyrosine at position 73. In another embodiment, the amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise asparagine at position 109 and at least one of the amino acids selected from the group consisting of: lysine at position 71, threonine at position 72, and tyrosine at position 73.

In another embodiment, the amino acids involved in binding the novel anti-hTNFSF13b human antibodies comprise lysine at position 71, threonine at position 72, tyrosine at position 73, and glutamic acid at position 105.

The following examples are intended to illustrate but not to limit the invention.

Example 1: Generation of Anti-hTNFSF13b Human Monoclonal Antibodies

Monoclonal antibodies were generated using the HuMAb-Mouse™ technology at Medarex by immunising the mice with soluble hTNFSF13b (amino acids 133–285, purchased from RDI, Flanders, NJ). Both the HCo7 and HCo12 mice were used. Mice were immunised with 15µg to 50µg soluble hTNFSF13b in RIBI, Freund's complete adjuvant or Freund's incomplete adjuvant. Eight mice producing serum antibody titres to hTNFSF13b were injected i.v. with 10µg hTNFSF13b in PBS. The spleen was harvested three days later from each mouse and fused with myeloma cells according to the method described in Zola (Zola, H. Monoclonal antibodies: A Manual of Techniques. CRC Press, Boca Raton, FL. (1987)).

Hybridomas were tested for binding to hTNFSF13b and to make sure they were expressing human immunoglobulin heavy and light chains. Antibody binding to hTNFSF13b was detected by ELISA as follows:

Plates were coated with 50µL of 5µg/mL hTNFSF13b in PBS overnight at 4°C. Plates were then emptied and blocked with 100µL PBS + 0.05% Tween 20 (PBST) + 5% chicken serum for 1 hour at room temperature. After washing three times with PBST, the plates were drained and 100µL diluted secondary reagents (HRP-HuIgGFc, Jackson cat#109-036-098 or HRP-HuKappa, Bethyl cat#A80-115P; 1:5000 in blocking buffer) was added per well. After an 1 hour incubation at room temperature plates were washed three times as described above. Plates were developed using 10mL citrate phosphate buffer pH 4.0, 80µL ABTS, 8µL H_2O_2 per plate. After incubating 30 min. to 1 hour at room temperature, absorbance of the plates was read A415-A490. Hybridomas that showed binding

to hTNFSF13b and that were hulgG heavy chain and human kappa light chain were selected for subcloning.

Cell culture media of subcloned hybridomas was concentrated in Amicon ProFlux M12 tangential filtration systems using an Amicon S3Y30 UF membranes. The concentrated media was passed over protein-A Sephaose columns (5 to 20mL column) at a flow rate of 5mL/min. The columns were washed with buffer A (PBS, pH7.4) until the absorbance returned to baseline and the bound polypeptides were eluted with 50mM citric acid, pH3.2. Fractions were immediately neutralised with 1M Tris, pH8.0. Fractions were then analysed by SDS-PAGE. Fractions containing antibody were pooled and concentrated using an Ultrafree centrifugal filter unit (Millipore, 10kDa molecular weight cut-off).

Example 2: Functional Activity of Anti-hTNFSF13b Human Antibodies

Neutralising activity of the anti-hTNFSF13b human antibodies of the invention was measured using a murine Il-1 dependent B cell line, T1165.17. The cells were washed three times with assay media (RPMI1640 containing 10% FBS, 1mM sodium pyruvate, 5 x 10^{-5}M 2-mercaptoethanol and penicillin, streptomycin and fungizone) to remove IL-1. The cells were resuspended at 100 000 cells/mL in assay media containing 2.5ng/mL soluble huTNFSF13b and plated at 5000 cells/well in a 96 well plate and incubated at 37°C in 5% CO_2. Supernatants from ELISA positive hybridomas were included at a 1:4 dilution. Forty-eight hours later, 20µL of Promega CellTiter 96 Aqueous One Solution (Madison, WI) was added and the plate incubated for 5 more hours at 37°C in 5% CO_2. Absorbance was read at A490, to measure proliferation. An example of neutralisation activity for one of the hybridoma supernatants, 4A5-3.1.1-B4, is shown in Figure 1. As a control, the antibodies were added to IL-1 stimulated cells. There was no evidence of inhibition of IL-1 stimulated proliferation, only the hTNFSF13b stimulated proliferation.

The neutralising antibodies were tested for the ability to inhibit TNFSF13b augmented primary human B cell proliferation in response to anti-IgM stimulation. Primary human B cells were isolated from human blood using CD19 positive selection using the MACS magnetic isolation system (Miltenyi Biotec, Auburn, CA). The B cells were added to wells of a 96-well plate at 2 x 10^5 cells per well in complete RPMI containing 10% FCS (complete RPMI is RPMI1640 containing 10mM L-glutamine, 100U/mL penicillin, 100µg/mL streptomycin, 1mM sodium puruvate, 0.1mM non-essential amino acids, and 1 x 10^{-5}M β-mercaptoethanol). Some of the wells were coated with 10µg/mL anti-human IgM in PBS (BD PharMingen, Clone G20-127), overnight at 4°C and washed four times with PBS before use. Some of the cells were stimulated with soluble hTNFSF13b (25ng/mL) in the presence or absence of neutralising anti-hTNFSF13b antibody (2.5µg/mL). Figure 2 illustrates the ability of 4A5-3.1.1-B4 to neutralise the stimulatory effect of hTNFSF13b.

Example 3: Characterisation of Monoclonal Antibodies

All of the neutralising anti-hTNFSF13b antibodies were either human IgG1 or human IgG4. They were also assayed for their ability to bind to hTNFSF13b in a denatured state, ie., hTNFSF13b separated on SDS-PAGE and blotted onto nitrocellulose. All of the neutralising antibodies failed to bind hTNFSF13b in a Western blot while several of the non-neutralising antibodies were able to do so.

Experiments utilising the BIACore system were performed to determine if non-neutralising antibodies and neutralising antibodies bound to the same site on hTNFSF13b. First, 4A5-3.1.1-B4 was coated onto a chip followed by injection of hTNFSF13b and then a saturating amount of non-neutralising antibody. Once saturation was achieved, a high concentration of 4A5-3.1.1-B4 was run over the chip. Eleven of the non-neutralising monoclonal antibodies were unable to compete for the same binding site as 4A5-3.1.1-B4. One non-neutralising hybridoma was able to block the binding of 4A5-3.1.1-B4 by approximately 45%, indicating that it may have an epitope near the 4A5-3.1.1-B4 epitope.

Using the same experimental design, it was also determined that the neutralising mAb, 4A5-3.1.1-B4, could compete for the same binding site as one of the receptors for hTNFSF13b, TACI. These experiments suggest that TACI-Fc and 4A5-3.1.1-B4 may have overlapping epitopes on hTNFSF13b.

4A5-3.1.1-B4 was immobilised on a solid phase by passing the antibody solution over an IMAC resin loaded with Co^{+2}. Following binding, the cobalt was oxidised to the +3 state by incubation of the resin with a dilute peroxide solution. After washing the resin, native hTNFSF13b and hTNFSF13b that was modified (by reduction/alkylation or by thermal denaturation) was passed over the column. After washing, the bound protein was eluted with an acidic solution and the eluted proteins were analysed by MALDI MS. 4A5-3.1.1-B4 bound native recombinant hTNFSF13b, but did not bind either the chemically or thermally modified hTNFSF13b. Therefore, the 4A5-3.1.1-B4 appears to recognise a conformational epitope on soluble hTNFSF13b.

Recombinant soluble hTNFSF13b (RDI) was incubated with 4A5-3.1.1-B4 or anti-TNFSF13b rabbit polyclonal antibody (MoBiTec, Marco Island, FL; against amino acids 254 to 269 of hTNFSF13b) on ice for 2 hours and the protein mixture was applied to a size-exclusion HPLC (two, tandem TosoHaas TSK-GEL G3000PW columns) equilibrated in PBS at a flow rate of 0.25mL/min. Proteins were eluted with PBS. As controls, antibody solutions and the solution of hTNFSF13b were analysed separately. Human TNFSF13b eluted from the size exclusion column in a position consistent with a trimer of TNFSF13b molecules. The elution of trimeric hTNFSF13b shifted to an earlier timepoint in the presence of 4A5-3.1.1-B4 but not in the presence of anti-TNFSF13b polyclonal antibodies indicating the binding of trimeric hTNFSF13b to the 4A5-3.1.1-B4 antibody. This data suggests that the neutralising mAb 4A5-3.1.1-B4 binds to a conformational epitope on hTNFSF13b.

Example 4: Affinity Measurement of Monoclonal Antibodies by BIAcore

The affinity of various anti-hTNFSF13b human antibodies for hTNFSF13b was measured using a BIAcore 2000 instrument system. The system utilises the optical properties of Surface Plasmon Resonance to detect alteration in protein concentration of interacting molecules within a dextran biosensor matrix. Except where noted, all reagents and materials were purchased from BIAcore AB (Upsala, Sweden). All measurements were performed at 25°C. Samples were dissolved in HBS-EP buffer (150mM NaCl, 3mM EDTA, 0.005% (w/v) surfactant P-20, and 10mM HEPES, pH 7.4). Goat anti-mouse IgG (Fc specific; Jackson Immunoresearch, West Grove, PA) was immobilised on flow cell 1 on a CM5 sensor chip using the amine coupling kit. Goat anti-human IgG (Fc specific; Jackson

Immunoresearch) was immobilised on flow cell 2 also by amine coupling. Both antibodies were immobilised to reach 700 response units each.

Binding of recombinant hTNFSF13b (Research Diagnostics, Inc., Flanders, NJ) was evaluated using multiple analytical cycles. Each cycle was performed at a flow rate of 30µL/min. and consisted of the following steps: injection of 150µL of 4A5-3.1.1-B4 at 20µg/mL, injection of 250µL of hTNFSF13b (starting at 50nM and using 2 fold serial dilutions for each cycle) followed by 15 minutes for dissociation, and regeneration using 90µL of 10mM glycine HCl, pH1.5.

Association and dissociation rates for each cycle were evaluated using a Langmuir 1:1 binding model in the BIAevaluation software. The K_D of 4A5-3.1.1-B4 for hTNFSF13b was determined to be 38pM.

Example 5: Cloning and Sequencing of Heavy and Light Chain Antigen Binding Regions

The variable region for the heavy and light chain for the neutralising human mAb 4A5-3.1.1-B4 were cloned and sequenced using the following protocols.

mRNA was prepared from 2 x 10^6 hybridoma cells using the Micro-Fast Track protocol (Invitrogen) supplied with the kit. cDNA was prepared from 200µL of ethanol precipitate of mRNA using cDNA Cycle kit (Invitrogen) by spinning the aliquot of mRNA for 30 min. at 14 000rpm at 4°C followed by washing the pellet with 70% ethanol. The air dried pellet was resuspended in 11.5µL of sterile water and cDNA was prepared following the kit's instructions. The optional second round of cDNA synthesis was omitted but the cDNA was cleaned using the pheno/chloroform extraction step and ethanol precipitation. The cDNA pellet was resuspended in 30µL of TE for use in PCR.

The PCR reactions were set up with degenerate primers at the 5' end of the variable region for the heavy and light chain paired with 3' primers in the constant region. For each 50µL reaction, 1µL of cDNA was used. The reaction was set up as directed for use with Pful followed by 20 cycles. The PCR products were checked by running 5µL of each reaction on a 1% agarose gel. The positive reactions were cloned using the Zero Blunt TOPO PCR cloning kit (Invitrogen). Minipreps from the positive clones were sequenced and analysed for productive gene rearrangements. Results from independent PCR reactions and sequencing of multiple clones revealed the sequences as described below.

Human antibody 4A5-3.1.1-B4 light chain sequences (CDRs are in bold).

```
        E ·I  V   L  T  Q  S   P  A  T   L  S  L   S  P  G  E
    1   GAAATTGTGT TGACGCAGTC TCCAGCCACC CTGTCTTTGT CTCCAGGGGA
        CTTTAACACA ACTGCGTCAG AGGTCGGTGG GACAGAAACA GAGGTCCCCT
                                          CDR1
        R ·A  T   L  S  C  R  A  S  Q   S  V  S   R  Y  L
   51   AAGAGCCACC CTCTCCTGCA GGGCCAGTCA GAGTGTTAGC CGCTACTTAG
        TTCTCGGTGG GAGAGGACGT CCCGGTCAGT CTCACAATCG GCGATGAATC

        A  W  Y  Q   Q  K  P   G  Q  A   P  R  L  L   I  Y  D
  101   CCTGGTACCA GCAGAAACCT GGCCAGGCTC CCAGGCTCCT CATCTATGAT
```

GGACCATGGT CGTCTTTGGA CCGGTCCGAG GGTCCGAGGA GTAGATACTA

CDR2

A ·S N R A T G I P A R F S G S G S

151 GCATCCAACA GGGCCACTGG CATCCCAGCC AGGTTCAGTG GCAGTGGGTC

CGTAGGTTGT CCCGGTGACC GTAGGGTCGG TCCAAGTCAC CGTCACCCAG

G T D S T L T I S S L E P E D F

201 TGGGACAGAC TCCACTCTCA CCATCAGCAG CCTAGAGCCT GAAGATTTTG

ACCCTGTCTG AGGTGAGAGT GGTAGTCGTC GGATCTCGGA CTTCTAAAAC

CDR3

A V Y Y C **Q Q R S N W P R T** F G Q

251 CAGTTTATTA CTGTCAGCAG CGTAGCAACT GGCCTCGGAC GTTCGGCCAA

GTCAAATAAT GACAGTCGTC GCATCGTTGA CCGGAGCCTG CAAGCCGGTT

Ck

G ·T K V E I K R T V A A P S V F I

301 GGGACCAAGG TGGAAATCAA ACGAACTGTG GCTGCACCAT CTGTCTTCAT

CCCTGGTTCC ACCTTTAGTT TGCTTGACAC CGACGTGGTA GACAGAAGTA

· F P

351 CTTCCCG

GAAGGGC

Human antibody 4A5-3.1.1-B4 heavy chain sequences (CDRs are in bold, signal sequence is italicised).

M ·K H L W F F L L L V A A P R W V

1 ATGAAACACC TGTGGTTCTT CCTCCTCCTG GTGGCAGCTC CCAGATGGGT

TACTTTGTGG ACACCAAGAA GGAGGAGGAC CACCGTCGAG GGTCTACCCA

L S Q V Q L Q Q W G A G L L K P

51 CCTGTCCCAG GTGCAACTAC AGCAGTGGGG CGCAGGACTG TTGAAGCCTT

GGACAGGGTC CACGTTGATG TCGTCACCCC GCGTCCTGAC AACTTCGGAA

S E T L S L T C A V Y **G G S F S** G

101 CGGAGACCCT GTCCCTCACC TGCGCTGTCT ATGGTGGGTC CTTCAGTGGT

GCCTCTGGGA CAGGGAGTGG ACGCGACAGA TACCACCCAG GAAGTCACCA

CDR1

Y ·Y W S W I R Q P P G K G L E W I

151 TACTACTGGA GCTGGATCCG CCAGCCCCCA GGGAAGGGGC TGGAGTGGAT

ATGATGACCT CGACCTAGGC GGTCGGGGGT CCCTTCCCCG ACCTCACCTA

CDR2

```
        G· E  I   N  H  S   G  S  T  N   Y  N  P   S  L  K
201     TGGGGAAATC AATCATAGTG GAAGCACCAA CTACAACCCG TCCCTCAAGA
        ACCCCTTTAG TTAGTATCAC CTTCGTGGTT GATGTTGGGC AGGGAGTTCT

        S  R  V  T   I  S  V   D  T  S   K  N  Q  F   S  L  K
251     GTCGAGTCAC CATATCAGTA GACACGTCCA AGAACCAGTT CTCCCTGAAA
        CAGCTCAGTG GTATAGTCAT CTGTGCAGGT TCTTGGTCAA GAGGGACTTT

        L ·S  S   V  T  A  A   D  T  A   V  Y  Y   C  A  R  G
301     CTGAGCTCTG TGACCGCCGC GGACACGGCT GTGTATTACT GTGCGAGAGG
        GACTCGAGAC ACTGGCGGCG CCTGTGCCGA CACATAATGA CACGCTCTCC
                   [CDR3]

        Y  Y  D   I  L  T   G  Y  Y  Y   Y  F  D   Y  W  G
351     GTATTACGAT ATTTTGACTG GTTATTATTA CTACTTTGAC TACTGGGGCC
        CATAATGCTA TAAAACTGAC CAATAATAAT GATGAAACTG ATGACCCCGG
                                                  [Cγ1]
                                                  ------->
        Q  G  T  L   V  T  V   S  S  A   S  T  K  G   P  S  V
401     AGGGAACCCT GGTCACCGTC TCCTCAGCCT CCACCAAGGG CCCATCGGTC
        TCCCTTGGGA CCAGTGGCAG AGGAGTCGGA GGTGGTTCCC GGGTAGCCAG

        F  P  L   A
451     TTCCCCCTGG CA
        AAGGGGGACC GT
```

Example 6: Species Crossreactivity of Anti-hTNFSF13b Human Antibodies with non-human TNFSF13b

In order to determine the species crossreactivity of the neutralising mAbs, an ELISA was set up utilising 4A5-3.1.1-B4 as both the capture and detecting mAb. Human recombinant TNFSF13b was used as the standard curve. Human TNFSF13b could be detected in the culture supernatant from CHO cells transfected with a vector expressing hTNFSF13b, supernatants from cultured human monocytes or human serum or plasma. Supernatants from CHO cells expressing murine TNFSF13b were tested for reactivity in the ELISA and were negative. 4A5-3.1.1-B4 was also unable to immunopreciptate murine TNFSF13b but was able to immunoprecipitate human TNFSF13b. Murine TNFSF13b was used in the proliferation assay described in Example 2. Using this proliferation assay, 4A5-3.1.1-B4 was unable to neutralise the proliferation induced by murine TNFSF13b. This indicates that 4A5-3.1.1-B4 is unable to recognise murine TNFSF13b.

Example 7: Amino Acid sequence of Heavy Chain 4A5-3.1.1-B4

Below is the amino acid sequence (SEQ ID NO:17) of the heavy chain 4A5-3.1.1-B4 antibody which comprises the HCVR and the IgG4 constant region. The human IgG4 constant region has a

serine at position 231. However, this position at 231 was substituted from a serine to a proline which introduces a structural change in the hinge region for obtaining optimal inter-chain disulfide bonds. This reduces the generation of half antibodies. Half antibodies are formed from one heavy chain and one light chain.

```
  1   QVQLQQWGAG LLKPSETLSL TCAVYGGSFS GYYWSWIRQP PGKGLEWIGE
 51   INHSGSTNYN PSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARGYY
101   DILTGYYYYF DYWGQGTLVT VSSASTKGPS VFPLAPCSRS TSESTAALGC
151   LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG
201   TKTYTCNVDH KPSNTKVDKR VESKYGPPCP PCPAPEFLGG PSVFLFPPKP
251   KDTLMISRTP EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN
301   STYRVVSVLT VLHQDWLNGK EYKCKVSNKG LPSSIEKTIS KAKGQPREPQ
351   VYTLPPSQEE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
401   LDSDGSFFLY SRLTVDKSRW QEGNVFSCSV MHEALHNHYT QKSLSLSLGK
```

In addition, an alanine for phenylalanine substitution at position 237 and an alanine or glutamic acid substitution for leucine at position 238 can be made to lessen the effector function of the antibody.

Example 8: Amino Acid sequence of Heavy Chain 4A5-3.1.1-B4

Below is the amino acid sequence (SEQ ID NO: 18) of the heavy chain 4A5-3.1.1-B4 antibody which comprises the HCVR and the IgG1 constant region.

```
  1   QVQLQQWGAG LLKPSETLSL TCAVYGGSFS GYYWSWIRQP PGKGLEWIGE
 51   INHSGSTNYN PSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARGYY
101   DILTGYYYYF DYWGQGTLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC
151   LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG
201   TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP
251   PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
301   QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
351   EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT
401   PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
451   PGK
```

Example 9: Amino Acid sequence of Light Chain 4A5-3.1.1-B4

Below is the amino acid sequence (SEQ ID NO: 19) of the light chain 4A5-3.1.1-B4 antibody which comprises the LCVR and the kappa constant region.

```
  1   EIVLTQSPAT LSLSPGERAT LSCRASQSVS RYLAWYQQKP GQAPRLLIYD
 51   ASNRATGIPA RFSGSGSGTD STLTISSLEP EDFAVYYCQQ RSNWPRTFGQ
101   GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
151   DNALQSGNSQ ESVTEQDSKD STYSLSNTLT LSKADYEKHK VYACEVTHQG
201   LSSPVTKSFN RGEC
```

Example 10: Identification of the Epitope for 4A5-3.1.1-B4

The epitope to which 4A5-3.1.1-B4 bound and neutralised human TNFSF13b was determined.

Human and murine TNFSF13b sequences were aligned as shown below:

```
Mouse TNFSF13b   1 AFQGPEETEQ DVDLSAPPAP CLPGCRHSQH DDNGMNLRNI IQDCLQLIA 49
Human TNFSF13b   1 AVQGPEE--- ---------- ---------- --------TV TQDCLQLIA 18
Mouse TNFSF13b  50 DSDTPTIRKG TYTFVPWLLS FKRGNALEEK ENKIVVRQTG YFFIYSQVLY 99
Human TNFSF13b  19 DSETPTIQKG SYTFVPWLLS FKRGSALEEK ENKILVKETG YFFIYGQVLY 68
Mouse TNFSF13b 100 TDPIFAMGHV IQRKKVHVFG DELSLVTLFR CIQNMPKTLP NNSCYSAGIA 149
Human TNFSF13   69 TDKTYAMGHL IQRKKVHVFG DELSLVTLFR CIQNMPETLP NNSCYSAGIA 118
Mouse TNFSF13b 150 RLEEGDEIQL AIPRENAQIS RNGDDTFFGA LKLL 183
Human TNFSF13b 119 KLEEGDELQL AIPRENAQIS LDGDVTFFGA LKLL 152
```

A homology model was created for human TNFSF13b based on the known crystal structure for several TNF family members. Exposed residues that are different between mouse and human TNFSF13b are potential binding sites for 4A5-3.1.1-B4 since 4A5-3.1.1-B4 neutralises human but not mouse TNFSF13b.

Three potential epitopes were identified: 1) K71, T72, Y73, E105; 2) Q26, S29, L139, D140; and 3) L53, K55, E56, K119. Mutagenesis was performed to make chimeric molecules by changing the amino acid sequence from human to mouse. Chimera A was L139R, D140N; Chimera B was K71P, T72I, Y73F; Chimera C was K71P, T72I, Y73F, E105K; Chimera D was L53V, K55R, E56Q; Chimera E was E105K.

Using the proliferation assay as described in Example 2, all of the chimeras were tested for functional activity and neutralisation by 4A5-3.1.1-B4. Initial assays were performed using supernatants from 293 transient transfections for each of the chimeras and both human TNFSF13b and murine TNFSF13b parent molecules. All of the chimeras induced similar proliferation indicating that the chimeras produced were functional. Using 6µg/mL of 4A5-3.1.1-B4, 100% neutralisation was observed with human TNFSF13b and chimeras A, B, D and E. No neutralisation was observed for murine TNFSF13b or chimera C. Purified TNFSF13b mutants were produced for chimera A, B, and C and the assay was repeated using 11 ng/mL of each parent TNFSF13b or chimera TNFSF13b and 1µg/mL of 4A5-3.1.1-B4. The results showed 100% neutralisation was observed with human TNFSF13b and chimera A, 88% neutralisation with chimera B, and no neutralisation was observed for murine TNFSF13b or chimera C.

Example 11: In vivo studies using 4A5-3.1.1-B4

Transgenic mice overexpressing soluble human TNFSF13b are generated using established techniques as described by Hogan, B. et al. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual*. Cold Spring Harbor Laboratory, NY] as modified by Fox and Solter (Mol. Cell. Biol. 8: 5470, 1988). Briefly, a DNA fragment encompassing the hTNFSF13b gene is microinjected into the male pronuclei of newly fertilised one-cell-stage embryos (zygotes) of the FVB/N strain. The embryos are cultured in vitro overnight to allow development to the two-cell-stage. Two-cell embryos are then transplanted into the oviducts of pseudopregnant CD-1 strain mice to allow development to term. To test for the presence of the transgene in the newborn mice, a small piece of toe is removed from each animal and digested with proteinase K to release the nucleic acids. A sample of the toe extract is subsequently subjected to PCR analysis to identify transgene-containing mice.

The hTNFSF13b transgenic mice had a dramatic increase in peripheral B cells, generally about three fold compared to age and sex matched littermates. There was a slight increase in peripheral T cells as well. The hTNFSF13b transgenic mice were treated with 4A5-3.1.1-B4 to determine if neutralisation of hTNFSF13b would result in a reduction in B cell numbers back to normal levels. At 15 weeks old, female hTNFSF13b mice were injected subcutaneously twice a week for three weeks with either 25 ug of 4A5-3.1.1-B4 or isotype control antibody. Four days after the last injection of antibody, the mice were sacrificed and the spleen removed for analysis. B and T cell numbers were calculated by determining the percentage of CD19+ cells, for B cells, and CD3+ cells, for T cells using flow cytometry and absolute white blood cell count for each spleen. The results are shown below demonstrate that in vivo administration of 4A5-3.1.1-B4 to hTNFSF13b transgenic mice is able to restore the normal numbers of T and B cells (average ± standard deviation)

	B cells (x10^6)	T cells (x10^6)
Treatment Group		
Wild type littermates	29 ± 11	46 ± 15
Transgenic + Isotype mAb	122 ± 30	75 ± 14
Transgenic + 4A5 mAb	29± 5	46 ± 12

Sequences of the present invention:

SEQ ID NO:1 → polynucleotide sequence encoding light chain variable region

GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCTTTGTCTCCAGG
GGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCCGCTACT
TAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT
GATGCATCCAACAGGGCCACTGGCATCCCAGCCAGGTTCAGTGGCAGTGG
GTCTGGGACAGACTCCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATT
TTGCAGTTTATTACTGTCAGCAGCGTAGCAACTGGCCTCGGACGTTCGGC
CAAGGGACCAAGGTGGAAATCAAACGAACT

SEQ ID NO:2 → amino acid sequence encoding light chain variable region

eivltqspatlslspgeratlscrasqsvsrylawyqqkpgqaprlliydasnratgiparfsgsgsgtdstlt
isslepedfavyycqqrsnwprtfgqgtkveikrt

SEQ ID NO:3 → polynucleotide sequence encoding light chain CDR1

AGGGCCAGTCAGAGTGTTAGCCGCTACTTAGCC

SEQ ID NO:4 → amino acid sequence encoding light chain CDR1

RASQSVSRYLA

SEQ ID NO:5 → polynucleotide sequence encoding light chain CDR2

GATGCATCCAACAGGGCCACT

SEQ ID NO:6 → amino acid sequence encoding light chain CDR2

DASNRAT

SEQ ID NO:7 → polynucleotide sequence encoding light chain CDR3

CAGCAGCGTAGCAACTGGCCTCGGACG

SEQ ID NO:8 → amino acid sequence encoding light chain CDR3

QQRSNWPRT

SEQ ID NO:9 → polynucleotide sequence encoding heavy chain variable region

ATGAAA
CACCTGTGGTTCTTCCTCCTCCTGGTGGCAGCTCCCAGATGGGTCCTGTC
CCAGGTGCAACTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGA
CCCTGTCCCTCACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTAC
TGGAGCTGGATCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGA
AATCAATCATAGTGGAAGCACCAACTACAACCCGTCCCTCAAGAGTCGAG
TCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAACTGAGC
TCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGGGTATTA
CGATATTTTGACTGGTTATTATTACTACTTTGACTACTGGGGCCAGGGAA
CCCTGGTCACCGTCTCCTCA

SEQ ID NO:10 → amino acid sequence encoding heavy chain variable region

mkhlwffllvaaprwvlsqvqlqqwgagllkpsetlsltcavyggsfsgyywswirqppgkglewigeinhsg
stnynpslksrvtisvdtsknqfslklssvtaadtavyycargyydiltgyyyyfdywgqgtlvtvss

SEQ ID NO:11 → polynucleotide sequence encoding heavy chain CDR1

GGTGGGTCCTTCAGTGGTTACTACTGGAGC

SEQ ID NO:12 → amino acid sequence encoding heavy chain CDR1

GGSFSGYYWS

SEQ ID NO:13 → polynucleotide sequence encoding heavy chain CDR2

GAAATCAATCATAGTGGAAGCACCAACTACAACCCGTCCCTCAAGAGT

SEQ ID NO:14 → amino acid sequence encoding heavy chain CDR2

EINHSGSTNYNPSLKS

SEQ ID NO:15 → polynucleotide sequence encoding heavy chain CDR3

GGGTATTACGATATTTTGACTGGTTATTATTACTACTTTGACTAC

SEQ ID NO:16 → amino acid sequence encoding heavy chain CDR3

GYYDILTGYYYYFDY

SEQ ID NO: 17 → heavy chain 4A5-3.1.1-B4 antibody

```
   1   QVQLQQWGAG LLKPSETLSL TCAVYGGSFS GYYWSWIRQP PGKGLEWIGE
  51   INHSGSTNYN PSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARGYY
 101   DILTGYYYYF DYWGQGTLVT VSSASTKGPS VFPLAPCSRS TSESTAALGC
 151   LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG
 201   TKTYTCNVDH KPSNTKVDKR VESKYGPPCP PCPAPEFLGG PSVFLFPPKP
 251   KDTLMISRTP EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN
 301   STYRVVSVLT VLHQDWLNGK EYKCKVSNKG LPSSIEKTIS KAKGQPREPQ
 351   VYTLPPSQEE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
 401   LDSDGSFFLY SRLTVDKSRW QEGNVFSCSV MHEALHNHYT QKSLSLSLGK
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SEQ ID NO: 18 → heavy chain 4A5-3.1.1-B4 antibody

```
   1   QVQLQQWGAG LLKPSETLSL TCAVYGGSFS GYYWSWIRQP PGKGLEWIGE
  51   INHSGSTNYN PSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARGYY
 101   DILTGYYYYF DYWGQGTLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC
 151   LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG
 201   TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP
 251   PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
 301   QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 351   EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT
 401   PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
 451   PGK
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SEQ ID NO: 19 → light chain 4A5-3.1.1-B4 antibody

```
   1   EIVLTQSPAT LSLSPGERAT LSCRASQSVS RYLAWYQQKP GQAPRLLIYD
  51   ASNRATGIPA RFSGSGSGTD STLTISSLEP EDFAVYYCQQ RSNWPRTFGQ
 101   GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
 151   DNALQSGNSQ ESVTEQDSKD STYSLSNTLT LSKADYEKHK VYACEVTHQG
 201   LSSPVTKSFN RGEC
```

The claims defining the invention are as follows:

1. An anti-hTNFSF13b human antibody comprising at least three of the polypeptides selected from the group consisting of:

a. SEQ ID NO: 4 located at CDR1 of the light chain variable region (LCVR);

b. SEQ ID NO: 6 located at CDR2 of the LCVR;

c. SEQ ID NO: 8 located at CDR3 of the LCVR;

d. SEQ ID NO: 12 located at CDR1 of the heavy chain variable region (HCVR);

e. SEQ ID NO: 14 located at CDR2 of the HCVR; and

f. SEQ ID NO: 16 located at CDR3 of the HCVR.

2. The antibody of claim 1 comprising at least four of the polypeptides selected from the group consisting of:

a. SEQ ID NO: 4 located at CDR1 of the light chain variable region (LCVR);

b. SEQ ID NO: 6 located at CDR2 of the LCVR;

c. SEQ ID NO: 8 located at CDR3 of the LCVR;

d. SEQ ID NO: 12 located at CDR1 of the heavy chain variable region (HCVR);

e. SEQ ID NO: 14 located at CDR2 of the HCVR; and

f. SEQ ID NO: 16 located at CDR3 of the HCVR.

3. The antibody of claim 1 comprising at least five of the polypeptides selected from the group consisting of:

a. SEQ ID NO: 4 located at CDR1 of the light chain variable region (LCVR);

b. SEQ ID NO: 6 located at CDR2 of the LCVR;

c. SEQ ID NO: 8 located at CDR3 of the LCVR;

d. SEQ ID NO: 12 located at CDR1 of the heavy chain variable region (HCVR);

e. SEQ ID NO: 14 located at CDR2 of the HCVR; and

f. SEQ ID NO: 16 located at CDR3 of the HCVR.

4. The antibody of claim 1 comprising the polypeptides of:

a. SEQ ID NO: 4 located at CDR1 of the light chain variable region (LCVR);

b. SEQ ID NO: 6 located at CDR2 of the LCVR;

c. SEQ ID NO: 8 located at CDR3 of the LCVR;

d. SEQ ID NO: 12 located at CDR1 of the heavy chain variable region (HCVR);

e. SEQ ID NO: 14 located at CDR2 of the HCVR; and

f. SEQ ID NO: 16 located at CDR3 of the HCVR.

5. An anti-hTNFSF13b human antibody comprising a light chain variable region (LCVR) polypeptide as shown in SEQ ID NO: 2 or a heavy chain variable region (HCVR) polypeptide as shown in SEQ ID NO: 10.

6. The antibody of claim 5 comprising the LCVR polypeptide as shown in SEQ ID NO: 2 and the HCVR polypeptide as shown in SEQ ID NO: 10.

7. The antibody of claim 6 further comprising an IgG4 constant region with a proline substituted for serine at position 231.

8. An antibody that neutralizes TNFSF13b activity by binding an epitope of TNFSF13b, wherein the epitope comprises lysine at position 71, threonine at position 72, tyrosine at position 73, and glutamic acid at position 105.

9. An antibody or fragment thereof comprising an amino acid sequence of heavy chain 4A5-3.1.1-B4 represented by SEQ ID NO 17 and an amino acid sequence of light chain 4A5-3.1.1-B4 represented SEQ ID NO 19.

10. A pharmaceutical composition comprising the antibody of any one of claims 1-9.

11. A use of the antibody of any one of claims 1-9 in the manufacture of a medicament for administration to subject suffering from a disorder selected from the group consisting of systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, Lyme arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, asthma, allergic diseases, psoriasis, acute or chronic immune disease associated with organ transplantation, organ transplant rejection, graft-versus-host disease, sarcoidosis, infectious diseases, parasitic diseases, female infertility, autoimmune thrombocytopenia, autoimmune thyroid disease, Hashimoto's disease, Sjogren's syndrome, and cancer.

12. An anti-hTNFSF13b human antibody substantially as hereinbefore described with reference to any one of the examples.

13. An antibody that neutralizes TNFSF13b activity substantially as hereinbefore described with reference to any one of the examples.

14. A pharmaceutical composition comprising the antibody of claims 12 or 13.

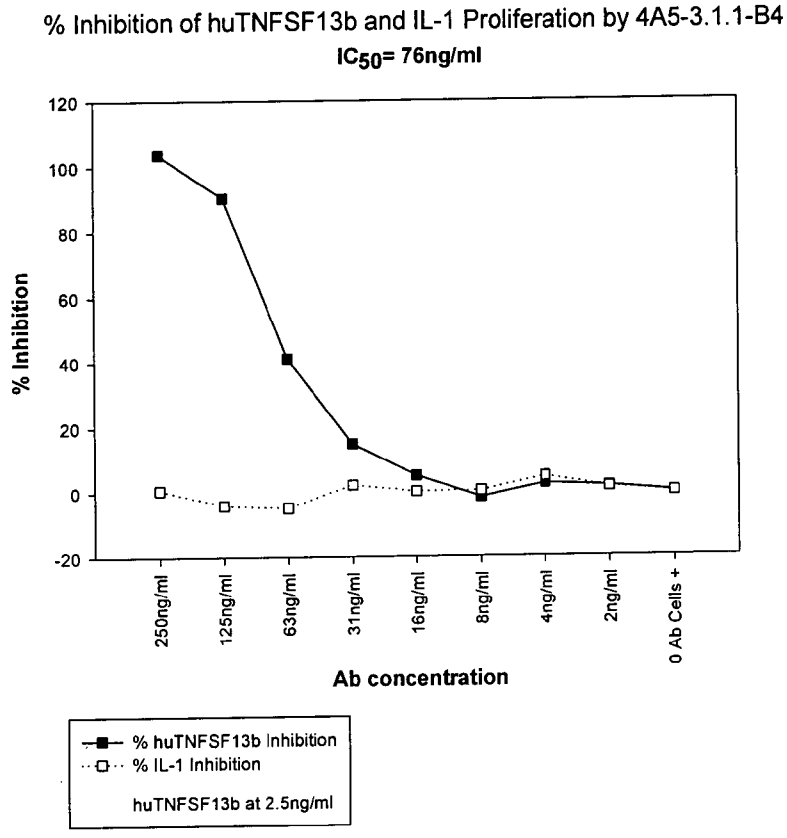
15. A use of the antibody of claim 12 or 13 for the manufacture of a medicament for administration to subject suffering from a disorder selected from the group consisting of systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, Lyme arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, asthma, allergic diseases, psoriasis, acute or chronic immune disease associated with organ transplantation, organ transplant rejection, graft-versus-host disease, sarcoidosis,

infectious diseases, parasitic diseases, female infertility, autoimmune thrombocytopenia, autoimmune thyroid disease, Hashimoto's disease, Sjogren's syndrome, and cancer.

Dated 11 November, 2008
Eli Lilly and Company

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

Fig. 1/2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IC50= 76ng/ml
% Inhibition
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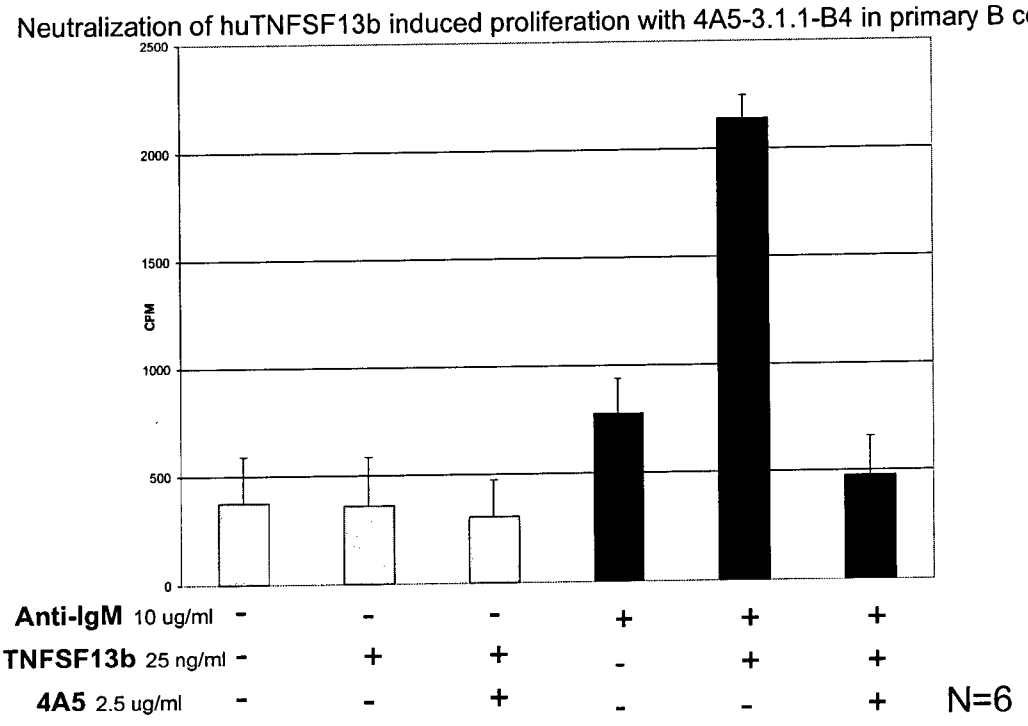
100
80
60
40
20
0
-20
250ng/ml
125ng/ml
63ng/ml
31ng/ml
16ng/ml
8ng/ml
4ng/ml
2ng/ml
0 Ab Cells +
Ab concentration
% huTNFSF13b Inhibition
% IL-1 Inhibition
huTNFSF13b at 2.5ng/ml

Fig. 2/2


Neutralization of huTNFSF13b induced proliferation with 4A5-3.1.1-B4 in primary B cells
2500
2000
1500
CPM
1000
500
0
Anti-IgM 10 ug/ml - - - + + +
TNFSF13b 25 ng/ml - + + - + +
4A5 2.5 ug/ml - - + - - +
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Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Ser Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
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            20                  25                  30
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        35                  40                  45
Ser Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu
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Glu Trp Ile Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro
65                  70                  75                  80
Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln
                85                  90                  95
Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
            100                 105                 110
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Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu
        35                  40                  45
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    50                  55                  60
Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys
65                  70                  75
Asn Gln Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr
                80                  85                  90
Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Tyr Asp Ile Leu Thr Gly
            95                  100                 105
Tyr Tyr Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
        110                 115                 120
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
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Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys
140                 145                 150
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
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Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
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Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
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Lys Pro Ser Gln Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr
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Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
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Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
        290                 295                 300
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
    305                 310                 315
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
320                 325                 330
Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
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Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu
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Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
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His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
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Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
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Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
395                 400                 405
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
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Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
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        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Ser Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Arg
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
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Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175
Asn Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205
Phe Asn Arg Gly Glu Cys
    210                 214
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