# Antagonists of IL17C for the treatment of inflammatory disorders

## Cross Reference to Related Applications

This application claims the benefit of U.S. provisional application serial number 61/548,744 filed October 19, 2011, which is incorporated by reference in its entirety.

## Field of the invention

The present application relates to antagonists of IL17C for use in the treatment of inflammatory disorders, such as arthritis. Exemplary IL17C antagonists are IL17C-specific antibodies or fragments thereof, such as human anti-IL17C antibodies.

## Background

IL17C is a secreted disulfide-linked homodimer of the IL17 protein family. *In vitro* it has been shown that IL17C stimulates the release of TNF-α and IL-1β from the monocytic cell line THP-1 (Li et al. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 773-8). IL17C can induce the mRNA expression of inflammatory cytokines such as IL-1β, IL-6 and IL-23 in peritoneal exudates cells (PECS) and the 3T3 cell line (Yamaguchi et al. (2007) J. Immunol 179, 7128-36. *In vivo* CD4+ T cells transduced with IL17C exacerbated collagen induced arthritis (CIA) in mice and mice reconstituted with bone marrow cells transduced with IL17C suffered from severe collagen induced arthritis. IL17C was reported to bind IL-17 receptor E (IL17RE/Fc) and seemed to activate NF-κB (Calhoun (2007) Bachelor's Thesis: Generation of proof of concept molecules: Neutralizing monoclonal antibodies to IL17C; Oregon State University, University Honours College; Gaffen (2009) Nat Rev. Immunol 9, 556-67) and was also reported to affect nuclear IkappaB family member, IkBζ in Th17 cells activation in IL17C deficient mice (Chang et al. (2011) Immunity 35, 1-11). IL17C expression in normal tissue seems to be restricted to adult and fetal kidney. The mRNA expression of IL17C in the arthritic paws of CIA mice is highly elevated. Hwang et al. described IL17C expression in mononuclear cells of synovial fluid and peripheral blood of rheumatoid arthritis patients (Hwang & Kim (2005) Mol Cells 19, 180-184).

WO 99/060127 describes the cloning of IL17C (PRO1122). WO 99/060127 loosely associated certain disorders with IL17C, including arthritis (e.g., osteoarthritis, rheumatoid

arthritis, psoriatic arthritis), sepsis, ulcerative colitis, psoriasis, multiple sclerosis, type I diabetes, giant cell arthritis, systemic lupus erythematosus and Sjogren's syndrome. It is also contemplated that the compounds of WO 99/060127 may be used to treat various conditions, including those characterized by overexpression and/or activation of the disease-associated genes identified herein. However, no experimental proof is provided for this speculative function of IL17C. The same holds true for WO 2005/065711.

The present invention for the first time demonstrates in *in vivo* experiments that antagonists of IL17C are highly effective in the treatment of inflammatory disorders.

## Summary of the invention

The present invention provides antagonists of IL17C for use in the treatment of an inflammatory disorder.

The inflammatory disorder treated with the antagonists of the present invention may be any inflammatory disorder selected from arthritis, such as rheumatoid arthritis, asthma, sepsis, an autoimmune disease, such as inflammatory bowel disease, COPD (chronic obstructive pulmonary disease), systemic lupus erythematosus (SLE) and sarcoidosis. In particular embodiments, said inflammatory disorder is arthritis.

The antagonists of IL17C of the present invention may be any antagonist. Preferably, said antagonist is an antibody or antibody fragment, such as a monoclonal antibody. Said antibody may be an antibody or fragment thereof specific for IL17C or an antibody or fragment thereof specific for the receptor of IL17C.

## Figure legends

Figure 1A shows the assay set up for a receptor interaction assay. ECD fusion proteins of IL17 receptors were coated on a Multi-array® 384-well plate. Biotinylated mouse IL17C was applied and detected via Streptavidin.

Figure 1B shows results of the receptor interaction assay shown in Figure 1A. IL17C was clearly found to bind to mouse IL17RE/Fc, but not to mouse IL-17RB or another irrelevant receptor.

Figure 1C shows results of the receptor interaction assay shown in Figure 1A. None of the three prior art antibodies inhibits binding of mouse IL17C to mouse IL17RE/Fc.

Figure 2 shows quality control results based on size exclusion chromatography (SEC).

Figure 3 shows results of $EC_{50}$ determination in ELISA. All purified chimeric human-mouse chimeric IgG2a antibodies were titrated on mouse IL17C starting with a concentration of 100 nM.

Figure 4 shows monovalent affinities of anti-IL17C antibodies. Affinities were determined by solution equilibrium titration (SET) using Fab fragments.

Figure 5 shows $IC_{50}$ values determined in the IL-17 receptor E inhibition assay described in example 11.

Figure 6 shows results of a mouse serum stability assay. 11 purified IgGs showed acceptable production yields and specific binding to mouse IL17C after 24h incubation with mouse serum.

Figure 7 shows the results of MOR12743 and MOR12762 administration on the clinical score in the treatment protocol of the mouse CIA model compared to vehicle, Enbrel® (etanercept) and a control antibody (MOR03207).

Figure 8 shows the results of MOR12743 and MOR12762 administration on the AUC of the clinical score in the treatment protocol of the mouse CIA model compared to vehicle, Enbrel® (etanercept) and a control antibody (MOR03207).

Figure 9 shows the results of MOR12762 administration on the Larsen score in the prophylaxis protocol of the mouse CIA model compared to a control antibody (MOR03207).

Figure 10 shows the results of MOR12762 or MOR12743 administration on the recruitment of inflammatory cells into the BALF in a mouse model of acute lung neutrophilia compared to a control antibody (MOR03207).

Figure 11 shows the results of IL17C expression profiling using qRT-PCR. Increased IL17C expression levels were detected in lung tissue samples derived from donors with

diagnosed inflammatory respiratory diseases in comparison to IL17C expression levels in the control samples. Samples from 5 individual donors were analyzed for each group.

Figure 12 shows effect of MOR1243 neutralizing antibodies on epidermal thickness in Imiquimod (IMQ) psoriasis-like mouse model.

Figure 13A shows the results of quantitative RT-PCR analysis of IL17C mRNA expression in human epidermal keratinocytes treated for 2h, 6h, 24h or 48h with medium alone, IL-1 (10 ng/mL), TNF (10 ng/mL), IL-17A (250 ng/mL) or combinations of these triggers.

Figure 13B shows results of quantitative RT-PCR analysis of IL-17C mRNA expression in human epidermal keratinocytes treated for 2h, 6h or 24h with medium alone or various TLR ligands: TLR4 ligand LPS (1 µg/mL), TLR5 ligand flagellin (1 µg/mL), TLR7 ligand guardiquimod (4 µg/mL), TLR7/8 ligand CL097 (10 µg/mL) and TLR9 ligand CpG ODN 2116 (10 µM).

## Detailed description of the invention

The present invention demonstrates that IL17C is a valid target for the treatment of inflammatory disorders. In this respect, the invention provides, in one aspect, methods of using an IL17C antagonist to bring about a prophylactic or therapeutic benefit in the treatment of inflammatory disorders.

The present invention provides therapeutic methods comprising the administration of a therapeutically effective amount of an IL17C antagonist to a subject in need of such treatment. A "therapeutically effective amount" or "effective amount", as used herein, refers to the amount of an IL17C antagonist necessary to elicit the desired biological response. In accordance with the subject invention, the therapeutic effective amount is the amount of an IL17C antagonist necessary to treat and/or prevent an inflammatory disorder.

The terms "inflammatory disorder" or "inflammatory disease" are used interchangeably and as used herein refer to any abnormality associated with inflammation. Examples of disorders associated with inflammation include acne vulgaris, arthritis, such as rheumatoid arthritis, asthma, autoimmune diseases, chronic prostatitis, COPD (chronic obstructive pulmonary disease), glomerulonephritis, hypersensitivities, inflammatory bowel diseases,

pelvic inflammatory disease, reperfusion injury, sarcoidosis, transplant rejection, vasculitis, interstitial cystitis and sepsis. Inflammatory disorders may be chronic or acute. Examples of autoimmune diseases include ankylosing spondylitis, Crohn's Disease, Diabetes mellitus type I, gastritis, Guillain-Barré syndrome, idiopathic thrombocytopenic purpura, Lupus erythematosus, multiple sclerosis, psoriasis, psoratic arthritis, restless leg syndrome, rheumatoid arthritis, sarcoidosis, scleroderma, systemic lupus erythematosus and ulcerative colitis.

Arthritis can manifest itself as the primary form of a disease. This is for example the case for osteoarthritis, rheumatoid arthritis, septic arthritis, gout and pseudo-gout, juvenile idiopathic arthritis, Still's disease and ankylosing spondylitis. Other forms of arthritis are secondary to other disease, e.g. Ehlers-Danlos syndrome, sarcoidosis, Henoch-Schönlein purpura, psoriatic arthritis, reactive arthritis, haemochromatosis, hepatitis, Wegener's granulomatosis (and other vasculitis syndromes), Lyme disease, familial Mediterranean fever, hyperimmunoglobulinemia D with recurrent fever, TNF receptor associated periodic syndrome and inflammatory bowel disease (including Crohn's disease and ulcerative colitis).

The term "pulmonary inflammation" encompasses any inflammatory lung disease, acute chronic bronchitis, chronic obstructive lung disease, pulmonary fibrosis, Goodpasture's syndrome, and any pulmonary condition in which white blood cells may play a role including but not limited to idiopathic pulmonary fibrosis and any other autoimmune lung disease.

The term "IL17C" refers to a protein known as interleukin 17C (identified in HUGO Gene Nomenclature Committee (HGNC) by ID 5983 and in Mouse genome Informatics (MGI) database by ID 2446486). IL17C is some older publications referred to as CX2 or IL-21, however, it should not be confused with IL-21 cytokine, which is specifically expressed in activated $CD4^+$ T cells, but not most of other tissues (Parrish-Novak et al (2000). Nature 408 (6808): 57–63). Human IL-21 is located on Chromosome 4 and is identified in HGNC database by ID 6005. Human IL17C is located on Chromosome 16 and has the amino acid sequence of (UniProt Q9P0M4):

MTLLPGLLFLTWLHTCLAHHDPSLRGHPHSHGTPHCYSAEELPLGQAPPHLLA
RGAKWGQALPVALVSSLEAASHRGRHERPSATTQCPVLRPEEVLEADTHQRSI
SPWRYRVDTDEDRYPQKLAFAECLCRGCIDARTGRETAALNSVRLLQSLLVLR
RRPCSRDGSGLPTPGAFAFHTEFIHVPVGCTCVLPRSV (SEQ ID No.:181)

The term “IL17RA” refers to a protein known as interleukin 17 receptor A. Human IL17RA has the amino acid sequence of (UniProt Q96F46):

MGAARSPPSAVPGPLLGLLLLLLGVLAPGGASLRLLDHRALVCSQPGLNCTVKNSTCL
DDSWIHPRNLTPSSPKDLQIQLHFAHTQQGDLFPVAHIEWTLQTDASILYLEGAELSVL
QLNTNERLCVRFEFLSKLRHHHRRWRFTFSHFVVDPDQEYEVTVHHLPKPIPDGDPN
HQSKNFLVPDCEHARMKVTTPCMSSGSLWDPNITVETLEAHQLRVSFTLWNESTHY
QILLTSFPHMENHSCFEHMHHIPAPRPEEFHQRSNVTLTLRNLKGCCRHQVQIQPFFS
SCLNDCLRHSATVSCPEMPDTPEPIPDYMPLWVYWFITGISILLVGSVILLIVCMTWRL
AGPGSEKYSDDTKYTDGLPAADLIPPPLKPRKVWIIYSADHPLYVDVVLKFAQFLLTAC
GTEVALDLLEEQAISEAGVMTWVGRQKQEMVESNSKIIVLCSRGTRAKWQALLGRGA
PVRLRCDHGKPVGDLFTAAMNMILPDFKRPACFGTYVVCYFSEVSCDGDVPDLFGAA
PRYPLMDRFEEVYFRIQDLEMFQPGRMHRVGELSGDNYLRSPGGRQLRAALDRFRD
WQVRCPDWFECENLYSADDQDAPSLDEEVFEEPLLPPGTGIVKRAPLVREPGSQAC
LAIDPLVGEEGGAAVAKLEPHLQPRGQPAPQPLHTLVLAAEEGALVAAVEPGPLADGA
AVRLALAGEGEACPLLGSPGAGRNSVLFLPVDPEDSPLGSSTPMASPDLLPEDVREH
LEGLMLSLFEQSLSCQAQGGCSRPAMVLTDPHTPYEEEQRQSVQSDQGYISRSSPQ
PPEGLTEMEEEEEEEQDPGKPALPLSPEDLESLRSLQRQLLFRQLQKNSGWDTMGS
ESEGPSA (SEQ ID No.:182)

The term “IL17RE” refers to a protein known as interleukin 17 receptor E. Human IL17RE has the amino acid sequence of (UniProt Q8NFR9):

MGSSRLAALLLPLLLIVIDLSDSAGIGFRHLPHWNTRCPLASHTDDSFTGSSAYIPCRT
WWALFSTKPWCVRVWHCSRCLCQHLLSGGSGLQRGLFHLLVQKSKKSSTFKFYRR
HKMPAPAQRKLLPRRHLSEKSHHISIPSPDISHKGLRSKRTQPSDPETWESLPRLDSQ
RHGGPEFSFDLLPEARAIRVTISSGPEVSVRLCHQWALECEELSSPYDVQKIVSGGHT
VELPYEFLLPCLCIEASYLQEDTVRRKKCPFQSWPEAYGSDFWKSVHFTDYSQHTQM
VMALTLRCPLKLEAALCQRHDWHTLCKDLPNATARESDGWYVLEKVDLHPQLCFKFS
FGNSSHVECPHQTGSLTSWNVSMDTQAQQLILHFSSRMHATFSAAWSLPGLGQDTL
VPPVYTVSQARGSSPVSLDLIIPFLRPGCCVLVWRSDVQFAWKHLLCPDVSYRHLGLL
ILALLALLTLLGVVLALTCRRPQSGPGPARPVLLLHAADSEAQRRLVGALAELLRAALG
GGRDVIVDLWEGRHVARVGPLPWLWAARTRVAREQGTVLLLWSGADLRPVSGPDP
RAAPLLALLHAAPRPLLLLAYFSRLCAKGDIPPPLRALPRYRLLRDLPRLLRALDARPFA
EATSWGRLGARQRRQSRLELCSRLEREAARLADLG (SEQ ID No.:183)

An "antagonist of IL17C" and an "IL17C antagonist", as used herein, refer to IL17C antagonists in the broadest sense. Any molecule which inhibits the activity or function of IL17C, or which by any other way exerts an effect on IL17C is included. The term IL17C antagonist includes, but is not limited to, antibodies or antibody fragments specifically binding to IL17C, inhibitory nucleic acids specific for IL17C or small organic molecules specific for IL17C. Also within the meaning of the term IL17C antagonist are antibodies or antibody fragments specifically binding to the receptor of IL17C, inhibitory nucleic acids specific for the receptor of IL17C or small organic molecules specific for the receptor of IL17C. The term IL17C antagonist also refers to non-antibody scaffold molecules, such as fibronectin scaffolds, ankyrins, maxybodies/avimers, protein A-derived molecules, anticalins, affilins, protein epitope mimetics (PEMs) or the like.

Inhibitory nucleic acids include, but are not limited to, antisense DNA, triplex-forming oligonucleotides, external guide sequences, siRNA and microRNA. Useful inhibitory nucleic acids include those that reduce the expression of RNA encoding IL17C by at least 20, 30, 40, 50, 60, 70, 80, 90 or 95 percent compared to controls. Inhibitory nucleic acids and methods of producing them are well known in the art. siRNA design software is available.

Small organic molecules (SMOLs) specific for IL17C or the receptor of IL17C may be identified via natural product screening or screening of chemical libraries. Typically the molecular weight of SMOLs is below 500 Dalton, more typically from 160 to 480 Daltons. Other typical properties of SMOLs are one or more of the following:

- The partition coefficient log P is in the range from -0.4 to +5.6
- The molar refractivity is from 40 to 130
- The number of atoms is from 20 to 70

For reviews see Ghose et al. (1999) J Combin Chem: 1, 55-68 and Lipinski et al (1997) Adv Drug Del Rev: 23, 3-25.

Preferably, an IL17C antagonist for use in the present invention is an antibody specific for IL17C or specific for the receptor of IL17C. Such an antibody may be of any type, such as a murine, a rat, a chimeric, a humanized or a human antibody. A "human" antibody or functional human antibody fragment is hereby defined as one that is not chimeric (e.g., not "humanized") and not from (either in whole or in part) a non-human species. A human antibody or functional antibody fragment can be derived from a human or can be a synthetic human antibody. A "synthetic human antibody" is defined herein as an antibody having a sequence derived, in

whole or in part, *in silico* from synthetic sequences that are based on the analysis of known human antibody sequences. *In silico* design of a human antibody sequence or fragment thereof can be achieved, for example, by analyzing a database of human antibody or antibody fragment sequences and devising a polypeptide sequence utilizing the data obtained therefrom. Another example of a human antibody or functional antibody fragment is one that is encoded by a nucleic acid isolated from a library of antibody sequences of human origin (i.e., such library being based on antibodies taken from a human natural source).

A “humanized antibody” or functional humanized antibody fragment is defined herein as one that is (i) derived from a non-human source (e.g., a transgenic mouse which bears a heterologous immune system), which antibody is based on a human germline sequence; or (ii) chimeric, wherein the variable domain is derived from a non-human origin and the constant domain is derived from a human origin or (iii) CDR-grafted, wherein the CDRs of the variable domain are from a non-human origin, while one or more frameworks of the variable domain are of human origin and the constant domain (if any) is of human origin.

The term "chimeric antibody" or functional chimeric antibody fragment is defined herein as an antibody molecule which has constant antibody regions derived from, or corresponding to, sequences found in one species and variable antibody regions derived from another species. Preferably, the constant antibody regions are derived from, or corresponding to, sequences found in humans, e.g. in the human germ line or somatic cells, and the variable antibody regions (e.g. VH , VL , CDR or FR regions) are derived from sequences found in a non-human animal, e.g. a mouse, rat, rabbit or hamster.

In one aspect antigen binding can be performed by “fragments” of an intact antibody. Examples of binding fragments encompassed within the term "antibody fragment" of an antibody include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and an isolated complementary determining region (CDR).

A “single chain Fragment (scFv)” is a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see, e.g., Bird *et al.*, (1988) *Science* 242:423-426; and Huston *et al.*, (1988) *Proc. Natl. Acad. Sci.* 85:5879-5883). Although the two domains VL and VH are coded for by separate genes, they can be

joined, using recombinant methods, by an artificial peptide linker that enables them to be made as a single protein chain. Such single chain antibodies include one or more antigen binding moieties. These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The term "isolated" refers to a compound which can be e.g. an antibody or antibody fragment that is substantially free of other antibodies or antibody fragments having different antigenic specificities. Moreover, an isolated antibody or antibody fragment may be substantially free of other cellular material and/or chemicals.

The term "monoclonal antibody" as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a unique binding site having a unique binding specificity and affinity for particular epitopes.

As used herein, an antibody "binds specifically to", "specifically binds to", is "specific to/for" or "specifically recognizes" an antigen (here, IL17C or, alternatively, the receptor of IL17C) if such antibody is able to discriminate between such antigen and one or more reference antigen(s), since binding specificity is not an absolute, but a relative property. The reference antigen(s) may be one or more closely related antigen(s), which are used as reference points, e.g. IL17A or IL17B. In its most general form (and when no defined reference is mentioned), "specific binding" is referring to the ability of the antibody to discriminate between the antigen of interest and an unrelated antigen, as determined, for example, in accordance with one of the following methods. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-tests and peptide scans. For example, a standard ELISA assay can be carried out. The scoring may be carried out by standard color development (e.g. secondary antibody with horseradish peroxide and tetramethyl benzidine with hydrogen peroxide). The reaction in certain wells is scored by the optical density, for example, at 450 nm. Typical background (=negative reaction) may be 0.1 OD; typical positive reaction may be 1 OD. This means the difference positive/negative can be more than 10-fold. Typically, determination of binding specificity is performed by using not a single reference antigen, but a set of about three to five unrelated antigens, such as milk powder, BSA, transferrin or the like. Additionally, "specific binding" may relate to the ability of an antibody to discriminate between different parts of its target antigen, e.g. different domains or regions of IL17C or the receptor of IL17C, or between one or more key amino acid residues or stretches of amino acid residues of IL17C or the receptor of IL17C.

"Cross competes" means the ability of an antibody, antibody fragment or other antigen-binding moieties to interfere with the binding of other antibodies, antibody fragments or antigen-binding moieties to a specific antigen in a standard competitive binding assay. The ability or extent to which an antibody, antibody fragment or other antigen-binding moieties is able to interfere with the binding of another antibody, antibody fragment or antigen-binding moieties to a specific antigen, and, therefore whether it can be said to cross-compete according to the invention, can be determined using standard competition binding assays. One suitable assay involves the use of the Biacore technology (e.g. by using the BIAcore 3000 instrument (Biacore, Uppsala, Sweden)), which can measure the extent of interactions using surface plasmon resonance technology. Another assay for measuring cross-competing uses an ELISA-based approach. A high throughput process for "epitope binning" antibodies based upon their cross-competition is described in International Patent Application No. WO 2003/48731. Cross-competition is present if the antibody or antibody fragment under investigation reduces the binding of one of the antibodies described in Table 1 to IL17C by 60% or more, specifically by 70% or more and more specifically by 80% or more and if one of the antibodies described in Table 1 reduces the binding of said antibody or antibody fragment to IL17C by 60% or more, specifically by 70% or more and more specifically by 80% or more.

The term "epitope" includes any proteinacious region which is specifically recognized by an immunoglobulin or T-cell receptor or otherwise interacts with a molecule. Generally epitopes are of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and generally may have specific three-dimensional structural characteristics, as well as specific charge characteristics. As will be appreciated by one of skill in the art, practically anything to which an antibody can specifically bind could be an epitope. An epitope can comprise those residues to which the antibody binds and may be "linear" or "conformational." The term "linear epitope" refers to an epitope wherein all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein (continuous). The term "conformational epitope" refers to an epitope in which discontinuous amino acids that come together in three dimensional conformations. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another.

"Binds the same epitope as" means the ability of an antibody, antibody fragment or other antigen-binding moiety to bind to a specific antigen and having the same epitope as the exemplified antibody. The epitopes of the exemplified antibody and other antibodies can be determined using epitope mapping techniques. Epitope mapping techniques are well known in

the art. For example, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., hydrogen/deuterium exchange, x-ray crystallography and two-dimensional nuclear magnetic resonance.

Also, as used herein, an "immunoglobulin" (Ig) hereby is defined as a protein belonging to the class IgG, IgM, IgE, IgA, or IgD (or any subclass thereof), and includes all conventionally known antibodies and functional fragments thereof. A "functional fragment" of an antibody/immunoglobulin hereby is defined as a fragment of an antibody/immunoglobulin (e.g., a variable region of an IgG) that retains the antigen-binding region. An "antigen binding region" of an antibody typically is found in one or more hypervariable region(s) of an antibody, i.e., the CDR-1, -2, and/or –3 regions; however, the variable "framework" regions can also play an important role in antigen binding, such as by providing a scaffold for the CDRs. Preferably, the "antigen-binding region" comprises at least amino acid residues 4 to 103 of the variable light (VL) chain and 5 to 109 of the variable heavy (VH) chain, more preferably amino acid residues 3 to 107 of VL and 4 to 111 of VH, and particularly preferred are the complete VL and VH chains (amino acid positions 1 to 109 of VL and 1 to 113 of VH; numbering according to WO 97/08320). A preferred class of immunoglobulins for use in the present invention is IgG. "Functional fragments" of the invention include the domain of a F(ab')2 fragment, a Fab fragment, scFv or constructs comprising single immunoglobulin variable domains or single domain antibody polypeptides, e.g. single heavy chain variable domains or single light chain variable domains. The F(ab')2 or Fab may be engineered to minimize or completely remove the intermolecular disulphide interactions that occur between the CH1 and CL domains.

An antibody of the invention may be derived from a recombinant antibody library that is based on amino acid sequences that have been designed *in silico* and encoded by nucleic acids that are synthetically created. *In silico* design of an antibody sequence is achieved, for example, by analyzing a database of human sequences and devising a polypeptide sequence utilizing the data obtained therefrom. Methods for designing and obtaining *in silico*-created sequences are described, for example, in Knappik et al., J. Mol. Biol. (2000) 296:57; Krebs et al., J. Immunol. Methods. (2001) 254:67, Rothe et al., J. Mol. Biol. (2008) 376:1182; and U.S. Patent No. 6,300,064 issued to Knappik et al., which are hereby incorporated by reference in their entirety.

Any antibody specific for IL17C may be used with the present invention. Exemplary antibodies include antibodies in the prior art, such as
A: rat $IgG_{2A}$ monoclonal anti-mouse IL17C antibody (R&D Systems; clone 311522,# MAB23061),

B: rat $IgG_{2A}$ monoclonal anti-mouse IL17C antibody (R&D Systems; clone 311523,# MAB2306), and

C: rat anti-mouse IL17C (US Biological; clone: 8B28, #I8439-20R3) (take from Example 4).

Other antibodies that may be used to practice the present invention include the anti-IL17C antibodies available from Abnova (Walnut, CA, USA; Catalog #H00027189-B01P, #H00027189-D01 and #H00027189-D01P) and the anti-IL17C antibodies available from antibodies-online GmbH (Aachen, Germany; Catalog #ABIN525892, #ABIN327411, #ABIN525893, #ABIN221340, #ABIN221341, #ABIN221342 and #ABIN525891). Other antibodies specific for IL17C that may be used with the present invention are those isolated and described in the present invention itself, i.e. those listed in Table 1.

Compositions of the invention may be used for therapeutic or prophylactic applications. The invention, therefore, includes a pharmaceutical composition containing an inventive antibody (or functional antibody fragment) and a pharmaceutically acceptable carrier or excipient therefor. In a related aspect, the invention provides a method for treating an inflammatory disorder. Such method contains the steps of administering to a subject in need thereof an effective amount of the pharmaceutical composition that contains an inventive antibody as described or contemplated herein.

In certain aspects, the present invention provides methods for the treatment of an inflammatory disorder in a subject, said method comprising the step of administering an IL17C antagonist to said subject. “Subject”, as used in this context refers to any mammal, including rodents, such as mouse or rat, and primates, such as cynomolgus monkey (*Macaca fascicularis*), rhesus monkey (*Macaca mulatta*) or humans (*Homo sapiens*). Preferably the subject is a primate, most preferably a human.

In certain aspects, the present invention provides methods for the treatment of an inflammatory disorder, said method comprising the step of administering to a subject an IL17C antagonist, wherein said IL17C antagonist can bind to IL17C with an affinity of about less than 100 nM, more preferably less than about 60 nM, and still more preferably less than about 30 nM. Further preferred are antibodies or antibody fragments that bind to IL17C with an affinity of less than about 10 nM, and more preferably less than about 3 nM.

In certain aspects said IL17C antagonist is an antibody or antibody fragment specific for IL17C and said antibody or antibody fragment is cross-reactive with IL17C of another species, such as IL17C from mouse, rat, rhesus monkey and/or cynomolgus monkey. In certain aspects said IL17C antibody or antibody fragment is an isolated antibody or antibody fragment specific for IL17C. In another embodiment said isolated antibody or antibody fragment specific for IL17C is a monoclonal antibody or antibody fragment. In a further embodiment said isolated monoclonal antibody or antibody fragment is an isolated monoclonal antibody specific for a polypeptide comprising the amino acid sequence of SEQ ID No.: 181. In a further embodiment said isolated monoclonal antibody or antibody fragment is an isolated monoclonal antibody specific for a polypeptide consisting of the amino acid sequence of SEQ ID No.: 181. In a further embodiment said isolated monoclonal antibody or antibody fragment is cross-reactive with IL17C of another species, such as IL17C from mouse, rat, rhesus monkey and/or cynomolgus monkey.

In certain aspects, said antibody or antibody fragment specific for IL17C is a human, humanized or chimeric antibody. In certain aspects, said antibody or antibody fragment specific for IL17C is a human synthetic antibody. In certain aspects the present invention provides an isolated monoclonal antibody or antibody fragment specific for a polypeptide comprising the amino acid sequence of SEQ ID No.: 181 wherein said antibody or antibody fragment is a human, humanized or chimeric antibody. In certain aspects the present invention provides an isolated monoclonal antibody or antibody fragment specific for a polypeptide consisting of the amino acid sequence of SEQ ID No.: 181 wherein said antibody or antibody fragment is a human, humanized or chimeric antibody. In certain aspects, said antibody or antibody fragment specific for a polypeptide consisting of the amino acid sequence of SEQ ID No.: 181 is a human synthetic antibody or antibody fragment.

In a certain aspect, the present invention provides a composition comprising an IL17C antagonist capable of antagonizing IL17C in an inflammatory disorder, said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents. In certain aspects, antagonists of IL17C, such as antibodies specific for IL17C, may antagonize any of the roles of IL17C in an inflammatory disorder.

In another aspect, the present invention provides a method for the prophylaxis of an inflammatory disorder in a subject, said method comprising administering an IL17C antagonist to said subject. “Prophylaxis” as used in this context refers to methods which aim to prevent the onset of a disease or which delay the onset of a disease.

In certain aspects, the present invention provides a composition comprising an IL17C antagonist useful in the treatment of an inflammatory disorder, said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

In certain aspects, the present invention provides IL17C antagonists for use in the treatment of an inflammatory disorder.

In other aspects, the present invention provides the use of an IL17C antagonist in the preparation of a medicament for the treatment of an inflammatory disorder.

In other aspects, the present invention provides a method for the treatment of an inflammatory disorder in a subject, comprising administering to the subject an antagonist of IL17C.

In particular aspects, the IL17C antagonists of the present invention are administered subcutaneously. In other aspects, the IL17C antagonists of the present invention are administered intra-venously, intra-articularly or intra-spinally.

The compositions of the present invention are preferably pharmaceutical compositions comprising an IL17C antagonist and a pharmaceutically acceptable carrier, diluent or excipient, for the treatment of an inflammatory disorder. Such carriers, diluents and excipients are well known in the art, and the skilled artisan will find a formulation and a route of administration best suited to treat a subject with the IL17C antagonists of the present invention.

In certain aspects, the present invention provides a method for the treatment or prophylaxis of an inflammatory disorder in a subject, comprising the step of administering to the subject an effective amount of an antagonist of IL17C. In certain aspects said subject is a human. In alternative aspects said subject is a rodent, such as a rat or a mouse.

In certain aspects, said antagonist of IL17C is an antibody or antibody fragment specific for IL17C. In certain aspects said antagonist is an antibody or antibody fragment specific for a polypeptide comprising the amino acid sequence of SEQ ID No.: 181. In

alternative aspects, said antagonist of IL17C is an antibody or antibody fragment specific for the receptor of IL17C.

In certain aspects, said antibody or antibody fragment specific for IL17C blocks the binding of IL17C to the receptor of IL17C. In alternative aspects, said antibody or antibody fragment specific for the receptor of IL17C blocks the binding of IL17C to the receptor of IL17C.

In certain aspects, said antibody or antibody fragment specific for IL17C blocks the binding of IL17C to the receptor of IL17, wherein said receptor is IL17RE. In alternative aspects, said antibody or antibody fragment specific for the receptor of IL17C blocks the binding of IL17C to IL17RE.

In certain aspects, said antibody or antibody fragment specific for IL17C blocks the binding of IL17C to IL17RE with an $IC_{50}$ concentration of less than 100nM, 90nM, 80nM, 70nM, 60nM, 50nM, 40nM, 30nM, 20nM, 10nM, 9nM, 8nM, 7nM, 6nM, 5nM, 4nM, 3nM, 2nM, 1nM, 100pM, 90pM, 80pM, 70pM, 60pM, 50pM, 40pM, 30pM, 20pM, 10pM, 9pM, 8pM, 7pM, 6pM, 5pM, 4pM, 3pM, 2pM or 1pM. In certain aspects the $IC_{50}$ concentration can be determined by ELISA; SET, FACS or MSD (Meso Scale Discovery).

In certain aspects, said antibody or antibody fragment specific for IL17C blocks the binding of IL17C to one or more receptors of IL17C. In alternative aspects, said antibody or antibody fragment specific for the receptor of IL17C blocks the binding of IL17C to receptors of IL17C, wherein the receptors of IL17 include IL17RE and IL17RA. In alternative aspects, said antibody or antibody fragment specific for the receptor of IL17C blocks the binding of IL17C to IL17RE and IL17RA.

In certain aspects, the present invention provides an antagonist of IL17C for use in the treatment or prophylaxis of an inflammatory disorder. In certain aspects, said treatment or prophylaxis comprises the step of administering to a subject an effective amount of the antagonist of IL17C. In certain aspects, said subject is a human. In alternative aspects, said subject is a rodent, such as a rat or a mouse.

In another aspect, the disclosure pertains to an isolated monoclonal antibody or fragment thereof that cross-competes with an antibody described in Table 1. In a certain embodiment, the disclosure pertains to an isolated monoclonal antibody or fragment thereof that cross-competes with an antibody comprising 6 CDRs of one of the antibodies described in Table 1. In a certain embodiment, the disclosure pertains to an isolated monoclonal antibody or fragment thereof that cross-competes with an antibody described in Table 1 and reduces the specific binding of one of the antibodies described in Table 1 by at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% in an ELISA-based cross-competition. In a certain embodiment, the disclosure pertains to an isolated monoclonal antibody or fragment thereof that cross-competes with an antibody described in Table 1 and reduces the specific binding of one of the antibodies described in Table 1 to IL17C by at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% in an ELISA-based cross-competition.

In another aspect, the disclosure pertains to an isolated monoclonal antibody or fragment thereof that interacts with (e.g., by binding, stabilizing, spatial distribution) the same epitope as an antibody described in Table 1.

In one aspect, the disclosure pertains to an isolated monoclonal antibody or fragment thereof comprising 6 CDRs defined by Kabat of any of the antibodies in Table 1. In another aspect, the disclosure pertains to an isolated monoclonal antibody or fragment thereof comprising 6 CDRs defined by Kabat of each of the antibodies in Table 1.

In one aspect, the disclosure pertains to an isolated monoclonal antibody or fragment thereof comprising a VH and a VL of any of the antibodies in Table 1.

In another aspect, the disclosure pertains to a nucleic acid encoding an isolated monoclonal antibody or fragment thereof wherein the nucleic acid comprises a VH and a VL of any of the antibodies in Table 1.

In another aspect, the disclosure pertains to a nucleic acid encoding an isolated monoclonal antibody or fragment thereof having at least 75%, 80%, 85%, 90%, 95%, 98%, 99% sequence identity to nucleic acids described in Table 1.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

**Table 1:**

| **MOR#** | | **Seq. ID:** | **[aa] / DNA** |
|---|---|---|---|
| **12740** | HCDR1 | Seq. ID: 1 | GGTFSIYAIS |
| | HCDR2 | Seq. ID: 2 | WMGGIIPILGIANYAQKFQG |
| | HCDR3 | Seq. ID: 3 | DATHSYYHDY |
| | LCDR1 | Seq. ID: 4 | TGTSSDVGSYETVS |
| | LCDR2 | Seq. ID: 5 | VMIYEVSDRPS |
| | LCDR3 | Seq. ID: 6 | GSFAHWGSW |
| | VL | Seq. ID: 7 | DIALTQPASVSGSPGQSITISCTGTSSDVGSYETVSWY QQHPGKAPKVMIYEVSDRPSGVSNRFSGSKSGNTAS LTISGLQAEDEADYYCGSFAHWGSWVFGGGTKLTVL GQ |
| | VH | Seq. ID: 8 | QVQLVQSGAEVKKPGSSVKVSCKASGGTFSIYAISWV RQAPGQGLEWMGGIIPILGIANYAQKFQGRVTITADES TSTAYMELSSLRSEDTAVYYCARDATHSYYHDYWGQ GTLVTVSS |
| | VL (DNA) | Seq. ID: 9 | GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCG GTAGCCCGGGCCAGAGCATTACCATTAGCTGCACC GGCACCAGCAGCGATGTGGGCTCTTACGAAACTGT GTCTTGGTACCAGCAGCATCCGGGCAAGGCGCCGA AAGTTATGATCTACGAAGTTTCTGACCGTCCGAGCG GCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGC AACACCGCGAGCCTGACCATTAGCGGCCTGCAAGC GGAAGACGAAGCGGATTATTACTGCGGTTCTTTCGC TCATTGGGGTTCTTGGGTGTTTGGCGGCGGCACGA AGTTAACCGTTCTTGGCCAG |
| | VH (DNA) | Seq. ID: 10 | CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAA AAAACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAG CATCCGGAGGGACGTTTTCTATCTACGCTATCTCTT GGGTGCGCCAGGCCCCGGGCCAGGGCCTCGAGTG GATGGGCGGTATCATCCCGATCCTGGGCATCGCGA ACTACGCCCAGAAATTTCAGGGCCGGGTGACCATT ACCGCCGATGAAAGCACCAGCACCGCCTATATGGA ACTGAGCAGCCTGCGCAGCGAAGATACGGCCGTGT ATTATTGCGCGCGTGACGCTACTCATTCTTACTACC ATGATTACTGGGGCCAAGGCACCCTGGTGACTGTT AGCTCA |
| **12741** | HCDR1 | Seq. ID: 11 | GGTFSSYAIS |
| | HCDR2 | Seq. ID: 12 | WMGMIMPEVGMADYAQKFQG |
| | HCDR3 | Seq. ID: 13 | DFIAVGSLEIWHYYYGLDV |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | LCDR1 | Seq. ID: 14 | SGDNIGEHYAS |
| | LCDR2 | Seq. ID: 15 | LVISYDNERPS |
| | LCDR3 | Seq. ID: 16 | QSWTSQKPDY |
| | VL | Seq. ID: 17 | DIELTQPPSVSVSPGQTASITCSGDNIGEHYASWYQQ KPGQAPVLVISYDNERPSGIPERFSGSNSGNTATLTIS GTQAEDEADYYCQSWTSQKPDYVFGGGTKLTVLGQ |
| | VH | Seq. ID: 18 | QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGMIMPEVGMADYAQKFQGRVTITA DESTSTAYMELSSLRSEDTAVYYCARDFIAVGSLEIWH YYYGLDVWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 19 | GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGT GAGCCCGGGCCAGACCGCGAGCATTACCTGTAGCG GCGATAACATCGGTGAACATTACGCTTCTTGGTACC AGCAGAAACCGGGCCAGGCGCCGGTGCTGGTGAT CTCTTACGACAACGAACGTCCGAGCGGCATCCCGG AACGTTTTAGCGGATCCAACAGCGGCAACACCGCG ACCCTGACCATTAGCGGCACCCAGGCGGAAGACGA AGCGGATTATTACTGCCAGTCTTGGACTTCTCAGAA ACCGGACTACGTGTTTGGCGGCGGCACGAAGTTAA CCGTTCTTGGCCAG |
| | VH (DNA) | Seq. ID: 20 | CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAA AAAACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAG CATCCGGAGGGACGTTTAGCAGCTATGCGATTAGC TGGGTGCGCCAGGCCCCGGGCCAGGGCCTCGAGT GGATGGGCATGATCATGCCGGAAGTTGGCATGGCT GACTACGCCCAGAAATTTCAGGGCCGGGTGACCAT TACCGCCGATGAAAGCACCAGCACCGCCTATATGG AACTGAGCAGCCTGCGCAGCGAAGATACGGCCGTG TATTATTGCGCGCGTGACTTCATCGCTGTTGGTTCT CTGGAAATCTGGCATTACTACTACGGTCTGGATGTT TGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA |
| **12742** | HCDR1 | Seq. ID: 21 | GGTFSSYGIS |
| | HCDR2 | Seq. ID: 22 | WMGRIIPIFGTAYYAQKFQG |
| | HCDR3 | Seq. ID: 23 | DMRYHDYWPYYYGSDQFDV |
| | LCDR1 | Seq. ID: 24 | SGSSSNIGSDIVS |
| | LCDR2 | Seq. ID: 25 | LLIYYNNLRPS |
| | LCDR3 | Seq. ID: 26 | QSWDWASLAM |
| | VL | Seq. ID: 27 | DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSDIVSWYQ QLPGTAPKLLIYYNNLRPSGVPDRFSGSKSGTSASLAI TGLQAEDEADYYCQSWDWASLAMVFGGGTKLTVLG Q |
| | VH | Seq. ID: 28 | QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYGISW VRQAPGQGLEWMGRIIPIFGTAYYAQKFQGRVTITADE STSTAYMELSSLRSEDTAVYYCARDMRYHDYWPYYY GSDQFDVWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 29 | GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGG TGCACCGGGCCAGCGCGTGACCATTAGCTGTAGCG GCAGCAGCAGCAACATTGGTTCTGACATCGTGTCTT GGTACCAGCAGCTGCCGGGCACGGCGCCGAAACT GCTGATCTACTACAACAACCTGCGCCCGAGCGGCG TGCCGGATCGCTTTAGCGGATCCAAAAGCGGCACC |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | | | AGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGA<br>AGACGAAGCGGATTATTACTGCCAGTCTTGGGACTG<br>GGCTTCTCTGGCTATGGTGTTTGGCGGCGGCACGA<br>AGTTAACCGTTCTTGGCCAG |
| | VH (DNA) | Seq. ID: 30 | CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAA<br>AAAACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAG<br>CATCCGGAGGGACGTTTTCTTCTTACGGTATCTCTT<br>GGGTGCGCCAGGCCCCGGGCCAGGGCCTCGAGTG<br>GATGGGCCGTATCATCCCGATCTTCGGCACTGCGT<br>ACTACGCCCAGAAATTTCAGGGCCGGGTGACCATT<br>ACCGCCGATGAAAGCACCAGCACCGCCTATATGGA<br>ACTGAGCAGCCTGCGCAGCGAAGATACGGCCGTGT<br>ATTATTGCGCGCGTGACATGCGTTACCATGACTACT<br>GGCCGTACTACTACGGTTCTGACCAGTTCGATGTTT<br>GGGGCCAAGGCACCCTGGTGACTGTTAGCTCA |
| **12743** | HCDR1 | Seq. ID: 31 | GYTFTSNFIH |
| | HCDR2 | Seq. ID: 32 | WMGWISPYNGDTNYAQKFQG |
| | HCDR3 | Seq. ID: 33 | ESVYYGSDYGYNGMDI |
| | LCDR1 | Seq. ID: 34 | SGDNLGEEYVS |
| | LCDR2 | Seq. ID: 35 | LVIYDDTKRPS |
| | LCDR3 | Seq. ID: 36 | ASWDLWSVE |
| | VL | Seq. ID: 37 | DIELTQPPSVSVSPGQTASITCSGDNLGEEYVSWYQQ<br>KPGQAPVLVIYDDTKRPSGIPERFSGSNSGNTATLTIS<br>GTQAEDEADYYCASWDLWSVEVFGGGTKLTVLGQ |
| | VH | Seq. ID: 38 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSNFIHWV<br>RQAPGQGLEWMGWISPYNGDTNYAQKFQGRVTMTR<br>DTSISTAYMELSRLRSEDTAVYYCARESVYYGSDYGY<br>NGMDIWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 39 | GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGT<br>GAGCCCGGGCCAGACCGCGAGCATTACCTGTAGCG<br>GCGATAACCTGGGTGAAGAATACGTTTCTTGGTACC<br>AGCAGAAACCGGGCCAGGCGCCGGTGCTGGTGAT<br>CTACGACGACACTAAACGTCCGAGCGGCATCCCGG<br>AACGTTTTAGCGGATCCAACAGCGGCAACACCGCG<br>ACCCTGACCATTAGCGGCACCCAGGCGGAAGACGA<br>AGCGGATTATTACTGCGCTTCTTGGGACCTGTGGTC<br>TGTTGAAGTGTTTGGCGGCGGCACGAAGTTAACCG<br>TTCTTGGCCAG |
| | VH (DNA) | Seq. ID: 40 | CAGGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAA<br>AAAACCGGGTGCCAGCGTGAAAGTTAGCTGCAAAG<br>CGTCCGGATATACCTTCACTTCTAACTTCATCCATTG<br>GGTGCGCCAGGCCCCGGGCCAGGGCCTCGAGTGG<br>ATGGGCTGGATCTCTCCGTACAACGGCGACACGAA<br>CTACGCGCAGAAATTTCAGGGCCGGGTGACCATGA<br>CCCGTGATACCAGCATTAGCACCGCGTATATGGAAC<br>TGAGCCGTCTGCGTAGCGAAGATACGGCCGTGTAT<br>TATTGCGCGCGTGAATCTGTTTACTACGGTTCTGAC<br>TACGGTTACAACGGTATGGATATCTGGGGCCAAGG<br>CACCCTGGTGACTGTTAGCTCA |
| **12744** | HCDR1 | Seq. ID: 41 | GYTFTSNFIH |
| | HCDR2 | Seq. ID: 42 | WMGWISPYNGDTNYAQKFQG |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | HCDR3 | Seq. ID: 43 | ESVYYGSDYGYNGMDI |
| | LCDR1 | Seq. ID: 44 | SGDNLGEEYVS |
| | LCDR2 | Seq. ID: 45 | LVIYDDTKRPS |
| | LCDR3 | Seq. ID: 46 | ASWAFYSSQ |
| | VL | Seq. ID: 47 | DIELTQPPSVSVSPGQTASITCSGDNLGEEYVSWYQQ KPGQAPVLVIYDDTKRPSGIPERFSGSNSGNTATLTIS GTQAEDEADYYCASWAFYSSQVFGGGTKLTVLGQ |
| | VH | Seq. ID: 48 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSNFIHWV RQAPGQGLEWMGWISPYNGDTNYAQKFQGRVTMTR DTSISTAYMELSRLRSEDTAVYYCARESVYYGSDYGY NGMDIWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 49 | GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGT GAGCCCGGGCCAGACCGCGAGCATTACCTGTAGCG GCGATAACCTGGGTGAAGAATACGTTTCTTGGTACC AGCAGAAACCGGGCCAGGCGCCGGTGCTGGTGAT CTACGACGACACTAAACGTCCGAGCGGCATCCCGG AACGTTTTAGCGGATCCAACAGCGGCAACACCGCG ACCCTGACCATTAGCGGCACCCAGGCGGAAGACGA AGCGGATTATTACTGCGCTTCTTGGGCTTTCTACTC TTCTCAGGTGTTTGGCGGCGGCACGAAGTTAACCG TTCTTGGCCAG |
| | VH (DNA) | Seq. ID: 50 | CAGGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAA AAAACCGGGTGCCAGCGTGAAAGTTAGCTGCAAAG CGTCCGGATATACCTTCACTTCTAACTTCATCCATTG GGTGCGCCAGGCCCCGGGCCAGGGCCTCGAGTGG ATGGGCTGGATCTCTCCGTACAACGGCGACACGAA CTACGCGCAGAAATTTCAGGGCCGGGTGACCATGA CCCGTGATACCAGCATTAGCACCGCGTATATGGAAC TGAGCCGTCTGCGTAGCGAAGATACGGCCGTGTAT TATTGCGCGCGTGAATCTGTTTACTACGGTTCTGAC TACGGTTACAACGGTATGGATATCTGGGGCCAAGG CACCCTGGTGACTGTTAGCTCA |
| **12745** | HCDR1 | Seq. ID: 51 | GYTFTSNFIH |
| | HCDR2 | Seq. ID: 52 | WMGWISPYNGDTNYAQKFQG |
| | HCDR3 | Seq. ID: 53 | ESVYYGSDYGYNGMDI |
| | LCDR1 | Seq. ID: 54 | SGDNLGEEYVS |
| | LCDR2 | Seq. ID: 55 | LVIYDDTKRPS |
| | LCDR3 | Seq. ID: 56 | SAWATWSVA |
| | VL | Seq. ID: 57 | DIELTQPPSVSVSPGQTASITCSGDNLGEEYVSWYQQ KPGQAPVLVIYDDTKRPSGIPERFSGSNSGNTATLTIS GTQAEDEADYYCSAWATWSVAVFGGGTKLTVLGQ |
| | VH | Seq. ID: 58 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSNFIHWV RQAPGQGLEWMGWISPYNGDTNYAQKFQGRVTMTR DTSISTAYMELSRLRSEDTAVYYCARESVYYGSDYGY NGMDIWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 59 | GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGT GAGCCCGGGCCAGACCGCGAGCATTACCTGTAGCG GCGATAACCTGGGTGAAGAATACGTTTCTTGGTACC AGCAGAAACCGGGCCAGGCGCCGGTGCTGGTGAT CTACGACGACACTAAACGTCCGAGCGGCATCCCGG |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | | | AACGTTTTAGCGGATCCAACAGCGGCAACACCGCG<br>ACCCTGACCATTAGCGGCACCCAGGCGGAAGACGA<br>AGCGGATTATTACTGCTCTGCTTGGGCTACTTGGTC<br>TGTTGCTGTGTTTGGCGGCGGCACGAAGTTAACCG<br>TTCTTGGCCAG |
| | VH (DNA) | Seq. ID: 60 | CAGGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAA<br>AAAACCGGGTGCCAGCGTGAAAGTTAGCTGCAAAG<br>CGTCCGGATATACCTTCACTTCTAACTTCATCCATTG<br>GGTGCGCCAGGCCCCGGGCCAGGGCCTCGAGTGG<br>ATGGGCTGGATCTCTCCGTACAACGGCGACACGAA<br>CTACGCGCAGAAATTTCAGGGCCGGGTGACCATGA<br>CCCGTGATACCAGCATTAGCACCGCGTATATGGAAC<br>TGAGCCGTCTGCGTAGCGAAGATACGGCCGTGTAT<br>TATTGCGCGCGTGAATCTGTTTACTACGGTTCTGAC<br>TACGGTTACAACGGTATGGATATCTGGGGCCAAGG<br>CACCCTGGTGACTGTTAGCTCA |
| **12746** | HCDR1 | Seq. ID: 61 | GYTFTSNFIH |
| | HCDR2 | Seq. ID: 62 | WMGWISPYNGDTNYAQKFQG |
| | HCDR3 | Seq. ID: 63 | ESVYYGSDYGYNGMDI |
| | LCDR1 | Seq. ID: 64 | SGDNLGEEYVS |
| | LCDR2 | Seq. ID: 65 | LVIYDDTKRPS |
| | LCDR3 | Seq. ID: 66 | SSWTHFSNI |
| | VL | Seq. ID: 67 | DIELTQPPSVSVSPGQTASITCSGDNLGEEYVSWYQQ<br>KPGQAPVLVIYDDTKRPSGIPERFSGSNSGNTATLTIS<br>GTQAEDEADYYCSSWTHFSNIVFGGGTKLTVLGQ |
| | VH | Seq. ID: 68 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSNFIHWV<br>RQAPGQGLEWMGWISPYNGDTNYAQKFQGRVTMTR<br>DTSISTAYMELSRLRSEDTAVYYCARESVYYGSDYGY<br>NGMDIWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 69 | GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGT<br>GAGCCCGGGCCAGACCGCGAGCATTACCTGTAGCG<br>GCGATAACCTGGGTGAAGAATACGTTTCTTGGTACC<br>AGCAGAAACCGGGCCAGGCGCCGGTGCTGGTGAT<br>CTACGACGACACTAAACGTCCGAGCGGCATCCCGG<br>AACGTTTTAGCGGATCCAACAGCGGCAACACCGCG<br>ACCCTGACCATTAGCGGCACCCAGGCGGAAGACGA<br>AGCGGATTATTACTGCTCTTCTTGGACTCATTTCTCT<br>AACATCGTGTTTGGCGGCGGCACGAAGTTAACCGT<br>TCTTGGCCAG |
| | VH (DNA) | Seq. ID: 70 | CAGGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAA<br>AAAACCGGGTGCCAGCGTGAAAGTTAGCTGCAAAG<br>CGTCCGGATATACCTTCACTTCTAACTTCATCCATTG<br>GGTGCGCCAGGCCCCGGGCCAGGGCCTCGAGTGG<br>ATGGGCTGGATCTCTCCGTACAACGGCGACACGAA<br>CTACGCGCAGAAATTTCAGGGCCGGGTGACCATGA<br>CCCGTGATACCAGCATTAGCACCGCGTATATGGAAC<br>TGAGCCGTCTGCGTAGCGAAGATACGGCCGTGTAT<br>TATTGCGCGCGTGAATCTGTTTACTACGGTTCTGAC<br>TACGGTTACAACGGTATGGATATCTGGGGCCAAGG<br>CACCCTGGTGACTGTTAGCTCA |
| **12751** | HCDR1 | Seq. ID: 71 | GDSVSSNSAAWN |
| | HCDR2 | Seq. ID: 72 | WLGVIYYRSKWYINYADSVKS |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | HCDR3 | Seq. ID: 73 | EGIVGGWFAY |
| | LCDR1 | Seq. ID: 74 | SGDKLGSKIAH |
| | LCDR2 | Seq. ID: 75 | LVIYDDNERPS |
| | LCDR3 | Seq. ID: 76 | QSWDYLSWSV |
| | VL | Seq. ID: 77 | DIELTQPPSVSVSPGQTASITCSGDKLGSKIAHWYQQK PGQAPVLVIYDDNERPSGIPERFSGSNSGNTATLTISG TQAEDEADYYCQSWDYLSWSVVFGGGTKLTVLGQ |
| | VH | Seq. ID: 78 | QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWN WIRQSPSRGLEWLGVIYYRSKWYINYADSVKSRITINP DTSKNQFSLQLNSVTPEDTAVYYCAREGIVGGWFAY WGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 79 | GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGT GAGCCCGGGCCAGACCGCGAGCATTACCTGTAGCG GCGATAAACTGGGTTCTAAAATCGCTCATTGGTACC AGCAGAAACCGGGCCAGGCGCCGGTGCTGGTGAT CTACGACGACAACGAACGTCCGAGCGGCATCCCGG AACGTTTTAGCGGATCCAACAGCGGCAACACCGCG ACCCTGACCATTAGCGGCACCCAGGCGGAAGACGA AGCGGATTATTACTGCCAGTCTTGGGACTACCTGTC TTGGTCTGTTGTGTTTGGCGGCGGCACGAAGTTAAC CGTTCTTGGCCAG |
| | VH (DNA) | Seq. ID: 80 | CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGT GAAACCGAGCCAGACCCTGAGCCTGACCTGCGCGA TTTCCGGAGATAGCGTGAGCAGTAACTCTGCTGCTT GGAACTGGATTCGTCAGAGCCCGAGCCGTGGCCTC GAGTGGCTGGGCGTTATCTACTACCGTAGCAAATG GTACATCAACTATGCCGACAGCGTGAAAAGCCGCAT TACCATTAACCCGGATACTTCGAAAAACCAGTTTAG CCTGCAACTGAACAGCGTGACCCCGGAAGATACGG CCGTGTATTATTGCGCGCGTGAAGGTATCGTTGGTG GTTGGTTCGCTTACTGGGGCCAAGGCACCCTGGTG ACTGTTAGCTCA |
| 12753 | HCDR1 | Seq. ID: 81 | GDSVSSSSAAWN |
| | HCDR2 | Seq. ID: 82 | WLGRIEYRSKWYNDYAVSVKS |
| | HCDR3 | Seq. ID: 83 | EMYYYSGYGVFDV |
| | LCDR1 | Seq. ID: 84 | SGDALGGEYVH |
| | LCDR2 | Seq. ID: 85 | LVIYDDDKRPS |
| | LCDR3 | Seq. ID: 86 | SSFDTWTSY |
| | VL | Seq. ID: 87 | DIELTQPPSVSVSPGQTASITCSGDALGGEYVHWYQQ KPGQAPVLVIYDDDKRPSGIPERFSGSNSGNTATLTIS GTQAEDEADYYCSSFDTWTSYVFGGGTKLTVLGQ |
| | VH | Seq. ID: 88 | QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSSSAAWN WIRQSPSRGLEWLGRIEYRSKWYNDYAVSVKSRITINP DTSKNQFSLQLNSVTPEDTAVYYCAREMYYYSGYGVF DVWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 89 | GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGT GAGCCCGGGCCAGACCGCGAGCATTACCTGTAGCG GCGATGCTCTGGGTGGTGAATACGTTCATTGGTACC AGCAGAAACCGGGCCAGGCGCCGGTGCTGGTGAT CTACGACGACGACAAACGTCCGAGCGGCATCCCGG AACGTTTTAGCGGATCCAACAGCGGCAACACCGCG |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | | | ACCCTGACCATTAGCGGCACCCAGGCGGAAGACGA AGCGGATTATTACTGCTCTTCTTTCGACACTTGGAC TTCTTACGTGTTTGGCGGCGGCACGAAGTTAACCGT TCTTGGCCAG |
| | VH (DNA) | Seq. ID: 90 | CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGT GAAACCGAGCCAGACCCTGAGCCTGACCTGCGCGA TTTCCGGAGATAGCGTGAGCTCCTCTTCTGCTGCTT GGAACTGGATTCGTCAGAGCCCGAGCCGTGGCCTC GAGTGGCTGGGCCGTATCGAATACCGTAGCAAATG GTACAACGACTATGCCGTGAGCGTGAAAAGCCGCA TTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACG GCCGTGTATTATTGCGCGCGTGAAATGTACTACTAC TCTGGTTACGGTGTTTTCGATGTTTGGGGCCAAGGC ACCCTGGTGACTGTTAGCTCA |
| 12754 | HCDR1 | Seq. ID: 91 | GFTFSDYAMT |
| | HCDR2 | Seq. ID: 92 | WVSVISYDGSLTYYADSVKG |
| | HCDR3 | Seq. ID: 93 | DPGVWWLSYLDY |
| | LCDR1 | Seq. ID: 94 | RASQDIISYLA |
| | LCDR2 | Seq. ID: 95 | LLIYGASNLQG |
| | LCDR3 | Seq. ID: 96 | QQYMIAPPN |
| | VL | Seq. ID: 97 | DIQMTQSPSSLSASVGDRVTITCRASQDIISYLAWYQQ KPGKAPKLLIYGASNLQGGVPSRFSGSGSGTDFTLTIS SLQPEDFAVYYCQQYMIAPPNTFGQGTKVEIKRT |
| | VH | Seq. ID: 98 | EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYAMTW VRQAPGKGLEWVSVISYDGSLTYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCARDPGVWWLSYLD YWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 99 | GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAG CGCCAGCGTGGGCGATCGCGTGACCATTACCTGCA GAGCCAGCCAGGACATTATCTCTTACCTGGCTTGGT ACCAGCAGAAACCGGGCAAAGCGCCGAAACTATTA ATCTACGGTGCTTCTAACCTGCAAGGCGGCGTGCC GAGCCGCTTTAGCGGCAGCGGATCCGGCACCGATT TCACCCTGACCATTAGCTCTCTGCAACCGGAAGACT TTGCGGTGTATTATTGCCAGCAGTACATGATCGCTC CACCGAACACCTTTGGCCAGGGCACGAAAGTTGAA ATTAAACGTACG |
| | VH (DNA) | Seq. ID: 100 | GAAGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGT GCAGCCGGGTGGCAGCCTGCGTCTGAGCTGCGCG GCGTCCGGATTCACCTTTTCTGACTACGCTATGACT TGGGTGCGCCAGGCCCCGGGCAAAGGTCTCGAGT GGGTTTCCGTTATCTCTTACGACGGTTCTCTGACCT ACTATGCGGATAGCGTGAAAGGCCGCTTTACCATCA GCCGCGATAATTCGAAAAACACCCTGTATCTGCAAA TGAACAGCCTGCGTGCGGAAGATACGGCCGTGTAT TATTGCGCGCGTGACCCGGGTGTTTGGTGGCTGTC TTACCTGGATTACTGGGGCCAAGGCACCCTGGTGA CTGTTAGCTCA |
| 12755 | HCDR1 | Seq. ID: 101 | GDSVSSNSAAWN |
| | HCDR2 | Seq. ID: 102 | WLGKTYYRSTWSNDYAESVKS |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | HCDR3 | Seq. ID: 103 | EMDSLTRSASSIAFDY |
| | LCDR1 | Seq. ID: 104 | SGDNLREHYVH |
| | LCDR2 | Seq. ID: 105 | LVIYDDTERPS |
| | LCDR3 | Seq. ID: 106 | ATRDWSNV |
| | VL | Seq. ID: 107 | DIELTQPPSVSVSPGQTASITCSGDNLREHYVHWYQQ KPGQAPVLVIYDDTERPSGIPERFSGSNSGNTATLTIS GTQAEDEADYYCATRDWSNVVFGGGTKLTVLGQ |
| | VH | Seq. ID: 108 | QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWN WIRQSPSRGLEWLGKTYYRSTWSNDYAESVKSRITIN PDTSKNQFSLQLNSVTPEDTAVYYCAREMDSLTRSAS SIAFDYWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 109 | GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGT GAGCCCGGGCCAGACCGCGAGCATTACCTGTAGCG GCGATAACCTGCGTGAACATTACGTTCATTGGTACC AGCAGAAACCGGGCCAGGCGCCGGTGCTGGTGAT CTACGACGACACTGAACGTCCGAGCGGCATCCCGG AACGTTTTAGCGGATCCAACAGCGGCAACACCGCG ACCCTGACCATTAGCGGCACCCAGGCGGAAGACGA AGCGGATTATTACTGCGCTACTCGTGACTGGTCTAA CGTTGTGTTTGGCGGCGGCACGAAGTTAACCGTTC TTGGCCAG |
| | VH (DNA) | Seq. ID: 110 | CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGT GAAACCGAGCCAGACCCTGAGCCTGACCTGCGCGA TTTCCGGAGATAGCGTGAGCAGTAACTCTGCTGCTT GGAACTGGATTCGTCAGAGCCCGAGCCGTGGCCTC GAGTGGCTGGGCAAAACCTACTACCGTAGCACTTG GTCTAACGACTATGCCGAAAGCGTGAAAAGCCGCA TTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACG GCCGTGTATTATTGCGCGCGTGAAATGGACTCTCTG ACTCGTTCTGCTTCTTCTATCGCTTTCGATTACTGGG GCCAAGGCACCCTGGTGACTGTTAGCTCA |
| 12756 | HCDR1 | Seq. ID: 111 | GDSVSDNSVAWN |
| | HCDR2 | Seq. ID: 112 | WLGRIYYRSKWYNDYAVSVKS |
| | HCDR3 | Seq. ID: 113 | EVLLFPARSYGTGMDV |
| | LCDR1 | Seq. ID: 114 | SGDNLPSKYVH |
| | LCDR2 | Seq. ID: 115 | LVIYDDNERPS |
| | LCDR3 | Seq. ID: 116 | GVADMPRQMK |
| | VL | Seq. ID: 117 | DIELTQPPSVSVSPGQTASITCSGDNLPSKYVHWYQQ KPGQAPVLVIYDDNERPSGIPERFSGSNSGNTATLTIS GTQAEDEADYYCGVADMPRQMKVFGGGTKLTVLGQ |
| | VH | Seq. ID: 118 | QVQLQQSGPGLVKPSQTLSLTCAISGDSVSDNSVAW NWIRQSPSRGLEWLGRIYYRSKWYNDYAVSVKSRITI NPDTSKNQFSLQLNSVTPEDTAVYYCAREVLLFPARS YGTGMDVWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 119 | GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGT GAGCCCGGGCCAGACCGCGAGCATTACCTGTAGCG GCGATAACCTGCCGTCTAAATACGTTCATTGGTACC AGCAGAAACCGGGCCAGGCGCCGGTGCTGGTGAT CTACGACGACAACGAACGTCCGAGCGGCATCCCGG AACGTTTTAGCGGATCCAACAGCGGCAACACCGCG |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | | | ACCCTGACCATTAGCGGCACCCAGGCGGAAGACGA<br>AGCGGATTATTACTGCGGTGTTGCTGACATGCCGC<br>GTCAGATGAAAGTGTTTGGCGGCGGCACGAAGTTA<br>ACCGTTCTTGGCCAG |
| | VH (DNA) | Seq. ID: 120 | CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGT<br>GAAACCGAGCCAGACCCTGAGCCTGACCTGCGCGA<br>TTTCCGGAGATAGCGTGAGCGACAACTCTGTTGCTT<br>GGAACTGGATTCGTCAGAGCCCGAGCCGTGGCCTC<br>GAGTGGCTGGGCCGTATCTACTACCGTAGCAAATG<br>GTACAACGACTATGCCGTGAGCGTGAAAAGCCGCA<br>TTACCATTAACCCGGATACTTCGAAAAACCAGTTTA<br>GCCTGCAACTGAACAGCGTGACCCCGGAAGATACG<br>GCCGTGTATTATTGCGCGCGTGAAGTTCTGCTGTTC<br>CCGGCTCGTTCTTACGGTACTGGTATGGATGTTTGG<br>GGCCAAGGCACCCTGGTGACTGTTAGCTCA |
| 12757 | HCDR1 | Seq. ID: 121 | GFTFSSYAMS |
| | HCDR2 | Seq. ID: 122 | WVSFISSGGSETFYADSVKG |
| | HCDR3 | Seq. ID: 123 | VSYIYYYSWVLFDV |
| | LCDR1 | Seq. ID: 124 | RASQGIGTALN |
| | LCDR2 | Seq. ID: 125 | LLIYDVSSLQS |
| | LCDR3 | Seq. ID: 126 | QQGLFLPF |
| | VL | Seq. ID: 127 | DIQMTQSPSSLSASVGDRVTITCRASQGIGTALNWYQ<br>QKPGKAPKLLIYDVSSLQSGVPSRFSGSGSGTDFTLTI<br>SSLQPEDFATYYCQQGLFLPFTFGQGTKVEIKRT |
| | VH | Seq. ID: 128 | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSW<br>VRQAPGKGLEWVSFISSGGSETFYADSVKGRFTISRD<br>NSKNTLYLQMNSLRAEDTAVYYCARVSYIYYYSWVLF<br>DVWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 129 | GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAG<br>CGCCAGCGTGGGCGATCGCGTGACCATTACCTGCA<br>GAGCCAGCCAGGGTATTGGTACTGCTCTGAACTGG<br>TACCAGCAGAAACCGGGCAAAGCGCCGAAACTATT<br>AATCTACGACGTTTCTTCTCTGCAAAGCGGCGTGCC<br>GAGCCGCTTTAGCGGCAGCGGATCCGGCACCGATT<br>TCACCCTGACCATTAGCTCTCTGCAACCGGAAGACT<br>TTGCGACCTATTATTGCCAGCAGGGTCTGTTCCTGC<br>CGTTCACCTTTGGCCAGGGCACGAAAGTTGAAATTA<br>AACGTACG |
| | VH (DNA) | Seq. ID: 130 | GAAGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGT<br>GCAGCCGGGTGGCAGCCTGCGTCTGAGCTGCGCG<br>GCGTCCGGATTCACCTTTTCTTCTTACGCTATGTCTT<br>GGGTGCGCCAGGCCCCGGGCAAAGGTCTCGAGTG<br>GGTTTCCTTCATCTCTTCTGGTGGTTCTGAAACCTTC<br>TATGCGGATAGCGTGAAAGGCCGCTTTACCATCAG<br>CCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT<br>GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATT<br>ATTGCGCGCGTGTTTCTTACATCTACTACTACTCTTG<br>GGTTCTGTTCGATGTTTGGGGCCAAGGCACCCTGG<br>TGACTGTTAGCTCA |
| 12758 | HCDR1 | Seq. ID: 131 | GYSFTDYWIS |
| | HCDR2 | Seq. ID: 132 | WMGAIDPTDSYTRYSPSFQG |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | HCDR3 | Seq. ID: 133 | WYTSHPYYEGRYPMDV |
| | LCDR1 | Seq. ID: 134 | TGTSSDVGHYNYVS |
| | LCDR2 | Seq. ID: 135 | LMIYGVTKRPS |
| | LCDR3 | Seq. ID: 136 | ASADEWPTLH |
| | VL | Seq. ID: 137 | DIALTQPASVSGSPGQSITISCTGTSSDVGHYNYVSWY QQHPGKAPKLMIYGVTKRPSGVSNRFSGSKSGNTASL TISGLQAEDEADYYCASADEWPTLHVFGGGTKLTVLG Q |
| | VH | Seq. ID: 138 | EVQLVQSGAEVKKPGESLKISCKGSGYSFTDYWISWV RQMPGKGLEWMGAIDPTDSYTRYSPSFQGQVTISAD KSISTAYLQWSSLKASDTAMYYCARWYTSHPYYEGRY PMDVWGQGTLVTVSS |
| | VL (DNA) | Seq. ID: 139 | GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCG GTAGCCCGGGCCAGAGCATTACCATTAGCTGCACC GGCACCAGCAGCGATGTGGGCCATTACAACTACGT GTCTTGGTACCAGCAGCATCCGGGCAAGGCGCCGA AACTGATGATCTACGGTGTTACTAAACGTCCGAGCG GCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGC AACACCGCGAGCCTGACCATTAGCGGCCTGCAAGC GGAAGACGAAGCGGATTATTACTGCGCTTCTGCTGA CGAATGGCCGACTCTGCATGTGTTTGGCGGCGGCA CGAAGTTAACCGTTCTTGGCCAG |
| | VH (DNA) | Seq. ID: 140 | GAAGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAA AAAACCGGGCGAAAGCCTGAAAATTAGCTGCAAAG GCTCCGGATATAGCTTCACTGACTACTGGATCTCTT GGGTGCGCCAGATGCCGGGCAAAGGTCTCGAGTG GATGGGCGCTATCGACCCGACTGACAGCTACACCC GTTATAGCCCGAGCTTTCAGGGCCAGGTGACCATTA GCGCGGATAAAAGCATCAGCACCGCGTATCTGCAA TGGAGCAGCCTGAAAGCGAGCGATACCGCGATGTA TTATTGCGCGCGTTGGTACACTTCTCATCCGTACTA CGAAGGTCGTTACCCGATGGATGTTTGGGGCCAAG GCACCCTGGTGACTGTTAGCTCA |
| 12759 | HCDR1 | Seq. ID: 141 | GYSFNNYWIA |
| | HCDR2 | Seq. ID: 142 | WMGFIYPSNSATQYSPSFQG |
| | HCDR3 | Seq. ID: 143 | DNEYSDSYFDV |
| | LCDR1 | Seq. ID: 144 | RASQIVSSYLA |
| | LCDR2 | Seq. ID: 145 | LLIYDASSRAT |
| | LCDR3 | Seq. ID: 146 | QQSVNFPT |
| | VL | Seq. ID: 147 | DIVLTQSPATLSLSPGERATLSCRASQIVSSYLAWYQQ KPGQAPRLLIYDASSRATGIPARFSGSGSGTDFTLTISS LEPEDFAVYYCQQSVNFPTTFGQGTKVEIKRT |
| | VH | Seq. ID: 148 | EVQLVQSGAEVKKPGESLKISCKGSGYSFNNYWIAWV RQMPGKGLEWMGFIYPSNSATQYSPSFQGQVTISAD KSISTAYLQWSSLKASDTAMYYCARDNEYSDSYFDVW GQGTLVTVSS |
| | VL (DNA) | Seq. ID: 149 | GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAG CCTGAGCCCGGGTGAACGTGCCACCCTGAGCTGCA GAGCGAGCCAGATCGTTTCTTCTTACCTGGCTTGGT ACCAGCAGAAACCGGGCCAGGCCCCGCGTCTATTA ATCTACGACGCTTCTTCTCGTGCGACCGGCATTCCG |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | | | GCGCGTTTTAGCGGCAGCGGATCCGGCACCGATTT CACCCTGACCATTAGCAGCCTGGAACCGGAAGACT TTGCGGTGTATTATTGCCAGCAGTCTGTTAACTTCC CGACTACCTTTGGCCAGGGCACGAAAGTTGAAATTA AACGTACG |
| | VH (DNA) | Seq. ID: 150 | GAAGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAA AAAACCGGGCGAAAGCCTGAAAATTAGCTGCAAAG GCTCCGGATATAGCTTCAACAACTACTGGATCGCTT GGGTGCGCCAGATGCCGGGCAAAGGTCTCGAGTG GATGGGCTTCATCTACCCGTCTAACAGCGCTACCCA GTATAGCCCGAGCTTTCAGGGCCAGGTGACCATTA GCGCGGATAAAAGCATCAGCACCGCGTATCTGCAA TGGAGCAGCCTGAAAGCGAGCGATACCGCGATGTA TTATTGCGCGCGTGACAACGAATACTCTGACTCTTA CTTCGATGTTTGGGGCCAAGGCACCCTGGTGACTG TTAGCTCA |
| 12760 | HCDR1 | Seq. ID: 151 | GYSFNNYWIA |
| | HCDR2 | Seq. ID: 152 | WMGFIYPSNSATQYSPSFQG |
| | HCDR3 | Seq. ID: 153 | DNEYSDSYFDV |
| | LCDR1 | Seq. ID: 154 | RASQIVSSYLA |
| | LCDR2 | Seq. ID: 145 | LLIYDASSRAT |
| | LCDR3 | Seq. ID: 156 | QQSVKSN |
| | VL | Seq. ID: 157 | DIVLTQSPATLSLSPGERATLSCRASQIVSSYLAWYQQ KPGQAPRLLIYDASSRATGIPARFSGSGSGTDFTLTISS LEPEDFATYYCQQSVKSNTFGQGTKVEIKRT |
| | VH | Seq. ID: 158 | EVQLVQSGAEVKKPGESLKISCKGSGYSFNNYWIAWV RQMPGKGLEWMGFIYPSNSATQYSPSFQGQVTISAD KSISTAYLQWSSLKASDTAMYYCARDNEYSDSYFDVW GQGTLVTVSS |
| | VL (DNA) | Seq. ID: 159 | GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAG CCTGAGCCCGGGTGAACGTGCCACCCTGAGCTGCA GAGCGAGCCAGATCGTTTCTTCTTACCTGGCTTGGT ACCAGCAGAAACCGGGCCAGGCCCCGCGTCTATTA ATCTACGACGCTTCTTCTCGTGCGACCGGCATTCCG GCGCGTTTTAGCGGCAGCGGATCCGGCACCGATTT CACCCTGACCATTAGCAGCCTGGAACCGGAAGACT TTGCGACCTATTATTGCCAGCAGTCTGTTAAATCTAA CACCTTTGGCCAGGGCACGAAAGTTGAAATTAAACG TACG |
| | VH (DNA) | Seq. ID: 160 | GAAGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAA AAAACCGGGCGAAAGCCTGAAAATTAGCTGCAAAG GCTCCGGATATAGCTTCAACAACTACTGGATCGCTT GGGTGCGCCAGATGCCGGGCAAAGGTCTCGAGTG GATGGGCTTCATCTACCCGTCTAACAGCGCTACCCA GTATAGCCCGAGCTTTCAGGGCCAGGTGACCATTA GCGCGGATAAAAGCATCAGCACCGCGTATCTGCAA TGGAGCAGCCTGAAAGCGAGCGATACCGCGATGTA TTATTGCGCGCGTGACAACGAATACTCTGACTCTTA CTTCGATGTTTGGGGCCAAGGCACCCTGGTGACTG TTAGCTCA |
| 12761 | HCDR1 | Seq. ID: 161 | GYSFNNYWIA |
| | HCDR2 | Seq. ID: 162 | WMGFIYPSNSATQYSPSFQG |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | HCDR3 | Seq. ID: 163 | DNEYSDSYFDV |
| | LCDR1 | Seq. ID: 164 | RASQIVSSYLA |
| | LCDR2 | Seq. ID: 165 | LLIYDASSRAT |
| | LCDR3 | Seq. ID: 166 | QQSNGWLP |
| | VL | Seq. ID: 167 | DIVLTQSPATLSLSPGERATLSCRASQIVSSYLAWYQQ KPGQAPRLLIYDASSRATGIPARFSGSGSGTDFTLTISS LEPEDFATYYCQQSNGWLPTFGQGTKVEIKRT |
| | VH | Seq. ID: 168 | EVQLVQSGAEVKKPGESLKISCKGSGYSFNNYWIAWV RQMPGKGLEWMGFIYPSNSATQYSPSFQGQVTISAD KSISTAYLQWSSLKASDTAMYYCARDNEYSDSYFDVW GQGTLVTVSS |
| | VL (DNA) | Seq. ID: 169 | GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAG CCTGAGCCCGGGTGAACGTGCCACCCTGAGCTGCA GAGCGAGCCAGATCGTTTCTTCTTACCTGGCTTGGT ACCAGCAGAAACCGGGCCAGGCCCCGCGTCTATTA ATCTACGACGCTTCTTCTCGTGCGACCGGCATTCCG GCGCGTTTTAGCGGCAGCGGATCCGGCACCGATTT CACCCTGACCATTAGCAGCCTGGAACCGGAAGACT TTGCGACCTATTATTGCCAGCAGTCTAACGGTTGGC TGCCGACCTTTGGCCAGGGCACGAAAGTTGAAATTA AACGTACG |
| | VH (DNA) | Seq. ID: 170 | GAAGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAA AAAACCGGGCGAAAGCCTGAAAATTAGCTGCAAAG GCTCCGGATATAGCTTCAACAACTACTGGATCGCTT GGGTGCGCCAGATGCCGGGCAAAGGTCTCGAGTG GATGGGTTTCATCTACCCGTCTAACAGCGCTACCCA GTATAGCCCGAGCTTTCAGGGCCAGGTGACCATTA GCGCGGATAAAAGCATCAGCACCGCGTATCTGCAA TGGAGCAGCCTGAAAGCGAGCGATACCGCGATGTA TTATTGCGCGCGTGACAACGAATACTCTGACTCTTA CTTCGATGTTTGGGGCCAAGGCACCCTGGTGACTG TTAGCTCA |
| 12762 | HCDR1 | Seq. ID: 171 | GYSFNNYWIA |
| | HCDR2 | Seq. ID: 172 | WMGFIYPSNSATQYSPSFQG |
| | HCDR3 | Seq. ID: 173 | DNEYSDSYFDV |
| | LCDR1 | Seq. ID: 174 | RASQIVSSYLA |
| | LCDR2 | Seq. ID: 175 | LLIYDASSRAT |
| | LCDR3 | Seq. ID: 176 | QQSEQVPT |
| | VL | Seq. ID: 177 | DIVLTQSPATLSLSPGERATLSCRASQIVSSYLAWYQQ KPGQAPRLLIYDASSRATGIPARFSGSGSGTDFTLTISS LEPEDFAVYYCQQSEQVPTTFGQGTKVEIKRT |
| | VH | Seq. ID: 178 | EVQLVQSGAEVKKPGESLKISCKGSGYSFNNYWIAWV RQMPGKGLEWMGFIYPSNSATQYSPSFQGQVTISAD KSISTAYLQWSSLKASDTAMYYCARDNEYSDSYFDVW GQGTLVTVSS |
| | VL (DNA) | Seq. ID: 179 | GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAG CCTGAGCCCGGGTGAACGTGCCACCCTGAGCTGCA GAGCGAGCCAGATCGTTTCTTCTTACCTGGCTTGGT ACCAGCAGAAACCGGGCCAGGCCCCGCGTCTATTA ATCTACGACGCTTCTTCTCGTGCGACCGGCATTCCG |

| MOR# | | Seq. ID: | [aa] / DNA |
|---|---|---|---|
| | | | GCGCGTTTTAGCGGCAGCGGATCCGGCACCGATTT CACCCTGACCATTAGCAGCCTGGAACCGGAAGACT TTGCGGTGTATTATTGCCAGCAGTCTGAACAGGTTC CGACTACCTTTGGCCAGGGCACGAAAGTTGAAATTA AACGTACG |
| | VH (DNA) | Seq. ID: 180 | GAAGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAA AAAACCGGGCGAAAGCCTGAAAATTAGCTGCAAAG GCTCCGGATATAGCTTCAACAACTACTGGATCGCTT GGGTGCGCCAGATGCCGGGCAAAGGTCTCGAGTG GATGGGCTTCATCTACCCGTCTAACAGCGCTACCCA GTATAGCCCGAGCTTTCAGGGCCAGGTGACCATTA GCGCGGATAAAAGCATCAGCACCGCGTATCTGCAA TGGAGCAGCCTGAAAGCGAGCGATACCGCGATGTA TTATTGCGCGCGTGACAACGAATACTCTGACTCTTA CTTCGATGTTTGGGGCCAAGGCACCCTGGTGACTG TTAGCTCA |

Example 1: Mouse IL17C

Mouse IL17C was purchased from R&D Systems (#2306-ML/CF; R&D Systems, Inc., Minneapolis, USA). Biotinylated mouse IL17C was prepared using the ECLTM biotinylation module (GE Healthcare; #1061918). After biotinlyation the product was purified using ZebaTM Desalt spin columns (Pierce; #89889).

Quality control of the biotinylated IL17C was performed using dynamic light scattering (DLS), size exclusion chromatography (SEC) and SDS-PAGE. As expected, SDS-PAGE revealed an apparent molecular weight of about 22 kDa under reducing conditions and of about 40 kDa under non-reducing conditions. In addition no high molecular weight species or aggregates could be detected. The predicted size of the antigen was confirmed in SEC, where biotinylated mouse IL17C was visible as one peak with a molecular weight of about 43 kDa. In DLS, no aggregates could be detected. Additionally it was confirmed that mouse IL17C was biotinylated quantitatively.

Only material that passed the quality control was used for panning and binding assays.

Example 2: Mouse IL17 receptor E

The extracellular domain (ECD) of mouse IL-17 receptor E (GeneID: 57890, isoform 1) was cloned as a C-terminal Fc fusion protein (referred to as “IL17RE/Fc”). A construct containing a vκ-Leader followed by the ECD of mouse IL17C was transiently expressed in

HKB11 cells (Cho et al. (2002) J. Biomed Sci. Nov-Dec; 9(6 Pt 2):631-8). The products were purified via protein A affinity chromatography. Purity was analyzed under denaturing, reducing and denaturing, non-reducing conditions in SDS-PAGE and in native state by High Pressure-SEC and DLS.

Example 3: IL17C – IL17 receptor E interaction assay

To test binding of mouse IL17 to its putative receptor, IL17 receptor E an interaction assay was set up. The assay setup is depicted in Figure 1A. In brief, IL17 receptors B and E were coated on a Multi-array® 384-well plate Standard plate (Meso Scale Discovery; #L21XA-4) and biotinylated mouse IL17C was added. Binding of mouse IL17C to its receptor was measured via binding of Streptavidin in a MSD Sector Imager 6000 (Meso Scale Discovery, Gaithersburg, MD, USA).

Results are shown in Figure 1B. IL17C was clearly found to bind to mouse IL17RE/Fc, but not to mouse IL-17RB or another irrelevant receptor. Also, an irrelevant biotinylated ligand did not show binding to any of the three receptors tested. The interaction assay is therefore highly specific and well suited for the analysis of IL17C – IL17 receptor E interactions.

Example 4: Effect of prior art antibodies on the IL17C – IL17 receptor E interaction

Three prior art antibodies were tested for their ability to inhibit binding of mouse IL17C to mouse IL17RE/Fc in the interaction assay of Example 3. The following antibodies were tested:

A: rat $IgG_{2A}$ monoclonal anti-mouse IL17C antibody (R&D Systems; clone 311522,# MAB23061)

B: rat $IgG_{2A}$ monoclonal anti-mouse IL17C antibody (R&D Systems; clone 311523,# MAB2306)

C: rat anti-mouse IL17C (US Biological; clone: 8B28, #I8439-20R3)

Antibodies were pre-incubated with biotinylated mouse IL17C and the pre-formed complex was then added to the coated mouse IL17RE/Fc.

Results are shown in Figure 1C. None of the prior art anti-mouse IL17C antibodies did show any effect on the binding of mouse IL17C to its receptor IL17RE/Fc.

Example 5: Panning strategy

The HuCAL PLATINUM® library was used to select specific Fab fragments against mouse IL17C. This phagemid library is based on the HuCAL® concept disclosed in Knappik et al. (Knappik et al. (2000) J. Mol. Biol. 296:57-86) and employs the CysDisplay® technology for displaying the Fab on the phage surface (Lohning et al., WO2001/05950).

Different panning strategies were performed, solution panning, including various maturation strategies, as well as conventional solid phase panning. For solid phase panning mouse IL17C was directly coated on MaxisorpTM Immuno plates (Nunc; #442404). A total of three rounds of panning were performed for solid phase panning. Three selection rounds were performed with a successive increase of washing stringency and reduction of antigen concentration from round to round. For solution panning biotinylated antigen was exposed to the phage library in solution with subsequent capture of phage-antigen complexes on streptavidin beads. Again, three selection rounds were performed with a successive increase of washing stringency and reduction of antigen concentration from round to round. Phages were isolated using streptavidin-coupled magnetic beads (Invitrogen, #112-05D). To select high affinity antibodies, HCDR2 and LCDR3 libraries were generated after the second round of selection (average library size ~$1x10^8$) followed by two more selection rounds with further increased stringency and further decreased antigen concentrations.

Example 6: Initial characterization of panning output via ELISA

To facilitate rapid expression of soluble Fab fragments in crude bacterial lysates periplasmatic extracts were prepared as previously described (Rauchenberger et al. (2003) J. Biol. Chem. 278.40: 38194-205). Fab containing *E. coli* lysates were used for ELISA screening of the initial hits.

Specificity of the binders were investigates via ELISA screening on the directly coated antigen or on the biotinylated antigen. For ELISA screening on the directly coated antigen Maxisorp™ 384 well plates (Nunc; #460518) were coated with 2.5 µg/mL mouse IL17C in PBS. After blocking of plates with 5% skimmed milk powder in PBS, Fab-containing *E. coli* lysates were added. Binding of Fabs was detected by $F(ab)_2$ specific goat anti-human IgG conjugated to alkaline phosphatase (diluted 1:5000) using Attophos fluorescence substrate (Roche: #11681982001). Fluorescence emission at 535 nm was recorded with excitation at 430 nm. For ELISA screening on biotinylated antigen Maxisorp™ 384 well plates were coated

with Fd fragment specific sheep anti-human IgG (Binding site, #PC075) diluted 1:1000 in PBS. After blocking with 5% skim milk powder in PBS, Fab-containing *E. coli* lysates were added. Subsequently the captured HuCAL®-Fab fragments were allowed to bind to 1 µg/ml biotinylated mouse IL17C, which was detected by incubation with streptavidin conjugated to alkaline phosphatase followed by addition of AttoPhos fluorescence substrate (Roche: #11681982001). Fluorescence emission at 535 nm was recorded with excitation at 430 nm. Almost 9000 Fab fragments isolated from the panning procedure were tested in these ELISA assays, and about 2900 were positive in all ELISA tests.

Example 7: Characterization of the binders via the IL17C – IL17 receptor E interaction assay

To test Fab-containing crude bacterial lysates for neutralizing activity in high throughput screening mode a slightly modified assay as outlined in Example 3 was used. Maxisorp™ 384 well MSD plates (Nunc; #460518) were coated with 30 µL mouse IL17RE/Fc at 0.6 µg/mL in PBS overnight at 4°C. The next day 20 µL Fab-containing *E. coli* lysates were pre-incubated for 30 min at RT with an equal volume of biotinylated mouse IL17C at 2 nM. After blocking of plates for 1h with 5% BSA in PBS, preformed antibody – ligand complexes were added for 1h to coated IL17RE/Fc and receptor binding was detected via Streptavidin-ECL using MSD Sector Imager 6000 (Meso Scale Discovery, Gaithersburg, MD, USA).

To determine inhibitory activity of purified anti-IL17C human-mouse chimeric IgG2a in a mIL-17 RE interaction assay, Maxisorp™ 384 well MSD plates were coated with 30 µL mouse IL17RE/Fc at 75 ng/mL in PBS at 4°C overnight. The next day 25 µL of a serial antibody dilution (concentrations from 0.001 to 100 nM) were pre-incubated for 30 min at RT with an equal volume of biotinylated mouse IL17C at 0.125 nM. After blocking of plates for 1h with 2.5% BSA in PBST, preformed antibody – ligand complexes were added for 1h to coated IL17RE/Fc and receptor binding was detected via Streptavidin-ECL using MSD Sector Imager.

Of all ELISA-positive binders, the IL17C – IL17 receptor E interaction assay, followed by sequencing of the positive clones, revealed merely 141 sequence-unique clones belonging to 33 different HCDR3 families. This number was again reduced by confirmatory screening in ELISA and mouse IL17RE/Fc inhibition assay. To select candidates with best inhibitory activity, BEL lysates were pre-diluted up to 1:500 for use in IL17RE/Fc inhibition assay. Finally, 18 sequence-unique clones belonging to 8 different HCDR3 families could be identified which consistently and repeatedly were positive in the IL17C – IL17 receptor E interaction assay.

Example 8: Polyclonal IgG conversion

Conversion of the Fab fragments into an IgG format was performed by polyclonal cloning of the Fab fragments into the desired IgG format. A human-mouse chimeric IgG format was used to avoid immunogenicity reactions directed against administered anti-mouse IL17C antibodies in *the in vivo* proof of concept study with wild-type mice. Two different constant regions were used - the IgG2a and the IgG1 isotype. Potential N-linked glycosylation sites were removed via site-directed mutagenesis using the QuickChange II Site-directed Mutagenesis Kit (Stratagene; #0200524). The sequence diversity of all IgG's recovered from this procedure is depicted in Table 1.

Example 9: Exploratory scale production of IgG's

All IgGs recovered from the procedure described in Example 8 were produced in exploratory-scale in HKB11 cells (Cho et al. (2002) J. Biomed Sci. Nov-Dec; 9(6 Pt 2):631-8) in both chimeric human/mouse IgG2a and chimeric human/mouse IgG1 isotype format, in order to assess production yields and monomeric portion of the antibodies with two different constant regions. The highest quantities could be produced with antibodies of the chimeric human/mouse IgG2a format. This format also successfully passed quality control in SEC (> 90% monomer content). The chimeric human/mouse IgG2a format was therefore chosen for further functional testing. Purification yield and the portion of monomer as determined in SEC is shown in Figure 2.

Example 10: Affinity determination

All purified chimeric human-mouse chimeric IgG2a antibodies were titrated on mouse IL17C for $EC_{50}$ determination in ELISA starting with a concentration of 100 nM. $EC_{50}$ values on mouse IL17C were determined in 1 to 3 independent experiments. Results are shown in Figure 3.

$EC_{50}$ values ranged between 200 and 1200 pM, with most $EC_{50}$ values were around 300 pM. Binding activity of 4 IgGs (MOR12755, MOR12756, 12757, 12758) could not be confirmed with purified IgG2a, therefore these IgGs were excluded from further characterization. None of the antibodies showed cross-reactivity to human IL17C (sequence identity 77%), to mouse IL-17B (sequence identity 30%) or to the negative control antigen lysozyme.

Monovalent affinities of anti-IL17C antibodies were determined by solution equilibrium titration (SET) using Fab fragments. Affinity determination in solution was basically performed as described in the literature (Friquet et al., (1985) J. Immunol. Meth. 77: 305-19). In order to improve the sensitivity and accuracy of the SET method, it was transferred from classical ELISA to ECL based technology (Haenel et al. (2005) Anal Biochem. 339.1: 182-84). Binders were expressed and purified in Fab_FH format. Some Fabs did either not bind or showed no sigmoidal binding curve in SET, therefore affinities could only be determined for 10 candidates. Monovalent affinities ranged between 48 and 4100 pM with most $K_D$ values of the Fabs in the low pM range (≤ 100 pM**Error! Reference source not found.**). Results are shown in Figure 4.

Example 11: IL17C – IL17 receptor E interaction assay in with binders in IgG format

To characterize the antibodies in human/mouse IgG2a format in more detail, for each candidate $IC_{50}$ values were determined in the IL-17 receptor E inhibition assay as described herein above in up to 4 independent experiments.

Results are depicted in Figure 5. 11 out of the 12 IgGs showed inhibitory activity with mean $IC_{50}$ values ranging between 9 and 8442 pM. 9 out of these 11 inhibitory IgGs even had $IC_{50}$ values in the low picomolar range. The best candidates belonged to different HCDR3 families and were of different VL subtypes (kappa or lambda).

Example 12: Stability in mouse serum

In order to analyse whether the selected anti-mouse IL17C antibodies are suitable for *in vivo* administration in mice, stability in mouse serum was determined for a subset of 11 purified IgGs that showed acceptable production yields and specific binding to mouse IL17C (see hereinabove).

96 well Maxisorp plates (Nunc; #442404) were coated with avidin at a concentration of 1 µg/ml in PBS overnight at 4 °C. The next day, anti-IL17 chimeric IgG2a were incubated in mouse serum for 24 h at 37°C at a final concentration 100 µg/mL. After the incubation step the antibodies were diluted 1:100 in LowCross buffer (Candor Bioscience; #100500). As a reference the same set of antibodies were freshly diluted in LowCross buffer + 1% mouse serum and incubated for 30 min at RT. Avidin coated plates were incubated with blocking buffer (Superblock blocking buffer from Pierce, #37515) and subsequently 100 µL of 0.1µg/mL biotin-IL17C in LowCross buffer was added to blocked wells. After a washing step a serial

dilution of the serum-incubated and freshly diluted IgGs was added. Binding of IgGs was detected by anti-mouse-IgG2a POD conjugated detection antibody (diluted 1:5000 in LowCross buffer) using TMB One Component HRP as substrate. The reaction was stopped by adding 1M HCl and absorbance was measured at 450 nm.

With exception of one IgG candidate (MOR12760) which exhibited only moderate serum stability, all other antibodies showed a very good stability in mouse serum (coefficient of variation ≤ 20%) after incubation for 24 h at 37°C. Results are depicted in Figure 6.

Example 13: Selection of the binders for the *In Vivo* Proof of Concept study

Based on their favorable properties with respect to productivity, stability, binding and functional activity in IL-17RE receptor inhibition assay and in a cell-based NFkB reporter gene assay, MOR12743 and MOR12762 were selected as candidates for the *in vivo* proof of concept study.

Example 14: *In vivo* CIA model

*14.1 Materials*

Completed Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) were purchased from Difco (MI, US). Bovine collagen type II (CII), lipopolysaccharide (LPS), and Enbrel was obtained from MD Biosciences (Germany); Sigma (P4252, L'Isle d'Abeau, France), Whyett (25mg injectable syringe, France), respectively. All other reagents used were of reagent grade and all solvents were of analytical grade.

*14.2 Animals*

DBA1/J mice (male, 6-7 weeks old, approx 20 gram) were obtained from Centre d'Elevage Regional Janvier (Laval, France). Mice were kept on a 12 hr light/dark cycle (0700 - 1900). Temperature was maintained at 22°C, and food and water were provided *ad libitum*.

*14.3 Collagen induced arthritis (CIA)*

One day before the experiment, CII solution (2 mg/mL) was prepared with 0.05 M acetic acid and stored at 4ºC. Just before the immunization, equal volumes of adjuvant (IFA) and CII were mixed by a homogenizer in a pre-cooled glass bottle in an ice water bath. Extra adjuvant and prolonged homogenization may be required if an emulsion is not formed. 0.1 mL of the emulsion was injected intradermally at the base of the tail of each mouse on day 1, a

second booster intradermal injection (CII solution at 2 mg/mL in CFA 0.1 mLemulsion) was performed on day 21. This immunization method was modified from published methods (Brand *et al*, 2007, Collagen-induced arthritis. Nature Protocols; vol 2 (5): 1269- 1275; Lin *et al*., 2007, Anti-rheumatic activities of histone deacetylase (HDAC) inhibitors in vivo in collagen-induced arthritis in rodents. Br J Pharmacol. Apr; 150 (7):829-31).

*14.4 Study design*

14.4.1 Therapeutic protocol

The therapeutic effects of the antibodies were tested in the mouse CIA model. Mice were randomly divided into equal groups and each group contained 10 mice. All mice were immunized on day 1 and boosted on day 21. Therapeutic dosing lasted from day 31 to day 46. The negative control group was treated with vehicle (PBS) and the positive control group with Enbrel (10 mg/kg, 3x week., i.p.). An antibody of interest was tested at 10 mg/kg, i.p. 3 times per week.

Therefore, five different treatment groups were used, wherein each group consisted of ten mice.

Group 1: Vehicle (PBS)
Group 2: Enbrel 10mg/kg/3x week, i.p.
Group 3: MOR03207 10mg/kg/3 x week, i.p.
Group 4: MOR12743 10mg/kg/3 x week, i.p.
Group 5: MOR12762 10mg/kg/3 x week, i.p.

Anti-IL17C antibodies were administered i.p. three times a week at 10 mg/kg. Blood samples (approx 250 µL) were taken on days 31 and 46 for pharmacokinetic analysis.

14.4.2 Preventative protocol

The preventative effects of the antibodies were tested in the mouse CIA model. Mice were randomly divided into two equal groups containing 20 mice. All mice were immunized on day 1 and boosted on day 21. Prophylactic dosing lasted from day 21 to day 46. The negative control group was treated with negative antibody (MOR03207, 10 mg/kg, 3x week., i.p.) An antibody of interest (MOR12762) was tested at 10 mg/kg, 3x week, i.p.

Therefore, two different treatment groups were used, wherein each group consisted of twenty mice.

Group 1: MOR03207 10mg/kg/3 x week, i.p.
Group 2: MOR12762 10mg/kg/3 x week, i.p.

Anti-IL17C antibodies were administered i.p.three times a week at 10 mg/kg. Blood sample (approx 250 µL) were taken on days 21, 36 and 46 for pharmacokinetic analysis.

*14.5 Clinical assessment of arthritis*

Arthritis was scored according to the method of Khachigian 2006 (Collagen antibody-induced arthritis. (2006) Nature Protocols 1, 2512-6), Lin et al 2007 (*supra*); Nishida *et al.* 2004 (Histone deacetylase inhibitor suppression of autoantibody-mediated arthritis in mice via regulation of p16INK4a and p21(WAF1/Cip1) expression. Arthritis Rheum. 10: 3365-76); and Brand et al. 2007 (*supra*). The swelling of each of the four paws was ranked with the arthritic score as follows: 0-no symptoms; 1-mild, but definite redness and swelling of one type of joint such as the ankle or wrist, or apparent redness and swelling limited to individual digits, regardless of the number of affected digits; 2-moderate redness and swelling of two or more types of joints; 3-severe redness and swelling of the entire paw including digits; 4-maximally inflamed limb with involvement of multiple joints (maximum cumulative clinical arthritis score 16 per animal) (Nishida *et al.*, 2004 (*supra*)).

If required, to permit the meta-analysis of multiple therapeutic studies the clinical score values may be normalised as follows:

*AUC of clinical score (AUC score)*:

The area under the curve (AUC) from day 31(21) to day 46 was calculated for each individual animal. The AUC of each animal was divided by the average AUC obtained for the vehicle in the study from which the data on that animal was obtained and multiplied by 100 (i.e. the AUC was expressed as a percentage of the average vehicle AUC per study).

*Clinical score increase from day 21 to day 46 (End point score)*:

The clinical score difference for each animal was divided by the average clinical score difference obtained for the vehicle in the study from which the data on that animal was obtained and multiplied by 100 (i.e. the difference was expressed as a percentage of the average clinical score difference for the vehicle per study).

*14.6 Radiology (Larsen's score)*

X-ray photos were taken of the hind paws of each individual animal. A random blind identity number was assigned to each of the photos, and the severity of bone erosion was

ranked by three independent scorers with the radiological Larsen's score system as follows: 0-normal with intact bony outlines and normal joint space; 1- slight abnormality with any one or two of the exterior metatarsal bones showing slight bone erosion; 2-definite early abnormality with any three to five of the exterior metatarsal bones showing bone erosion; 3-medium destructive abnormality with all the exterior metatarsal bones as well as any one or two of the interior metatarsal bones showing definite bone erosions; 4-severe destructive abnormality with all the metatarsal bones showing definite bone erosion and at least one of the inner metatarsal joints completely eroded leaving some bony joint outlines partly preserved; 5-mutilating abnormality without bony outlines. This scoring system is a modification from Salvemini *et al.*, 2001 (Amelioration of joint disease in a rat model of collagen-induced arthritis by M40403, a superoxide dismutase mimetic. Arthritis Rheum. 44:2909-21); Bush *et al.*, 2002 (Reduction of joint inflammation and bone erosion in rat adjuvant arthritis by treatment with interleukin-17 receptor IgG1 Fc fusion protein. Arthritis Rheum. 46: 802-5); Sims *et al.*, 2004, (Targeting osteoclasts with zoledronic acid prevents bone destruction in collagen-induced arthritis. Arthritis Rheum., 50: 2338-46) and Jou *et al.*, 2005 (Thrombospondin 1 as an effective gene therapeutic strategy in collagen-induced arthritis. Arthritis Rheum. 52:339-44).

*14.7 Results*

In the treatment model, treatment was assessed via the clinical score and the Larsen score. Results are depicted in Figure 7 and Figure 8. Strikingly, both anti-IL17C antibodies tested (MOR12743 and MOR12762) demonstrated a significant inhibition of inflammation. The negative control antibody, MOR03207, did not inhibit inflammation, whereas the positive control, Enbrel, did inhibit disease progression.

In the preventative model, treatment was assessed via the clinical score and the Larsen score. Results are depicted in Figure 9. Strikingly, the anti-IL17C antibody tested (MOR12762) demonstrated a significant inhibition of inflammation and bone degradation. The negative control antibody, MOR03207, did not inhibit neither inflammation nor bone erosion.

*14.8 Steady State PK*

At pre-dose, days 31 and 46 (treatment protocol) or days 21, 36 and 46 (preventive protocol), blood samples were collected at the retro-orbital sinus with lithium heparin as anti-coagulant. Whole blood samples were centrifuged and the resulting plasma samples were stored at -20°C pending analysis.

Example 15 – Tobacco Smoke model

Daily exposures of mice (C57BL/6J, Charles River) to tobacco smoke (TS) for 11 consecutive days resulted in pulmonary inflammation, as indicated by an increase in the total number of cells recovered in the bronchoalveolar lavage (BAL), when compared with a similarly treated air-exposed group, 24h after the final exposure. The response consisted of significant increases in the numbers of macrophages, epithelial cells, neutrophils and lymphocytes recovered in BAL.

MOR12743 was administered by the intra-peritoneal route (i.p.), 1h prior to TS-exposure on days 1, 4, 7 and 10 of exposure. This resulted in significant inhibition of the total number of cells recovered in the BAL and specifically the numbers of epithelial cells and neutrophils.

MOR03207 was administered by the intra-peritoneal route (i.p.), 1h prior to TS-exposure on days 1, 4, 7 and 10 of exposure. This did not result in any significant inhibition of total cell numbers or the numbers of any specifically identified cell types recovered in the BAL.

Roflumilast (ChemPharmaServe Ltd. Ref. 0010206) was administered 5 mg/kg orally (p.o.), 1h prior to each TS-exposure. This significantly inhibited the total number of cells recovered in the BAL and specifically the numbers of epithelial cells neutrophils and lymphocytes.

All TS-exposed groups showed some bodyweight loss but this was not significant when compared with the air-exposed group at sacrifice on day 12.

*15.1 Materials*

Vehicle for i.p. administration: D-PBS pH7.4 (PAA Product Ref. H15-002, Lot. H00208-2353)

Vehicle for p.o. administration: PEG 200/water for injection (60%/40% v/v)

Phosphate buffered saline (PBS), for the bronchoalveolar lavage (BAL), was obtained from Gibco. Euthatal (sodium pentobarbitone) was obtained from Merial Animal Health Ltd. The tobacco smoke was generated using 'Marlboro 100' cigarettes purchased from a commercial supplier.

Formulations:

MOR12743 and MOR03207 were frozen, at 1mg/mL. Both test substances were allowed to thaw at 4ºC overnight prior to administration.

Roflumilast was formulated by placing a pre-weighed amount in a mortar and grinding gently while adding vehicle (PEG200/water, 60%/40% v/v) drop-wise to form a suspension. Suspensions were vortex-mixed prior to administration.

*15.2 Methods*

Previous studies have established that the total numbers of cells recovered in the BAL are significantly elevated 24h following the last of 11 daily TS-exposures. In this study, a time point of 24h after the final air or TS-exposure was used for analysis.

Vehicle (PBS), MOR12743 and MOR03207 were administered i.p., 1h prior to TS-exposure on days 1, 4, 7 and 10 of the study. Roflumilast was administered p.o., 1h prior to each TS-exposure.

*15.2.1 Exposure of animals to TS daily for 11 consecutive days*

In this exposure protocol, mice were exposed in groups of 5 in clear polycarbonate chambers (27cm x 16cm x 12cm). The TS from 'Marlboro 100' cigarettes was allowed to enter the exposure chambers at a flow rate of 100mL/min. In order to minimise any potential problems caused by repeated exposure to a high level of TS, the exposure period to TS was increased initially from 25 minutes at the start of the study (day 1) to a maximum of 45 minutes on day 3. The exposure schedule used in this study was as follows:

Day 1: 25min exposure (~ 5 cigarettes).

Day 2: 35min exposure (~ 7 cigarettes).

Days 3-11: 45min exposure (~ 9 cigarettes).

Exposure boxes were vented after 10min and every 5min thereafter.

One further group of mice was exposed to air on a daily basis for equivalent lengths of time as sham controls (no TS-exposure).

*15.2.2 Bronchoalveolar lavage and cytospin analysis*

Bronchoalveolar lavage was performed as follows:

The trachea was cannulated using a 10mm long Luer fitting stainless steel cannula. Phosphate buffered saline (PBS) was used as the lavage fluid. A volume of 0.4mL was gently instilled and withdrawn 3 times using a 1mL syringe and then placed in an Eppendorf tube and kept on ice prior to subsequent determinations.

Total cell-counts were performed as follows:

Lavage fluid was separated from cells by centrifugation (6min at 3400rpm, RCF = 3070 x g – 'Eppendorf Mini Spin') and the supernatant decanted and frozen for possible subsequent analysis. The cell pellet was re-suspended in a known volume of PBS and total cell numbers calculated by counting a stained (Turks stain) aliquot under a microscope using a haemocytometer.

Differential cell-counts were performed as follows:

The residual cell pellet was diluted to approximately 105 cells per mL. A volume of 500µl was placed in the funnel of a cytospin slide and centrifuged for 8min at 800rpm, RCF = 72.26 x g (Shandon Cytospin 3). The slide was air-dried and stained using Wrights/Giemsa stain as per the proprietary instructions. When dried and cover-slipped, differential cell-counts were performed using light microscopy. Approximately 400 cells were counted for each slide. Cells were identified using standard morphometric techniques.

*15.3 Treatment regimes*

In this study, 4 groups of mice were subjected to daily TS-exposure for 11 days and were sacrificed on the 12$^{th}$ day, 24h after the final TS-exposure. Three groups received either vehicle (D-PBS), MOR12743 or MOR03207, i.p., 1h prior to TS-exposure on days 1, 4, 7 and 10 of the study. One group received Roflumilast, p.o., 1h prior to each TS-exposure. A further group was exposed to air for 11 consecutive days and sacrificed on the 12$^{th}$ day, 24h after the final air-exposure. This group received vehicle (D-PBS), i.p., 1h prior to exposure on days 1, 4, 7 and 10 of the study. For all groups n = 10.

*15.4 Sampling procedures*

All mice were killed on day 12, by intra-peritoneal barbiturate anaesthetic overdose, 24h after final exposure to air or TS. A blood sample was taken over heparin from the sub-clavian vein and the plasma separated by centrifugation and stored at -40ºC. A BAL was performed using 0.4mL of phosphate buffered saline (PBS). Cells recovered from the BAL were used for the total cell and differential cell counts. The BAL supernatants and remaining

cell pellet were stored at -40ºC and -80ºC respectively for possible future analysis. Following BAL, the cannula was left tied in place. The heart and lungs were removed after gently opening the thorax and cutting down either side of the sternum and ribs. The left lobe was tied off, removed, snap-frozen and stored at -80°C. The right lobe was inflated with 10% phosphate buffered formalin (PBF) to a pressure of 18cm of PBF for 20 minutes. The trachea was then ligated below the cannula and the cannula removed. The heart, lung & trachea were immersed in PBF.

*15.5 Data Measurement and Statistical Analysis*

Results are presented as individual data points for each animal and the mean value calculated for each group.

The data were therefore initially subjected to a one-way analysis of variance test (ANOVA), followed by a Bonferroni correction for multiple comparisons in order to test for statistically significant differences between treatment groups. A "p" value of <0.05 was considered to be statistically significant.

Percentage inhibitions were calculated using the formula below:

$$\% \text{ Inhibition} = \left(1-\left(\frac{Treatment\ \ group\ \ result\ -\ sham\ \ group\ \ result}{TS\ vehicle\ \ group\ \ result\ -\ sham\ \ group\ \ result}\right)\right)\times 100$$

*15.6 Results*

*15.6.1 Pulmonary inflammation, as indicated by the increase in cell numbers recovered in the BAL, induced by daily exposures to TS*

One group was exposed to TS daily for 11 days and received vehicle (D-PBS), i.p., 1h prior TS-exposure on days 1, 4, 7 and 10 of the study. When compared to a similarly treated air-exposed group, mice exhibited pulmonary inflammation presented as a significant increase ($p<0.001$) in the total number of cells recovered in BAL at sacrifice on day 12 (24h after the last TS-exposure). This inflammation consisted of significant increases in the numbers of macrophages, epithelial cells, neutrophils and lymphocytes ($p<0.001$ for all), when compared with the air-exposed (sham) animals (Table 2).

**Table 2:** Summary of the effects of TS-exposure for 11 consecutive days on pulmonary inflammatory responses in mice.

| Inflammatory markers (BAL) | TS-exposure Days 1-11 | |
|---|---|---|
| | Fold increase* | p |
| **Total Cells** | 9.5 | <0.001 |
| **Macrophages** | 7.3 | <0.001 |
| **Epithelial cells** | 6.6 | <0.001 |
| **Neutrophils** | 551.8 | <0.001 |
| **Eosinophils** | 80.1 | ns |
| **Lymphocytes** | 96.7 | <0.001 |

* When compared to the air-exposed control group at the same time point.

Data were subjected to ANOVA. A "p" value of <0.05 was considered to be statistically significant. ns = not statistically significant.

*15.6.2 Effect of i.p. administration of MOR12743 on pulmonary inflammation, as indicated by the increase in cell numbers recovered in the BAL, induced by daily exposures to TS*

MOR12743, 5mg/kg, administered i.p., 1h prior to TS-exposure on days 1, 4, 7 and 10, significantly inhibited total cell numbers recovered in the BAL (26%, p<0.001) and specifically epithelial cells (52%, p<0.001) and neutrophils (48% , p<0.001). Degree and significance of inhibition are summarised in Table 3.

*15.6.3 Effect of i.p. administration of MOR03207 on pulmonary inflammation, as indicated by the increase in cell numbers recovered in the BAL, induced by daily exposures to TS*

MOR12743, 5mg/kg, administered i.p., 1h prior to TS-exposure on days 1, 4, 7 and 10, did not significantly inhibit total cell numbers recovered in the BAL, or the numbers of any

specifically identified cell-type elevated following TS-exposure. Data are summarised in Table 3.

15.6.4 *Effect of oral administration of Roflumilast on pulmonary inflammation, as indicated by the increase in cell numbers recovered in the BAL, induced by daily exposures to TS*

Roflumilast, 5mg/kg, administered p.o., 1h prior to each TS-exposure, significantly inhibited the total cell numbers recovered in the BAL (32% $p<0.001$) and specifically epithelial cells (50% $p<0.001$), neutrophils (56% $p<0.001$) and lymphocytes (54% $p<0.01$). Degree and significance of inhibition are summarised in Table 3.

**Table 3:** Summary of the effects of MOR12743, MOR03207 and Roflumilast on the TS-induced inflammatory responses in mice.

| **Compound** | **MOR12743** | | **MOR03207** | | **Roflumilast** | |
|---|---|---|---|---|---|---|
| **Treatment** | **5mg/kg i.p. on days 1, 4, 7, & 10.** | | **5mg/kg i.p. on days 1, 4, 7, & 10.** | | **5mg/kg p.o. q.d.** | |
| **Inhibition** | % | p value | % | p value | % | p value |
| **Total cells** | 26 | <0.001 | 0 | ns | 32 | <0.001 |
| **Macrophages** | 6 | ns | -3 | ns | 13 | <0.001 |
| **Epithelial Cells** | 52 | <0.001 | 8 | ns | 50 | <0.001 |
| **Neutrophils** | 48 | <0.001 | -1 | ns | 56 | <0.001 |
| **Lymphocytes** | 24 | ns | 20 | ns | 54 | <0.01 |
| **Eosinophils** | No statistically significant increase following TS-exposure | | | | | |

All data were subjected to an ANOVA test for comparisons in order to test for significant differences between treatment groups. A "p" value of <0.05 was considered to be statistically significant. ns = not statistically significant.

*15.6.5 Effect of treatment on bodyweights of mice throughout the eleven daily exposures to TS*

In order to monitor the health of the mice throughout the duration of the exposure protocol, mice were weighed at the start of the study, on day 6 and on day 12 prior to sacrifice.

Over the 12 days of the study, sham-exposed mice showed little or no change in bodyweight. All TS-exposed groups showed some loss of bodyweight over this period but these losses were not statistically significant.

*15.7 Conclusion*

MOR12743 (5mg/kg), administered i.p., on days 1, 4, 7 and 10, significantly inhibited the total number of cells recovered in BAL and specifically epithelial cells and neutrophils. MOR03207 (5mg/kg), administered in the same way, had no effect on total cell numbers recovered in BAL, or on the numbers of any specifically identified cell type.

The reference compound, Roflumilast (5mg/kg), administered p.o., 1h prior to each TS-exposure, significantly inhibited the total number of cells recovered in BAL and specifically epithelial cells neutrophils and lymphocytes. The response was similar to that seen in previous studies

All TS-exposed groups showed some bodyweight loss over the 12 day study period but there were no significant differences between any of the treatment groups.

Example 16 - Intranasal instillation of LPS: a mouse model of acute lung neutrophilia

Two independent studies were conducted with one negative mAb and two positive mAbs in the intranasal instillation of Lipopolysaccharide (LPS) mouse model of acute lung neutrophilia, a model which mimics some relevant aspects of COPD (Chronic Obstructive Pulmonary Disease). The effects were measured by Broncho Alveolar Lavage (BAL) inflammatory cell counting.

*16.1 Study groups*

Several groups of mice were treated with different antibodies and Dexamethasone and compared to the mice subjected to LPS only. The summary of the groups is provided in Table 4.

**Table 4**

| | Number of animals in the group | Dose | Route | Frequency | Vehicle |
|---|---|---|---|---|---|
| **Saline solution** | 5 | - | - | - | - |
| **LPS** | 10 | 10 µg/mouse | Intranasal | - | saline |
| **LPS+DEX** | 10 | 30 mg/kg | Per os | Twice per day | MC 0.5% |
| **LPS+MOR03207** | 10 | 5 mg/kg | Intra-peritoneally | ONCE | D-PBS |
| **LPS+MOR12743** | 10 | 5 mg/kg | Intra-peritoneally | ONCE | D-PBS |
| **LPS+MOR12762** | 10 | 5 mg/kg | Intra-peritoneally | ONCE | D-PBS |

*16.2 Materials*

- Lipopolysaccharide (LPS): from *Escherichia coli* 055:B5, ref: L4524-25MG, purified by affinity chromatography lot: 018K4077 – Sigma. LPS was prepared by Volume administered by intranasal instillation: 50 µL/mouse (10 µg/50 µL)
- Saline solution: sodium chloride 0.9%, lot 9F0191 (endotoxin free, Lavoisier)
- Ketamine: Imalgene MERIAL 1000, 10 mL
- Xylazine: Rompun BAYER PHARMA 2%, 25 mL
- Isoflurane: Aerrane, batch 10E28A35
- Methylcellulose (MC): VWR, ref AX021233 batch M1395
- Dexamethasone (DEX) preparation: 30 mg/kg, 10 mL/kg, 0.2 mL/mouse, po
- Antibodies were frozen, at 1 mg/mL. Test substances were allowed to thaw at 4ºC overnight prior to administration. All antibodies were ready to use upon defrosting (or were diluted extemporaneously).

*16.3 Animals*

BALB/c female mice from Harlan (France,) were used in the study. Mice weight was around 20g.

*16.4 Experimental procedures*

Intranasal administration

Mice were anesthetized by isoflurane inhalation. During the breathing, LPS was instilled intranasally. After 24h, mice were anesthetized intra-peritoneally (IP) and Broncho Alveolar Lavage procedure was performed. Mice were anesthetized by injection of anaesthetic

solution of 0.1 mL per 10 g of the mouse weight. The anaesthetic solution was composed of 18 ml 0.9% NaCl, 0.5 mL xylazine (5 mg/Kg) and 1.5 mL ketamine (75 mg/Kg).

Bronchoalveolar Lavage (BAL)

The trachea was exposed through midline incision and canulated (with a mice catheter). BAL was performed twice using 1 mL of sterile PBS buffer. Lavage fluid was removed and centrifuged at 1500 rpm for 10 min at 4°C. The cell pellet was resuspended in 200µL of PBS buffer. The cells were counted using a cell counter (Vet abc, France). The lavage fluid supernatant was kept at -20°C for inflammation mediators dosing.

*16.5 Study design*

Dexamethasone: Treatment with dexamethasone was performed 24, 16 and 1 hour prior to LPS instillation and 6 hours after LPS administration

Antibodies: 24 hours before LPS instillation mice were treated with one of the mAbs (MOR03207, MOR12762 or MOR12743).

For all groups, BAL was performed 24 hours after LPS administration and cell counts were measured.

Results

The results were analyzed using Student t-test. Cell counts from BAL from mice treated with either antibody or Dexamethasone were compared to the cell counts in mice subjected only to LPS. The averages +/- sem of cell counts from the mice in each group were considered. The results are presented in Fig. 10.

Conclusion

Single treatment with MOR03207 antibody (negative control) at 5mg/kg did not inhibit significantly (ns trend) the recruitment of inflammatory cells into the BALF. At the same time a single treatment with MOR12762 or MOR12743 at 5mg/kg inhibited significantly the recruitment of inflammatory cells into the BALF. This indicates that IL17C regulates acute neutrophilia and is a therapeutic target for lung diseases such as COPD.

Therefore it is demonstrated for the first time that IL17C antagonists, e.g. IL17C antibodies, are effective in the treatment of inflammatory disorders and diseases.

Example 17 – IL17C expression profiling in human respiratory tissue

In this study, IL17C expression was measured via quantitative Real Time PCR (qRT-PCR) in different human respiratory tissue types (tertiary bronchus, quaternary bronchus and pulmonary artery) from both control and diseased samples. Control samples were derived from non-smoking and smoking-donors whereas diseased samples were derived from COPD patients, acute/chronic bronchitis patients and patients having lung emphysema.

*17.1. RNA Purification and QC*

Total RNA was isolated from the frozen tissues using standard methodologies according to the suppliers' protocols, or with in-house adaptations.

QC criteria met (data not shown) were:
Presence of 18S ribosomal RNA
Minimum copy numbers of control gene mRNA transcripts, determined using qRT-PCR, as follows:

- β-actin (amplicon length 295 bp) > 3,800 transcript copies/100ng total RNA
- glyceraldehyde-3-phosphate dehydrogenase (GAPDH, amplicon length 71 bp) >10,000 transcript copies /100ng total RNA
- no DNA contamination

*17.2 Treatment of RNA samples with DNase*

Total RNA was treated with RNase-free DNase I to remove any residual genomic DNA (gDNA). To test for successful removal of DNA from RNA samples, qPCR was done without prior reverse transcription. The absence of an amplification signal confirmed that the RNA samples were free of DNA.

*17.3 Primer probe sets*

The primer probe sets used for qRT-PCR are shown below:

IL17C Amplicon size: 72bp

| | |
|---|---|
| Forward primer: | 5' – ATGAGGACCGCTATCCACAGA – 3' (SEQ ID No.:184) |
| Reverse primer: | 5' – CCCGTCCGTGCATCGA – 3' (SEQ ID No.:185) |
| Probe: | 5' – TGGCCTTCGCCGAGTGCCTG – 3' (SEQ ID No.:186) |

The probe was labeled at the 5'-end with 6-carboxyfluorescein (FAM) and at the 3'-end with

6-carboxy-tetramethyl-rhodamine (TAMRA).

*17.4 cDNA synthesis*

DNased RNA was incubated with the reverse primers for beta 1, beta 2, beta 3 and GAPDH in reverse transcription buffer, with the samples being heated to 72°C (to remove secondary structure) and then cooled to 55°C (to anneal the primers). MuLV reverse transcriptase and nucleotides were added and the reaction mixes were incubated for 30 minutes at 37°C to allow cDNA synthesis to occur. The samples were then heated to 90°C for 5 minutes to denature the reverse transcriptase.

*17.5 qRT-PCR*

Multiplexing methodology-using qRT-PCR for simultaneous measurement of mRNA levels of the genes of interest and GAPDH was used. The simultaneous measurement of GAPDH in each assay tube provided a QC check for successful reverse transcription and qRT-PCR. The reactions were performed with cDNA derived from 50ng of total RNA. Forward and reverse primers and probes for each target and GAPDH were added to the reaction mix along with nucleotides, buffer and AmpliTaq GoldTM Taq polymerase. The PCR conditions were: 94°C for 12 minutes (enzyme activation step), followed by 40 cycles of 94°C for 15 seconds (denaturing step) and 60°C for 30 seconds (to anneal and extend). Following sensitivity testing, the initial PCR temperature for beta 3 was increased to 97°C.

Results

The data presented here show that IL17C, a member of the interleukin 17 family of pro-inflammatory cytokines, is constitutively expressed in human lung. In addition, increased expression levels of IL17C were observed in lung tissue samples derived from donors with diagnosed inflammatory respiratory diseases like COPD, bronchitis or lung emphysema in comparison to the control samples (Figure 11).

Example 18 - Imiquimod (IMQ) psoriasis-like mouse model

The pro-inflammatory function of IL17C in the skin was examined in a non-infectious cutaneous inflammation mouse model of psoriasis where topical TLR7-TLR8 agonist Imiquimod induces psoriatic skin lesions characterized by epidermal proliferation and leukocyte infiltration, which are dependent on pathogenic TH17 cytokines (Van der Fits et al., J of Immunol. 2009 May 1;182(9):5836-45.). The role of IL17C in this particular model was recently documented (Ramirez-Carrozzi et al, Nat Immunol. 2011 12(12):1159-66) and a convincing genetic proof of the IL17C role in the disease model was provided with IL17C$^{-/-}$

mice. The same psoriasis–like model was used to further study the IL17C response to IMQ in the skin and identify the IL17C producing cells.

*18.1 Reagents*

Vaseline (Vaseline officinale, Cooper) and Imiquimod cream (Aldara, 5% cream, MEDA) were used. The antibodies used were anti-mouse IL17A/F (R&D System, clone 50104, ref MAB421) and anti-mouse IL23p40 (eBioscience, clone C17.8, ref 16-7123-85)

*18.2 Animals*

Balb/c N mice (female, 18-20gr, approx. 10 weeks old) were obtained from CERJ or Halan (France). Mice were kept on a 12 hr light/dark cycle (0700 -1900). Temperature was maintained at 22°C, and food and water were provided *ad libitum*.

*18.3 Experimental procedures*

In order to induce a psoriatic-like response, a daily topical dose of 62.5mg of Imiquimod cream on the shaved back and the right ear for 5 consecutive days (D0-D4), translating in a daily dose of 3.13 mg of the active compound. The control group was constituted with mice receiving the same quantity of Vaseline cream. Severity of skin inflammation (erythema, scaling and thickening) was observed every day. Body weight was daily recorded. At necropsy and at the days indicated, the ears and the back skin thicknesses were measured using a micrometer (Mitutoyo). Samples from back and ear skin were collected for histology and gene expression. Spleen and thymus weight was measured.

*18.4 Study design*

Mice were randomly divided into equal groups (n=10). The IMQ group received a daily topical dose of 62.5mg of Imiquimod cream on the shaved back and ear for 5 consecutive days (D0-D4), translating in a daily dose of 3.13 mg of the active compound. The control group was constituted with control mice receiving the same quantity of Vaseline. Antibodies were formulated in PBS, tested at 10mg/kg (200ug/mice) and administered i.p., 3 days before and at start of the experiment (D0), therefore, 6 different treatment groups were used:

- Control (Vaseline)
- IMQ (Aldara 5% cream)
- IMQ + MOR03207_h/m 10mg/kg i.p. (negative control Ab)
- IMQ + MOR12743_h/m 10mg/kg i.p.
- anti-mouse IL17A/F 10mg/kg i.p
- anti-mouse IL23p40 10mg/kg i.p

*18.5 Results*

IL17C protein expression was detected using biotinylated MOR12743 antibodies and IHC. IL17C was expressed in the mast cells of the dermis both in control and IMQ treated groups (data not shown). In response to IMQ, IL17C expression was increased in keratinocytes of the epidermis from D2 to D4- with the higher expression observed at D3- as well as in some smaller inflammatory cells of the dermis and the stratum. MOR03207 (isotype contol) displayed no staining whatever the conditions.

This observation was in line with IL17C gene expression. Basal levels were undetectable for IL17A and IL17F and low for IL17C and IL23p19. IL17A, IL17F were maximally increased by IMQ in the back skin after 96h whereas IL23p19 and IL17C were increased earlier with a maximum at 48h. IL17RA and IL17RE were well expressed with very moderate expression changes in response to IMQ.

Effects of neutralizing antibodies relevant to the pathway or specific to IL17C were assessed on the IMQ induced psoriasis by a histological measure of epidermal thickness. IL-17A and IL-23p40 antibodies showed partial preventive effects in line with the common knowledge in the field. The effect of MOR1243 neutralizing antibodies was significant thus demonstrating that IL17C neutralization can significantly prevent the epidermal thickness induced by IMQ in the mouse ear skin(Figure 12).

Example 19 - IL17C expression and function in primary human epidermal keratinocytes

To further explore function of IL17C in psoriasis, we focused on IL17C expression and function in primary human epidermal keratinocytes

*19.1 Adult normal human epidermal keratinocytes (NHEK-Ad) cultures*

Cryopreserved primary normal human epidermal keratinocytes from adult were obtained from Lonza and cultured in Keratinocyte Growth Medium-gold (KGM-Gold™) that was made by supplementing the Keratinocyte Basal Medium-gold (KBM-Gold™) with the various SingleQuots™ of growth factor supplements including bovine pituitary extract, hydrocortisone, hEGF, epinephrine, transferrin, insulin and GA-1000 (media & supplements all from Lonza). Cells that were expanded for 2 more passages were seeded in 96-well plates (25 000 c/well) in KGM-Gold™. After overnight culture, medium was removed and changed to KGM-Gold™ w/o hydrocortisone prior to addition of various cytokine triggers. Total RNA was extracted at various time points and expression of IL-17RE, IL17C and ß-defensin-2 (DEF4B) was determined by quantitative RT-PCR. Cell supernatant harvested was kept at -20°C until analyzed for levels of secreted ß-defensin-2 (hBD2) using ELISA. Recombinant human IL17C was from Novus Biologicals. Recombinant human IL-1ß and TNFα were from PeproTech.

Recombinant human IL-17A and IL-22 were from R&D Systems. The following Toll-Like receptor (TLR) agonists were acquired from InvivoGen: flagellin (FLA) purified from *S. typhimurium* (TLR5 agonist), guardiquimod (TLR7 agonist), CL097 (TLR7/8 agonist) and CpG oligonucleotide ODN 2611 (TLR9 agonist). The TLR4 agonist lipopolysacccharide (LPS, from *E. Coli* serotype 026:B6) was obtained from Sigma.

*19.2 Quantitative RT-PCR*

Total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen) and reverse-transcribed using Taqman® Reverse Transcription Reagents (Applied Biosystems). Twenty-five µl PCR reactions were prepared using Taqman® universal PCR master mix/No AmpErase® UNG and predesigned Assay-on-Demand Gene Expression primer/probe sets (all Applied Biosystems). qPCR was performed on the ABI Prism® 7000 (Applied Biosystems). Gene expression was normalized to the housekeeping gene GAPDH and expressed as ΔCt values, with ΔCt = Ctgene-Ct(GAPDH) or expressed as relative mRNA level of specific gene expression as obtained using the 2-ΔCt method.

*19.3 hBD2 ELISA*

The following protocol was developed and validated to measure hBD2 levels using capture and detection antibodies obtained from PeproTech (catno. 900-K172). White Lumitrac 600 384-well plates (Greiner) were coated with 40 µL of anti-hBD2 capture antibody solution (0.5 µg/mL in PBS). After overnight incubation at 4°C, plates were washed once with PBST (PBS + 0.05% Tween-20 (Sigma)) and once with PBS and blocked by a 4 hr incubation at room temperature with 100 uL/well blocking buffer (PBS + 1% BSA + 1% sucrose + 0.05% $NaN_3$). Blocking buffer was removed by inverting the plate and tapping it on an absorbent paper. The plate was washed with 100 µL PBST and 100 µL PBS and 35 uL of hBD2 standard or sample was added. After overnight incubation at 4°C, the plates were washed twice with PBST and once with PBS. Subsequently, 35 uL of biotinylated anti-hBD2 detection antibody solution (0.1 µg/mL in PBS containing 1% BSA) was added. After 2hr incubation at room temperature, plates were washed as described above and incubated with 35 µL Streptavidin-HRP conjugate (Invitrogen, catno. SNN2004) diluted 1/2000 in PBS + 1% BSA. After 45 min, plates were washed as described above and incubated for 5 min at room temperature with 50 µL/well BM Chem ELISA Substrate (Roche). Readout was performed on the Luminoscan Ascent Luminometer (Labsystems) with an integration time of 100 msec.

*19.4 Results*

While IL-17RA is ubiquitously expressed, expression of IL-17RE is more restricted and its expression is particularly high on cells of epithelial origin. We analyzed the expression of IL-

17RE mRNA on primary human epidermal keratinocytes and observed high expression of IL-17RE in these cells with ΔCT (IL-17RE, GAPDH) ~ 4-6. Expression of IL-17RE was not modulated by any of the tested inflammatory triggers (data not shown).

We further extended these initial findings and also analyzed the regulation of IL17C expression in keratinocytes by IL-17A, a cytokine produced by Th17 cells and known to play an important role in psoriasis. The obtained data confirm induction of IL17C mRNA by IL-1 and by flagellin, a TLR5 agonist (Figures 13A and 13B). Ligand of other TLRs (TLR4, TLR7, TLR8 or TLR9) did not significantly induce IL17C mRNA. Kinetic analysis showed that the induction of IL17C mRNA by IL-1 or Flagelin was rapid and transient. Interestingly, while IL-17A did not significantly induce IL17C mRNA on its own, it synergistically boosted and sustained the expression of IL17C over time when combined with the pro-inflammatory cytokines TNF or IL-1 (Figure 13A).

As keratinocytes express high levels of IL-17RE, function of IL17C in these cells was further examined. Although human primary keratinocytes did not respond to IL17C alone, IL17C did stimulate expression of ß-defensin-2 in synergy with other tested pro-inflammatory genes i.e. IL-1ß, TNFα and IL-22. Synergistic stimulation of ß-defensin-2 mRNA expression was observed both at level of mRNA and protein.

*19.5 Summary*

Overall, data indicate that IL17C produced by proinflammatory cytokines in keratinocytes could play a role in a positive feed forward loop that amplifies and sustains inflammatory gene expression in keratinocytes contributing to psoriasis skin inflammation.

Example 20: ELISA-based cross-competition assay

Cross-competition of an anti-IL17C antibody or another IL17C binding agent may be detected by using an ELISA assay according to the following standard procedure. Likewise, cross-competition of an anti-IL17C antibody or another IL17C binding agent may be detected.

The general principle of the ELISA-assay involves coating of an anti-IL17C antibody onto the wells of an ELISA plate. An excess amount of a second, potentially cross-competitive, anti-IL17C antibody is then added in solution (i.e. not bound to the ELISA plate). Subsequently a limited amount of IL17C-Fc is then added to the wells.

The antibody which is coated onto the wells and the antibody in solution will compete for binding of the limited number of IL17C molecules. The plate is then washed to remove IL17C molecules that has not bound to the coated antibody and to also remove the second,

solution phase antibody as well as any complexes formed between the second, solution phase antibody and IL17C. The amount of bound IL17C is then measured using an appropriate IL17C detection reagent. Therefore, IL17C may be fused with a tag, e.g. Fc, Flag, etc. which can be detected via an appropriate tag-specific antibody.

An antibody in solution that is cross-competitive to the coated antibody will be able to cause a decrease in the number of IL17C molecules that the coated antibody can bind relative to the number of IL17C molecules that the coated antibody can bind in the absence of the second, solution phase antibody.

This assay is described in more detail further below for two antibodies termed Ab-X and Ab-Y. In the instance where Ab-X is chosen to be the immobilized antibody, it is coated onto the wells of the ELISA plate, after which the plates are blocked with a suitable blocking solution to minimize non-specific binding of reagents that are subsequently added. An excess amount of Ab-Y is then added to the ELISA plate such that the moles of Ab-Y IL17C binding sites per well are at least 10 fold higher than the moles of Ab-X IL17C binding sites that are used, per well, during the coating of the ELISA plate. IL17C is then added such that the moles of IL17C added per well were at least 25-fold lower than the moles of Ab-X IL17C binding sites that are used for coating each well. Following a suitable incubation period, the ELISA plate is washed and a IL17C detection reagent is added to measure the amount of IL17C molecules specifically bound by the coated anti-IL17C antibody (in this case Ab-X). The background signal for the assay is defined as the signal obtained in wells with the coated antibody (in this case Ab-X), second solution phase antibody (in this case Ab-Y), buffer only (i.e. no IL17C) and IL17C detection reagents. The positive control signal for the assay is defined as the signal obtained in wells with the coated antibody (in this case Ab-X), second solution phase antibody buffer only (i.e. no second solution phase antibody), IL17C detection reagents. The ELISA assay needs to be run in such a manner so as to have the positive control signal be at least 6 times the background signal.

To avoid any artifacts (e.g. significantly different affinities between Ab-X and Ab-Y for IL17C) resulting from the choice of which antibody to use as the coating antibody and which to use as the second (competitor) antibody, the cross-blocking assay needs to be run in two formats: 1) format 1 is where Ab-X is the antibody that is coated onto the ELISA plate and Ab-Y is the competitor antibody that is in solution and 2) format 2 is where Ab-Y is the antibody that is coated onto the ELISA plate and Ab-X is the competitor antibody that is in solution.

## Claims

1. An isolated antibody or antibody fragment specific for IL17C for use in the treatment of an inflammatory disorder.

2. The isolated antibody or antibody fragment according to claim 1, wherein IL17C comprises the amino acid of SEQ. ID No 181.

3. An isolated antibody or antibody fragment of claims 1 or 2 wherein said treatment is the treatment of arthritis, asthma, sepsis, an autoimmune disease, pulmonary inflammation, psoriasis, inflammatory bowel disease, rheumatoid arthritis, COPD (chronic obstructive pulmonary disease), systemic lupus erythematosus and/or sarcoidosis.

4. An isolated antibody or antibody fragment according to claims 1-3, wherein said antibody blocks the binding of IL17C to IL17RE.

5. An isolated antibody or antibody fragment according to one of the claims 1-4, wherein said antibody or antibody fragment cross-competes with an antibody or antibody fragment comprising 6 CDRs defined by Kabat of any of the antibodies in Table 1.

6. An isolated antibody or antibody fragment according to one of the claims 1-5 wherein said antibody or fragment thereof is a monoclonal antibody.

7. The antibody or antibody fragment of claim 6, wherein said antibody or antibody fragment is a human, humanized or chimeric antibody or antibody fragment.

8. The antibody or antibody fragment of claim 6, wherein said antibody or antibody fragment comprises a human heavy chain constant region and a human light chain constant region.

9. The antibody or antibody fragment of claim 6, wherein said antibody is of the IgG isotype.

10. The antibody or antibody fragment of claim 6, wherein said antibody fragment is selected from the group consisting of a Fab, F(ab2)', F(ab)2', scFV.