# Immunoglobulins

## Field of the Invention

The present invention relates to immunoglobulins that specifically bind Interleukin 13 (IL-13) and in particular human IL-13 (hIL-13). One embodiment of the invention relates to antibodies that specifically bind hIL-13. The present invention also concerns methods of treating diseases or disorders with said immunoglobulins, pharmaceutical compositions comprising said immunoglobulins and methods of manufacture. Other aspects of the present invention will be apparent from the description below.

## Background of the Invention

### Interleukin-13 (IL-13)

IL-13 is a 12kDa secreted cytokine originally described as a T cell-derived cytokine that inhibits inflammatory cytokine production. Structural studies indicate that it has a four-helical bundle arrangement held by two disulphide bonds. Although IL-13 has four potential glycosylation sites, analysis of native IL-13 from rat lung has indicated that it is produced as an unglycosylated molecule. Expression of human IL-13 from NSO and COS-7 cells confirms this observation (Eisenmesser et al, J. Mol. Biol. 2001 310(1):231-241; Moy et al, J. Mol. Biol 2001 310(1):219-230; Cannon-Carlson et al, Protein Expression and Purification 1998 12(2):239-248).

IL-13 is a pleiotropic cytokine produced by a variety of cell types including activated Th2 cells, mast cells, basophils, dendritic cells, keratinocytes and NKT cells. It can also be produced by Th0, Th1, CD8 and naïve $CD45RA^+$ T cells. IL-13 has immunoregulatory activities that partially overlap with those of IL4, this redundancy may be explained by shared components in the receptors for IL4 and IL-13. IL-13 signals through the type II IL4 receptor which is a heterodimer composed of the IL4Rα and the IL-13Rα1 chains. IL-13Rα1 binds IL-13 with low affinity (Kd = 2-10 nM), but when paired with IL4Rα it binds with a high affinity (Kd = 400 pM) and forms a functional IL-13 receptor (the human receptor is referred to herein as "hIL-13R") that signals, resulting in activation of JAK/STAT and IRS-1/IRS-2 pathways. An additional IL-13 receptor chain has also been characterised (IL-13Rα2) which binds IL-13 with high affinity (Kd = 250 pM). IL-13Rα2 is believed to act as a decoy receptor modulating IL-13 activity. IL-13 can also signal through the IL13Rα2 chain (Fichter-Feigl 2006 Nature Medicine 12:99-106) to induce TGFbeta1, and as such may contribute towards the fibrosis associated with asthma pathology. Functional receptors for IL-13 are expressed on a wide range of cells including the airway

epithelium, smooth muscle, mast cells, eosinophils, basophils, B cells, fibroblasts, monocytes and macrophages. T cells do not have functional receptors for IL-13 (Hilton et al, PNAS 1996 93(1):497-501; Caput et al, J. Biol. Chem. 1996 271(28):16921-16926; Hershey GK, J.Allergy Clin. Immunol. 2003 111(4):677-690).

Both IL-13 and IL-4 act to modify immune and inflammatory responses by promoting allergy associated inflammation and suppressing inflammation due to bacteria, viruses and intracellular pathogens. The principal biological effects of IL-13 include; induction of B cell proliferation and regulation of isotype switching to IgE; induction of MHC II and CD23 expression on B cells and monocytes; up-regulation of VCAM-1 on endothelial cells; regulation of chemokine production; activation of mast cell, eosinophil and neutrophil function as well as inhibition of pro-inflammatory gene expression in monocyte and macrophage populations. IL-13 does not have any proliferative effects on T cells. Thus unlike IL4, IL-13 does not appear to be important in the initial differentiation of CD4 T cells into Th2-type cells, but rather appears to be important in the effector phase of allergic inflammation (McKenzie et al, PNAS 1993 90(8):3735-3739; Wynn TA, Annu. Rev. Immunol. 2003 21:425-456).

**IL-13 and Asthma**

Asthma is a chronic lung disease, caused by inflammation of the lower airways and is characterised by recurrent breathing problems. Airways of patients are sensitive and swollen or inflamed to some degree all the time, even when there are no symptoms. Inflammation results in narrowing of the airways and reduces the flow of air in and out of the lungs, making breathing difficult and leading to wheezing, chest tightness and coughing. Asthma is triggered by super-sensitivity towards allergens (e.g. dust mites, pollens, moulds), irritants (e.g. smoke, fumes, strong odours), respiratory infections, exercise and dry weather. The triggers irritate the airways and the lining of the airways swell to become even more inflamed, mucus then clogs up the airways and the muscles around the airways tighten up until breathing becomes difficult and stressful and asthma symptoms appear.

There is strong evidence from animal models and patients that asthmatic inflammation and other pathologies are driven by dysregulated Th2 responses to aeroallergens and other stimuli (Busse et al, Am. J. Resp. Crit. Care Med.1995 152(1):388-393). In particular, IL-13 is believed to be the major effector cytokine driving a variety of cellular responses in the lung, including airway hyperreactivity, eosinophilia, goblet cell metaplasia and mucus hyper-secretion.

**Clinical Evidence for the role of IL-13 in asthma**

The gene encoding IL-13 is located on chromosome 5q31. This region also contains genes encoding IL-3, IL-4, IL-5, IL-9 and GM-CSF, and has been linked with asthma. Genetic variants of IL-13 that are associated with asthma and atopy have been found both in the promoter and coding regions (Vercelli D, Curr. Opin. Allergy Clin. Immunol. 2002 2(5):389-393). Functional study data are available for the coding variant, Q130 IL-13 (referred to herein as "Q130 IL-13"). The +2044 G to A single nucleotide polymorphism (SNP) found in the fourth exon, results in a substitution of an arginine with a glutamine at position 130 (Q130 IL-13). Also note that in SEQ.ID.NO: 9, this is equivalent to position 110, where the first 'G' amino acid residue at the start of the mature human IL-13 amino acid sequence is position 1. This variant has been found to be associated with asthma, increased IgE levels and atopic dermatitis in Japanese and European populations. Q130 IL-13 is believed to have enhanced stability compared with wild-type IL-13. It also has slightly lower affinity for the IL-13Rα2 decoy receptor and consistent with these observations, higher median serum IL-13 levels are found in patients homozygous for the Q130 IL-13 variant compared with non-homozygous patients. These results indicate that Q130 IL-13 could influence the local and systemic concentrations of IL-13 (Kazuhiko et al, J. Allergy Clin. Immunol. 2002 109(6):980-987).

Elevated IL-13 levels have been measured in both atopic and non-atopic asthmatics. In one study, average serum IL-13 levels of 50 pg/ml were measured in asthmatic patients compared to 8 pg/ml in normal control patients (Lee et al, J. Asthma 2001 38(8):665-671). Increased IL-13 levels have also been measured in plasma, bronchio-alveolar lavage fluid, lung biopsy samples and sputum (Berry et al, J Allergy Clin. Immunol 2004 114(5):1106-1109; Kroegel et al, Eur Respir. J. 1996 9(5):899-904; Huang et al, J. Immunol. 1995 155(5):2688-2694; Humbert et al, J. Allergy Clin. Immunol. 1997 99(5):657-665).

**In vivo evidence for involvement of IL-13 in asthma**

A number of studies have defined a critical effector role for IL-13 in driving pathology in both acute and chronic mouse models of allergic asthma. The high affinity IL-13 receptor (IL-13Rα2) or anti-IL-13 polyclonal antibodies have been used to neutralize mouse IL-13 bioactivity in these models. Blockade of IL-13 at the time of allergen challenge completely inhibited OVA-induced airway hyper-reponsiveness, eosinophilia and goblet cell metaplasia. In contrast, administration of antibody to IL-4 after sensitisation and during the allergen challenge phase only partially reduced the asthma phenotype. Thus although exogenous IL-4 and IL-13 are both capable of inducing an asthma-like phenotype, the effector activity for IL-13 appears to be superior to that for IL-4. These data suggest a primary role for IL-4 in immune induction (particularly for Th2 cell development and recruitment to airways, and IgE production), whereas IL-13 is believed to be principally engaged in various effector outcomes, including airway hyper-responsiveness, mucus overproduction and cellular inflammation (Wills-Karp et al, Science 1998 282:2258-2261; Grunig et al,

Science 1998 282:2261-2263; Taube et al, J. Immunol. 2002 169:6482-6489; Blease at al, J. Immunol 2001 166(8):5219-5224).

In complementary experiments, lung IL-13 levels have been raised by over-expression in a transgenic mouse or by instillation of IL-13 protein into the trachea of wild-type mice. In both settings, asthma-like characteristics were induced: non-specific airway hyper-responsiveness to cholinergic stimulation, pulmonary eosinophilia, epithelial cell hyperplasia, mucus cell metaplasis, sub-epithelial fibrosis, airways obstruction and Charcot-Leyden-like crystals. In addition, IL-13 was found to be a potent stimulator of matrix metalloproteinases and cathepsin proteases in the lung, resulting in emphysematous changes and mucus metaplasia. Therefore IL-13 may be an important effector molecule both in asthma and COPD disease phenotypes (Zhu et al, J. Clin. Invest. 1999 103(6):779-788; Zheng et al, J. Clin. Invest. 2000 106(9):1081-1093).

These data indicate that IL-13 activity is both necessary and sufficient to produce several of the major clinical and pathological features of allergic asthma in well-validated animal models.

**Chronic Obstructive Pulmonary Disease (COPD)**

COPD is a generic term covering several clinical syndromes including emphysema and chronic bronchitis. Symptoms are similar to asthma and COPD can be treated with the same drugs. COPD is characterised by a chronic, progressive and largely irreversible airflow obstruction. The contribution of the individual to the course of the disease is unknown, but smoking cigarettes is thought to cause 90% of the cases. Symptoms include coughing, chronic bronchitis, breathlessness and respiratory infections. Ultimately the disease will lead to severe disability and death. Chronic bronchitis is diagnosed in patients with a history of cough or sputum production on most days for at least 3 months over 2 years without any other explanation. Emphysema of the lung is characterised by an abnormal permanent enlargement of the air spaces and destruction of alveolar walls.

IL-13 may play a role in the development of COPD. Human smokers who develop COPD have many inflammatory cell types (neutrophils, macrophages, eosinophils) in the lung parenchyma. IL-13 is a proinflammatory Th2 cytokine therefore to model the progression of emphysema; Zheng et al targeted IL-13 over-expression to the airway epithelium in IL-13 transgenic mice. These animals developed airway and lung parenchymal inflammation and emphysema. They also developed mucus metaplasia reminiscent of chronic bronchitis (J. Clin. Invest. 2000 106(9):1081-1093).

The IL-13 promoter polymorphism (-1055 C to T) that is associated with allergic asthma has also been reported to have an increased frequency in COPD patients

compared to healthy controls. This implies a functional role for the IL-13 promoter polymorphism in the enhanced risk to develop COPD (Kraan et al, Genes and Immunity 2002 3:436-439). In addition, an increased number of IL-13 and IL-4 positive cells were observed in smokers with chronic bronchitis compared to asymptomatic smokers (Miotto et al, Eur. Resp. J. 2003 22:602-608). However a recent study to assess the level of IL-13 expression in the lungs of severe emphysema patients did not find an association between IL-13 levels and disease (Boutten et al, Thorax 2004 59:850-854).

**Allergic disease including atopic dermatitis and allergic rhinitis**

IL-13 has also been implicated in atopic disorders such as atopic rhinitis and atopic dermatitis. Allergic rhinitis is the most common atopic disease in the United States and is estimated to affect up to 25% of adults and more than 40% of children. There is a close relationship between allergic rhinitis and asthma. Both conditions share common immunopathology and pathophysiology; they have similar immunologic processes in which eosinophils and Th2 lymphocytes in nasal and bronchial tissue play a role. Excessive production of Th2 cytokines, particularly IL-4 and IL-5, is thought to be fundamental in the pathogenesis of allergic disease. IL-13 shares several characteristics and effector functions with IL-4 and this, combined with the functional overlap in IL-4 and IL-13 receptor usage, intracellular signaling components, and genetic organization provides compelling (albeit indirect) evidence for a role of IL-13 in promoting or maintaining human immediate hypersensitivity in vivo. This has been corroborated by Li *et al* (Li et al. *J Immunol* 1998;161:7007) who demonstrated that atopic subjects with seasonal allergic rhinitis exhibited significantly stronger IL-13 responses in response to Ag-dependent but not polyclonal activation.

Atopic dermatitis is a common, chronic, relapsing, highly pruritic inflammatory skin disease. The lesional skin of atopic dermatitis patients is histologically characterized by an inflammatory T-cell infiltrate, which during acute phases is associated with a predominance of IL-4, IL-5 and IL-13 expression (Simon et al, *J Allergy Clin Immunol* 2004;114:887; Hamid et al. *J Allergy Clin Immunol* 1996; 98: 225) In addition, Tazawa et al have demonstrated that IL-13 mRNA (but not IL-4) is significantly upregulated in subacute and chronic skin lesions of atopic dermatitis patients (Tazawa et al, Arch Derm Res 2004;296:459). The frequency of IL-13 expressing circulating CD4+ and CD8+ T-cells is also significantly increased in these patients (Aleksza et al British J Dermatol 2002;147;1135). This increased IL-13 activity is thought to result in raised levels of serum IgE, thereby contributing to the pathogenesis of atopic dermatitis. Furthermore, increased production of IL-13 by neonatal CD4+ T cells is a useful marker for identifying newborns at high risk for subsequent development of allergic diseases, esp. atopic dermatitis (Ohshima et al. *Pediatr Res* 2002; 51:195). Additional evidence for the importance of IL-13 in the etiology of atopic dermatitis was provided by Simon et al (Simon et al, *J Allergy Clin*

*Immunol* 2004; 114:887); topical treatment with tacrolimus ointment (an immunosuppressive drug that inhibits intracellular signaling pathways for cytokine production) resulted in significant clinical and histological improvement of the atopic skin lesions accompanied by significant reductions in local expression of Th2 cytokines, including IL-13. Furthermore, IL-13 Rα1 (a cell surface protein that together with IL-4Rα forms a functional receptor for IL-13) has been shown to be over-expressed on the suprabasal keratinocytes in the skin of atopic dermatitis patients, and IL-13 was able to upregulate IL-13 Rα1 mRNA in vitro (Wongpiyabovorn et al., *J Dermatol Science* 2003;33:31).

These data collectively indicate that IL-13 targeted interventions, including an IL-13 monoclonal antibody, may provide an effective approach for treatment of human allergic disease.

**Esophagal eosinophilia**

The accumulation of eosinophils in the esophagus is a common medical problem in patients with diverse diseases, including gastro-esophageal reflux disease, eosinophilic esophagitis, eosinophilic gastroenteritis, and parasitic infections. Esophageal eosinophilia is associated with allergic responses, and repeated challenging of mice with aeroallergens established a link between allergic airway inflammation and esophagal eosinophilia. Th2 cells are thought to induce eosinophil-associated inflammation through the secretion of an array of cytokines including IL-4 and IL-13 that activate inflammatory and effector pathways both directly and indirectly. IL-13 appears to be particularly important because it is produced in high quantities by Th2-cells and regulates multiple features of allergic disease (e.g. IgE production, mucus over-production, eosinophil recruitment and survival, and airway hyperreactivity. Eosinophils can generate functionally active IL-13 after exposure to GM-CSF and/or IL-5 under in vitro, ex vivo, and in vivo conditions in eosinophilic inflammatory responses. (Schmid-Grendelmeier *J Immunology,* 2002, 169: 1021–1027). IL-13 delivered to the lung of wild-type, STAT-6, eotaxin-1 or IL-5 deficient mice by intratracheal administration, established that pulmonary inflammation, triggered by IL-13, is associated with the development of esophagal eosinophilia (Mishra et al. *Gastroenterol* 2003;125:1419). Taken together, these data provide evidence for a role of IL-13 in esophagal eosinophilia.

**Oncology Indications**

Another important area of interest is in targeting IL-13 or IL-13 receptors to inhibit growth of certain types of tumors. Type 1 T cell–mediated host defenses are believed to mediate optimal tumor rejection in vivo, and deviation to a Th2-type response may contribute to blocking tumor rejection and/or promotion of tumor recurrence

(Kobayashi M et al. *J. Immunol.* 1998; 160:5869). Several animal studies using transplantable tumor cell lines support this notion by demonstrating that Stat6, IL-4, and IL-13 (produced in part by NKT cells) were capable of inhibiting tumor rejection (Terabe et al. *Nat. Immunol.* 2000;1:515; Kacha et al. *J. Immunol.* 2000;165:6024–28; Ostrand-Rosenberg et al. *J. Immunol.* 2000;165:6015). The potent anti-tumor activity in the absence of Stat-6 was thought to be due to enhancement of tumor-specific IFNg production and CTL activity. In addition, a loss of NKT cells has been shown to reduce IL-13 production with a concomitant rise in tumor recurrence, indicating that IL-13, produced in part by NKT cells is important for immunosurveillance (Terabe et al. *Nat. Immunol.* 2000; 1:515). As such, these findings suggest that IL-13 inhibitors or novel IL-13 antagonists, including IL-13 mAb, may be effective as cancer immunotherapeutics by interfering with the negative regulatory IL-13 plays in downregulating immune responses to tumor cells.

In addition to boosting Th-type-1-associated anti-tumor defenses, IL-13 inhibitors may also be able to block tumor cell growth more directly. For example, in B-cell chronic lymphocytic leukemia (B-CLL) and Hodgkin's disease, IL-13 either blocks apoptosis or promotes tumor cell proliferation (Chaouchi et al. *Blood* 1996; 87:1022; Kapp et al. *J. Exp Med.* 1999; 189:1939). B-CLL is a clinically heterogeneous disease originating from B lymphocytes that involves apoptotic defect in the leukemic cells. IL-13 is not thought to act as a direct growth factor but protects tumor cells from in vitro spontaneous apoptosis (Chaouchi et al. *Blood* 1996; 87:1022; Lai et al. *J. Immunol.*1999; 162:78) and may contribute to B-CLL by preventing neoplastic cell death.

Hodgkin's disease is a type of lymphoma that primarily affects young adults and accounts for about 7,500 cases a year in the United States. The cancer is characterized by the presence of large multi-nucleated Hodgkin/Reed-Sternberg cells (H/RS). In a large majority of cases, the malignant cell population arises from B cells. Several Hodgkin's disease-derived cell lines, as well as lymph node tissue taken from Hodgkin's lymphoma patients, overexpress IL-13 and/or IL-13 receptors. (Kapp et al. *J. Exp Med.* 1999;189:1939, Billard et al. *Eur Cytokine Netw* 1997;8:19; Skinnider et al. *Blood* 2001; 97:250; Oshima et al, *Cell Immunol* 2001 ;211:37). Neutralizing anti-IL-13 mAbs or IL-13 antagonists have been shown to inhibit H/RS cell proliferation in a dose-dependent manner (Kapp et al. *J. Exp Med.* 1999; 189:1939; Oshima et al, *Cell Immunol* 2001; 211:37). Similarly, delivery of soluble IL-13Ra2 decoy receptor to NOD/SCID mice with an implanted Hodgkin's disease-derived cell line delayed tumor onset and growth, and enhanced survival, demonstrating that IL-13 neutralization can suppress Hodgkin's lymphoma growth *in vitro* and *in vivo* (Trieu et al. Cancer Research 2004;64:3271). Collectively, these studies indicate that IL-13 stimulates the proliferation of H/RS cells in an autocrine fashion (Kapp et al. *J. Exp Med.* 1999; 189:1939; Ohshima et al. *Histopathology* 2001; 38:368).

Neutralization of IL-13 may therefore represent an attractive and effective treatment for Hodgkin's disease and other B cell–associated cancers by inhibiting tumor cell growth while at the same time enhancing anti-tumor defenses.

**Inflammatory Bowel Diseases**

There is a possible role for IL-13 in the pathogenesis of inflammatory bowel disease (IBD). Inflammatory bowel disease comprises a number of diseases clinically classified as ulcerative colitis, Crohn's disease and indeterminate colitis. Its main manifestation is chronic intestinal inflammation due to an exaggerated immune response with an imbalance in the activation of Th1 and Th2 lymphocytes in the intestinal mucosa. This has been demonstrated in animal models of crohn's disease (Bamias et al. Gastroenterol 2005; 128:657) and ulcerative colitis (Heller et al, Immunity 2002; 17:629). Neutralization of IL-13 by IL-13Rα2-Fc administration prevented colitis in a murine Th2 model of human ulcerative colitis (Heller et al, Immunity 2002; 17:629). Furthermore, IL-13 production rapidly supersedes that of IL-4 in this model, and IL-13 production can be induced by stimulation of NKT cells, suggesting that tissue damage may result from toxic activity of IL-13 on the epithelium cells. There are some human data to support these findings: the frequency of IL-13 positive rectal biopsy specimens from patients with ulcerative colitis was significantly higher than of inflammatory and non-inflammatory control subjects, and a higher rate IL-4 and IL-13 expression was observed in acute than non-acute ulcerative colitis (Inoue et al. Am J Gastroenterol 1999;94:2441). In addition Akido *et al* characterized the immune activity in the muscularis externa from intestinal segments of Crohn's disease patients and found that IL-4 and IL-13 mediate hypercontractility of the intestinal smooth muscle cells via a STAT-6 pathway. The authors concluded that this pathway may contribute to the hypercontractility of intestinal muscles in Crohn's disease (Akiho et al., *Am J Physiol Gastrointest Liver Physiol* 2005; 288:619).

Thus, an IL-13 mAb, possibly in combination with molecules directed at other cytokines, may provide an approach to stop or slow the progression of IBDs.

**Psoriasis and Psoriatic Arthritis**

Psoriasis is a chronic skin disease characterized by hyper-proliferation of keratinocytes and an immunologic cellular infiltrate, including activated T cells, producing various cytokines that can influence the phenotype of epidermal keratinocytes. CDw60 is a carbohydrate-bearing molecule that is upregulated on the surface of psoriatic basal and suprabasal keratinocytes of psoriatic skin. IL-4 and IL-13 secreted from T cells derived from psoriatic lesions have been shown to strongly up-regulate the expression of CDw60 on keratinocytes, (Skov et al., *Am J Pathol* 1997;15:675), whereas interferon-gamma blocked IL-4/IL-13 mediated induction of

CDw60 on cultured keratinocytes (Huang et al., *J Invest Dermatol* 2001;116:305). Thus, CDw60 expression on psoriatic epidermal keratinocytes is thought to be induced at least in part by IL-13 secreted by activated T cells within the lesion. In addition, IL-13 Rα1 and IL-4Rα, cell surface proteins that together form a receptor complex for IL-13, are differently expressed in skin biopsies from patients with and without psoriasis (Cancino-Diaz et al., *J Invest Dermatol* 2002;119:1114; Wongpiyabovorn et al., *J Dermatol Science* 2003;33:31), and in vitro experiments demonstrated that IL-13 (but not IL-4) could upregulate the expression of IL-13Rα1 (Wongpiyabovorn et al., *J Dermatol Science* 2003;33:31). Since IL-13 has an effect on a variety of cell types, these studies suggest that the IL-13 receptor may play a part in the early inflammatory process of psoriasis.

Psoriatic arthritis is characterized by synovitis which is mediated by both pro-inflammatory and anti-inflammatory cytokines. The role of IL-13 in various forms of arthritis has been receiving increased interest. Spadaro et al have observed significantly higher levels of IL-13 in synovial fluid of patients with psoriatic arthritis and rheumatoid arthritis than in patients with osteoarthritis. In addition, synovial fluid levels of IL-13 were significantly higher than those in serum in patients with psoriatic arthritis, and the IL-13 synovial fluid/serum ratio was markedly higher in the psoriatic arthritis group than in the rheumatoid arthritis group, suggesting a possible role for the locally produced IL-13 in synovial tissues of patients with psoriatic arthritis (Spadaro et al., *Ann Rheum Dis* 2002; 61:174).

**Potential Role of IL-13 in other conditions**

Acute graft-versus-host disease is a serious cause of morbidity and mortality following stem cell transplantation and is directly related to the degree of human leukocyte antigen (HLA) incompatibility between donor and recipient. Jordan et al first identified IL-13 as a typical Th2 cytokine that is abundantly produced during unrelated, unmatched MLRs (mixed lymphocyte reaction; an in vitro assay for fine-tuning donor selection after initial HLA typing) (Jordan et al. J Immunol Methods; 2002;260:1). The same group subsequently showed that IL-13 production by donor T-cells is predictive of acute graft-versus-host-disease (aGVHD) following unrelated donor stem cell transplantation (Jordan et al. *Blood* 2004; 103:717). All patients with severe, grade III aGVHD following stem cell transplantation had donors who produced very high pre-transplantation IL-13 responses, demonstrating a significant link between IL-13 levels and aGVHD and raising the possibility that IL-13 may be directly responsible for some of the aGVHD associated pathology. Consequently, a therapy based on specific blocking of IL-13 may be useful for the treatment of post-stem cell transplantation aGVHD.

Diabetic nephropathy is one of the major causes of end stage renal disease in the Western world. Although the incidence of nephropathy owing to type 1 diabetes is

declining, diabetes mellitus type 2 is now the most common single cause of renal insufficiency in the USA, Japan and Europe. Furthermore, this group of patients has a very poor prognosis on maintenance dialysis owing to extremely high mortality caused by cardiovascular events. It is now increasingly clear that hemodynamic, metabolic and structural changes are interwoven, and various enzymes, transcription factors and growth factors have been identified that play a role in the pathogenesis of this disease. Particularly, TGF-β is important in the development of renal hypertrophy and accumulation of extracellular matrix components, and is considered the pivotal cytokine in mediating collagen formation in the kidney (Cooper. *Diabetologia* 2001; 44:1957; Wolf. *Eur J Clin Invest* 2004; 34 (12): 785). In experimental and human diabetic nephropathy TGF-1 bioactivity is increased and administration of TGF-β1 antibodies to diabetic mouse led to improvement in renal function and reduced extra-cellular matrix accumulation. IL-13 was recently shown in a transgenic mouse model of lung fibrosis to mediate its effects at least in part by regulating the production and activation of TGF-β1 and collagen deposition (Lee et al. *J. Exp. Med.* 2001; 194:809; Zhu et al. *J. Clin. Invest.* 1999; 103:779), thereby establishing a direct functional link between IL-13 and TGF-β. Consequently a similar role for IL-13 in regulating TGF-b1 activity in the diabetic kidney can be envisioned and IL-13 targeted interventions could potentially have a role in the management of diabetic nephropathy.

**Fibrotic Conditions**

Pulmonary fibrosis is a condition of inappropriate and harmful scarring of the lungs, leading to disability and often death. The term encompasses a variety of different conditions with distinct etiologies, pathologies and responses to treatment. In some cases the cause of the fibrosis is identified. Causes include: (1) inhaled profibrotic material such as asbestos or silicon, or hard metal dust (2) inhaled organic material to which the patient has an idiosyncratic immunological response leading to fibrosis (e.g. farmer's lung) (3) drugs, such as nitrofurantoin, amiodarone and methotrexate (4) in association with a systemic inflammatory disease, such as Systemic Sclerosis or Rheumatoid Arthritis.

However, in many instances no cause or underlying condition is identified. Many such patients are diagnosed with Idiopathic Pulmonary Fibrosis (IPF). This is a relative rare condition (prevalence 20/100 000). The diagnosis is based on the absence of an identified cause combined with certain radiological and pathological features, particularly honeycombing on the CT or lung biopsy. The disease is usually seen in older patients (>50) and often follows a relentless course of progressive lung impairment leading to death, with the median survival quoted as 2-5 years. Moreover, the patients have the most unpleasant experience of breathlessness progressing over months or years. This initially restricts physical activity, but in the terminal phase – which may last several months – the patient is breathless even at rest and is furthermore oxygen dependent.

At present there is no satisfactory treatment for this disease. Current treatment generally takes the form of corticosteroids and immunosuppressives such as azathioprine. However, corticosteroids may be ineffective in many of patients and their side effects may make the situation worse. There are many potential treatments under investigation including Interferon gamma, which has shown a trend to improved survival in a recent large study, and perfenidone.

There is evidence that IL-13 and cytokines associated with the Th2 phenotype are involved in the process of fibrosis in tissue repair (Wynn TA, Nat. Rev. Immunol. 2004 4:583-594; Jakubzick et al, Am. J. Pathol. 2004 164(6):1989-2001; Jakubzick et al, Immunol. Res. 2004 30(3):339-349; Jakubzick et al, J. Clin. Pathol. 2004 57:477-486). IL-13 and IL-4 have been implicated in a variety of fibrotic conditions. Hepatic fibrosis induced by *Schistosoma* appears to be IL-13 dependent and there is limited evidence that IL-13 is involved in the pathogenesis of scleroderma (Hasegawa et al, J. Rheumatol. 1997 24:328-332; Riccieri et al, Clin. Rheumatol. 2003 22:102-106)

In terms of pulmonary fibrosis, in vitro studies have shown that IL-13 promotes a fibrogenic phenotype. Animal studies have shown elevated levels of IL-13 expression in artificially induced models of fibrosis, and that fibrosis can be reduced by elimination of IL-13.

IL-13 promotes a profibrotic phenotype. At a cellular level, there are several mechanisms by which IL-13 may promote fibrosis. The signal pathways and importance of these various mechanisms are not well defined.

There is evidence that IL-13 acts on the fibroblast both to promote the production of collagen, and to inhibit its breakdown, thus favouring a fibrotic phenotype. Skin fibroblasts possess IL-13 receptors (= the type II IL-4 receptor) and exposure of cultured skin fibroblasts to IL-13 leads to upregulation of collagen generation (Oriente et al, J. Pharmacol. Exp. Ther. 2000 292:988-994). IL-4 also has a similar, but more transitory effect. A human lung fibroblast cell line (ICIG7) expresses the type II IL-4 receptor (Jinnin et al, J. Biol. Chem 2004 279:41783-41791). Exposure of these cells to IL-13 promotes secretion of a variety of inflammatory and profibrotic mediators: GM-CSF, G-CSF, VCAM beta1 integrin (Doucet et al, Int. Immunol. 1998 10(10):1421-1433).

IL-13 inhibits IL-1a-induced matrix metalloproteinases 1 and 3 protein production by skin fibroblasts which would tend to reduce breakdown of EC matrix (Oriente et al, J. Pharmacol. Exp. Ther. 2000 292:988-994). IL-13 acts synergistically with TGF-β on human fibroblasts obtained by biopsy of asthma airways to promote expression of tissue inhibitor of metalloproteinase 1 (TIMP-1). Breakdown of extracellular matrix is effected by matrix metalloproteinases, which are inhibited by TIMP-1. This action of

IL-13 would thus tend to reduce matrix degradation (Zhou et al, Am. J. Physiol. Cell Physiol. 2005 288:C435-C442)

Over-expression of IL-13 in transgenic mice leads to subepithelial fibrosis, epithelial cell hypertrophy, goblet cell hyperplasia, crystal deposition (acidic mammalian chitinase), airway hyper-responsiveness, interstitial fibrosis, type 2 cell hypertrophy and surfactant accumulation (Zhu et al, J. Clin. Invest. 1999 103(6):779-788).

Different strains of mice have different susceptibilities to bleomycin-induced pulmonary fibrosis. C57B1/6J mice, which are susceptible, exhibit rapid up regulation of IL-13, IL-13Rα and IL-4 (as well as TGFβ, TNFRα and IL1Rs) in response to bleomycin. BALB/c mice, which are not susceptible, do not show upregulation of IL-13.

Belperio et al (Am. J. Respir. Cell Mol. Biol. 2002 27:419-427) studied the expression and role of IL-13, IL-4 and the CC chemokine C10 in a mouse bleomycin fibrosis model. Lung tissue levels of both IL-13 and IL-4 increased in response to bleomycin. Prior neutralisation of IL-13 using polyclonal anti IL-13 antibodies significantly reduced lung fibrosis in response to bleomycin as assessed by lung hydroxyproline levels. Despite the increased expression of IL-4 in the same model, neutralisation of IL-4 had no effect on lung fibrosis.
In another model of acute lung fibrosis induced by FITC in the BALB/c mouse, absence of IL-13 (in knockouts), but not IL-4, protected against lung fibrosis. There is no added protection of knockout of IL-4 in IL-13 knockouts (Kolodsick et al, J. Immunol. 2004 172:4068-4076). The protective effect of IL-13 absence is not due to a difference in cell recruitment into the lung: in all knockouts and BALB/c total cell numbers recruited are similar, so the initial inflammatory component seems to be the unaffected. Eosinophil recruitment is lower in IL-4 and IL-13 knockouts compared with BALB/c, but since IL-4 -/- were not protected against fibrosis this cannot explain the difference in fibrosis. Perhaps surprisingly, there was no difference in the levels of cytokines between IL-13 +/+ and -/-, including for IL10, MCP-1, gamma interferon, TGF-í. In addition, the same number of fibroblasts were isolated from lungs of the different animals post FITC, but in the IL-13 -/- mice the production of collagen I is reduced. This indicates the loss of IL-13 is not simply preventing the inflammatory response, but rather is having a more specific anti-fibrotic role. It has been suggested that IL-13 might exert its fibrotic effect via TGF-í (Lee et al, J. Exp. Med. 2001 194:809-821). However in this FITC model, expression of TGF-í was not reduced in IL-13 knock-out mice.

Interleukin 4 may be expected to exert a similar effect as IL-13 as both act via the same receptor. IL-4 is significantly upregulated in the lungs of mice with bleomycin induced lung fibrosis (Gharaee-Kermani et al, Cytokine 2001 15:138-147). However, comparing bleomycin-induced lung fibrosis in C57BL6/J mice which overexpress IL-

4, IL-4 knockouts and wild type, Izbicki et al (Am. J. Physiol. Lung Cell Mol. Physiol 2002 283(5):L1110-L1116) did not find evidence that IL-4 was involved in lung fibrosis. Fibrosis was not reduced in IL-4 knockouts, and IL-4 over-expressing mice had increased levels of fibrosis.

BAL cytokine levels of IL-13 are significantly elevated in patients with a variety of forms of pulmonary fibrosis, though with considerable variability. Expression of IL-13 is significantly upregulated in alveolar macrophages obtained from patients with lung fibrosis.

The strongest clinical evidence comes from research at the University of Michigan. Jakubzick and colleagues have studied gene expression of IL-13 and IL-4 and their receptors in surgical lung biopsies from patients with pulmonary fibrosis. IL-13 gene expression is markedly greater in specimens from IPF affected lung than lung from normals or other lung fibrotic conditions. Fibroblasts cultured from patients with IPF/UIP show heightened expression of the IL-13 and IL-4 receptor, compared with tissue and fibroblasts obtained biopsies from patients with normal lungs or other forms of lung fibrosis. In particular, the fibroblastic foci, which are presumably the epicentre of disease activity, stain particularly strongly for these receptors (Jakubzick et al, J. Immunol 2003 171:2684-2693; Jakubzick et al, Am. J. Pathol. 2003 162:1475-1486; Jakubzick et al, Am. J. Pathol. 2004 164(6):1989-2001; Jakubzick et al, Immunol. Res. 2004 30(3):339-349; Jakubzick et al, J. Clin. Pathol. 2004 57:477-486).

There is good in vitro evidence that Th2 cytokines in general and IL-13 in particular promote a profibrotic phenotype. In at least 2 animal models it has been shown that chemically-induced fibrosis can be reduced by elimination of IL-13 (either in gene knock-out or by anti-IL-13 antibodies). Some evidence indicates that IL-13 is more important at promoting pulmonary fibrosis than IL-4. Clinical evidence for the role of IL-13 in pulmonary fibrosis suggests that IL-13 and its receptors are unregulated in the lungs of patients with IPF.

A growing body of data suggests an important role for IL-13 based therapies for the treatment of a variety of fibrotic conditions, including schistosomiasis-induced hepatic fibrosis, and various forms of pulmponary fibrosis (e.g. IPF [discussed elsewhere], scleroderma).

Experiments in which IL-4 and IL-13 were inhibited independently identified IL-13 as the dominant effector cytokine of fibrosis in several models (Chiaramonte et al *J. Clin. Invest.* 1999;104: 777–785; Blease *et al. J. Immunol.* 2001; 166:5219; Kumar *et al. Clin. Exp. Allergy* 2002; 32:1104). In schistosomiasis, although the egg-induced inflammatory response was unaffected by IL-13 blockade, collagen deposition decreased by more than 85% in chronically infected animals (Chiaramonte et al *J.*

*Clin. Invest.* 1999; 104: 777; Chiaramonte et al *Hepatology* 2001; 34:273) despite continued and undiminished production of IL-4.

The amino acid sequence for hIL-13 is set forth as SEQ.I.D.NO: 9. (This is the mature protein sequence, that is, no signal sequence is present).

A polynucleotide encoding hIL-13 is set forth in SEQ.I.D.NO:10. (This is the DNA sequence for the mature protein sequence, that is, no signal sequence is present).

All patent and literature references disclosed within the present specification (including any patent application to which this application claims priority) are expressly and entirely incorporated herein by reference.

Recently vaccines raising immune responses against IL-13 for the treatment of asthma have been described (WO 02/070711). A role for IL-13 in the sensitisation of the skin to environmental allergens has also been recently described (Herrick et al., The Journal of Immunology, 2003, 170:2488-2495).

The present invention provides an antibody that binds hIL-13 and inhibits the binding of hIL-13 with both chains of hIL-13R i.e. IL-13Rα1 and IL-13Rα2.

## Summary of the Invention

The present invention therefore provides an antibody or antigen binding fragment thereof which specifically binds hIL-13 and neutralises the activity of hIL-13. The present invention provides an antibody or antigen binding fragment thereof which specifically binds hIL-13 and comprises a CDRH3 which is a variant of the sequence set forth in SEQ.I.D.NO:3 or a variant in which one or two amino acid residues within said CDRH3 of said variant differs from the amino acid residue in the corresponding position in SEQ.I.D.NO:3. In one embodiment of the present invention these differences in amino acid residues are conservative substitutions.

The term "specifically binds" as used throughout the present specification in relation to antibodies and antigen binding fragments thereof of the invention means that the antibody binds hIL-13 with no or insignificant binding to other human proteins and in particular human IL-4. The term however does not exclude the fact that antibodies of the invention may also be cross-reactive with cynomolgus IL-13.

The term "neutralises" as used throughout the present specification in relation to antibodies and antigen binding fragments thereof of the invention means that the biological activity of IL-13 is reduced in the presence of the antibodies and antigen binding fragments of the present invention in comparison to the activity of IL-13 in the absence of such antibodies and antigen binding fragments thereof. Levels of

neutralisation can be measured in several ways, for example by use of the assays as set out in the examples below, for example in a TF-1 cell proliferation assay which may be carried out for example as described in Example 3.3-3.5. The neutralisation of IL-13 in this assay is measured by assessing the decreased TF-1 cell proliferation in the presence of neutralising antibody.

If an antibody or antigen binding fragment thereof is capable of neutralisation then this is indicative of inhibition of the interaction between hIL-13 and its receptor. Antibodies which are considered to have neutralising activity against human IL-13 would have an $ND_{50}$ of less than 100micrograms/ml, or less than 80micrograms/ml in the TF-1 cell proliferation assay as set out in Example 3.3, 3.4 or 3.5.

In an alternative aspect of the present invention there is provided antibodies or antigen binding fragments thereof which have equivalent neutralising activity to the antibodies exemplified herein, for example antibodies which retain the neutralising activity of H2L1 in the TF-1 cell proliferation assay as set out in Example 3.3, 3.4 or 3.5.

As used herein the term "modulates" means inhibition of the binding of IL-13 to its receptor, and/or blocking of the interaction between IL-13 and its receptor thereby decoupling the hIL-13/hIL-13R signalling pathway. This can be inhibition and/or blocking of either or both of IL-13Rα1 and IL-13Rα2. The hIL-13 receptor as used herein means one or both of these receptors. Inhibition of binding of IL-13 to its receptor can be measured in several ways, for example by use of the assays as set out in the examples below, for example an ELISA method such as that described in Example 6.5 and 6.6.

The antibodies and antigen binding fragments thereof of the present invention may be therapeutic antibodies and antigen binding fragments thereof i.e.suitable for use in therapy.

In one aspect, there is provided an antibody or antigen binding fragment thereof which specifically binds hIL-13, and comprises a CDRH3 comprising the sequence set forth in SEQ.I.D.NO:3.

In one embodiment the antibody or antigen binding fragment of the present invention neutralises human IL-13.

In another embodiment the antibody or antigen binding fragment of the present invention modulates the binding of human IL-13 to its receptor.

In another aspect of the present invention there is provided an antibody or antigen binding fragment thereof which specifically binds hIL-13 and modulates the interaction between hIL-13 and hIL-13R.

In certain embodiments, antibodies of the invention at least inhibit the interaction between hIL-13 and hIL-13R but may also block the interaction between hIL-13 and hIL-13R thereby decoupling the hIL-13/hIL-13R signalling pathway.

In another embodiment the present invention provides an antibody or antigen binding fragment wherein the CDRH3 comprises the sequence of SEQ ID NO:3. In a further embodiment the antibody or antigen binding fragment thereof of the present invention further comprises one or more of the following sequences CDRH2: SEQ.I.D.NO:2, CDRH1: SEQ.I.D.NO:1, CDRL1: SEQ.I.D.NO:4, CDRL2: SEQ.I.D.NO:5 and CDRL3: SEQ.I.D.NO:6. In a further embodiment the present invention further comprises these CDR sequences in the context of a human framework, for example as a humanised antibody or fragment thereof.

In another aspect of the present invention there is provided an antibody or antigen binding fragment thereof which specifically binds hIL-13, and comprises the following CDRs:

CDRH1: SEQ.I.D.NO: 1
CDRH2: SEQ.I.D.NO: 2
CDRH3: SEQ.I.D.NO: 3
CDRL1: SEQ.I.D.NO: 4
CDRL2: SEQ.I.D.NO: 5
CDRL3: SEQ.I.D.NO: 6

In one embodiment of the present invention one or more of the CDRs of the antibody or antigen binding fragment may comprise variants of the CDRs set out in the sequences listed above. Each variant CDR will comprise one or two amino acid residues which differ from the amino acid residue in the corresponding position in the sequence listed above. Such substitutions in amino acid residues may be conservative substitutions, for example substituting one hydrophobic amino acid for an alternative hydrophobic amino acid, for example substituting Leucine with Valine, or Isoleucine.

Throughout this specification, amino acid residues in antibody sequences are numbered according to the Kabat scheme. Similarly, the terms "CDR", "CDRL1", "CDRL2", "CDRL3", "CDRH1", "CDRH2", "CDRH3" follow the Kabat numbering system as set forth in Kabat *et al; Sequences of proteins of Immunological Interest* NIH, 1987 with the exception that position 30 of the heavy chain is taken to be part of a CDR.

As used herein the term "comprising" and "comprises" incorporates "consisting of" and "consists of".

In another aspect of the invention there is provided an antibody or antigen binding fragment thereof comprising a VH domain comprising the sequence set forth in SEQ.I.D.NO:7 and a VL domain comprising the sequence set forth in SEQ.I.D.NO:8.

In another aspect of the invention there is provided an isolated VH domain of an antibody comprising the sequence selected from the group consisting of SEQ.I.D.NO: 7, 11, 12, 13 and 14. In one embodiment the isolated VH domain of an antibody consists of or consists essentially of an isolated VH domain of an antibody selected from the group consisting of SEQ.I.D.NO: 7, 11, 12, 13 and 14.

In another aspect of the invention there is provided an antibody or antigen binding fragment thereof comprising a VH domain selected from the group consisting of; SEQ.I.D.NO:7, 11,12,13 and 14.

In another aspect of the invention there is provided an antibody which specifically binds hIL-13 and at least inhibits the interaction between hIL-13 and hIL-13R which antibody comprises a heavy chain of SEQ.I.D.NO:18 and a light chain selected from the group consisting of; SEQ.I.D.NO:22, 23 and 24.

In another aspect of the invention there is provided an antibody which specifically binds hIL-13 and at least inhibits the interaction between hIL-13 and hIL-13R which antibody comprises a heavy chain of SEQ.I.D.NO:19 and a light chain selected from the group consisting of; SEQ.I.D.NO:22, 23 and 24.

In another aspect of the invention there is provided an antibody which specifically binds hIL-13 and at least inhibits the interaction between hIL-13 and hIL-13R which antibody comprises a heavy chain of SEQ.I.D.NO:20 and a light chain selected from the group consisting of; SEQ.I.D.NO:22, 23 and 24.

In another aspect of the invention there is provided an antibody which specifically binds hIL-13 and at least inhibits the interaction between hIL-13 and hIL-13R which antibody comprises a heavy chain of SEQ.I.D.NO:21 and a light chain selected from the group consisting of; SEQ.I.D.NO:22, 23 and 24.

In another aspect of the invention there is provided an antibody or antigen binding fragment thereof which inhibits the binding of the antibody comprising a heavy chain of SEQ.I.D.NO: 18 and a light chain of SEQ.I.D.NO:22 to hIL-13.

In accordance with the present invention there is provided a humanised antibody which antibody comprises a VH domain of: SEQ.I.D.NO:11 and a VL domain selected from the group consisting of: SEQ.I.D.NO:15, 16 and 17.

In accordance with the present invention there is provided a humanised antibody which antibody comprises a VH domain of: SEQ.I.D.NO:12 and a VL domain selected from the group consisting of: SEQ.I.D.NO:15, 16 and 17.

In accordance with the present invention there is provided a humanised antibody which antibody comprises a VH domain of: SEQ.I.D.NO:13 and a VL domain selected from the group consisting of: SEQ.I.D.NO:15, 16 and 17.

In accordance with the present invention there is provided a humanised antibody which antibody comprises a VH domain of: SEQ.I.D.NO:14 and a VL domain selected from the group consisting of: SEQ.I.D.NO:15, 16 and 17.

In accordance with the present invention there is provided an antibody or antigen binding fragment thereof which binds to the peptides set out in SEQ ID NO:90, 99, 102, 103, 105, 106, 107, 108, 109, 110, 111, 112 and 114 but does not bind the peptides set out in SEQ ID NO:100, 101, 104 and 113, wherein binding is defined as having an equivalent binding activity to the antibodies exemplified herein, for example antibodies which retain similar binding activity to 3G4 binding to human IL-13 peptides in the ELISA assay as set out in Example 6.4.

In another aspect of the invention there is provided a method of treating a human patient afflicted with a disease or disorder responsive to modulation of the interaction between hIL-13 and hIL-13R (such as asthma, COPD, allergic rhinitis, atopic dermatitis) which method comprises the step of administering to said patient a therapeutically effective amount of the antibody or antigen binding fragment thereof as described herein.

Use of an antibody of the invention in the manufacture of a medicament for the treatment of a disease or disorder responsive to modulation of the interaction between hIL-13 and hIL-13R is also provided.

In a further aspect the invention provides a method of selecting an anti-IL13 antibody suitable for use in therapy, which method comprises i) providing an antibody which specifically binds to IL-13Rα1, ii) determining whether the antibody binds specifically to IL-13Rα2, and selecting an antibody which binds in step ii) for further development.

**Detailed Description of the Invention**

The antibodies of the present invention may be intact antibodies or fragments thereof; human, chimaeric or humanised antibodies; and mono or bispecific.

## 1. Antibody Structures

### 1.1 Intact Antibodies

Intact antibodies include heteromultimeric glycoproteins comprising at least two heavy and two light chains. Aside from IgM, intact antibodies are usually heterotetrameric glycoproteins of approximately 150Kda, composed of two identical light (L) chains and two identical heavy (H) chains. Typically, each light chain is linked to a heavy chain by one covalent disulfide bond while the number of disulfide linkages between the heavy chains of different immunoglobulin isotypes varies. Each heavy and light chain also has intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant regions. Each light chain has a variable domain (VL) and a constant region at its other end; the constant region of the light chain is aligned with the first constant region of the heavy chain and the light chain variable domain is aligned with the variable domain of the heavy chain. The light chains of antibodies from most vertebrate species can be assigned to one of two types called Kappa and Lambda based on the amino acid sequence of the constant region. Depending on the amino acid sequence of the constant region of their heavy chains, human antibodies can be assigned to five different classes, IgA, IgD, IgE, IgG and IgM. IgG and IgA can be further subdivided into subclasses, IgG1, IgG2, IgG3 and IgG4; and IgA1 and IgA2. Species variants exist with mouse and rat having at least IgG2a, IgG2b. The variable domain of the antibody confers binding specificity upon the antibody with certain regions displaying particular variability called complementarity determining regions (CDRs). The more conserved portions of the variable region are called Framework regions (FR). The variable domains of intact heavy and light chains each comprise four FR connected by three CDRs. The CDRs in each chain are held together in close proximity by the FR regions and with the CDRs from the other chain contribute to the formation of the antigen binding site of antibodies. The constant regions are not directly involved in the binding of the antibody to the antigen but exhibit various effector functions such as participation in antibody dependent cell-mediated cytotoxicity (ADCC), phagocytosis via binding to Fcγ receptor, half-life/clearance rate via neonatal Fc receptor (FcRn) and complement dependent cytotoxicity via the C1q component of the complement cascade.

In one embodiment therefore we provide an intact antibody that specifically binds hIL-13, which antibody modulates the interaction between hIL-13 and hIL-13R, for example the antibody inhibits the interaction between hIL-13 and its receptor. The intact antibody may comprise a constant region of any isotype or subclass thereof described *supra*. In one embodiment, the antibody is of the IgG isotype, particularly

IgG1. The antibody may be rat, mouse, rabbit, primate or human. In one typical embodiment, the antibody is primate (such as cynomolgus, Old World monkey or Great Ape, see e.g. WO99/55369, WO93/02108) or human.

In another embodiment there is provided an isolated intact antibody comprising a CDRH3 of SEQ.I.D.NO: 3. In another embodiment there is provided an intact antibody comprising a variable region comprising CDRs of SEQ.I.D.NO: 1, 2,3,4,5 and 6.

In another embodiment, there is provided an isolated murine intact antibody or antigen binding fragment thereof comprising a VH domain comprising the sequence of SEQ.I.D.NO: 7 and a VL domain of the sequence of SEQ.I.D.NO: 8.

### 1.1.2 Human antibodies

Human antibodies may be produced by a number of methods known to those of skill in the art. Human antibodies can be made by the hybridoma method using human myeloma or mouse-human heteromyeloma cells lines see Kozbor J.Immunol 133, 3001, (1984) and Brodeur, Monoclonal Antibody Production Techniques and Applications, pp51-63 (Marcel Dekker Inc, 1987). Alternative methods include the use of phage libraries or transgenic mice both of which utilize human V region repertories (see Winter G, (1994), Annu.Rev.Immunol 12,433-455, Green LL (1999), J.Immunol.methods 231, 11-23).
Several strains of transgenic mice are now available wherein their mouse immunoglobulin loci has been replaced with human immunoglobulin gene segments (see Tomizuka K, (2000) PNAS 97,722-727; Fishwild D.M (1996) Nature Biotechnol. 14,845-851, Mendez MJ, 1997, Nature Genetics, 15,146-156). Upon antigen challenge such mice are capable of producing a repertoire of human antibodies from which antibodies of interest can be selected. Of particular note is the Trimera™ system (see Eren R *et al*, (1998) Immunology 93:154-161) where human lymphocytes are transplanted into irradiated mice, the Selected Lymphocyte Antibody System (SLAM, see Babcook *et al*, PNAS (1996) 93:7843-7848) where human (or other species) lymphocytes are effectively put through a massive pooled *in vitro* antibody generation procedure followed by deconvulated, limiting dilution and selection procedure and the Xenomouse II™ (Abgenix Inc). An alternative approach is available from Morphotek Inc using the Morphodoma™ technology.

Phage display technology can be used to produce human antibodies (and fragments thereof), see McCafferty; Nature, 348, 552-553 (1990) and Griffiths AD *et al* (1994) EMBO 13:3245-3260. According to this technique antibody V domain genes are cloned in frame into either a major or minor coat of protein gene of a filamentous bacteriophage such as M13 or fd and displayed (usually with the aid of a helper phage) as functional antibody fragments on the surface of the phage particle.

Selections based on the functional properties of the antibody result in selection of the gene encoding the antibody exhibiting those properties. The phage display technique can be used to select antigen specific antibodies from libraries made from human B cells taken from individuals afflicted with a disease or disorder described above or alternatively from unimmunized human donors (see Marks; J.Mol.Bio. 222,581-597, 1991). Where an intact human antibody is desired comprising a Fc domain it is necessary to reclone the phage displayed derived fragment into a mammalian expression vectors comprising the desired constant regions and establishing stable expressing cell lines.
The technique of affinity maturation (Marks; Bio/technol 10,779-783 (1992)) may be used to improve binding affinity wherein the affinity of the primary human antibody is improved by sequentially replacing the H and L chain V regions with naturally occurring variants and selecting on the basis of improved binding affinities. Variants of this technique such as "epitope imprinting" are now also available see WO 93/06213. See also Waterhouse; Nucl.Acids Res 21, 2265-2266 (1993).

Thus in another embodiment there is provided an isolated human intact antibody or antigen binding fragment thereof which specifically binds hIL-13 and modulates the interaction between hIL-13 and hIL-13R, for example where it inhibits the interaction between hIL-13 and its receptor.

In another aspect there is provided an isolated human intact antibody or antigen binding fragment thereof comprising a CDRH3 of SEQ.I.D.NO: 3 which specifically binds hIL-13 and modulates the interaction between hIL-13 and hIL-13R, for example where it inhibits the interaction between hIL-13 and its receptor. In another aspect there is provided an isolated human intact antibody or antigen binding fragment thereof comprising a variable region comprising CDRs of SEQ.I.D.NO: 1, 2, 3, 4, 5 and 6 as defined *supra*.

## 1.2 Chimaeric and Humanised Antibodies

The use of intact non-human antibodies in the treatment of human diseases or disorders carries with it the potential for the now well established problems of immunogenicity, that is the immune system of the patient may recognise the non-human intact antibody as non-self and mount a neutralising response. This is particularly evident upon multiple administration of the non-human antibody to a human patient. Various techniques have been developed over the years to overcome these problems and generally involve reducing the composition of non-human amino acid sequences in the intact antibody whilst retaining the relative ease in obtaining non-human antibodies from an immunised animal e.g. mouse, rat or rabbit. Broadly two approaches have been used to achieve this. The first are

chimaeric antibodies, which generally comprise a non-human (e.g. rodent such as mouse) variable domain fused to a human constant region. Because the antigen-binding site of an antibody is localised within the variable regions the chimaeric antibody retains its binding affinity for the antigen but acquires the effector functions of the human constant region and are therefore able to perform effector functions such as described *supra*. Chimaeric antibodies are typically produced using recombinant DNA methods. DNA encoding the antibodies (e.g. cDNA) is isolated and sequenced using conventional procedures (e.g. by using oligonucleotide probes that are capable of binding specifically to genes encoding the H and L chains of the antibody of the invention, e.g. DNA encoding SEQ.I.D.NO 1,2,3,4,5 and 6 described *supra*). Hybridoma cells serve as a typical source of such DNA. Once isolated, the DNA is placed into expression vectors which are then transfected into host cells such as *E.Coli*, COS cells, CHO cells or myeloma cells that do not otherwise produce immunoglobulin protein to obtain synthesis of the antibody. The DNA may be modified by substituting the coding sequence for human L and H chains for the corresponding non-human (e.g. murine) H and L constant regions see e.g. Morrison; PNAS 81, 6851 (1984).

The second approach involves the generation of humanised antibodies wherein the non-human content of the antibody is reduced by humanizing the variable regions. Two techniques for humanisation have gained popularity. The first is humanisation by CDR grafting. CDRs build loops close to the antibody's N-terminus where they form a surface mounted in a scaffold provided by the framework regions. Antigen-binding specificity of the antibody is mainly defined by the topography and by the chemical characteristics of its CDR surface. These features are in turn determined by the conformation of the individual CDRs, by the relative disposition of the CDRs, and by the nature and disposition of the side chains of the residues comprising the CDRs. A large decrease in immunogenicity can be achieved by grafting only the CDRs of a non-human (e.g. murine) antibodies ("donor" antibodies) onto human framework ("acceptor framework") and constant regions (see Jones *et al* (1986) Nature 321,522-525 and Verhoeyen M *et al* (1988) Science 239, 1534-1536). However, CDR grafting *per se* may not result in the complete retention of antigen-binding properties and it is frequently found that some framework residues (sometimes referred to as "back-mutations") of the donor antibody need to be preserved in the humanised molecule if significant antigen-binding affinity is to be recovered (see Queen C *et al* (1989) PNAS 86, 10,029-10,033, Co, M *et al* (1991) Nature 351, 501-502). In this case, human V regions showing the greatest sequence homology to the non-human donor antibody are chosen from a database in order to provide the human framework (FR). The selection of human FRs can be made either from human consensus or individual human antibodies. Where necessary key residues from the donor antibody are substituted into the human acceptor framework to preserve CDR conformations. Computer modelling of the antibody maybe used to help identify such structurally important residues, see WO99/48523.

Alternatively, humanisation maybe achieved by a process of "veneering". A statistical analysis of unique human and murine immunoglobulin heavy and light chain variable regions revealed that the precise patterns of exposed residues are different in human and murine antibodies, and most individual surface positions have a strong preference for a small number of different residues (see Padlan E.A. *et al*; (1991) Mol.Immunol.28, 489-498 and Pedersen J.T. *et al* (1994) J.Mol.Biol. 235; 959-973). Therefore it is possible to reduce the immunogenicity of a non-human Fv by replacing exposed residues in its framework regions that differ from those usually found in human antibodies. Because protein antigenicity may be correlated with surface accessibility, replacement of the surface residues may be sufficient to render the mouse variable region "invisible" to the human immune system (see also Mark G.E. *et al* (1994) in *Handbook of Experimental Pharmacology vol.113: The pharmacology of monoclonal Antibodies*, Springer-Verlag, pp105-134). This procedure of humanisation is referred to as "veneering" because only the surface of the antibody is altered, the supporting residues remain undisturbed.

The skilled man will be aware that other methods of antibody humanisation exist and are available in the literature.

Thus another embodiment of the invention there is provided a chimaeric antibody comprising a non-human (e.g. rodent) variable domain fused to a human constant region (which maybe of a IgG isotype e.g. IgG1) which specifically binds hIL-13 and modulates the interaction between hIL-13 and hIL-13R, for example where it inhibits the interaction between hIL-13 and its receptor.

In another embodiment there is provided a chimaeric antibody comprising a non-human (e.g. rodent) variable region and a human constant region (which maybe of an IgG isotype e.g. IgG1) which specifically binds hIL-13, which antibody further comprises a CDRH3 of SEQ.I.D.NO3. Such antibodies may further comprise a human constant region of the IgG isotype, e.g. IgG1

In another embodiment there is chimaeric antibody comprising a non-human (e.g. rodent) variable region and a human constant region (which maybe of a IgG isotype, for example IgG1) which specifically binds hIL-13 comprising the CDRs of SEQ.I.D.NO: 1, 2,3,4,5 and 6.

In another embodiment there is provided a chimaeric antibody comprising a VH domain of SEQ.I.D.NO:7 and a VL domain of SEQ.I.D.NO:8 and a human constant region of an IgG isotype, e.g. IgG1 which specifically binds hIL-13 and modulates the interaction between hIL-13 and hIL-13R, for example where it inhibits the interaction between hIL-13 and its receptor.

In another embodiment there is provided a humanised antibody or antigen binding fragment thereof which specifically binds hIL-13 and modulates the interaction between hIL-13 and hIL-13R, for example where it inhibits the interaction between hIL-13 and its receptor.

In another embodiment there is provided a humanised antibody or antigen binding fragment thereof which specifically binds hIL-13 and comprises a CDRH3 of SEQ.I.D.NO: 3. Such antibodies may comprise a human constant region of the IgG isotype, e.g. IgG1.

In another embodiment there is provided a humanised antibody or antigen binding fragment thereof which specifically binds hIL-13 and comprises CDRs of SEQ.I.D.NO1, 2,3,4,5 and 6. Such antibodies may comprise a human constant region of the IgG isotype, e.g. IgG1.

In accordance with the present invention there is provided a humanised antibody which antibody comprises a VH domain selected from the group of: SEQ.I.D.NO:11, and a VL domain selected from the group of: SEQ.I.D.NO:15,16,17. Such antibodies may comprise a human constant region of the IgG isotype e.g. IgG1.

In accordance with the present invention there is provided a humanised antibody which antibody comprises a VH domain selected from the group of: SEQ.I.D.NO:12, and a VL domain selected from the group of: SEQ.I.D.NO:15,16,17. Such antibodies may comprise a human constant region of the IgG isotype e.g. IgG1.

In accordance with the present invention there is provided a humanised antibody which antibody comprises a VH domain selected from the group of: SEQ.I.D.NO:13, and a VL domain selected from the group of: SEQ.I.D.NO:15,16,17. Such antibodies may comprise a human constant region of the IgG isotype e.g. IgG1.

In accordance with the present invention there is provided a humanised antibody which antibody comprises a VH domain selected from the group of: SEQ.I.D.NO:14, and a VL domain selected from the group of: SEQ.I.D.NO:15,16,17. Such antibodies may comprise a human constant region of the IgG isotype e.g. IgG1

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 11 and a VL domain of SEQ.I.D.NO:15.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 12 and a VL domain of SEQ.I.D.NO:15.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 13 and a VL domain of SEQ.I.D.NO:15.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 14 and a VL domain of SEQ.I.D.NO:15.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 11 and a VL domain of SEQ.I.D.NO:16.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 12 and a VL domain of SEQ.I.D.NO:16.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 13 and a VL domain of SEQ.I.D.NO:16.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 14 and a VL domain of SEQ.I.D.NO:16.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 11 and a VL domain of SEQ.I.D.NO:17.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 12 and a VL domain of SEQ.I.D.NO:17.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 13 and a VL domain of SEQ.I.D.NO:17.

In another embodiment there is provided a humanised antibody which antibody comprises a VH domain of SEQ.I.D.NO: 14 and a VL domain of SEQ.I.D.NO:17.

In another embodiment, there is provided a humanised antibody or antigen binding fragment thereof which specifically binds hIL-13 wherein said antibody or fragment thereof comprises CDRH3 of SEQ.I.D.NO:3 optionally further comprising one or more of CDRs of SEQ.I.D.NO:1, 2, 4, 5, and 6 wherein one or more of the residue(s) selected from the group consisting of position 10, 30, 67, 69, 71, 73 and 93 of the human acceptor heavy chain framework and one or both residue(s) at position 76 and 98 of the human acceptor light chain framework are substituted by the corresponding residues found in the donor antibody framework from which CDRH3 is derived (which is set out in SEQ.I.D.NO:7).

In another embodiment there is provided a humanised antibody or antigen binding fragment thereof which specifically binds hIL-13 wherein said antibody or fragment thereof comprises CDRH3 of SEQ.I.D.NO:3, optionally further comprising one or more CDRs of SEQ.I.D.NO:1,2,4,5 and 6 wherein the human heavy chain framework

comprises one or more (e.g. all) of the following residues (or conservative substitution thereof);

| **Position** | **Residue** |
|---|---|
| 10 | D |
| 30 | I |
| 67 | A |
| 69 | L |
| 71 | A |
| 73 | K |
| 93 | T |

and a light chain framework comprising either or both of the following residues (or conservative substitute thereof);

| | |
|---|---|
| 76 | N |
| 98 | L |

It will be apparent to those skilled in the art that the term "derived" is intended to define not only the source in the sense of it being the *physical* origin for the material but also to define material which is structurally identical (in terms of primary amino acid sequence) to the material but which does not originate from the reference source. Thus "residues found in the donor antibody from which CDRH3 is derived" need not necessarily have been purified from the donor antibody.

It is well recognised in the art that certain amino acid substitutions are regarded as being "conservative". Amino acids are divided into groups based on common side-chain properties and substitutions within groups that maintain all or substantially all of the binding affinity of the antibody of the invention or antigen binding fragment thereof are regarded as conservative substitutions, see table 1:

Table 1

| Side chain | Members |
|---|---|
| Hydrophobic | met, ala,val,leu,ile |
| neutral hydrophilic | cys, ser, thr |

| | |
|---|---|
| Acidic | asp, glu |
| Basic | asn, gln, his, lys, arg |
| residues that influence chain orientation | gly, pro |
| Aromatic | trp, tyr, phe |

In accordance with the present invention there is provided a humanised antibody comprising a heavy chain selected from the group consisting of: SEQ.I.D.NO: 18,19,20,21 and a light chain selected from the group consisting of; SEQ.I.D.NO:22, 23, 24.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:18 and a light chain of SEQ.I.D.NO:22.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:19 and a light chain of SEQ.I.D.NO:22.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:20 and a light chain of SEQ.I.D.NO:22.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:21 and a light chain of SEQ.I.D.NO:22.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:18 and a light chain of SEQ.I.D.NO:23.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:19 and a light chain of SEQ.I.D.NO:23.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:20 and a light chain of SEQ.I.D.NO:23.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:21 and a light chain of SEQ.I.D.NO:23.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:18 and a light chain of SEQ.I.D.NO:24.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:19 and a light chain of SEQ.I.D.NO:24.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:20 and a light chain of SEQ.I.D.NO:24.

In one embodiment of the invention there is provided a humanised antibody which specifically binds hIL-13 comprising a heavy chain of SEQ.I.D.NO:21 and a light chain of SEQ.I.D.NO:24.

In one embodiment of the present invention there is provided a human or humanised heavy chain variable region comprising each of the CDRs listed in SEQ ID NO 1-3. In another embodiment of the present invention there is provided a humanised heavy chain variable region comprising the CDRs listed in SEQ ID NO 1-3 within the larger sequence of a human heavy chain variable region. In yet another embodiment the humanised heavy chain variable region comprises the CDRs listed in SEQ ID NO 1-3 within an acceptor antibody framework having greater than 40% identity in the framework regions, or greater than 50%, or greater than 60%, or greater than 65% identity to the murine 3G4 donor antibody heavy chain variable region (SEQ ID NO. 7).

In one aspect of the present invention the antibodies comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO. 44 further comprising a number of substitutions at one or more of positions 10, 30, 67, 69, 71, 73, 93 (Kabat numbering system); wherein each substituted amino acid residue is replaced with the amino acid residue at the equivalent position in SEQ ID NO. 7 (the heavy chain variable region of the donor antibody 3G4) and the number of substitutions is between 0 and 7. In other embodiments the number of substitutions is 0, or 1, or 2, or 3, or 4, or 5, or 6, or 7.

In one embodiment of the present invention there is provided a human or humanised light chain variable region comprising each of the CDRs listed in SEQ ID NO 4-6. In another embodiment of the present invention there is provided a humanised light chain variable region comprising the CDRs listed in SEQ ID NO 4-6 within the larger sequence of a human light chain variable region. In yet another embodiment the humanised light chain variable region comprises the CDRs listed in SEQ ID NO 4-6 within an acceptor antibody framework having greater than 40% identity in the

framework regions, or greater than 50%, or greater than 60%, or greater than 65% identity to the murine 3G4 donor antibody heavy chain variable region (SEQ ID NO. 8).

In one aspect of the present invention the antibodies comprise a light chain variable region comprising the amino acid sequence of SEQ ID NO. 45 further comprising a number of substitutions at one or more of positions 76, 98 (Kabat numbering system); wherein each substituted amino acid residue is replaced with the amino acid residue at the equivalent position in SEQ ID NO. 8 (the light chain variable region of the donor antibody 3G4) and the number of substitutions is between 0 and 2. In other embodiments the number of substitutions is 0 or 1 or 2.

**1.3 Bispecific antibodies**

A bispecific antibody is an antibody having binding specificities for at least two different epitopes. Methods of making such antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin H chain-L chain pairs, where the two H chains have different binding specificities see Millstein *et al*, Nature 305 537-539 (1983), WO93/08829 and Traunecker *et al* EMBO, 10, 1991, 3655-3659. Because of the random assortment of H and L chains, a potential mixture of ten different antibody structures are produced of which only one has the desired binding specificity. An alternative approach involves fusing the variable domains with the desired binding specificities to heavy chain constant region comprising at least part of the hinge region, CH2 and CH3 regions. It is preferred to have the CH1 region containing the site necessary for light chain binding present in at least one of the fusions. DNA encoding these fusions, and if desired the L chain are inserted into separate expression vectors and are then cotransfected into a suitable host organism. It is possible though to insert the coding sequences for two or all three chains into one expression vector. In one preferred approach, the bispecific antibody is composed of a H chain with a first binding specificity in one arm and a H-L chain pair, providing a second binding specificity in the other arm, see WO94/04690. Also see Suresh *et al* Methods in Enzymology 121, 210, 1986.

In one embodiment of the invention there is provided a bispecific antibody wherein at least one binding specificity of said antibody is for hIL-13, wherein said antibody modulates the interaction between hIL-13 and IL-13R, for example where it inhibits the interaction between hIL-13 and its receptor. Such antibodies may further comprise a human constant region of the IgG isotype, e.g. IgG1. In some embodiments, the bispecific therapeutic antibody has a first binding specificity for hIL-13 and modulates the interaction between hIL-13 and hIL-13R, for example where it inhibits the interaction between hIL-13 and its receptor and a second binding

specificity for hIL-4 and modulates the interaction between hIL-4 and a receptor for hIL-4, for example where it inhibits the interaction between hIL-4 and its receptor.

In one embodiment of the invention there is provided a bispecific antibody wherein at least one binding specificity of said antibody is for hIL-13, wherein said antibody comprises a CDRH3 of SEQ.I.D.NO: 3. Such antibodies may further comprise a human constant region of the IgG isotype, e.g. IgG1.

In one embodiment of the invention there is provided a bispecific antibody wherein at least one binding specificity of said antibody is for hIL-13, wherein said antibody comprises at least CDRs of SEQ.I.D.NO: 1, 2,3,4,5 and 6. Such antibodies may further comprise a human constant region of the IgG isotype, e.g. IgG1.

**1.4 Antibody Fragments**

In certain embodiments of the invention there is provided antibody fragments which modulate the interaction between hIL-13 and hIL-13R, for example where the fragments inhibit the interaction between hIL-13 and its receptor. Such fragments may be functional antigen binding fragments of intact and/or humanised and/or chimaeric antibodies such as Fab, Fab', $F(ab')_2$, Fv, ScFv fragments of the antibodies described *supra*. Traditionally such fragments are produced by the proteolytic digestion of intact antibodies by e.g. papain digestion (see for example, WO 94/29348) but may be produced directly from recombinantly transformed host cells. For the production of ScFv, see Bird *et al* ;(1988) Science, 242, 423-426. In addition, antibody fragments may be produced using a variety of engineering techniques as described below.

Fv fragments appear to have lower interaction energy of their two chains than Fab fragments. To stablise the association of the VH and VL domains, they have been linked with peptides (Bird *et al*, (1988) Science 242, 423-426, Huston *et al*, PNAS, 85, 5879-5883), disulphide bridges (Glockshuber *et al*, (1990) Biochemistry, 29, 1362-1367) and "knob in hole" mutations (Zhu *et al* (1997), Protein Sci., 6, 781-788). ScFv fragments can be produced by methods well known to those skilled in the art see Whitlow *et al* (1991) Methods companion Methods Enzymol, 2, 97-105 and Huston *et al* (1993) Int.Rev.Immunol 10, 195-217. ScFv may be produced in bacterial cells such as *E.Coli* but are more preferably produced in eukaryotic cells. One disadvantage of ScFv is the monovalency of the product, which precludes an increased avidity due to polyvalent binding, and their short half-life. Attempts to overcome these problems include bivalent $(ScFv')_2$ produced from ScFV containing an additional C terminal cysteine by chemical coupling ( Adams *et al* (1993) Can.Res 53, 4026-4034 and McCartney *et al* (1995) Protein Eng. 8, 301-314) or by spontaneous site-specific dimerization of ScFv containing an unpaired C terminal

cysteine residue (see Kipriyanov *et al* (1995) Cell. Biophys 26, 187-204). Alternatively, ScFv can be forced to form multimers by shortening the peptide linker to 3 to 12 residues to form "diabodies", see Holliger *et al* PNAS (1993), 90, 6444-6448. Reducing the linker still further can result in ScFV trimers ("triabodies", see Kortt *et al* (1997) Protein Eng, 10, 423-433) and tetramers ("tetrabodies", see Le Gall *et al* (1999) FEBS Lett, 453, 164-168). Construction of bivalent ScFV molecules can also be achieved by genetic fusion with protein dimerizing motifs to form "miniantibodies" (see Pack *et al* (1992) Biochemistry 31, 1579-1584) and "minibodies" (see Hu *et al* (1996), Cancer Res. 56, 3055-3061). ScFv-Sc-Fv tandems ((ScFV)2) may also be produced by linking two ScFv units by a third peptide linker, see Kurucz *et al* (1995) J.Immol.154, 4576-4582. Bispecific diabodies can be produced through the noncovalent association of two single chain fusion products consisting of VH domain from one antibody connected by a short linker to the VL domain of another antibody, see Kipriyanov *et al* (1998), Int.J.Can 77,763-772. The stability of such bispecific diabodies can be enhanced by the introduction of disulphide bridges or "knob in hole" mutations as described *supra* or by the formation of single chain diabodies (ScDb) wherein two hybrid ScFv fragments are connected through a peptide linker see Kontermann *et al* (1999) J.Immunol.Methods 226 179-188. Tetravalent bispecific molecules are available by e.g. fusing a ScFv fragment to the CH3 domain of an IgG molecule or to a Fab fragment through the hinge region see Coloma *et al* (1997) Nature Biotechnol. 15, 159-163. Alternatively, tetravalent bispecific molecules have been created by the fusion of bispecific single chain diabodies (see Alt *et al*, (1999) FEBS Lett 454, 90-94. Smaller tetravalent bispecific molecules can also be formed by the dimerization of either ScFv-ScFv tandems with a linker containing a helix-loop-helix motif (DiBi miniantibodies, see Muller *et al* (1998) FEBS Lett 432, 45-49) or a single chain molecule comprising four antibody variable domains (VH and VL) in an orientation preventing intramolecular pairing (tandem diabody, see Kipriyanov *et al*, (1999) J.Mol.Biol. 293, 41-56). Bispecific $F(ab')_2$ fragments can be created by chemical coupling of Fab' fragments or by heterodimerization through leucine zippers (see Shalaby *et al*, (1992) J.Exp.Med. 175, 217-225 and Kostelny *et al* (1992), J.Immunol. 148, 1547-1553). Also available are isolated VH and VL domains (Domantis plc), see US 6, 248,516; US 6,291,158; US 6, 172,197.

In one embodiment there is provided an antibody fragment (e.g. ScFv, Fab, Fab', $F(ab')_2$) or an engineered antibody fragment as described *supra* that specifically binds hIL-13 and modulates the interaction between hIL-13 and hIL-13R, for example where such fragments inhibit the interaction between hIL-13 and its receptor. The antibody fragment may comprise a CDRH3 comprising the sequence of SEQ.I.D.NO: 3 optionally together with further CDRs comprising one or more of the sequences as set out in SEQ.I.D.NO: 1, 2, 4, 5 and 6.

A ScFv may be made comprising the VH and VL regions of the antibodies of the present invention. For example a ScFv may comprise SEQ.I.D.NO: 12 and 15, or for example may comprise SEQ.I.D.NO: 13 and 15. This could be made by the polynucleotides according to the invention, for example the sequences set out in SEQ.I.D.NO: 93 and 94, or for example by the sequences set out in SEQ.I.D.NO: 28 and 31. In one embodiment of the invention is provided a ScFv comprising a protein encoded by the sequences as set out in SEQ.I.D.NO: 93 and 94.

**1.5 Heteroconjugate antibodies**

Heteroconjugate antibodies also form an embodiment of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies formed using any convenient cross-linking methods. See, for example, US 4,676,980.

**1.6 Other Modifications**.

The interaction between the Fc region of an antibody and various Fc receptors (FcγR) is believed to mediate the effector functions of the antibody which include antibody-dependent cellular cytotoxicity (ADCC), fixation of complement, phagocytosis and half-life/clearance of the antibody. Various modifications to the Fc region of antibodies of the invention may be carried out depending on the desired property. For example, specific mutations in the Fc region to render an otherwise lytic antibody, non-lytic is detailed in EP 0629 240B1 and EP 0307 434B2 or one may incorporate a salvage receptor binding epitope into the antibody to increase serum half life see US 5,739,277. There are five currently recognised human Fcγ receptors, FcγR (I), FcγRIIa, FcγRIIb, FcγRIIIa and neonatal FcRn. Shields *et al*, (2001) J.Biol.Chem 276, 6591-6604 demonstrated that a common set of IgG1 residues is involved in binding all FcγRs, while FcγRII and FcγRIII utilize distinct sites outside of this common set. One group of IgG1 residues reduced binding to all FcγRs when altered to alanine: Pro-238, Asp-265, Asp-270, Asn-297 and Pro-239. All are in the IgG CH2 domain and clustered near the hinge joining CH1 and CH2. While FcγRI utilizes only the common set of IgG1 residues for binding, FcγRII and FcγRIII interact with distinct residues in addition to the common set. Alteration of some residues reduced binding only to FcγRII (e.g. Arg-292) or FcγRIII (e.g. Glu-293). Some variants showed improved binding to FcγRII or FcγRIII but did not affect binding to the other receptor (e.g. Ser-267Ala improved binding to FcγRII but binding to FcγRIII was unaffected). Other variants exhibited improved binding to FcγRII or FcγRIII with reduction in binding to the other receptor (e.g. Ser-298Ala improved binding to FcγRIII and reduced binding to FcγRII). For FcγRIIIa, the best binding IgG1 variants had combined alanine substitutions at Ser-298, Glu-333 and Lys-334. The neonatal FcRn receptor is believed to be involved in both antibody clearance and the transcytosis across tissues (see Junghans R.P (1997) Immunol.Res 16. 29-57 and Ghetie *et al* (2000) Annu.Rev.Immunol. 18, 739-766). Human IgG1 residues

determined to interact directly with human FcRn includes Ile253, Ser254, Lys288, Thr307, Gln311, Asn434 and His435. Switches at any of these positions described in this Example may enable increased serum half-life and/or altered effector properties of antibodies of the invention.

Other modifications include glycosylation variants of the antibodies of the invention. Glycosylation of antibodies at conserved positions in their constant regions is known to have a profound effect on antibody function, particularly effector functioning such as those described above, see for example, Boyd *et al* (1996), Mol.Immunol. 32, 1311-1318. Glycosylation variants of the antibodies or antigen binding fragments thereof of the present invention wherein one or more carbohydrate moiety is added, substituted, deleted or modified are contemplated. Introduction of an asparagine-X-serine or asparagine-X-threonine motif creates a potential site for enzymatic attachment of carbonhydrate moieties and may therefore be used to manipulate the glycosylation of an antibody. In Raju *et al* (2001) Biochemistry 40, 8868-8876 the terminal sialyation of a TNFR-IgG immunoadhesin was increased through a process of regalactosylation and/or resialylation using beta-1, 4-galactosyltransferace and/or alpha, 2,3 sialyltransferase. Increasing the terminal sialylation is believed to increase the half-life of the immunoglobulin. Antibodies, in common with most glycoproteins, are typically produced as a mixture of glycoforms. This mixture is particularly apparent when antibodies are produced in eukaryotic, particularly mammalian cells. A variety of methods have been developed to manufacture defined glycoforms, see Zhang *et al* Science (2004), 303, 371, Sears *et al*, Science, (2001) 291, 2344, Wacker *et al* (2002) Science, 298 1790, Davis *et al* (2002) Chem.Rev. 102, 579, Hang *et al* (2001) Acc.Chem.Res 34, 727. Thus the invention contemplates a plurality of (monoclonal) antibodies (which maybe of the IgG isotype, e.g. IgG1) as herein described comprising a defined number (e.g. 7 or less, for example 5 or less such as two or a single) glycoform(s) of said antibodies or antigen binding fragments thereof.

Further embodiments of the invention include antibodies of the invention or antigen binding fragments thereof coupled to a non-proteinaeous polymer such as polyethylene glycol (PEG), polypropylene glycol or polyoxyalkylene. Conjugation of proteins to PEG is an established technique for increasing half-life of proteins, as well as reducing antigenicity and immunogenicity of proteins. The use of PEGylation with different molecular weights and styles (linear or branched) has been investigated with intact antibodies as well as Fab' fragments, see Koumenis I.L. *et al* (2000) Int.J.Pharmaceut. 198:83-95.

## 2. Production Methods

Antibodies of the invention maybe produced as a polyclonal population but are more preferably produced as a monoclonal population (that is as a substantially homogenous population of identical antibodies directed against a specific antigenic binding site). It will of course be apparent to those skilled in the art that a population implies more than one antibody entity. Antibodies of the present invention may be produced in transgenic organisms such as goats (see Pollock *et al* (1999), J.Immunol.Methods 231:147-157), chickens (see Morrow KJJ (2000) Genet.Eng.News 20:1-55, mice (see Pollock *et al*) or plants (see Doran PM, (2000) Curr.Opinion Biotechnol. 11, 199-204, Ma JK-C (1998), Nat.Med. 4; 601-606, Baez J *et al*, BioPharm (2000) 13: 50-54, Stoger E *et al*; (2000) Plant Mol.Biol. 42:583-590). Antibodies may also be produced by chemical synthesis. However, antibodies of the invention are typically produced using recombinant cell culturing technology well known to those skilled in the art. A polynucleotide encoding the antibody is isolated and inserted into a replicable vector such as a plasmid for further cloning (amplification) or expression. One useful expression system is a glutamate synthetase system (such as sold by Lonza Biologics), particularly where the host cell is CHO or NS0 (see below). Polynucleotide encoding the antibody is readily isolated and sequenced using conventional procedures (e.g. oligonucleotide probes). Vectors that may be used include plasmid, virus, phage, transposons, minichromsomes of which plasmids are a typical embodiment. Generally such vectors further include a signal sequence, origin of replication, one or more marker genes, an enhancer element, a promoter and transcription termination sequences operably linked to the light and/or heavy chain polynucleotide so as to facilitate expression. Polynucleotide encoding the light and heavy chains may be inserted into separate vectors and transfected into the same host cell or, if desired both the heavy chain and light chain can be inserted into the same vector for transfection into the host cell. Thus according to one aspect of the present invention there is provided a process of constructing a vector encoding the light and/or heavy chains of an antibody or antigen binding fragment thereof of the invention, which method comprises inserting into a vector, a polynucleotide encoding either a light chain and/or heavy chain of an antibody of the invention.

In other aspect of the invention there is provided a polynucleotide encoding a murine VH domain comprising the sequence set forth as SEQ.I.D.NO:25.

In another aspect of the invention there is provided polynucleotide encoding a murine VL domain comprising the sequence set forth as SEQ.I.D.NO: 26.

In another embodiment there is provided a polynucletotide encoding a VH domain comprising the sequence selected from the group consisting of SEQ.I.D.NO:27, 28, 29,30

In another embodiment there is provided a polynucletotide encoding a VL domain comprising the sequence selected from the group consisting of; SEQ.I.D.NO: 31, 32, 33

In accordance with the present invention there is provided a polynucleotide encoding a heavy chain of the invention which polynucleotide is selected from the group consisting of; SEQ.I.D.NO:34, 35, 36, 37.

In accordance with the present invention there is provided a polynucleotide encoding a light chain of the invention which polynucleotide is selected from the group consisting of; SEQ.I.D.NO:38, 39, 40.

It will be immediately apparent to those skilled in the art that due to the redundancy of the genetic code, alternative polynucleotides to those disclosed herein (particularly those codon optimised for expression in a given host cell) are also available that will encode the polypeptides of the invention.

**3.1 Signal sequences**

Antibodies of the present invention maybe produced as a fusion protein with a heterologous signal sequence having a specific cleavage site at the N terminus of the mature protein. The signal sequence should be recognised and processed by the host cell. For prokaryotic host cells, the signal sequence may be an alkaline phosphatase, penicillinase, or heat stable enterotoxin II leaders. For yeast secretion the signal sequences may be a yeast invertase leader, $\alpha$ factor leader or acid phosphatase leaders see e.g. WO90/13646. In mammalian cell systems, viral secretory leaders such as herpes simplex gD signal and a native immunoglobulin signal sequence are available. Typically the signal sequence is ligated in reading frame to DNA encoding the antibody of the invention.

**3.2 Origin of replication**

Origin of replications are well known in the art with pBR322 suitable for most gram-negative bacteria, 2$\mu$ plasmid for most yeast and various viral origins such as SV40, polyoma, adenovirus, VSV or BPV for most mammalian cells. Generally the origin of replication component is not needed for mammalian expression vectors but the SV40 may be used since it contains the early promoter.

**3.3 Selection marker**

Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins e.g. ampicillin, neomycin, methotrexate or tetracycline or (b) complement auxiotrophic deficiencies or supply nutrients not available in the complex media. The

selection scheme may involve arresting growth of the host cell. Cells, which have been successfully transformed with the genes encoding the antibody of the present invention, survive due to e.g. drug resistance conferred by the selection marker. Another example is the so-called DHFR selection marker wherein transformants are cultured in the presence of methotrexate. In typical embodiments, cells are cultured in the presence of increasing amounts of methotrexate to amplify the copy number of the exogenous gene of interest. CHO cells are a particularly useful cell line for the DHFR selection. A further example is the glutamate synthetase expression system (Lonza Biologics). A suitable selection gene for use in yeast is the trp1 gene, see Stinchcomb *et al* Nature 282, 38, 1979.

## 3.4 Promoters

Suitable promoters for expressing antibodies of the invention are operably linked to DNA/polynucleotide encoding the antibody. Promoters for prokaryotic hosts include phoA promoter, Beta-lactamase and lactose promoter systems, alkaline phosphatase, tryptophan and hybrid promoters such as Tac. Promoters suitable for expression in yeast cells include 3-phosphoglycerate kinase or other glycolytic enzymes e.g. enolase, glyceralderhyde 3 phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose 6 phosphate isomerase, 3-phosphoglycerate mutase and glucokinase. Inducible yeast promoters include alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, metallothionein and enzymes responsible for nitrogen metabolism or maltose/galactose utilization. Promoters for expression in mammalian cell systems include viral promoters such as polyoma, fowlpox and adenoviruses (e.g. adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus (in particular the immediate early gene promoter), retrovirus, hepatitis B virus, actin, rous sarcoma virus (RSV) promoter and the early or late Simian virus 40. Of course the choice of promoter is based upon suitable compatibility with the host cell used for expression. In one embodiment therefore there is provided a first plasmid comprising a RSV and/or SV40 and/or CMV promoter, DNA encoding light chain V region (VL) of the invention, κC region together with neomycin and ampicillin resistance selection markers and a second plasmid comprising a RSV or SV40 promoter, DNA encoding the heavy chain V region (VH) of the invention, DNA encoding the γ1 constant region, DHFR and ampicillin resistance markers

## 3.5 Enhancer element

Where appropriate, e.g. for expression in higher eukaroytics, an enhancer element operably linked to the promoter element in a vector may be used. Suitable mammalian enhancer sequences include enhancer elements from globin, elastase, albumin, fetoprotein and insulin. Alternatively, one may use an enhancer element from a eukaroytic cell virus such as SV40 enhancer (at bp100-270), cytomegalovirus

early promoter enhancer, polyma enhancer, baculoviral enhancer or murine IgG2a locus (see WO04/009823). The enhancer is preferably located on the vector at a site upstream to the promoter.

### 3.5.5 - Polyadenylation signals

In eukaryotic systems, polyadenylation signals are operably linked to DNA/polynucleotide encoding the antibody of this invention. Such signals are typically placed 3' of the open reading frame. In mammalian systems, non-limiting example include signals derived from growth hormones, elongation factor-1 alpha and viral (eg SV40) genes or retroviral long terminal repeats. In yeast systems non-limiting examples of polydenylation/termination signals include those derived from the phosphoglycerate kinase (PGK) and the alcohol dehydrogenase 1 (ADH) genes. In prokaryotic system polyadenylation signals are typically not required and it is instead usual to employ shorter and more defined terminator sequences. Of course the choice of polyadenylation/termination sequences is based upon suitable compatibility with the host cell used for expression.

### 3.6 Host cells

Suitable host cells for cloning or expressing vectors encoding antibodies of the invention are prokaroytic, yeast or higher eukaryotic cells. Suitable prokaryotic cells include eubacteria e.g. enterobacteriaceae such as *Escherichia* e.g. *E.Coli* (for example ATCC 31,446; 31,537; 27,325), *Enterobacter, Erwinia, Klebsiella Proteus, Salmonella* e.g. *Salmonella typhimurium*, Serratia e.g. *Serratia marcescans* and *Shigella* as well as Bacilli such as *B.subtilis* and *B.licheniformis* (see DD 266 710), Pseudomonas such as *P.aeruginosa* and *Streptomyces.* Of the yeast host cells, *Saccharomyces cerevisiae*, *schizosaccharomyces pombe*, Kluyveromyces (e.g. ATCC 16,045; 12,424; 24178; 56,500), yarrowia (EP402, 226), *Pichia Pastoris* (EP183, 070, see also Peng *et al* J.Biotechnol. 108 (2004) 185-192), *Candida, Trichoderma reesia* (EP244, 234), *Penicillin, Tolypocladium* and *Aspergillus* hosts such as *A.nidulans* and *A.niger* are also contemplated.

Although Prokaryotic and yeast host cells are specifically contemplated by the invention, preferably however, host cells of the present invention are higher eukaryotic cells. Suitable higher eukaryotic host cells include mammalian cells such as COS-1 (ATCC No.CRL 1650) COS-7 (ATCC CRL 1651), human embryonic kidney line 293, baby hamster kidney cells (BHK) (ATCC CRL.1632), BHK570 (ATCC NO: CRL 10314), 293 (ATCC NO.CRL 1573), Chinese hamster ovary cells CHO (e.g. CHO-K1, ATCC NO: CCL 61, DHFR-CHO cell line such as DG44 (see Urlaub *et al*, (1986) Somatic Cell Mol.Genet.12, 555-556)), particularly those CHO cell lines adapted for suspension culture, mouse sertoli cells, monkey kidney cells, African

green monkey kidney cells (ATCC CRL-1587), HELA cells, canine kidney cells (ATCC CCL 34), human lung cells (ATCC CCL 75), Hep G2 and myeloma or lymphoma cells e.g. NS0 (see US 5,807,715), Sp2/0, Y0.

Thus in one embodiment of the invention there is provided a stably transformed host cell comprising a vector encoding a heavy chain and/or light chain of the antibody or antigen binding fragment thereof as herein described. Preferably such host cells comprise a first vector encoding the light chain and a second vector encoding said heavy chain.

**Bacterial fermentation**

Bacterial systems are particularly suited for the expression of antibody fragments. Such fragments are localised intracellularly or within the periplasma. Insoluble periplasmic proteins can be extracted and refolded to form active proteins according to methods known to those skilled in the art, see Sanchez *et al* (1999) J.Biotechnol. 72, 13-20 and Cupit PM *et al* (1999) Lett Appl Microbiol, 29, 273-277.

**3.7 Cell Culturing Methods**.

Host cells transformed with vectors encoding the antibodies of the invention or antigen binding fragments thereof may be cultured by any method known to those skilled in the art. Host cells may be cultured in spinner flasks, roller bottles or hollow fibre systems but it is preferred for large scale production that stirred tank reactors are used particularly for suspension cultures. Preferably the stirred tankers are adapted for aeration using e.g. spargers, baffles or low shear impellers. For bubble columns and airlift reactors direct aeration with air or oxygen bubbles maybe used. Where the host cells are cultured in a serum free culture media it is preferred that the media is supplemented with a cell protective agent such as pluronic F-68 to help prevent cell damage as a result of the aeration process. Depending on the host cell characteristics, either microcarriers maybe used as growth substrates for anchorage dependent cell lines or the cells maybe adapted to suspension culture (which is typical). The culturing of host cells, particularly invertebrate host cells may utilise a variety of operational modes such as fed-batch, repeated batch processing (see Drapeau *et al* (1994) cytotechnology 15: 103-109), extended batch process or perfusion culture. Although recombinantly transformed mammalian host cells may be cultured in serum-containing media such as fetal calf serum (FCS), it is preferred that such host cells are cultured in synthetic serum –free media such as disclosed in Keen *et al* (1995) Cytotechnology 17:153-163, or commercially available media such as ProCHO-CDM or UltraCHO™ (Cambrex NJ, USA), supplemented where necessary with an energy source such as glucose and synthetic growth factors such as recombinant insulin. The serum-free culturing of host cells may require that those cells are adapted to grow in serum free conditions. One adaptation approach is to culture such host cells in serum containing media and repeatedly exchange 80% of

the culture medium for the serum-free media so that the host cells learn to adapt in serum free conditions (see e.g. Scharfenberg *K et al* (1995) in *Animal Cell technology: Developments towards the 21st century* (Beuvery E.C. *et al* eds), pp619-623, Kluwer Academic publishers).

Antibodies of the invention secreted into the media may be recovered and purified using a variety of techniques to provide a degree of purification suitable for the intended use. For example the use of antibodies of the invention for the treatment of human patients typically mandates at least 95% purity, more typically 98% or 99% or greater purity (compared to the crude culture medium). In the first instance, cell debris from the culture media is typically removed using centrifugation followed by a clarification step of the supernatant using e.g. microfiltration, ultrafiltration and/or depth filtration. A variety of other techniques such as dialysis and gel electrophoresis and chromatographic techniques such as hydroxyapatite (HA), affinity chromatography (optionally involving an affinity tagging system such as polyhistidine) and/or hydrophobic interaction chromatography (HIC, see US 5, 429,746) are available. In one embodiment, the antibodies of the invention, following various clarification steps, are captured using Protein A or G affinity chromatography followed by further chromatography steps such as ion exchange and/or HA chromatography, anion or cation exchange, size exclusion chromatography and ammonium sulphate precipitation. Typically, various virus removal steps are also employed (e.g. nanofiltration using e.g. a DV-20 filter). Following these various steps, a purified (preferably monoclonal) preparation comprising at least 75mg/ml or greater e.g. 100mg/ml or greater of the antibody of the invention or antigen binding fragment thereof is provided and therefore forms an embodiment of the invention. Suitably such preparations are substantially free of aggregated forms of antibodies of the invention.

## 4. Pharmaceutical Compositions

Purified preparations of antibodies of the invention (particularly monoclonal preparations) as described *supra*, may be incorporated into pharmaceutical compositions for use in the treatment of human diseases and disorders such as atopic diseases e.g. asthma, allergic rhinitis, COPD. Typically such compositions comprise a pharmaceutically acceptable carrier as known and called for by acceptable pharmaceutical practice, see e.g. Remingtons Pharmaceutical Sciences, 16th edition, (1980), Mack Publishing Co. Examples of such carriers include sterilised carrier such as saline, Ringers solution or dextrose solution, buffered with suitable buffers to a pH within a range of 5 to 8. Pharmaceutical compositions for injection (e.g. by intravenous, intraperitoneal, intradermal, subcutaneous, intramuscular or intraportal) or continuous infusion are suitably free of visible particulate matter and may comprise between 0.1ng to 100mg of antibody, preferably between 5mg and 25mg of antibody. Methods for the preparation of such

pharmaceutical compositions are well known to those skilled in the art. In one embodiment, pharmaceutical compositions comprise between 0.1ng to 100mg of antibodies of the invention in unit dosage form, optionally together with instructions for use. Pharmaceutical compositions of the invention may be lyophilised (freeze dried) for reconstitution prior to administration according to methods well known or apparent to those skilled in the art. Where embodiments of the invention comprise antibodies of the invention with an IgG1 isotype, a chelator of copper such as citrate (e.g. sodium citrate) or EDTA or histidine may be added to pharmaceutical composition to reduce the degree of copper-mediated degradation of antibodies of this isotype, see EP0612251. Anti-hIL-13 treatment maybe given orally, by inhalation, topically (for example, intraocular, intransnasal, rectal into wounds on the skin).

Effective doses and treatment regimes for administering the antibody of the invention are generally determined empirically and are dependent on factors such as the age, weight and health status of the patient and disease or disorder to be treated. Such factors are within the purview of the attending physician. Guidance in selecting appropriate doses may be found in e.g. Smith *et al* (1977) Antibodies in human diagnosis and therapy, Raven Press, New York but will in general be between 1mg and 1000mg.

Depending on the disease or disorder to be treated (but particularly asthma), pharmaceutical compositions comprising a therapeutically effective amount of the antibody of the invention may be used simultaneously, separately or sequentially with an effective amount of another medicament such as anti-inflammatory agents (e.g. corticosteroid or an NSAID), anticholinergic agents (particularly M1/M2/M3 receptor antagonists), $\beta_2$ adrenoreceptor agonists, antiinfective agents (e.g. antibiotics, antivirals), antihistamines, PDE4 inhibitor. Examples of $\beta_2$ adrenoreceptor agonists include salmeterol, salbutamol, formoterol, salmefamol, fenoterol, terbutaline. Preferred long acting $\beta_2$ adrenoreceptor agonists include those described in WO02/66422A, WO02/270490, WO02/076933, WO03/024439 and WO03/072539. Suitable corticosteroids include methyl prednisolone, prednisolone, dexamethasone, fluticasone propionate, 6α,9α-difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester, 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxy- androsta-1,4-diene-17β-carbothioic acid *S*-(2-oxo-tetrahydro-furan-3S-yl) ester, beclomethasone esters (eg. the 17-propionate ester or the 17,21-dipropionate ester), budesonide, flunisolide, mometasone esters (eg. the furoate ester), triamcinolone acetonide, rofleponide, ciclesonide (16α,17-[[(*R*)-cyclohexylmethylene]bis(oxy)]-11β,21-dihydroxy-pregna-1,4-diene-3,20-dione), butixocort propionate, RPR-106541, and ST-126. Preferred corticosteroids include fluticasone propionate, 6α,9α-difluoro-11β-hydroxy-16α-methyl-17α-[(4-methyl-1,3-thiazole-5-carbonyl)oxy]-3-oxo-androsta-1,4-diene-17β-

carbothioic acid *S*-fluoromethyl ester and 6α,9α-difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester, more preferably 6α,9α-difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid *S*-fluoromethyl ester.

Non-steroidal compounds having glucocorticoid agonism that may posess selectivity for transrepression over transactivation and that may be useful in combination therapy include those covered in the following patents: WO03/082827, WO01/10143, WO98/54159, WO04/005229, WO04/009016, WO04/009017, WO04/018429, WO03/104195, WO03/082787, WO03/082280, WO03/059899, WO03/101932, WO02/02565, WO01/16128, WO00/66590, WO03/086294, WO04/026248, WO03/061651, WO03/08277.

Suitable anti-inflammatory agents include non-steroidal anti-inflammatory drugs (NSAID's).

Suitable NSAID's include sodium cromoglycate, nedocromil sodium, phosphodiesterase (PDE) inhibitors (e.g. theophylline, PDE4 inhibitors or mixed PDE3/PDE4 inhibitors), leukotriene antagonists, inhibitors of leukotriene synthesis (eg. montelukast), iNOS inhibitors, tryptase and elastase inhibitors, beta-2 integrin antagonists and adenosine receptor agonists or antagonists (e.g. adenosine 2a agonists), cytokine antagonists (e.g. chemokine antagonists, such as a CCR3 antagonist) or inhibitors of cytokine synthesis, or 5-lipoxygenase inhibitors. Suitable other $\beta_2$-adrenoreceptor agonists include salmeterol (e.g. as the xinafoate), salbutamol (e.g. as the sulphate or the free base), formoterol (e.g. as the fumarate), fenoterol or terbutaline and salts thereof. An iNOS (inducible nitric oxide synthase inhibitor) is preferably for oral administration. Suitable iNOS inhibitors include those disclosed in WO93/13055, WO98/30537, WO02/50021, WO95/34534 and WO99/62875. Suitable CCR3 inhibitors include those disclosed in WO02/26722.

Of particular interest is use of the antibodies of the invention in combination with a phosphodiesterase 4 (PDE4) inhibitor. The PDE4-specific inhibitor useful in this aspect of the invention may be any compound that is known to inhibit the PDE4 enzyme or which is discovered to act as a PDE4 inhibitor, and which are only PDE4 inhibitors, not compounds which inhibit other members of the PDE family, such as PDE3 and PDE5, as well as PDE4.

Compounds of interest include *cis*-4-cyano-4-(3-cyclopentyloxy-4-methoxyphenyl)cyclohexan-1-carboxylic acid, 2-carbomethoxy-4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl)cyclohexan-1-one and *cis*-[4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl)cyclohexan-1-ol]. Also, *cis*-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylic acid (also known as

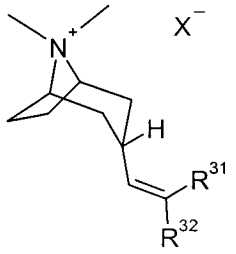
cilomilast) and its salts, esters, pro-drugs or physical forms, which is described in U.S. patent 5,552,438 issued 03 September, 1996; this patent and the compounds it discloses are incorporated herein in full by reference.

AWD-12-281 from Elbion (Hofgen, N. et al. 15th EFMC Int Symp Med Chem (Sept 6-10, Edinburgh) 1998, Abst P.98; CAS reference No. 247584020-9); a 9-benzyladenine derivative nominated NCS-613 (INSERM); D-4418 from Chiroscience and Schering-Plough; a benzodiazepine PDE4 inhibitor identified as CI-1018 (PD-168787) and attributed to Pfizer; a benzodioxole derivative disclosed by Kyowa Hakko in WO99/16766; K-34 from Kyowa Hakko; V-11294A from Napp (Landells, L.J. et al. Eur Resp J [Annu Cong Eur Resp Soc (Sept 19-23, Geneva) 1998] 1998, 12 (Suppl. 28): Abst P2393); roflumilast (CAS reference No 162401-32-3) and a pthalazinone (WO99/47505, the disclosure of which is hereby incorporated by reference) from Byk-Gulden; Pumafentrine, (-)-p-[(4*a*R*,10*b*S*)-9-ethoxy-1,2,3,4,4a,10b-hexahydro-8-methoxy-2-methylbenzo[c][1,6]naphthyridin-6-yl]-N,N-diisopropylbenzamide which is a mixed PDE3/PDE4 inhibitor which has been prepared and published on by Byk-Gulden, now Altana; arofylline under development by Almirall-Prodesfarma; VM554/UM565 from Vernalis; or T-440 (Tanabe Seiyaku; Fuji, K. et al. J Pharmacol Exp Ther,1998, 284(1): 162), and T2585.

Further compounds of interest are disclosed in the published international patent application WO04/024728 (Glaxo Group Ltd), PCT/EP2003/014867 (Glaxo Group Ltd) and PCT/EP2004/005494 (Glaxo Group Ltd).

Suitable anticholinergic agents are those compounds that act as antagonists at the muscarinic receptors, in particular those compounds which are antagonists of the $M_1$ or $M_3$ receptors, dual antagonists of the $M_1/M_3$ or $M_2/M_3$, receptors or pan-antagonists of the $M_1/M_2/M_3$ receptors. Exemplary compounds for administration via inhalation include ipratropium (e.g. as the bromide, CAS 22254-24-6, sold under the name Atrovent), oxitropium (e.g. as the bromide, CAS 30286-75-0) and tiotropium (e.g. as the bromide, CAS 136310-93-5, sold under the name Spiriva). Also of interest are revatropate (e.g. as the hydrobromide, CAS 262586-79-8) and LAS-34273 which is disclosed in WO01/04118. Exemplary compounds for oral administration include pirenzepine (CAS 28797-61-7), darifenacin (CAS 133099-04-4, or CAS 133099-07-7 for the hydrobromide sold under the name Enablex), oxybutynin (CAS 5633-20-5, sold under the name Ditropan), terodiline (CAS 15793-40-5), tolterodine (CAS 124937-51-5, or CAS 124937-52-6 for the tartrate, sold under the name Detrol), otilonium (e.g. as the bromide, CAS 26095-59-0, sold under the name Spasmomen), trospium chloride (CAS 10405-02-4) and solifenacin (CAS 242478-37-1, or CAS 242478-38-2 for the succinate also known as YM-905 and sold under the name Vesicare).

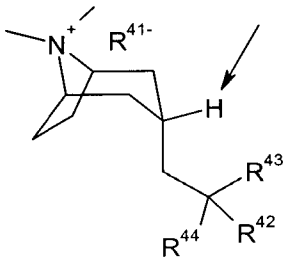
Other suitable anticholinergic agents include compounds of formula (XXI), which are disclosed in US patent application 60/487981:



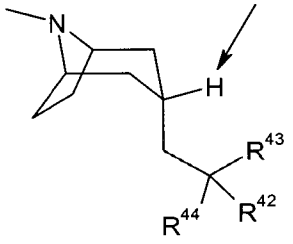
(XXI)

in which the preferred orientation of the alkyl chain attached to the tropane ring is endo;
$R^{31}$ and $R^{32}$ are, independently, selected from the group consisting of straight or branched chain lower alkyl groups having preferably from 1 to 6 carbon atoms, cycloalkyl groups having from 5 to 6 carbon atoms, cycloalkyl-alkyl having 6 to 10 carbon atoms, 2-thienyl, 2-pyridyl, phenyl, phenyl substituted with an alkyl group having not in excess of 4 carbon atoms and phenyl substituted with an alkoxy group having not in excess of 4 carbon atoms;
$X^-$ represents an anion associated with the positive charge of the N atom. $X^-$ may be but is not limited to chloride, bromide, iodide, sulfate, benzene sulfonate, and toluene sulfonate,
including, for example:
(3-*endo*)-3-(2,2-di-2-thienylethenyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide;
(3-*endo*)-3-(2,2-diphenylethenyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide;
(3-*endo*)-3-(2,2-diphenylethenyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane 4-methylbenzenesulfonate;
(3-*endo*)-8,8-dimethyl-3-[2-phenyl-2-(2-thienyl)ethenyl]-8-azoniabicyclo[3.2.1]octane bromide; and/or
(3-*endo*)-8,8-dimethyl-3-[2-phenyl-2-(2-pyridinyl)ethenyl]-8-azoniabicyclo[3.2.1]octane bromide.

Further suitable anticholinergic agents include compounds of formula (XXII) or (XXIII), which are disclosed in US patent application 60/511009:



(XXII)



(XXIII)

wherein:

the H atom indicated is in the exo position;
$R^{41-}$ represents an anion associated with the positive charge of the N atom. R1$^-$ may be but is not limited to chloride, bromide, iodide, sulfate, benzene sulfonate and toluene sulfonate;
$R^{42}$ and $R^{43}$ are independently selected from the group consisting of straight or branched chain lower alkyl groups (having preferably from 1 to 6 carbon atoms), cycloalkyl groups (having from 5 to 6 carbon atoms), cycloalkyl-alkyl (having 6 to 10 carbon atoms), heterocycloalkyl (having 5 to 6 carbon atoms) and N or O as the heteroatom, heterocycloalkyl-alkyl (having 6 to10 carbon atoms) and N or O as the heteroatom, aryl, optionally substituted aryl, heteroaryl, and optionally substituted heteroaryl;
$R^{44}$ is slected from the group consisting of $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_{12})$cycloalkyl, $(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_{12})$cycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_7)$heterocycloalkyl, aryl, heteroaryl, $(C_1\text{-}C_6)$alkyl-aryl, $(C_1\text{-}C_6)$alkyl-heteroaryl, $-OR^{45}$, $-CH_2OR^{45}$, $-CH_2OH$, $-CN$, $-CF_3$, $-CH_2O(CO)R^{46}$, $-CO_2R^{47}$, $-CH_2NH_2$, $-CH_2N(R^{47})SO_2R^{45}$, $-SO_2N(R^{47})(R^{48})$, $-CON(R^{47})(R^{48})$, $-CH_2N(R^{48})CO(R^{46})$, $-CH_2N(R^{48})SO_2(R^{46})$, $-CH_2N(R^{48})CO_2(R^{45})$, $-CH_2N(R^{48})CONH(R^{47})$;
$R^{45}$ is selected from the group consisting of $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_{12})$cycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl-aryl, $(C_1\text{-}C_6)$alkyl-heteroaryl;
$R^{46}$ is selected from the group consisting of $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_{12})$cycloalkyl, $(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_{12})$cycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_7)$heterocycloalkyl, aryl, heteroaryl, $(C_1\text{-}C_6)$alkyl-aryl, $(C_1\text{-}C_6)$alkyl-heteroaryl;
$R^{47}$ and $R^{48}$ are, independently, selected from the group consisting of H, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_{12})$cycloalkyl, $(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_{12})$cycloalkyl, $(C_1\text{-}C_6)$alkyl$(C_3\text{-}C_7)$heterocycloalkyl, $(C_1\text{-}C_6)$alkyl-aryl, and $(C_1\text{-}C_6)$alkyl-heteroaryl, including, for example:
(Endo)-3-(2-methoxy-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;
3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile;
(Endo)-8-methyl-3-(2,2,2-triphenyl-ethyl)-8-aza-bicyclo[3.2.1]octane;
3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionamide;
3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionic acid;
(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;
(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide;
3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propan-1-ol;
*N*-Benzyl-3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionamide;
(Endo)-3-(2-carbamoyl-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;
1-Benzyl-3-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea;
1-Ethyl-3-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea;

*N*-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-acetamide;
*N*-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzamide;
3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-di-thiophen-2-yl-propionitrile;
(Endo)-3-(2-cyano-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;
*N*-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzenesulfonamide;
[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea;
*N*-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-methanesulfonamide; and/or
(Endo)-3-{2,2-diphenyl-3-[(1-phenyl-methanoyl)-amino]-propyl}-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide.
More preferred compounds useful in the present invention include:
(Endo)-3-(2-methoxy-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;
(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;
(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide;
(Endo)-3-(2-carbamoyl-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;
(Endo)-3-(2-cyano-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide; and/or
(Endo)-3-{2,2-diphenyl-3-[(1-phenyl-methanoyl)-amino]-propyl}-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide.

Suitable antihistamines (also referred to as H1-receptor antagonists) include any one or more of the numerous antagonists known which inhibit H1-receptors, and are safe for human use. First generation antagonists, include derivatives of ethanolamines, ethylenediamines, and alkylamines, e.g diphenylhydramine, pyrilamine, clemastine, chlropheniramine. Second generation antagonists, which are non-sedating, include loratidine, desloratidine,terfenadine,astemizole,acrivastine, azelastine, levocetirizine fexofenadine and cetirizine.

Examples of preferred anti-histamines include loratidine, desloratidine, fexofenadine and cetirizine.

Other contemplated combinations include the use of antibodies of the invention in combination with an anti-IL-4 agent (e.g. anti-IL-4 antibody such as pascolizumab) and/or anti-IL-5 agent (e.g. anti-IL-5 antibody such as mepolizumab) and/or anti-IgE agent (e.g. anti-IgE antibody such as omalizumab (Xolair™) or talizumab).

Conveniently, a pharmaceutical composition comprising a kit of parts of the antibody of the invention or antigen binding fragment thereof together with such another medicaments optionally together with instructions for use is also contemplated by the present invention.

The invention furthermore contemplates a pharmaceutical composition comprising a therapeutically effective amount of monoclonal therapeutic antibody or antigen binding fragment thereof as herein described for use in the treatment of diseases responsive to modulation of the interaction between hIL-13 and hIL-13R.

In accordance with the present invention there is provided a pharmaceutical composition comprising a therapeutically effective amount of a monoclonal humanised therapeutic antibody which antibody comprises a VH domain selected from the group consisting of: SEQ.I.D.NO:11,12,13,14 and a VL domain selected from the group consisting of: SEQ.I.D.NO:15,16, 17.

In accordance with the present invention there is provided a pharmaceutical composition comprising a monoclonal antibody comprising a heavy chain selected from the group consisting of: SEQ.I.D.NO: 18,19,20,21 and a light chain selected from the group consisting of; SEQ.I.D.NO:22, 23,24

In accordance with the present invention there is provided a pharmaceutical composition comprising a monoclonal antibody comprising a heavy chain of SEQ.I.D.NO:18 and a light chain of SEQ.I.D.NO:22 and a pharmaceutically acceptable carrier.

In accordance with the present invention there is provided a pharmaceutical composition comprising a monoclonal antibody comprising (or consisting essentially of) a heavy chain of SEQ.I.D.NO:18 and a light chain of SEQ.I.D.NO:22 and a pharmaceutically acceptable carrier.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:18 and a light chain of SEQ.I.D.NO:22.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:18 and a light chain of SEQ.I.D.NO:23.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically

effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:18 and a light chain of SEQ.I.D.NO:24.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:19 and a light chain of SEQ.I.D.NO:22.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:19 and a light chain of SEQ.I.D.NO:23.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:19 and a light chain of SEQ.I.D.NO:24.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:20 and a light chain of SEQ.I.D.NO:22.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:20 and a light chain of SEQ.I.D.NO:23

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:20 and a light chain of SEQ.I.D.NO:24.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:21 and a light chain of SEQ.I.D.NO:22.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:21 and a light chain of SEQ.I.D.NO:23.

In accordance with the present invention there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a monoclonal population of antibody which antibody comprises a heavy chain of SEQ.I.D.NO:21 and a light chain of SEQ.I.D.NO:24.

**5. Clinical uses**.

Antibodies of the invention may be used in the treatment of atopic diseases/ disorders and chronic inflammatory diseases/disorders. Of particular interest is their use in the treatment of asthma, such as allergic asthma, particularly severe asthma (that is asthma that is unresponsive to current treatment, including systemically administered corticosteroids; see Busse WW et al, J Allergy Clin. Immunol 2000, 106: 1033-1042), "difficult" asthma (defined as the asthmatic phenotype characterised by failure to achieve control despite maximally recommended doses of prescribed inhaled steroids, see Barnes PJ (1998), Eur Respir J 12:1208-1218), "brittle" asthma (defines a subgroup of patients with severe, unstable asthma who maintain a wide peak expiratory flow (PEF) variability despite high doses of inhaled steroids, see Ayres JG et al (1998) Thorax 58:315-321), nocturnal asthma, premenstrual asthma, steroid resistant asthma (see Woodcock AJ (1993) Eur Respir J 6:743-747), steroid dependent asthma (defined as asthma that can be controlled only with high doses of oral steroids), aspirin induced asthma, adult-onset asthma, paediatric asthma . Antibodies of the invention maybe used to prevent, reduce the frequency of, or mitigate the effects of acute, asthmatic episodes (*status asthmaticus*). Antibodies of the invention may also be used to reduce the dosing required (either in terms of amount administered or frequency of dosing) of other medicaments used in the treatment of asthma. For example, antibodies of the invention may be used to reduce the dosing required for steroid treatment of asthma such as corticosteroid treatment ("steroid sparing"). Other diseases or disorders that may be treated with antibodies of the invention include atopic dermatitis, allergic rhinitis, Crohn's disease, chronic obstructive pulmonary disease (COPD), eosinophilic esophagitis, fibrotic diseases or disorders such as idiopathic pulmonary fibrosis, progressive systemic sclerosis (scleroderma), hepatic fibrosis, hepatic granulomas, schistosomiasis, leishmaniasis, and diseases of cell cycle regulation, e.g. Hodgkins disease, B cell chronic lymphocytic leukaemia. Further diseases or disorders that may be treated with antibodies of the invention are detailed in the Background of the invention Example above.

In one embodiment of the invention there is provided a method of treating a human patient afflicted with an asthmatic condition which is refractory to treatment with corticosteroids which method comprises the step of administering to said patient a therapeutically effective amount of an antibody of the invention.

In another embodiment there is provided a method of preventing an acute asthmatic attack in a human patient which method comprises the step of administering to said patient a therapeutically effective amount of an antibody of the invention.

In another embodiment there is provided a method of reducing the frequency of and/or mitigating the effects of an acute asthmatic attack in a human patient which method comprises the step of administering to said patient a therapeutically effective amount of an antibody of the invention.

In another embodiment of the invention there is provided a method of biasing T helper cell response towards a Th1 type response following an inflammatory and/or allergic insult in a human patient which method comprises administering to said patient a therapeutically effective amount of an antibody or antigen binding fragment thereof of the invention.

In another embodiment of the invention there is provided a method of treating a human patient having the Q130hIL-13 variant which patient is afflicted with asthma, such as severe asthma, said method comprising the step of administering to said patient a therapeutically effective amount of an antibody or antigen binding fragment thereof of the invention.

Although the present invention has been described principally in relation to the treatment of human diseases or disorders, the present invention may also have applications in the treatment of similar diseases or disorders in non-human mammals.

The present invention is now described by way of example only.

## Brief Description of the Drawings

Figure 1
Sandwich ELISA illustrating the binding of mouse monoclonal antibody 3G4 to recombinant E.coli-expressed human IL-13 at increasing concentrations.

Figure 2a
Neutralisation assay illustrating the ability of mouse monoclonal antibody 3G4 at increasing concentrations to inhibit the bioactivity of recombinant E.coli-expressed human IL-13 in a TF-1 cell proliferation assay.

Figure 2b
Neutralisation assay illustrating the ability of chimaeric 3G4 at increasing concentrations to inhibit the bioactivity of recombinant E.coli-expressed human IL-13 in a TF-1 cell proliferation assay.

Figure 3
Neutralisation assay illustrating the ability of mouse monoclonal antibody 3G4 (and chimaeric 3G4) at increasing concentrations to inhibit the bioactivity of recombinant E.coli-expressed cynomolgus IL-13 in a TF-1 cell proliferation assay. The curve labelled 'Campath' is that obtained using anti-CD52 humanised antibody alemtuzumab, acting as an irrelevant antibody control in this experiment. The line labelled 'anti-hIL13 poly' is that obtained using a neutralising polyclonal anti-IL13 preparation (R&D Systems, catalogue number AF-213-NA) as a positive control.

Figure 4
Neutralisation assay illustrating the ability of mouse monoclonal antibody 3G4 (and chimaeric 3G4) at increasing concentrations to inhibit the bioactivity of mammalian-expressed (CHO cell) human IL-13 in a TF-1 cell proliferation assay. Campath and anti-hIL13 are control reagents as described supra.

Figure 5
Neutralisation assay illustrating the ability of mouse monoclonal antibody 3G4 and (chimaeric 3G4) at increasing concentrations to inhibit the bioactivity of recombinant E.coli-expressed Q130 human IL-13 in a TF-1 cell proliferation assay. Campath and anti-hIL13 are control reagents as described supra.

Figure 6
An epitope mapping ELISA to determine the binding epitope for mouse monoclonal antibody 3G4 on human and cynomolgus IL-13.

Figure 7a
An epitope mapping ELISA to identify the fine binding specificity of mouse monoclonal antibody 3G4 on human IL-13

Figure 7b
An epitope mapping ELISA to identify the fine binding specificity of mouse monoclonal antibody 3G4 on cynomolgus IL-13

Figure 8
An epitope mapping ELISA to determine the key amino acid residues required for binding of mouse monoclonal antibody 3G4 to human IL-13.

Figure 9
An epitope mapping ELISA to determine the key amino acid residues required for binding of mouse monoclonal antibody 3G4 to human IL-13

Figure 10
Sandwich ELISA illustrating the binding of mouse monoclonal antibody 3G4, 3G4 chimaera and humanised mAbs H2L1, H3L1 to recombinant E.coli-expressed human IL-13 at increasing concentrations.

Figure 11
Neutralisation assay illustrating the ability of mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 at increasing concentrations to inhibit the bioactivity of recombinant E.coli-expressed human IL-13 in a TF-1 cell proliferation assay.

Figure 12
Neutralisation assay illustrating the ability of mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 at increasing concentrations to inhibit the bioactivity of recombinant E.coli-expressed cynomolgus IL-13 in a TF-1 cell proliferation assay.

Figure 13
Neutralisation assay illustrating the ability of mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 at increasing concentrations to inhibit the bioactivity of mammalian-expressed (CHO cell) human IL-13 in a TF-1 cell proliferation assay.

Figure 14
Neutralisation assay illustrating the ability of mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 at increasing concentrations to inhibit the bioactivity of recombinant E.coli-expressed Q130 human IL-13 in a TF-1 cell proliferation assay.

Figure 15
Sandwich ELISA demonstrating that mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 do not bind recombinant E.coli-expressed human IL-4.

Figure 16
Sandwich ELISA demonstrating that mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 do not bind recombinant E.coli-expressed human IL-5.

Figure 17

Direct binding ELISA demonstrating that mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 do not bind human GM-CSF.

Figure 18

Sandwich ELISA illustrating the binding of mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 to native human IL-13 at increasing concentrations.

Figure 19

ELISA illustrating the ability of mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 at increasing concentrations to inhibit recombinant E.coli-expressed human IL-13 binding to the human IL-13 receptor α 1 chain.

Figure 20

ELISA illustrating the ability of mouse monoclonal antibody 3G4, 3G4 chimera and humanised mAbs H2L1, H3L1 at increasing concentrations to inhibit recombinant E.coli-expressed human IL-13 binding to the human IL-13 receptor α 2 chain.

## Examples

### 1. GENERATION OF MONOCLONAL ANTIBODIES AND CHARACTERISATION OF MOUSE MONOCLONAL ANTIBODY 3G4

Five SJL mice were immunised by intraperitoneal injection each with 2µg recombinant human IL-13 derived from E.Coli (Cambridge Bioscience, Cat. No. CH-013). Spleen cells from the mice were removed and B lymphocytes fused with mouse myeloma cells derived from P3X cells using PEG1500 (Boehringer) to generate hybridomas. Individual hybridoma cell lines were cloned by limiting dilution (E Harlow and D Lane). Wells containing single colonies were identified microscopically and supernatants tested for activity. Cells from the most active clones were expanded for cryopreservation, antibody production etc.
Initially, hybridoma supernatants were screened for binding activity against an E.coli-expressed recombinant det-1 tagged human IL-13 protein (made in-house) in a sandwich assay format. A secondary screen of these positives was completed using a BIAcore™ method to detect for binding to the det-1 tagged human IL-13 protein. Samples from these hybridomas were then tested for ability to neutralise the bioactivity of E.coli-expressed recombinant human IL-13 (Cambridge Bioscience, cat. no CH-013) in a TF-1 cell bioassay.

Positives identified from the human IL-13 neutralising bioassay were subcloned by limiting dilution to generate stable monoclonal cell lines. Immunoglobulins from these hybridomas, grown in cell factories under serum free conditions, were purified using immobilised Protein A columns. These purified mAbs were then re-screened in the same three assay systems.

Monoclonal antibody 3G4 was identified as a potent antibody that neutralised human IL-13 bioactivity.

The 3G4 antibody has the $V_H$ region amino acid sequence as set out in SEQ.I.D.NO:7

The 3G4 antibody has the $V_L$ region amino acid sequence as set out in SEQ.I.D.NO:8

A chimaeric antibody was constructed by taking variable regions from the 3G4 murine monoclonal antibody and grafting these on to human IgG1/k wild type C regions. A human signal sequence (as shown in SEQ.I.D.NO: 43) was used in the construction of these constructs. This chimearic antibody was termed 3G4C.

#### 1.1 Binding to E.Coli-expressed recombinant human IL-13

3G4 bound E.Coli-expressed recombinant human IL-13 in a sandwich ELISA, the method was carried out as described in Example 6.1 (See Figure 1).

## 1.2 Neutralisation of E.Coli-expressed recombinant human and cynomolgus IL-13 in a TF-1 cell proliferation bioassay

TF-1 cells proliferate in response to human IL-13 and cynomolgus IL-13. A bioassay was developed to assess the neutralisation capacity of an anti-IL-13 mAb on human and cynomolgus IL-13-induced TF-1 cell proliferation. The method was carried out as described in Example 6.2.

The amino acid sequence and a cDNA sequence for cynomolgus IL-13 (not including signal sequence) is set forth as SEQ.I.D.NO:41 and SEQ.I.D.NO:42 respectively.

3G4 neutralised the bioactivity of recombinant human IL-13 in a TF-1 cell bioassay (see figure 2a).
3G4 neutralised cynomolgus IL-13 bioactivity less potently than human IL-13 (see figure 3).
An average $ND_{50}$ value of 0.13µg/ml was calculated for the neutralisation of approximately 10ng/ml E.coli-expressed recombinant human IL-13 bioactivity in a TF-1 cell bioassay by monoclonal antibody 3G4.
An $ND_{50}$ value of 29µg/ml was calculated for the neutralisation of approximately 10ng/ml E.coli-expressed recombinant cynomolgus IL-13 bioactivity in a TF-1 cell bioassay by monoclonal antibody 3G4. [The $ND_{50}$ (neutralisation dose) value is the concentration of monoclonal antibody required to reduce TF-1 cell proliferation by 50%, in response to a set concentration of IL-13].

## 1.3 Neutralisation of mammalian-expressed (CHO cell) human IL-13 in a TF-1 cell proliferation bioassay

The neutralisation capacity of monoclonal antibody 3G4 for human IL-13 expressed from CHO cells was assessed in a TF-1 cell proliferation assay. The method was carried out as described in Example 6.2. 3G4 neutralised mammalian-expressed human IL-13 more potently than a commercially available anti-human IL-13 polyclonal reagent. An $ND_{50}$ value of 0.31µg/ml was calculated for the neutralisation of ~ 20ng/ml mammalian-expressed human IL-13 in a TF-1 cell bioassay by monoclonal antibody 3G4. See Figure 4.

## 1.4 Neutralisation of recombinant Q130 human IL-13 variant in a TF-1 cell proliferation bioassay

The neutralisation capacity of monoclonal antibody 3G4 for E.coli-expressed recombinant Q130 human IL-13 (Peprotech, Cat. No. 200-13A) was assessed in a

TF-1 cell proliferation assay. The method was carried out as described in Example 6.2. 3G4 neutralised Q130 human IL-13 more potently than a commercially available anti-human IL-13 polyclonal reagent. An $ND_{50}$ value of 0.025µg/ml was calculated for the neutralisation of ~ 60ng/ml Q130 human IL-13 bioactivity in a TF-1 cell bioassay by monoclonal antibody 3G4. See Figure 5.

**1.5 BIAcore™ analysis**

The affinity of 3G4 mouse mAb for recombinant human and cynomolgus IL-13 was assessed by BIAcore™ (surface plasmon resonance) analysis. See Table 2.

BIAcore™ analyses were carried out using anti-mouse IgG capture. An anti-mouse IgG antibody was coupled onto a CM5 chip by primary amine coupling in accordance with the manufacturers recommendation. 3G4 parental mouse mAb was then captured onto this surface and human or cynomolgus IL-13 passed over at defined concentrations. The surface was regenerated back to the anti-mouse IgG surface using mild acid elution conditions, this did not significantly affect the ability to capture antibody for a subsequent IL-13 binding event. This work was carried out on the Biacore 3000 and analysed using the evaluation software inherent in the machine and the data analysed using the 1:1 model of binding. The data for human IL-13 binding was generated using recombinant E.coli-expressed Det-1 tagged human IL-13 as well as a commercial recombinant E.coli-expressed untagged human IL-13 reagent (supplied by Peprotech). Recombinant E.coli-expressed cynomolgus IL-13 was generated at GSK. All Biacore runs were carried out at 25 degrees C.

Table 2. Biacore 3000 data for parental 3G4 mouse mAb binding to human and cynomolgus IL-13

| 3G4 mouse mAb binding to | ka | Kd | KD (nM) |
|---|---|---|---|
| **Det-1 tagged human IL-13** | **5.61e6** (1.49e6) | **1.68e-4** (7.07e-7) | **0.031** (0.009) |
| **Human IL-13 (Peprotech)** | **2.25e6** | **1.37e-4** | **0.061** |
| **Cynomolgus IL-13** | **4.11e5** | **1.3e-3** | **3.15** |

For Det-1 tagged human IL-13, the data were produced from 2 independent experiments with both runs carried out in duplicate. The data are presented as the mean and standard deviation (in brackets) of these results.

For the human IL-13 (supplied by Peprotech) and cynomolgus IL-13 (made at GSK), the data are the result of one experiment, carried out in duplicate.

For the data described above, duplicates were analysed together to give one value for the run.

These data indicate that 3G4 mouse mAb has a very high binding affinity for human IL-13, the binding affinity of 3G4 mouse mAb for cynomolgus IL-13 is less potent in comparison.

## 2. HUMANISATION OF CLONE 3G4

### 2.1 Sequence analysis

A comparison was made between the sequences of the 3G4 variable regions and other murine and human immunoglobulin sequences. This was done using the FASTA and BLAST programs and by inspection.

The following framework residues in 3G4 were identified as being potentially important in the design of a CDR-grafted (humanised) version of the antibody:

| **Position** | **3G4 VH** |
|---|---|
| 10 | D |
| 30 | I |
| 93 | T |

Position is according to the Kabat *et al* numbering system.

| **Position** | **3G4 VL** |
|---|---|
| 98 | L |

A suitable human acceptor framework for the 3G4 $V_H$ was identified:
SEQ.I.D.NO:44
A suitable human acceptor framework for the 3G4 $V_L$ was identified:
SEQ.I.D.NO:45

In SEQ.I.D.NO:44 CRDH1 and H2 are present and CDRH3 is represented by XXXXXXXXXX. In SEQ.I.D.NO:45 CRDL1 and L2 are present and CDRL3 is represented by XXXXXXXXX.

In CDR grafting, it is typical to require one or more framework residues from the donor antibody to be included in place of their orthologues in the acceptor frameworks in order to obtain satisfactory binding. In addition to those listed above, the following murine framework residues were also considered for possible retention in a humanised antibody design:

| Position (Kabat#) | 3G4 $V_H$ | Human $V_H$ |
|---|---|---|
| 10 | D | E |
| 30 | I | T |
| 67 | A | V |
| 69 | L | M |
| 71 | A | R |
| 73 | K | T |
| 93 | T | A |

| Position (Kabat#) | mouse 3G4 $V_L$ | Human $V_L$ |
|---|---|---|
| 76 | N | S |
| 98 | L | F |

4 humanised $V_H$ constructs with different back-mutations were designed to obtain a humanised antibody with satisfactory activity. These are numbered H1 to H4.

H1 consists of a CDR graft of the 3G4 VH CDRs into the specified acceptor sequence. Additionally, H1 contains the murine residue at position 30 (isoleucine). This is outside the Kabat definition of CDR, but within the CDR H1 definition of Chothia. In this case, this residue is considered to be part of the CDR rather than a true framework back-mutation.

H2 is identical to H1, but with a back-mutation where the amino acid at position 93 is threonine instead of alanine.
H3 is identical to H2, but with an additional back-mutation where the amino acid at position 10 is aspartic acid instead of glutamic acid.
H4 is identical to H3, but contains four additional back-mutations at positions 67 (alanine in place of valine), 69 (leucine in place of methionine), 71 (alanine in place of arginine) and 73 (lysine in place of threonine).

3 humanised $V_L$ constructs with different back-mutations were designed to obtain a humanised antibody with satisfactory activity. These are numbered L1 to L3.

L1 consists of a CDR graft of the 3G4 $V_L$ CDRs into the specified acceptor sequence, using the Kabat definition of CDRs.
L2 is identical to L1, but with a back-mutation where the amino acid at position 98 is leucine in place of phenylalanine.
L3 is identical to L2, but with an additional back-mutation where the amino acid at position 76 is asparagine in place of serine.

**Humanised $V_H$ construct H1:**
SEQ.I.D.NO:11

**Humanised $V_H$ construct H2:**
SEQ.I.D.NO:12

**Humanised $V_H$ construct H3:**
SEQ.I.D.NO:13

**Humanised $V_H$ construct H4:**
SEQ.I.D.NO:14

**Humanised $V_L$ construct L1:**
SEQ.I.D.NO:15

**Humanised $V_L$ construct L2:**
SEQ.I.D.NO:16

**Humanised $V_L$ construct L3:**
SEQ.I.D.NO:17

## 2.2 Humanisation of 3G4

Humanised $V_H$ and $V_L$ constructs were prepared by *de novo* build up of overlapping oligonucleotides including restriction sites for cloning into RId and RIn mammalian expression vectors as well as a human signal sequence. Hind III and Spe I restriction sites were introduced to frame the $V_H$ domain containing the human signal sequence (SEQ.I.D.NO: 43) for cloning into RId containing the human γ1 wild type constant region. Hind III and BsiW I restriction sites were introduced to frame the $V_L$ domain containing the human signal sequence for cloning into RIn containing the human kappa constant region. This is essentially as described in WO 2004/014953.

## 3. EXPRESSION AND CHARACTERISATION OF HUMANISED ANTIBODIES

Humanised $V_H$ constructs (H1, H2, H3 and H4) and two humanised $V_L$ constructs (L1, and L2) were prepared in RId hCγ1wt and RIn hCκ mammalian expression vectors. Plasmid heavy chain-light chain combinations (H1L1, H1L2, H2L1, H2L2,, H3L1, H3L2, H4L1, H4L2) were transiently co-transfected into CHO cells and expressed at small scale to give eight different humanised antibodies.

The antibodies produced in the CHO cell supernatant and subsequent batches of purified antibodies were analysed for activity in the human IL-13 binding ELISA, in the human IL-13 neutralisation bioassay, and binding to human IL-13 by BIAcore™. All eight humanised mAbs showed binding and/or neutralisation of human IL-13 in each of these assays. H2L1 and H3L1 were selected for further analysis due to better

performance in the human IL-13 neutralisation bioassay, and binding to human IL-13 by BIAcore™, whilst offering a limited number of back-mutations.

**3.1 Binding to E-coli expressed recombinant human IL-13**

3G4C, H2L1 and H3L1 bound E.coli expressed recombinant human IL13 in a sandwich ELISA with similar profiles. The method was carried out as described in Example 6.1A. Table 3 shows average $ED_{50}$ values (see also figure 10).
[The $ED_{50}$ (effective dose) is the concentration of antibody required for half maximal binding to IL-13 in this ELISA]

Table 3

| mAb | ED50 (µg/ml) | Standard Error |
|---|---|---|
| chimaeric 3G4 | 0.04 | 0.021 |
| H2L1 | 0.04 | 0.001 |
| H3L1 | 0.05 | 0.007 |

The 3G4, 3G4C, H2L1, and H3L1 have also been shown to inhibit human IL-13 binding to human IL-13 receptor chains (IL13Rα1 and IL13Rα2) by ELISA. This method was carried out as described in Example 6.5 and 6.6.

Figure 19 shows the data demonstrating inhibition for binding to IL13Rα1.

Table 4 shows the average $IC_{50}$ values for the inhibition for binding to IL13Rα2.
[The $IC_{50}$ is the concentration of mAb required to inhibit binding of a fixed amount of human IL-13 to IL13Rα2 by 50%].

Table 4

| IL13Rα2 | | |
|---|---|---|
| **mAb** | **$IC_{50}$ (µg/ml)** | **Standard error** |
| Parental 3G4 mouse mAb | 0.025 | 0.0048 |
| chimaeric 3G4 | 0.063 | 0.0060 |
| H2L1 | 0.067 | 0.0097 |
| H3L1 | 0.068 | 0.0121 |

Figure 19 illustrates that 3G4, 3G4, H2L1 and H3L1 inhibit human IL-13 binding to IL13Rα1 with similar potency.
Figure 20 illustrates that 3G4, 3G4C, H2L1 and H3L1 inhibit human IL-13 binding to IL13Rα2 with similar potency.

**3.2 Binding to native human IL-13**

The HDLM-2 cell line (a Reed-Steinberg-like cell line, originally derived from bone marrow) makes human IL-13 and uses it in an autocrine fashion for growth. This native human IL-13 is secreted into HDLM-2 cell supernatant. This was used to assess the binding of 3G4, 3G4C, H2L1 and H3L1 to native human IL-13 using a method as described in Example 6.1B. By ELISA, all four antibodies bound native human IL-13 in the HDLM2 cell supernatant with very similar performance to that of the parental 3G4 mAb. See figure 18.

**3.3 Neutralisation of E-coli expressed recombinant human and cynomologus IL-13 in a TF-1 cell proliferation bioassay.**

3G4, 3G4C, H2L1 and H3L1 neutralised the bioactivity of E-coli expressed recombinant human and cynomomogus IL-13. The method was carried out as described in Example 6.2A.

3G4, 3G4C, H2L1 and H3L1 neutralised the bioactivity of E-coli expressed recombinant cynomomogus IL-13 less potently than human IL-

[$ND_{50}$ (neutralising dose) is the concentration of mAb required to reduce TF-1 cell proliferation by 50%, in response to a set concentration of IL-13].

See table 5 below and figures 11 and 12.

Table 5

| IL-13 variant | mAb | Mean $ND_{50}$ for minimum of 2 assays (µg/ml) | Standard Error |
|---|---|---|---|
| E.coli-expressed human IL-13 | chimaeric 3G4 | 0.089 | 0.029 |
| | H2L1 | 0.210 | 0.085 |
| | H3L1 | 0.217 | 0.061 |
| | 3G4 parental mAb | 0.049 | 0.018 |
| E.coli-expressed cynomolgus IL-13 | chimaeric 3G4 | 23.95 | 7.25 |
| | H2L1 | 36.59 | 4.61 |
| | H3L1 | 35.44 | 0.36 |
| | 3G4 parental mAb | 34.45 | 3.95 |

The average $ND_{50}$ value for 3G4 was 0.049µg/ml as calculated for the neutralisation of approximately 10ng/ml E.coli-expressed recombinant human IL-13 bioactivity in the TF-1 assay. The results in table 5 are the average of four separate repeats. The value obtained is comparable although approximately two fold lower than the $ND_{50}$ value previously obtained (see Example 1.2).

The level of neutralisation of E.coli expressed human IL13 achieved for parental 3G4 mouse mAb is comparable to that achieved by chimaeric 3G4. The potency of H2L1

and H3L1 were reduced in comparison to both parental 3G4 mouse mAb and chimaeric 3G4.

The average $ND_{50}$ value for 3G4 parental is 34µg/ml. This was calculated for the neutralisation of approximately 10ng/ml E.coli-expressed recombinant cyno IL-13 bioactivity in the TF-1 assay. This value was similar to the value previously reported for parental 3G4 (see Example 1.2). H2L1 and H3L1 also showed similar potency against cyno IL13 as 3G4.

**3.4 Neutralisation of mammalian expressed (CHO cell) human IL-13 in a TF-1 cell proliferation bioassay.**

The neutralisation capacity of monoclonal antibody 3G4,3G4C, H2L1, and H3L1 for human IL-13 expressed from CHO cells was assessed in a TF-1 cell proliferation assay according to the method as set out in Example 6.2A.
3G4, 3G4C H2L1 and H3L1 neutralised the bioactivity of recombinant CHO-expressed human IL13 (see table 6 and figure 13).
[$ND_{50}$ (neutralising dose) is the concentration of mAb required to reduce TF-1 cell proliferation by 50%, in response to a set concentration of IL-13]

Table 6

| IL-13 variant | mAb | Mean $ND_{50}$ of 4 assays (µg/ml) | Standard Error |
|---|---|---|---|
| Mammalian (CHO-cell) human IL-13 | chimaeric 3G4 | 0.12 | 0.05 |
| | H2L1 | 0.227 | 0.028 |
| | H3L1 | 0.215 | 0.003 |
| | 3G4 parental mAb | 0.0515 | 0.0015 |

The average ND50 value for 3G4 parental was 0.05µg/ml. This was calculated for the neutralisation of ~ 10ng/ml mammalian-expressed human IL-13 in a TF-1 cell bioassay. This value differs to that previously obtained (see Example 1.3). However the amount of human IL13 used to stimulate proliferation of the TF-1 cells in these experiments was lower than previously used (10ng/ml).
The level of neutralisation of CHO expressed human IL13 achieved by the parental 3G4 mAb was slightly better than the level for 3G4C. The potencies of H2L1 and H3L1 were reduced in comparison with both parental 3G4 mAb and chimaeric 3G4.

**3.5 Neutralisation of recombinant Q130 human IL-13 variant in a TF-1 cell proliferation bioassay.**

The neutralisation capacity of 3G4, 3G4C, H2L1 and H3L1 for E.coli-expressed recombinant Q130 human IL-13 was assessed in a TF-1 cell proliferation assay. The method was carried out as described in Example 6.2A.
An $ND_{50}$ value of 0.274µg/ml was obtained. This differs from the $ND_{50}$ value previously obtained (see Example 1.4). This assay was repeated several times to confirm these $ND_{50}$ values . The quality of the commercially-sourced Q130 human IL-13 preparation used in both sets of experiments (carried out at different times) may explain the differences observed for these 2 data sets.
Table 7 sets out the potencies of 3G4C, H2L1 and H3L1, which were all similar. See also Figure 14.

Table 7

| IL-13 variant | mAb | Mean $ND_{50}$ for a minimum of 2 assays (µg/ml) | Standard Error |
|---|---|---|---|
| E.coli-expressed Q130 human IL-13 | chimaeric 3G4 | 0.37925 | 0.151153 |
| | H2L1 | 0.385 | 0.160282 |
| | H3L1 | 0.416 | 0.102 |
| | 3G4 parental mAb | 0.274 | 0.089982 |

### 3.6 BIAcore™ analysis

The affinity of 3G4C, H2L1 and H3L1 for recombinant human and cynomolgus IL-13 was assessed by BIAcore™ analysis. See tables 8, 9 and 10.

Analyses were carried out using Protein A capture. Briefly, Protein A was coupled onto a CM5 chip by primary amine coupling in accordance with the manufactures recommendations. Chimeric antibody or humanised antibodies were then captured onto this surface and human or cynomolgus IL-13 passed over at defined concentrations. The surface was regenerated back to the Protein A surface using mild acid elution conditions, this did not significantly affect the ability to capture antibody for a subsequent IL-13 binding event. The work was carried out on the Biacore 3000 and the T100 Biacore machines, the data were analysed using the evaluation software inherent in the machines and fitted to the 1:1 model of binding. The data differed slightly between the two machines, though the differences seen between the kinetics for the chimera and humanised antibodies binding to human IL-13 was similar for both machines. The binding data for human IL-13 fitted well to the 1:1 model for all constructs, however the fit for binding to cynomolgus IL-13 was worse, raising the possibility that the actual values may be slightly worse (e.g. a 2- 3 fold difference) than reported due to this poorer fit and the difficulty in the analyses.

The data were generated using untagged recombinant human or cynomolgus IL-13 (made at GSK). All Biacore runs were carried out at 25 degrees C.

Table 8: Biacore 3000 data for chimeric and humanised antibodies binding to human IL-13

| Antibody | ka | kd | KD (nM) |
|---|---|---|---|
| **Chimeric 3G4** | **3.29e6** (6.31e5) | **1.92e-4** (3.68e-5) | **0.060** (0.00) |
| **H2L1** | **2.77e6** (1.43e5) | **3.30e-4** (9.19e-5) | **0.120** (0.03) |
| **H3L1** | **2.84e6** (9.11e4) | **3.77e-4** (1.94e-5) | **0.130** (0.01) |

For chimaeric 3G4, the data were produced from 4 independent experiments (two of which were carried out in duplicate, with each duplicate being analysed separately). For H2L1 and H3L1 humanised mAbs, the data were produced from two independent experiments carried out in duplicate (with each duplicate being analysed separately). The data are presented as the mean and standard deviation (in brackets) of these results.

Table 9: T100 data for chimeric and humanised antibodies binding to human IL-13

| Antibody | ka | kd | KD (nM) |
|---|---|---|---|
| **Chimeric 3G4** | **1.12e7** (7.07e4) | **2.5e-4** (2.4e-5) | **0.023** (0.002) |
| **H2L1** | **1.07e7** (7.76e6) | **5.68e-4** (1.38e-4) | **0.066** (0.035) |
| **H3L1** | **7.21e6** (2.09e6) | **5.31e-4** (3.25e-5) | **0.077** (0.018) |

The data were produced from 2 independent experiments and are presented as the mean and standard deviation (in brackets) of these results.

The 3G4 chimaeric antibody and the humanised mAbs H2L1 and H3L1 bind with high affinity to human IL-13, and these data are comparable to the bind affinity of the parental 3G4 mouse mAb for human IL-13.

Table 10: T100 data for chimeric and humanised antibody binding to cynomolgus IL-13

| Antibody | ka | kd | KD (nM) |
|---|---|---|---|
| **Chimeric 3G4** | **1.97e6** | **1.77e-3** | **0.899** |

| | | | |
|---|---|---|---|
| H2L1 | 3.18e5 | 1.12e-3 | 3.5 |
| H3L1 | 3.65e5 | 1.04e-3 | 2.9 |

The data were produced from a single experiment.
The 3G4 chimaeric antibody and the humanised mAbs H2L1 and H3L1 bind to cynomolgus IL-13 with a lower affinity in comparison to their binding affinity for human IL-13.

## 3.7 Specificity of 3G4C, H2L1 and H3L1 for binding to human IL-13.

The specificities of 3G4C, H2L1 and H3L1 for human IL-13 were assessed by analysis of the cross-reactivity potential against human IL-4, human IL-5 and human GM-CSF in binding ELISAs. These methods were carried as described in section 6.7, 6.8 and 6.9 respectively. See figures 15, 16 and 17. These mAbs were found to be specific for binding to IL-13, with no cross-reactivity for human IL-4 human IL-5 or human GM-CSF at mAb concentrations up to 30µg/ml. 3G4 chimera appeared to show some binding to human IL-5 at 30µg/ml, this is probably due to a pipetting error as no such observation was made for humanised mAbs H2L1 and H3L1 at a similar concentration.

## 4. EPITOPE MAPPING OF 3G4 USING BIOTINYLATED PEPTIDES

A series of epitope mapping experiments were performed to determine which amino acid residues in IL-13 were required for binding of the 3G4 mouse mAb.

### 4.1 Crude mapping of the 3G4 mouse mAb binding epitope on human and cynomolgus IL-13

Biotinylated 16 mer peptides offset by 4 were synthesised to map the location of the binding epitope recognised by mouse mAb 3G4 on human and cynomolgus IL-13. An ELISA method as described in Example 6.3 was used to detect binding of immobilised biotinylated peptide to the parental mouse mAb 3G4.
Details of 16 mer custom designed Peptides: 88 x 16 mers, offset by 4 (supplied by Mimotopes, Australia).

Format: Peptides 25 & 44 = Biotin-SGSG-PEPTIDE-acid
Peptides 2-24 & 27-43 = Biotin-SGSG-PEPTIDE-amide

| # | Hydro | MolWt | N-term | Sequence | C-term |
|---|---|---|---|---|---|
| 2 | 0.42 | 2,311.66 | Biotin- | SEQ.I.D.NO:46 | -NH2 |
| 3 | 0.27 | 2,453.82 | Biotin- | SEQ.I.D.NO:47 | -NH2 |
| 4 | 0.38 | 2,326.70 | Biotin- | SEQ.I.D.NO:48 | -NH2 |
| 5 | 0.31 | 2,231.58 | Biotin- | SEQ.I.D.NO:49 | -NH2 |
| 6 | 0.43 | 2,289.66 | Biotin- | SEQ.I.D.NO:50 | -NH2 |
| 7 | 0.59 | 2,190.57 | Biotin- | SEQ.I.D.NO:51 | -NH2 |
| 8 | 0.57 | 2,260.64 | Biotin- | SEQ.I.D.NO:52 | -NH2 |
| 9 | 0.62* | 2,255.64 | Biotin- | SEQ.I.D.NO:53 | -NH2 |
| 10 | 0.51 | 2,197.56 | Biotin- | SEQ.I.D.NO:54 | -NH2 |
| 11 | 0.56 | 2,144.52 | Biotin- | SEQ.I.D.NO:55 | -NH2 |
| 12 | 0.46 | 2,090.38 | Biotin- | SEQ.I.D.NO:56 | -NH2 |
| 13 | 0.29 | 2,219.54 | Biotin- | SEQ.I.D.NO:57 | -NH2 |
| 14 | 0.29 | 2,180.53 | Biotin- | SEQ.I.D.NO:58 | -NH2 |
| 15 | 0.36 | 2,318.70 | Biotin- | SEQ.I.D.NO:59 | -NH2 |
| 16 | 0.32 | 2,303.73 | Biotin- | SEQ.I.D.NO:60 | -NH2 |
| 17 | 0.47 | 2,209.57 | Biotin- | SEQ.I.D.NO:61 | -NH2 |
| 18 | 0.48 | 2,257.60 | Biotin- | SEQ.I.D.NO:62 | -NH2 |
| 19 | 0.17 | 2,273.57 | Biotin- | SEQ.I.D.NO:63 | -NH2 |
| 20 | 0.27 | 2,300.60 | Biotin- | SEQ.I.D.NO:64 | -NH2 |
| 21 | 0.29 | 2,383.77 | Biotin- | SEQ.I.D.NO:65 | -NH2 |
| 22 | 0.35 | 2,401.83 | Biotin- | SEQ.I.D.NO:66 | -NH2 |
| 23 | 0.45 | 2,407.92 | Biotin- | SEQ.I.D.NO:67 | -NH2 |
| 24 | 0.42 | 2,541.08 | Biotin- | SEQ.I.D.NO:68 | -NH2 |
| 25 | 0.33 | 2,513.97 | Biotin- | SEQ.I.D.NO:69 | -OH |
| 27 | 0.42 | 2,283.64 | Biotin- | SEQ.I.D.NO:70 | -NH2 |
| 28 | 0.27 | 2,425.81 | Biotin- | SEQ.I.D.NO:71 | -NH2 |
| 29 | 0.57 | 2,228.57 | Biotin- | SEQ.I.D.NO:72 | -NH2 |
| 30 | 0.62* | 2,223.57 | Biotin- | SEQ.I.D.NO:73 | -NH2 |
| 31 | 0.51 | 2,165.49 | Biotin- | SEQ.I.D.NO:74 | -NH2 |
| 32 | 0.56 | 2,112.45 | Biotin- | SEQ.I.D.NO:75 | -NH2 |
| 33 | 0.27 | 2,207.56 | Biotin- | SEQ.I.D.NO:76 | -NH2 |
| 34 | 0.33 | 2,345.73 | Biotin- | SEQ.I.D.NO:77 | -NH2 |
| 35 | 0.29 | 2,330.76 | Biotin- | SEQ.I.D.NO:78 | -NH2 |
| 36 | 0.45 | 2,236.60 | Biotin- | SEQ.I.D.NO:79 | -NH2 |
| 37 | 0.43 | 2,276.64 | Biotin- | SEQ.I.D.NO:80 | -NH2 |
| 38 | 0.12 | 2,292.62 | Biotin- | SEQ.I.D.NO:81 | -NH2 |
| 39 | 0.22 | 2,319.64 | Biotin- | SEQ.I.D.NO:82 | -NH2 |
| 40 | 0.24 | 2,402.82 | Biotin- | SEQ.I.D.NO:83 | -NH2 |
| 41 | 0.33 | 2,387.80 | Biotin- | SEQ.I.D.NO:84 | -NH2 |
| 42 | 0.43 | 2,393.90 | Biotin- | SEQ.I.D.NO:85 | -NH2 |

| | | | | | |
|---|---|---|---|---|---|
| 43 | 0.39 | 2,527.05 | Biotin- | SEQ.I.D.NO:86 | -NH2 |
| 44 | 0.35 | 2,471.88 | Biotin- | SEQ.I.D.NO:87 | -OH |

(* indicates a high hydrophobicity value)

The results indicated that 3G4 mouse mAb bound to the two peptides shown below (also see figure 6).

| | | |
|---|---|---|
| Peptide 25: | DLL**L**HLKKLFREG**R**FN | (SEQ.I.D.NO:88) |
| Peptide 44: | DLL**V**HLKKLFREG**Q**FN | (SEQ.I.D.NO:89) |

Peptide 25 is derived from human IL-13.
Peptide 44 is derived from cynomolgus IL-13.

NB: **BOLD** indicates residue differences between human IL-13 and the cynomolgus IL-13 orthologue.

## 4.2 Fine mapping of the 3G4 mouse mAb binding epitope on human and cynomolgus IL-13 using biotinylated peptides

The minimal binding epitope for mouse mAb 3G4 on human IL-13 was determined using a peptide set based around the peptide sequence, QFVKDLLLHLKKLFREGRFN (SEQ.I.D.NO:90). Peptides were obtained with 1 amino acid sequentially removed from either the N or C-terminus of this parental peptide sequence in order to define the precise linear binding epitope for mouse mAb 3G4. A similar approach was taken to map binding to cynomolgus IL-13.
An ELISA method (carried out as described in Example 6.4) was used to detect binding of immobilised biotinylated peptide to the parental mouse mAb 3G4 (figures 7a and 7b).

The results indicate that parental mouse mAb 3G4 binds to the minimal amino acid epitope LLHLKKLFREG (SEQ.I.D.NO:91) at the C-terminal region of human IL-13. However, the 2 amino acids (D and L) located adjacent to the N-terminal end of the above peptide sequence (in the human IL-13 sequence) may also be important for binding, as the binding signal is enhanced when these residues are present. Similarly, the 3 amino acids (R, F and N) located adjacent to the C-terminal end of the above peptide sequence (in the human IL-13 sequence) may also be important for binding, as the binding signal is lost when the R and F residues are present, but the binding signal is restored when the N residue is present.

Similar results were obtained for binding of mouse mAb 3G4 to the cynomolgus IL-13 peptide set. The results indicate that parental mouse mAb 3G4 binds to the minimal

amino acid epitope LLVHLKKLFREG (SEQ.I.D.NO:98) in the C-terminal region of cynomolgus IL-13. However, the 1 amino acid (D) located adjacent to the N-terminal end of the above peptide sequence (in the cynomolgus IL-13 sequence) may also be important for binding, as the binding signal is enhanced when this residue is present. Similarly, the 3 amino acids (Q, F and N) located adjacent to the C-terminal end of the above peptide sequence (in the cynomolgus IL-13 sequence) may also be important for binding, as the binding signal is lost when the Q and F residues are present, but the binding signal is restored when the N residue is present.

**4.3 Alanine scanning of the mouse mAb 3G4 binding epitope using biotinylated peptides**

In order to identify the key residues involved in the interaction of human IL-13 with mouse mAb 3G4, an alanine scanning approach was adopted using parental peptide sequence QFVKDLLLHLKKLFREGRFN (SEQ.I.D.NO:90). For this analysis, peptides as set out in Table 11 were obtained (AnaSpec Inc) where one amino acid was sequentially substituted for an alanine residue at each amino acid position in the LKKLFRE (SEQ.I.D.NO:92) portion of the parental QFVKDLLLHLKKLFREGRFN (SEQ.I.D.NO:90) epitope.

Table 11

| SEQ.I.D.NO | N-terminus | Peptide sequence |
|---|---|---|
| SEQ.I.D.NO:90 | Biotin–SGSG | QFVKDLLLHLKKLFREGRFN |
| SEQ.I.D.NO:99 | Biotin–SGSG | QFVKDLLLH**A**KKLFREGRFN |
| SEQ.I.D.NO:100 | Biotin–SGSG | QFVKDLLLHL**A**KLFREGRFN |
| SEQ.I.D.NO:101 | Biotin–SGSG | QFVKDLLLHLK**A**LFREGRFN |
| SEQ.I.D.NO:102 | Biotin–SGSG | QFVKDLLLHLKK**A**FREGRFN |
| SEQ.I.D.NO:103 | Biotin–SGSG | QFVKDLLLHLKKL**A**REGRFN |
| SEQ.I.D.NO:104 | Biotin–SGSG | QFVKDLLLHLKKLF**A**EGRFN |
| SEQ.I.D.NO:105 | Biotin–SGSG | QFVKDLLLHLKKLFR**A**GRFN |

An ELISA method was used (similar to that set out in Example 6.4) to detect binding of immobilised biotinylated peptide to the parental mouse mAb 3G4 (see Figure 8)

These data confirm that the key amino acid residues involved in the interaction of parental mouse mAb 3G4 with human IL-13 are at least, arginine (R) at position 107, lysine (K) at position 103 and lysine (K) at position 104. The numbering of these is as previously described in WO2006/003407.

As the above analysis had only scanned the LKKLFRE (SEQ ID NO:92) portion of the minimal binding epitope, a further set of peptides as set out in Table 12 were

obtained (Mimotopes) to expand the alanine scanning study to the other amino acid residues in the minimal binding epitope.

Table 12:

| SEQ.I.D.NO | N-terminus | Peptide sequence |
|---|---|---|
| SEQ.I.D.NO:90 | Biotin–SGSG | QFVKDLLLHLKKLFREGRFN |
| SEQ.I.D.NO:104 | Biotin–SGSG | QFVKDLLLHLKKLF**A**EGRFN |
| SEQ.I.D.NO:106 | Biotin–SGSG | QFVKDLLL**A**LKKLFREGRFN |
| SEQ.I.D.NO:107 | Biotin–SGSG | QFVKDLL**A**HLKKLFREGRFN |
| SEQ.I.D.NO:108 | Biotin–SGSG | QFVKDL**A**LHLKKLFREGRFN |
| SEQ.I.D.NO:109 | Biotin–SGSG | QFVKD**A**LLHLKKLFREGRFN |
| SEQ.I.D.NO:110 | Biotin–SGSG | QFVK**A**LLLHLKKLFREGRFN |
| SEQ.I.D.NO:111 | Biotin–SGSG | QFVKDLLLHLKKLFRE**A**RFN |
| SEQ.I.D.NO:112 | Biotin–SGSG | QFVKDLLLHLKKLFREG**A**FN |
| SEQ.I.D.NO:113 | Biotin–SGSG | QFVKDLLLHLKKLFREGR**A**N |
| SEQ.I.D.NO:114 | Biotin–SGSG | QFVKDLLLHLKKLFREGRF**A** |

Again, an ELISA method (similar to that used in Example 6.4) was used to detect binding of immobilised biotinylated peptide to the parental mouse mAb 3G4. See Figure 9 (in this experiment peptide QFVKDLLLHLKKLFREGRFN (SEQ.I.D.NO:90) is the positive control demonstrating maximal binding, and peptide QFVKDLLLHLKKLF**A**EGRFN (SEQ ID No 104) is the negative control demonstrating minimal binding).

These data suggest that the phenylalanine (F) residue at position 111 is also important for the interaction of parental mouse mAb 3G4 with human IL-13. The numbering of this residue is as previously described in WO2006/003407.

**5. Efficacy of a humanised anti-IL-13 mAb in cynomolgus asthma model.**

This Example is prophetic.

The model of Ascaris suum-induced (A.suum) pulmonary bronchoconstriction in cynomolgus monkeys (*Macaca fascicularis*) is widely recognised as the non-clinical model most similar and relevant to asthma in humans (Patterson R, *et al* Trans. Assoc. Am. Physicians 1980 93:317-325; Patterson R, *et al* J. Lab. Clin. Med. 1983 101:864-872).

In this model, animals having an innate pulmonary sensitivity to A.suum are exposed to nebulised A.suum to induce an asthmatic response. This asthmatic response can be characterised by measuring airways hyper-responsiveness (AHR), cellular infiltration as measured in broncho alveolar lavage (BAL) fluid and serum IgE levels. Experimental methods are similar to those previously described by Mauser P, *et al* in

Am. J. Resp. Crit. Care Med. 1995 204:467-472 and by Evanoff H, *et al* in Immunologic Investigation 1992 21:39.

This study uses 30 animals, pre-selected for entry having demonstrated a positive bronchoconstrictor response to a specific dose of A.suum antigen.
A.suum is administered at the optimal response dose (ORD) for each animal. It is a pre-determined dose of A.suum that produces an increase in $R_L$ (lung resistance) of at least 40% and a decrease in $C_{DYN}$ (dynamic compliance) of at least 35%, by aerosol inhalation (for a single dose given over 15 breaths using a neublizer).

The study takes place in 2 phases. During phase 1, AHR is assessed in response to intravenous (i/v) histamine challenge (that is a dose of histamine sufficient to induce an increase in $R_L$ of at least 30% above baseline ($PC_{30}$)) both before (the baseline pulmonary function assessment on day 1) and after (on day 11) administering A.suum antigen (on days 9 and 10, when A.suum is administered at an optimal pre-determined dose for each animal by aerosol inhalation).

Phase 2 is identical to phase 1 except that animals receive treatment with antibody (see below), each antibody is given as 3 doses of approximately 30mg/kg administered by i/v infusion on days 1, 5 and 9.

Group 1 (n=12): A humanised anti-IL-13 mAb (30mg/kg)

Group 2 (n=12): A humanised anti-IL-13 mAb (30mg/kg) and Pascolizumab (humanised anti-IL4 mAb, 30mg/kg)

Group 3 (n=6): vehicle alone negative control treatment

The AHR readouts from phases 1 and 2 are calculated by taking pressure and airflow readings - lung resistance ($R_L$) and dynamic compliance ($C_{DYN}$) – in response to histamine, using the Buxco pulmonary mechanics system. The maximum percentage change from the baseline compared to post A.suum antigen challenge [for lung resistance ($R_L$) and dynamic compliance ($C_{DYN}$)] is compared for phases 1 and 2 i.e. with or without antibody treatment, and these data are used to assess the AHR phenotype.

In addition BAL samples are taken at days 1 and 11 in phases 1 and 2, to measure cellular infiltration and in particular eosinophilia. Serum samples are also taken to monitor IgE levels.

## 6.1. Human or cynomolgus IL-13 binding ELISA

This assay describes an ELISA that detects binding of an antibody to human or cynomolgus IL-13. It is a sandwich ELISA format.

### 6.1.1 Materials

1. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
2. Human IL-13 (E.coli expressed from Cambridge Biosciences, cat. no. CH1-013)
3. Cynomolgus IL-13 (made by GlaxoSmithkline)
4. Goat anti-human IL-13 polyclonal antibody (R+D Systems, cat. no. AF-213-NA)
5. Anti-human IgG-HRP (Sigma, Cat No. A-6029)
6. Anti-mouse IgG-HRP (Sigma, Cat No. A-9309)
7. Carbonate/bicarbonate buffer (Sigma; cat. no. C-3041)
8. TBST [Tris buffered saline (6.06g Tris + 8.06g NaCl + 0.2g KCl + H20 to 1L) + 0.05% Tween 20]
9. BSA (Sigma A-7030)
10. OPD (Sigma, Cat. No. P-9187)
11. Sulphuric acid

### 6.1.2 Method

1. Blocking solution is 3% BSA+TBST
2. Washing solution is TBST
3. Coat 'Nunc Maxisorp' ELISA plates with 50ul of 5ug/ml goat anti-human IL-13 polyclonal antibody (R+D Systems, cat. no. AF-213-NA. Made up at a stock concentration of 500ug/ml according to maufacturers instructions, and stored in aliquots at –20C) in carbonate/bicarbonate buffer (Sigma; cat. no. C-3041, made up as per maufacturers instructions), cover with a plate sealer and incubate overnight at 4°C.
4. Block with 100ul of 3% BSA/TBST incubate at rtp for 1hr.
5. Wash X3 in TBST (at least 200ul wash solution per well per wash).
6. Add 20ng per well (in a 50ul volume) human IL-13 (Cambridge Bioscience, cat. no. CH1-013. Made up at a stock concentration of 100ng/ul according to maufacturers instructions, and stored in aliquots at –20C) or 20ng per well cynomolgus IL-13, in block solution and incubate at room temperature for 1hr.
7. Wash X3 in TBST.
8. Add 50ul antibody sample (titrate out to obtain end-point titre data, if required) in block solution, incubate at rtp for 1hr.
9. Wash X3 in TBST.
10. For 3G4 chimaeric antibody or humanised antibody, detect binding using 50ul per well anti-human IgG-HRP (Sigma, Cat No. A-6029) at a 1/2000 dilution in block solution for 1hr at rtp. For 3G4 mouse monoclonal antibody, detect binding

using 50ul per well anti-mouse IgG-HRP (Sigma, Cat No. A-9309) at a 1/1000 dilution in block solution for 1hr at rtp.

11. Wash X3 in TBST.
12. Develop with 100ul OPD (Sigma, Cat. No. P-9187. Made up as per maufacturers instructions), stop with 50ul 3M $H_2SO_4$, read at an absorbance of 490nm. Development time is ~ 12 minutes.

## 6.1A Human IL-13 binding ELISA

This assay describes an ELISA that detects binding of an antibody to human IL-13. It is a sandwich ELISA format and differs only slightly to that described in Example 6.1.

### 6.1A.1 Materials

1. Costar 96 well EIA plate (Corning Costar cat. no 3369)
2. Human IL-13 (Peprotech cat. no. 200-13)
3. Goat anti-human IL-13 polyclonal antibody (R+D Systems, cat. no. AF-213-NA)
4. Anti-human kappa light chain-HRP (Sigma, Cat No. A7164)
5. Carbonate/bicarbonate buffer (Sigma; cat. no. C-3041)
6. TBST [Tris buffered saline (6.06g Tris + 8.06g NaCl + 0.2g KCl + H20 to 1L) + 0.05% Tween 20]
7. BSA (Sigma A-7030)
8. OPD (Sigma, Cat. No. P-9187)
9. Sulphuric acid

### 6.1A.2 Method

1. Blocking solution is 3% BSA+TBST
2. Washing solution is TBST
3. Coat 'Costar E1A/RIA' ELISA plates with 50ul of 5ug/ml goat anti-human IL-13 polyclonal antibody (R+D Systems, cat. no. AF-213-NA. Made up at a stock concentration of 500ug/ml according to maufacturers instructions, and stored in aliquots at –20C) in carbonate/bicarbonate buffer (Sigma; cat. no. C-3041, made up as per maufacturers instructions), cover with a plate sealer and incubate overnight at 4°C.
4. Block with 100ul of 3% BSA/TBST incubate at rtp for 1hr or minimum of overnight 4°C.
5. Wash X2 in TBST (at least 200ul wash solution per well per wash).
6. Add 20ng per well (in a 50ul volume) human IL-13 (Peprotech cat. no. 200-13). Made up at a stock concentration of 100ng/ul according to maufacturers instructions, and stored in aliquots at –20C), dilute in block solution and incubate at room temperature for 1hr.
7. Wash X2 in TBST.

8. Add 50ul antibody sample (titrate out to obtain end-point titre data, if required) in block solution, incubate at rtp for 1hr.
9. Wash X2 in TBST.
10. For 3G4 chimaeric antibody or humanised antibody, detect binding using 50ul per well anti-human kappa light chain-HRP (Sigma, Cat No. A7164) at a 1/2000 dilution in block solution for 1hr at rtp.
11. Wash X2 in TBST.
12. Develop with 100ul OPD (Sigma, Cat. No. P-9187. Made up as per maufacturers instructions), stop with 50ul 3M $H_2SO_4$, read at an absorbance of 490nm.

**6.1B Native human IL-13 binding ELISA**

This assay describes an ELISA that detects binding of an antibody to native human IL-13. It is a sandwich ELISA format.

**6.1B.1Materials**

1. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
2. Native human IL-13 (HDLM-2 cell supernatant)
3. Anti-human IL13 antibody (Pharmingen, Cat. No. 554570)
4. Biotinylated Anti-human IL13 antibody (Pharmingen, Cat. No. 555054)
5. Streptavidin-HRP (Sigma cat no. E2886)
6. Carbonate/bicarbonate buffer (Sigma; cat. no. C-3041)
7. RPMI +20%FBS+2mMglutamine
8. PBST (PBS + 0.05% Tween 20)
9. BSA (Sigma A-7030)
10. OPD (Sigma, Cat. No. P-9187)
11. Sulphuric acid

**6.1B.2 Method**

1. Blocking solution is 1% BSA in PBST
2. Washing solution is PBST
3. Coat 'Nunc Maxisorp' ELISA plates with 50ul of 5ug/ml of 3G4 chimeric or humanised antibody or 2ug/ml anti human IL13 (Pharmingen cat no. 554570) dilution in Carbonate/bicarbonate buffer, cover with a plate sealer and incubate overnight at 4°C.
4. Block with 200ul of 1% BSA/PBST incubate at room temperature for 1hr.
5. Wash X3 in PBST.
6. Add 50ul native IL13 present in HDLM2 supernatent sample (titrate out) dilute in RPMI +20%FBS+2mMglutamine solution, incubate at rtp for 1hr.
7. Wash X3 in PBST.
8. Add 50ul per well biotinylated anti-human IL13 1ug/ml (Pharmingen, Cat. No. 555054) dilute in PBST + 1%BSA incubate at rtp for 1hr.
9. Wash X3 in PBST.

10. Add 50ul per well streptavidin-HRP conjugate at 1/1000 dilution dilute in PBST + 1%BSA. Incubate for 1hour at room temperature.
11. Wash x3 in TBST
12. Develop with 100ul per well OPD (Sigma, Cat. No. P-9187. Made up as per maufacturers instructions), stop with 50ul per well 3M $H_2SO_4$, read at an absorbance of 490nm.

**6.2. IL-13 neutralisation bioassay** (TF-1 cell proliferation assay)

This is an IL-13 bioassay that can be used to determine the neutralisation capacity of an anti-IL-13 antibody. The method described below uses recombinant human or cynomolgus IL-13. Mammalian-expressed human IL-13 or the Q130 human IL-13 variant can also be used in this assay too.

**6.2.1 Materials**

1. TF-1 cell line (TF-1 cell line obtained in-house, NB, ATCC version available)
2. 96 well tissue culture plates (Invitrogen)
3. Human IL-13 (Cambridge Bioscience, cat. no. CH1-013)
4. CellTiter 96 non-radioactive cell proliferation assay (Promega, Cat. No. G4000)

**6.2.2 Method**

1. Method to measure the ability of an anti-human IL-13 mAb to neutralise the bioactivity of recombinant human or cynomolgus IL-13 in a TF-1 cell bioassay.
2. This assay is performed in sterile 96 well tissue culture plates (Invitrogen), under sterile conditions. All tests are performed in triplicate.
3. Pre-incubate 10ng/ml human IL-13 (Cambridge Bioscience, cat. no. CH1-013. Make up at a stock concentration of 100ng/ul according to maufacturers instructions using sterile technique in a class 2 tissue culture hood, store in small aliquots at –20C) or 10ng/ml cynomolgus IL-13 (generated at GSK) with various dilutions of the anti-human IL-13 mAb (diluted from 6ug/ml in 3 fold dilutions down to 0.025ug/ml) in a total volume of 50ul for 1 hour at 37C. Also included will be positive control wells, having IL-13 present but no anti-human IL-13 mAb. In addition, negative control wells will have no IL-13 and no anti-human IL-13 mAb present. Use a sterile, low protein binding, round bottom 96 well plate for this pre-incubation. (Note that the concentration of IL-13 and anti-human IL-13 mAb will be halved at a later stage when cells are added).
4. Plate out 50ul of TF-1 cells at $2x10^5$ per ml in a sterile 96 well tissue culture plate. After the 1 hour pre-incubation, add the IL-13 and anti-human IL-13 mAb sample to the cells. The final 100ul assay volume, containing various anti-human IL-13 mAb dilutions, recombinant IL-13 and TF-1 cells, is incubated at $37^0$C for ~70 hours in a humidified $CO_2$ incubator.

5. At ~ 66hrs, scan the wells to confirm that they are sterile and that no bacterial contamination has occurred.
6. Add 15ul of filter sterilised MTT substrate per well (Cat. No. G4000, Promega. Made up as per maufacturers instructions) for the final 4 hours of incubation.
7. Stop the reaction with 100ul of stop solution (provided in the MTT kit) to solubilise the metabolised blue formazan product. Leave for at least 2 hours, then pipette up and down to help dissolve the crystals. Alternatively, cover with a plate sealer and leave at 4C overnight, then pipette up and down the next day (this is easier in terms of pipetting)
8. Read the absorbance of the solution in each well in a 96-well plate reader at 570nm wavelength.
9. The capacity of the anti-human IL-13 mAb to neutralise human or cynomolgus IL-13 bioactivity is expressed as, that concentration of anti-human IL-13 mAb required to neutralise the bioactivity of a defined amount of human or cynomolgus IL-13 (5ng/ml) by 50% (= $ND_{50}$). The lower the concentration required, the more potent the neutralisation capacity.

**6.2.A IL-13 neutralisation bioassay** (TF-1 cell proliferation assay)

This is an IL-13 bioassay that can be used to determine the neutralisation capacity of an anti-IL-13 antibody. The method described below uses recombinant human and cynomolgus IL-13. Mammalian-expressed human IL-13 or the Q130 human IL-13 variant can also be used in this assay too. Note that this method differs only slightly to that described in Example 6.2

**6.2.A.1 Materials**

1. TF-1 cell line (TF-1 cell line obtained in-house, NB, ATCC version available)
2. 96 well tissue culture plates (Corning costar, cat no. 3596)
3. Human IL-13 (Peprotech, cat. no. 200-13)
4. Human IL-13 (CHO cell expressed) generated at GSK.
5. Human IL-13 Q130 variant (Peprotech, cat. no. 200-13A)
6. Cynomolgus IL-13 (generated at GSK).
7. Polyclonal anti-human IL13 (R&D systems AF-213-NA)
8. 96 well tissue culture plates (Corning costar, cat no.3790)
9. CellTiter 96 non-radioactive cell proliferation assay (Promega, Cat. No. G4000)

**6.2.A.2 Method**

1. Method to measure the ability of an anti-human IL-13 mAb to neutralise the bioactivity of recombinant human and cynomolgus IL-13 in a TF-1 cell bioassay.
2. This assay is performed in sterile 96 well tissue culture plates (Corning costar, cat no. 3596), under sterile conditions. All tests are performed in triplicate.
3. Pre-incubate 10ng/ml human IL-13 (Peprotech, cat. no. 200-13), or 10ng/ml CHO expressed human IL13 (generated at GSK) or 60ng/ml human IL13 Q130 variant (Peprotech, cat. no. 200-13A), or 10ng/ml cynomolgus IL-13 (generated at GSK) make up commercial Ab at a stock concentration according to maufacturers instructions using sterile technique in a class 2 tissue culture hood, store in small aliquots at –20C. With various dilutions of the anti-human IL-13 mAb (diluted from 6ug/ml or 2ug/ml or 180 ug/ml in 3 fold dilutions down to 0.025ug/ml or 0.008 ug/ml or 0.74 ug/ml respectively) in a total volume of 50ul for 1 hour at 37C. Also included will be positive control wells, having IL-13 present but no anti-human IL-13 mAb. In addition, negative control wells will have no IL-13 and no anti-human IL-13 mAb present. Use a sterile, low protein binding, round bottom 96 well plate for this pre-incubation (Corning costar, cat no.3790). (Note that the concentration of IL-13 and anti-human IL-13 mAb will be halved at a later stage when cells are added).
4. Plate out 50ul of TF-1 cells at $2x10^5$ per ml in a sterile 96 well tissue culture plate (Corning costar, cat no. 3596). After the 1 hour pre-incubation, add the IL-13 and anti-human IL-13 mAb sample to the cells. The final 100ul assay volume, containing various anti-human IL-13 mAb dilutions, recombinant IL-13 and TF-1 cells, is incubated at $37^0C$ for 3 days in a humidified $CO_2$ incubator.
5. Scan the wells to confirm that they are sterile and that no bacterial contamination has occurred.
6. Add 15ul of MTT substrate per well (Cat. No. G4000, Promega) for the final 4 hours of incubation.
7. Stop the reaction with 100ul of stop solution (provided in the MTT kit) to solubilise the metabolised blue formazan product. Leave for at least 2 hours at RT, then shake plates on plate shaker for ~30min. Alternatively, cover with a plate sealer and leave at 4C overnight, then shake plates on plate shaker for ~30min.Read the absorbance of the solution in each well in a 96-well plate reader at 570nm wavelength.
8. The capacity of the anti-human IL-13 mAb to neutralise human and cynomolgus IL-13 bioactivity is expressed as, that concentration of anti-human IL-13 mAb required to neutralise the bioactivity of a defined amount of human or cynomolgus IL-13 by 50% (= $ND_{50}$). The lower the concentration required, the more potent the neutralisation capacity.

**6.3. Epitope crude mapping ELISA**

This assay describes an ELISA that detects binding of mouse mAb 3G4 to human or cynomolgus IL-13 peptides.

### 6.3.1 Materials

1. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
2. ImmunoPure© Streptavidin (Pierce, cat. no. 21125)
3. PBST (Phosphate buffered saline + 0.05% Tween 20)
4. BSA (Sigma A-7030)
5. Human and cynomolgus IL-13 16 mer peptides, offset = 4 (Mimotopes custom order)
6. Positive and negative control 20 mer peptides (Supplied with Mimotopes custom order)
7. 3G4 mouse mAb
8. Control Ab (Supplied with Mimotopes custom order)
9. Rabbit anti-mouse Ig HRP conjugated (DAKO, code no. P0260)
10. OPD (Sigma, Cat. No. P-9187)
11. 3M Sulphuric acid

### 6.3.2 Method

1. Blocking solution is 3% BSA+PBST.
2. Washing solution is PBST.
3. Coat 'Nunc Maxisorp' ELISA plates with 100µl of 5µg/ml ImmunoPure© Streptavidin (Pierce, cat. no. 21125 made up at a stock concentration of 1mg/ml according to manufacturers instructions, and stored in aliquots at +4°C) using PBST as a dilutent. Incubate overnight at 37°C to allow solution to dry.
4. Block with 200µl of 3% BSA/PBST. Add plate sealer and incubate at rtp for 1hr.
5. Wash X3 in PBST (at least 200µl wash solution per well per wash).
6. In duplicate and using PBST as a dilutent, add 100µl per well (except control wells) of 1,000-fold dilutions of each peptide (dissolved as per manufacturers instructions in 200µl 40% Acetonitrile 60% Water, then aliquoted in 10-fold dilutions in the same solvent and stored at –20°C).
7. In the control wells, in duplicate and using PBST as a dilutent add 100µl per well of 10-fold dilutions of control peptides (dissolved as per manufacturers instructions in 1ml 40% Acetonitrile 60% Water and stored at –20°C). Add plate sealer and incubate at rtp for 1 hr on a shaking table.
8. Wash X3 in PBST (at least 200µl wash solution per well per wash).
9. Add 100µl per well (except control wells) of 1.506µg/ml mouse mAb in PBST.
10. Add 100µl per well to control wells only, 4, 16 and 32-fold dilutions of control antibody (used as supplied by the manufacturer and stored at –20°C) using PBST as a dilutent. Add plate sealer and incubate at rtp (room temperature and pressure) for 1 hr on a shaking table.
11. Wash X3 in PBST (at least 200µl wash solution per well per wash).

12. Add 100µl per well of 2,000-fold dilution of rabbit anti-mouse Ig HRP-conjugated (DAKO, code no. P0260 used as supplied, stored at +4°C) using PBST as a dilutent. Add plate sealer and incubate at rtp for 1 hr on a shaking table.
13. Wash X3 in PBST (at least 200µl wash solution per well per wash).
14. Develop with 100µl OPD (Sigma, Cat. No. P-9187. Made up as per manufacturers instructions), stop with 50µl 3M $H_2SO_4$, read at an absorbency of 490nm. Development time is ~ 10 minutes.

## 6.4. Epitope fine mapping ELISA

This assay describes an ELISA that detects binding of mAb 3G4 to human or cynomolgus IL-13 peptides.

### 6.4.1 Materials

1. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
2. ImmunoPure© Streptavidin (Pierce, cat. no. 21125)
3. PBST (Phosphate buffered saline + 0.05% Tween 20)
4. BSA (Sigma A-7030)
5. Human and cynomolgus IL-13 partial window net peptides (14-mer truncated by one amino acid at a time from both the N- and C-terminal ends; Mimotopes custom order)
6. Positive control 16 mer peptide (Supplied with previous Mimotopes custom order)
7. 3G4 mAb (made in-house)
8. Goat anti-mouse IgG ($F_C$ specific) HRP conjugated antibody (Sigma A-9309)
9. OPD (Sigma, Cat. No. P-9187)
10. 3M Sulphuric acid

### 6.4.2 Method

1. Blocking solution is 3% BSA+PBST.
2. Washing solution is PBST.
3. Coat 'Nunc Maxisorp' ELISA plates with 100µl of 5µg/ml ImmunoPure© Streptavidin in ultra pure water (Pierce, cat. no. 21125 made up at a stock concentration of 1mg/ml according to manufacturer's instructions, and stored at +4°C). Incubate overnight at +37°C.
4. Block with 200µl of 3% BSA in PBST. Add plate sealer and incubate overnight at +4°C.
5. Wash X3 in PBST (at least 200µl wash solution per well per wash).
6. In duplicate and using 3% BSA in PBST as a dilutent, add 100µl per well of 1,000-fold dilutions of each peptide (dissolved as per manufacturers instructions in 200µl of 40% Acetonitrile 60% Water and stored at –20°C). Add plate sealer and incubate at room temperature for 1 hour on a shaking table.

7. Wash X3 in PBST (at least 200µl wash solution per well per wash).
8. Add 100µl per well of 3µg/ml 3G4 diluted in 3% BSA in PBST. Add plate sealer and incubate at room temperature for 1 hour on a shaking table.
9. Wash X3 in PBST (at least 200µl wash solution per well per wash).
10. Add 100µl per well of 1,000-fold dilution of goat anti-mouse IgG HRP-conjugated antibody (Sigma A-9309 used as supplied, stored at +4°C) using 3% BSA in PBST as a dilutent. Add plate sealer and incubate at room temperature for 1 hour on a shaking table.
11. Wash X3 in PBST (at least 200µl wash solution per well per wash).
12. Develop with 100µl OPD (Sigma, Cat. No. P-9187. Made up as per manufacturers instructions), stop with 50µl 3M $H_2SO_4$, read at an absorbency of 490nm. Development time is ~ 10 minutes.

## 6.5 Human IL-13 binding to the human IL-13Rα1 chain ELISA

This ELISA determines whether an antibody can inhibit human IL-13 binding to the human IL13Rα1 chain.

### 6.5.1 Materials

1. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
2. Human IL13Rα1-Fc (R&D Systems, cat.no. 146-IR)
3. human IL-13 (made in-house)
4. Biotinylated anti-human IL-13 (R&D Systems, cat. no. BAF213)
5. Streptavidin-HRP (Sigma cat no. E2886)
6. Carbonate/bicarbonate buffer (Sigma; cat. no. C-3041)
7. TBST [Tris buffered saline (6.06g Tris + 8.06g NaCl + 0.2g KCl + H20 to 1L) + 0.05% Tween 20]
8. BSA (Sigma A-7030)
9. OPD (Sigma, Cat. No. P-9187)
10. Sulphuric acid

### 6.5.2 Method

1. Blocking solution is 3% BSA+TBST
2. Washing solution is TBST
3. Coat 'Nunc Maxisorp' ELISA plates with 50ul of 5ng/ul human IL-13Rα1-Fc in carbonate/bicarbonate buffer. Cover with a plate sealer and incubate overnight at 4°C.
4. Block with 100ul of 3% BSA/TBST incubate at rtp for 1hr.
5. Wash X3 TBST (at least 200ul wash solution per well per wash).
6. In a total volume of 50ul, pre-incubate 0.4ng/ul human IL-13 with antibody sample (titrated) in block solution for 30 minutes. Add the pre-incubated sample to the receptor-coated ELISA plate and incubate at room temperature for 1hr.

7. Wash x3 in TBST
8. Detect any bound human IL-13 using 50ul per well biotinylated anti-human IL-13 diluted at 1ug/ml. Incubate for 1 hour at room temperature
9. Wash x3 in TBST
10. Add 50ul per well streptavidin-HRP conjugate at 1/1000 dilution. Incubate for 1hour at room temperature.
11. Wash x3 in TBST
12. Develop with 100ul per well OPD (Sigma, Cat. No. P-9187. Made up as per maufacturers instructions), stop with 50ul per well 3M $H_2SO_4$, read at an absorbance of 490nm.

## 6.6 Human IL-13 binding to the human IL13Rα2 chain ELISA

This ELISA determines whether an antibody can inhibit human IL-13 binding to the human IL13Rα2 chain.

### 6.6.1 Materials

1. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
2. Human IL13Rα2-Fc (R&D Systems, cat.no. 614-IR)
3. human IL-13 (generated at GSK)
4. Biotinylated anti-human IL-13 (R&D Systems, cat. no. BAF213)
5. Streptavidin-HRP
6. Carbonate/bicarbonate buffer (Sigma; cat. no. C-3041)
7. TBST [Tris buffered saline (6.06g Tris + 8.06g NaCl + 0.2g KCl + H20 to 1L) + 0.05% Tween 20]
8. BSA (Sigma A-7030)
9. OPD (Sigma, Cat. No. P-9187)
10. Sulphuric acid

### 6.6.2 Method

1. Blocking solution is 3% BSA+TBST
2. Washing solution is TBST
3. Coat 'Nunc Maxisorp' ELISA plates with 50ul of 5ng/ul human IL-13Rα2-Fc in carbonate/bicarbonate buffer. Cover with a plate sealer and incubate overnight at 4°C.
4. Block with 100ul of 3% BSA/TBST incubate at rtp for 1hr.
5. Wash X3 TBST (at least 200ul wash solution per well per wash).
6. In a total volume of 50ul, pre-incubate 0.01ng/ul human IL-13 with antibody sample (titrated) in block solution for 60 minutes. Add the pre-incubated sample to the receptor-coated ELISA plate and incubate at room temperature for 1hr.
7. Wash x3 in TBST
8. Detect any bound human IL-13 using 50ul per well biotinylated anti-human IL-13 diluted at 0.5ug/ml. Incubate for 1 hour at room temperature.

9. Wash x3 in TBST
10. Add 50ul per well streptavidin-HRP conjugate at 1/1000 dilution. Incubate for 1hour at room temperature.
11. Wash x3 in TBST
12. Develop with 100ul per well OPD (Sigma, Cat. No. P-9187. Made up as per maufacturers instructions), stop with 50ul per well 3M $H_2SO_4$, read at an absorbance of 490nm. Development time is ~ 2 minutes.

## 6.7 Human IL-4 binding ELISA

This assay describes an ELISA that detects binding of an antibody to human IL-4. It is a sandwich ELISA format.

### 6.7.1 Materials

12. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
13. Human IL-4 (R+D Systems, cat. no. 204IL)
14. Goat anti-human IL-4 polyclonal antibody (R+D Systems, Cat. No. AF-204-NA)
15. Biotinylated rat anti-human IL-4 antibody (R & D systems, Cat. No BAF204.)
16. Anti-mouse IgG-HRP (Dako, Cat No. P0260)
17. Anti-human kappa light chain-HRP (Sigma A7164)
18. Streptavidin-HRP (Sigma cat no. E2886)
19. Carbonate/bicarbonate buffer (Sigma; cat. no. C-3041)
20. TBST [Tris buffered saline (6.06g Tris + 8.06g NaCl + 0.2g KCl + H20 to 1L) + 0.05% Tween 20]
21. BSA (Sigma A-7030)
22. OPD (Sigma, Cat. No. P-9187)
23. Sulphuric acid

### 6.7.2 Method

13. Blocking solution is 3% BSA in TBST
14. Washing solution is TBST
15. Coat 'Nunc Maxisorp' ELISA plates with 50ul of 5ug/ml goat anti-human IL-4 polyclonal antibody (R+D Systems, cat. no. AF-204-NA. Made up at a stock concentration of 500ug/ml according to maufacturers instructions, and stored in aliquots at –20C) in carbonate/bicarbonate buffer (Sigma; cat. no. C-3041, made up as per manufacturers instructions), cover with a plate sealer and incubate overnight at 4°C.
16. Block with 100ul of 3% BSA/TBST incubate at room temperature pressure (rtp) for 1hr.
17. Wash X3 in TBST (at least 200ul wash solution per well per wash).
18. Add 1ng/ml (in a 50ul volume) human IL-4 in block solution and incubate at room temperature for 1hr.
19. Wash X3 in TBST.

20. Add 50ul antibody sample (titrate out to obtain end-point titre data, if required) in block solution, incubate at rtp for 1hr. As a positive control for binding to human IL-4, use a biotinylated anti-human IL-4 monoclonal antibody (titrated out).
21. Wash X3 in TBST.
22. For 3G4 mouse monoclonal antibody, detect binding using 50ul per well anti-mouse IgG-HRP (Dako, Cat No. P0260) at a 1/2000 dilution in block solution for 1hr at rtp. For 3G4 chimaeric antibody or humanised antibody, detect binding using 50ul per well anti-human kappa light chain-HRP (Sigma, Cat No. Sigma A7164) at a 1/2000 dilution in block solution for 1hr at rtp. For the positive control biotinylated rat anti-human IL-4 monoclonal antibody, detect using a streptavidin-HRP conjugated antibody (Sigma cat no. E2886) at 1/1000 dilution in block solution for 1hr at rtp.
23. Wash X3 in TBST.
24. Develop with 100ul OPD (Sigma, Cat. No. P-9187. Made up as per maufacturers instructions), stop with 50ul 3M $H_2SO_4$, read at an absorbance of 490nm.

## 6.8 Human IL-5 binding ELISA

This assay describes an ELISA that detects binding of an antibody to human IL-5. It is a sandwich ELISA format.

### 6.8.1 Materials

24. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
25. Human IL-5 (R+D Systems, cat. no. 205IL)
26. anti-human IL-5 polyclonal antibody (R+D Systems, Cat. No. AF-205-NA)
27. anti-human IL-5 Mepolizumab (in house clinical grade)
28. Anti-mouse IgG-HRP (Dako, Cat No. P0260 )
29. Anti-human kappa light chain-HRP (Sigma A7164)
30. Carbonate/bicarbonate buffer (Sigma; cat. no. C-3041)
31. TBST [Tris buffered saline (6.06g Tris + 8.06g NaCl + 0.2g KCl + H20 to 1L) + 0.05% Tween 20]
32. BSA (Sigma A-7030)
33. OPD (Sigma, Cat. No. P-9187)
34. Sulphuric acid

### 6.8.2 Method

25. Blocking solution is 3% BSA in TBST
26. Washing solution is TPBST
27. Coat 'Nunc Maxisorp' ELISA plates with 50ul of 5ug/ml goat anti-human IL-5 polyclonal antibody (R+D Systems, cat. no. AF-205-NA. Made up at a stock concentration of 500ug/ml according to maufacturers instructions, and stored in aliquots at –20C) in carbonate/bicarbonate buffer (Sigma; cat. no. C-3041, made up as per manufacturers instructions), cover with a plate sealer and incubate overnight at 4°C.

28. Block with 100ul of 3% BSA/TBST incubate at room temperature pressure (rtp) for 1hr.
29. Wash X3 in TBST (at least 200ul wash solution per well per wash).
30. Add 100ng/ml (in a 50ul volume) human IL-5 (R+D Systems, cat. no. 205IL)in block solution and incubate at room temperature for 1hr.
31. Wash X3 in TBST.
32. Add 50ul antibody sample (titrate out to obtain end-point titre data, if required) in block solution, incubate at rtp for 1hr. As a positive control for binding to human IL-5, use a anti-human IL-5 Mepolizumab antibody (titrated out).
33. Wash X3 in TBST.
34. For 3G4 mouse monoclonal antibody, detect binding using 50ul per well anti-mouse IgG-HRP (Dako, Cat No. P0260) at a 1/2000 dilution in block solution for 1hr at rtp. For 3G4 chimaeric antibody or humanised antibody and anti IL5 Mepolizumab, detect binding using 50ul per well anti-human kappa light chain-HRP (Sigma, Cat No. Sigma A7164) at a 1/2000 dilution in block solution for 1hr at rtp.
35. Wash X3 in TBST.
36. Develop with 100ul OPD (Sigma, Cat. No. P-9187. Made up as per maufacturers instructions), stop with 50ul 3M $H_2SO_4$, read at an absorbance of 490nm.

## 6.9 Human GM-CSF binding ELISA

This assay describes an ELISA that detects binding of an antibody to human GM-CSF. It is a direct binding ELISA format.

### 6.9.1 Materials

35. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
36. Human GM-CSF (clinical grade in house)
37. Anti-human GM-CSF monoclonal antibody (R+D Systems, Cat. No. MAB215)
38. Anti-mouse IgG-HRP (Dako, Cat No. P0260 )
39. Anti-human kappa light chain-HRP (Sigma A7164)
40. Carbonate/bicarbonate buffer (Sigma; cat. no. C-3041)
41. PBST (PBS + 0.05% Tween 20)
42. BSA (Sigma A-7030)
43. OPD (Sigma, Cat. No. P-9187)
44. Sulphuric acid

### 6.9.2 Method

37. Blocking solution is 3% BSA in PBST
38. Washing solution is PBST
39. Coat 'Nunc Maxisorp' ELISA plates with 50ul of 2ug/ml human GM-CSF dilution in PBS, cover with a plate sealer and incubate overnight at 4°C.
40. Block with 200ul of 3% BSA/PBST incubate at room temperature pressure (rtp) for 1hr.

41. Wash X3 in PBST.
42. Add 50ul antibody sample (titrate out to obtain end-point titre data) in block solution, incubate at rtp for 1hr. As a positive control for binding to human IL-GM-CSF, use anti-human GM-CSF (R&D systems cat no.MAB215 antibody (titrated out).
43. Wash X3 in PBST.
44. For anti GM CSF mouse monoclonal antibody, detect binding using 50ul per well anti-mouse IgG-HRP (Dako, Cat No. P0260) at a 1/2000 dilution in block solution for 1hr at rtp. For 3G4 chimaeric antibody or humanised antibody, detect binding using 50ul per well anti-human kappa light chain-HRP (Sigma, Cat No. Sigma A7164) at a 1/2000 dilution in block solution for 1hr at rtp.
45. Wash X3 in PBST.
46. Develop with 100ul OPD (Sigma, Cat. No. P-9187. Made up as per maufacturers instructions), stop with 50ul 3M $H_2SO_4$, read at an absorbance of 490nm.

Table 13:

| Protein or polynucleotide description | Sequence identifier (SEQ.I.D.NO:) |
|---|---|
| 3G4, CDRH1 | 1 |
| 3G4, CDRH2 | 2 |
| 3G4, CDRH3 | 3 |
| 3G4, CDRL1 | 4 |
| 3G4, CDRL2 | 5 |
| 3G4, CDRL3 | 6 |
| 3G4, VH (murine) | 7 |
| 3G4, VL (murine) | 8 |
| hIL-13 | 9 |
| Polynucleotide encoding hIL-13 | 10 |
| 3G4, VH, humanised construct H1 | 11 |
| 3G4, VH, humanised construct H2 | 12 |
| 3G4, VH, humanised construct H3 | 13 |
| 3G4, VH, humanised construct H4 | 14 |
| 3G4, VL, humanised construct L1 | 15 |
| 3G4, VL, humanised construct L2 | 16 |
| 3G4, VL, humanised construct L3 | 17 |
| 3G4, heavy chain, humanised construct H1 | 18 |
| 3G4, heavy chain humanised construct H2 | 19 |
| 3G4, heavy chain humanised construct H3 | 20 |
| 3G4, heavy chain humanised construct H4 | 21 |
| 3G4, light chain humanised construct L1 | 22 |
| 3G4, light chain humanised construct L2 | 23 |
| 3G4, light chain humanised construct L3 | 24 |
| Polynucleotide encoding 3G4 VH (murine) (SEQ.I.D.NO:7) | 25 |
| Polynucleotide encoding 3G4 VL (murine) (SEQ.I.D.NO:8) | 26 |
| Polynucleotide encoding 3G4 VH humanised construct H1 (SEQ.I.D.NO:11) | 27 |
| Polynucleotide encoding 3G4 VH humanised construct H2 (SEQ.I.D.NO:12) | 28 |
| Polynucleotide encoding 3G4 VH humanised construct H3 (SEQ.I.D.NO:13) | 29 |
| Polynucleotide encoding 3G4 VH humanised construct H4 (SEQ.I.D.NO:14) | 30 |
| Polynucleotide encoding 3G4 VL humanised construct L1 (SEQ.I.D.NO:15) | 31 |
| Polynucleotide encoding 3G4 VL humanised construct L2(SEQ.I.D.NO:16) | 32 |
| Polynucleotide encoding 3G4 VL humanised construct L3 | 33 |

| | |
|---|---|
| (SEQ.I.D.NO:17) | |
| Polynucleotide encoding 3G4 heavy chain, humanised construct H1 (SEQ.I.D.NO:18) | 34 |
| Polynucleotide encoding 3G4 heavy chain humanised construct H2 (SEQ.I.D.NO:19) | 35 |
| Polynucleotide encoding 3G4 heavy chain humanised construct H3 (SEQ.I.D.NO:20) | 36 |
| Polynucleotide encoding 3G4 heavy chain humanised construct H4 (SEQ.I.D.NO:21) | 37 |
| Polynucleotide encoding 3G4 light chain humanised construct L1 (SEQ.I.D.NO:22) | 38 |
| Polynucleotide encoding 3G4 light chain humanised construct L2 (SEQ.I.D.NO:23) | 39 |
| Polynucleotide encoding 3G4 light chain humanised construct L3 (SEQ.I.D.NO:24) | 40 |
| Cynomolgus IL-13 | 41 |
| Cynomolgus IL-13 (polynucleotide) | 42 |
| Human signal sequence | 43 |
| Human acceptor framework sequence for 3G4 VH | 44 |
| Human acceptor framework sequence for 3G4 VL | 45 |
| Alternative polynucleotide encoding 3G4 VH humanised construct H2 (SEQ.I.D.NO:12) | 93 |
| Alternative polynucleotide encoding 3G4 VL humanised construct L1 (SEQ.I.D.NO:15) | 94 |
| Alternative polynucleotide encoding heavy chain humanised construct H2 (SEQ.I.D.NO:19) | 95 |
| Alternative polynucleotide encoding heavy chain humanised construct H3 (SEQ.I.D.NO:20) | 96 |
| Alternative polynucleotide encoding light chain humanised construct L1 (SEQ.I.D.NO:22) | 97 |

Note: Protein or DNA polynucleotide sequence in SEQ.ID numbers from 11 to 24 and 27 to 40 (inclusive) also include the signal sequence.

SEQ.I.D.NO 1

DYEIH

SEQ.I.D.NO 2

AIDPETGGTAYNQKFKG

SEQ.I.D.NO 3

ILLYYYPMDY

SEQ.I.D.NO 4

RASQNISDYLH

SEQ.I.D.NO 5

YASQSIS

SEQ.I.D.NO 6

QNGHSFPLT

SEQ.I.D.NO 7

QVQLQQSGADLVRPGASVTLSCKASGYTFIDYEIHWMKQTPVHGLEWIGAIDPETGGTAYNQ
KFKGKAILTADKSSSTAYMELRSLTSEDSAVYYCTRILLYYYPMDYWGQGTSVTVSS

SEQ.I.D.NO 8

DIVMTQSPATLSVTPGDRVSLSCRASQNISDYLHWYQQKSHESPRLLIKYASQSISGIPSRF
SGSGSGSDFTLSINSVEPEDVGVYYCQNGHSFPLTLGAGTKLELK

SEQ.I.D.NO:9

GPVPPSTALRELIEELVNITQNQKAPLCNGSMVWSINLTAGMYCAALESLINVSGCSAIEKT
QRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLFREGRFN

SEQ.I.D.NO:10

GGCCCTGTGCCTCCCTCTACAGCCCTCAGGGAGCTCATTGAGGAGCTGGTCAACATCACCCA
GAACCAGAAGGCTCCGCTCTGCAATGGCAGCATGGTATGGAGCATCAACCTGACAGCTGGCA
TGTACTGTGCAGCCCTGGAATCCCTGATCAACGTGTCAGGCTGCAGTGCCATCGAGAAGACC
CAGAGGATGCTGAGCGGATTCTGCCCGCACAAGGTCTCAGCTGGGCAGTTTTCCAGCTTGCA
TGTCCGAGACACCAAAATCGAGGTGGCCCAGTTTGTAAAGGACCTGCTCTTACATTTAAAGA
AACTTTTTCGCGAGGGACGGTTCAACTGA

SEQ.I.D.NO 11

MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFIDYEIHWVRQAPGQ
GLEWMGAIDPETGGTAYNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARILLYYYP
MDYWGQGTLVTVSS

SEQ.I.D.NO 12

MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFIDYEIHWVRQAPGQ
GLEWMGAIDPETGGTAYNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCTRILLYYYP
MDYWGQGTLVTVSS

SEQ.I.D.NO 13

MGWSCIILFLVATATGVHSQVQLVQSGADVKKPGASVKVSCKASGYTFIDYEIHWVRQAPGQ
GLEWMGAIDPETGGTAYNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCTRILLYYYP
MDYWGQGTLVTVSS

SEQ.I.D.NO 14

MGWSCIILFLVATATGVHSQVQLVQSGADVKKPGASVKVSCKASGYTFIDYEIHWVRQAPGQ
GLEWMGAIDPETGGTAYNQKFKGRATLTADKSTSTAYMELRSLRSDDTAVYYCTRILLYYYP
MDYWGQGTLVTVSS

SEQ.I.D.NO 15

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQNISDYLHWYQQKPGQA
PRLLIYYASQSISGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQNGHSFPLTFGGGTKVE
IK

SEQ.I.D.NO 16

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQNISDYLHWYQQKPGQA
PRLLIYYASQSISGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQNGHSFPLTLGGGTKVE
IK

SEQ.I.D.NO 17

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQNISDYLHWYQQKPGQA
PRLLIYYASQSISGIPARFSGSGSGTDFTLTINSLEPEDFAVYYCQNGHSFPLTLGGGTKVE
IK

SEQ.I.D.NO 18

MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFIDYEIHWVRQAPGQ
GLEWMGAIDPETGGTAYNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARILLYYYP
MDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ.I.D.NO 19

MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFIDYEIHWVRQAPGQ
GLEWMGAIDPETGGTAYNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCTRILLYYYP
MDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ.I.D.NO 20

MGWSCIILFLVATATGVHSQVQLVQSGADVKKPGASVKVSCKASGYTFIDYEIHWVRQAPGQ
GLEWMGAIDPETGGTAYNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCTRILLYYYP
MDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ.I.D.NO 21

MGWSCIILFLVATATGVHSQVQLVQSGADVKKPGASVKVSCKASGYTFIDYEIHWVRQAPGQ
GLEWMGAIDPETGGTAYNQKFKGRATLTADKSTSTAYMELRSLRSDDTAVYYCTRILLYYYP

MDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ.I.D.NO 22

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQNISDYLHWYQQKPGQA
PRLLIYYASQSISGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQNGHSFPLTFGGGTKVE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ.I.D.NO 23

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQNISDYLHWYQQKPGQA
PRLLIYYASQSISGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQNGHSFPLTLGGGTKVE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ.I.D.NO 24

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCRASQNISDYLHWYQQKPGQA
PRLLIYYASQSISGIPARFSGSGSGTDFTLTINSLEPEDFAVYYCQNGHSFPLTLGGGTKVE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ.I.D.NO 25

CAGGTTCAACTGCAGCAGTCTGGGGCTGACCTGGTGAGGCCTGGGGCTTCAGTGACGCTGTC
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TGCATGGCCTGGAATGGATTGGAGCTATTGATCCTGAAACTGGTGGTACAGCCTATAATCAG
AAGTTCAAGGGCAAGGCCATTCTGACTGCAGACAAATCCTCCAGTACAGCCTACATGGAGCT
CCGCAGCCTGACATCTGAGGACTCTGCCGTCTATTACTGTACAAGAATTCTCTTATATTACT
ATCCTATGGACTACTGGGGTCAAGGGACCTCAGTCACAGTCTCCTCA

SEQ.I.D.NO 26

GACATTGTGATGACTCAGTCTCCAGCCACCCTGTCTGTGACTCCAGGAGATAGAGTCTCTCT
TTCCTGCAGGGCCAGCCAGAATATTAGCGACTACTTACACTGGTATCAACAAAAATCACATG
AGTCTCCAAGGCTTCTCATCAAATATGCTTCCCAATCCATCTCTGGGATCCCCTCCAGGTTC
AGTGGCAGTGGATCAGGGTCAGATTTCACTCTCAGTATCAACAGTGTGGAACCTGAAGATGT

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TGGAGCTGAAA

SEQ.I.D.NO 27

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCCAGGT
GCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAGCCCGGCGCCAGCGTGAAGGTGAGCTGCA
AGGCCAGCGGCTACACCTTCATCGACTACGAGATCCACTGGGTGCGCCAGGCCCCCGGCCAG
GGCCTGGAGTGGATGGGCGCCATCGACCCCGAGACCGGCGGCACCGCCTACAACCAGAAGTT
CAAGGGCCGCGTGACCATGACCACCGACACCAGCACCAGCACCGCCTACATGGAGCTGCGCA
GCCTGCGCAGCGACGACACCGCCGTGTACTACTGCGCCCGCATCCTGCTGTACTACTACCCC
ATGGACTACTGGGGCCAGGGCACACTAGTCACAGTCTCCTCA

SEQ.I.D.NO 28

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCCAGGT
GCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAGCCCGGCGCCAGCGTGAAGGTGAGCTGCA
AGGCCAGCGGCTACACCTTCATCGACTACGAGATCCACTGGGTGCGCCAGGCCCCCGGCCAG
GGCCTGGAGTGGATGGGCGCCATCGACCCCGAGACCGGCGGCACCGCCTACAACCAGAAGTT
CAAGGGCCGCGTGACCATGACCACCGACACCAGCACCAGCACCGCCTACATGGAGCTGCGCA
GCCTGCGCAGCGACGACACCGCCGTGTACTACTGCACCCGCATCCTGCTGTACTACTACCCC
ATGGACTACTGGGGCCAGGGCACACTAGTCACAGTCTCCTCA

SEQ.I.D.NO 29

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCCAGGT
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AGGCCAGCGGCTACACCTTCATCGACTACGAGATCCACTGGGTGCGCCAGGCCCCCGGCCAG
GGCCTGGAGTGGATGGGCGCCATCGACCCCGAGACCGGCGGCACCGCCTACAACCAGAAGTT
CAAGGGCCGCGTGACCATGACCACCGACACCAGCACCAGCACCGCCTACATGGAGCTGCGCA
GCCTGCGCAGCGACGACACCGCCGTGTACTACTGCACCCGCATCCTGCTGTACTACTACCCC
ATGGACTACTGGGGCCAGGGCACACTAGTCACAGTCTCCTCA

SEQ.I.D.NO 30

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCCAGGT
GCAGCTGGTGCAGAGCGGCGCCGACGTGAAGAAGCCCGGCGCCAGCGTGAAGGTGAGCTGCA
AGGCCAGCGGCTACACCTTCATCGACTACGAGATCCACTGGGTGCGCCAGGCCCCCGGCCAG
GGCCTGGAGTGGATGGGCGCCATCGACCCCGAGACCGGCGGCACCGCCTACAACCAGAAGTT
CAAGGGCCGCGCCACCCTGACCGCCGACAAGAGCACCAGCACCGCCTACATGGAGCTGCGCA
GCCTGCGCAGCGACGACACCGCCGTGTACTACTGCACCCGCATCCTGCTGTACTACTACCCC
ATGGACTACTGGGGCCAGGGCACACTAGTCACAGTCTCCTCA

SEQ.I.D.NO 31

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCGAGAT
CGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTGAGCCCCGGCGAGCGCGCCACCCTGAGCT
GCCGCGCCAGCCAGAACATCAGCGACTACCTGCACTGGTACCAGCAGAAGCCCGGCCAGGCC
CCCCGCCTGCTGATCTACTACGCCAGCCAGAGCATCAGCGGCATCCCCGCCCGCTTCAGCGG
CAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGGAGCCCGAGGACTTCGCCG
TGTACTACTGCCAGAACGGCCACAGCTTCCCCCTGACCTTCGGCGGCGGCACCAAGGTGGAG
ATCAAG

SEQ.I.D.NO 32

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCGAGAT
CGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTGAGCCCCGGCGAGCGCGCCACCCTGAGCT
GCCGCGCCAGCCAGAACATCAGCGACTACCTGCACTGGTACCAGCAGAAGCCCGGCCAGGCC
CCCCGCCTGCTGATCTACTACGCCAGCCAGAGCATCAGCGGCATCCCCGCCCGCTTCAGCGG
CAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGGAGCCCGAGGACTTCGCCG
TGTACTACTGCCAGAACGGCCACAGCTTCCCCCTGACCCTGGGCGGCGGCACCAAGGTGGAG
ATCAAG

SEQ.I.D.NO 33

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCGAGAT
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GCCGCGCCAGCCAGAACATCAGCGACTACCTGCACTGGTACCAGCAGAAGCCCGGCCAGGCC
CCCCGCCTGCTGATCTACTACGCCAGCCAGAGCATCAGCGGCATCCCCGCCCGCTTCAGCGG
CAGCGGCAGCGGCACCGACTTCACCCTGACCATCAACAGCCTGGAGCCCGAGGACTTCGCCG
TGTACTACTGCCAGAACGGCCACAGCTTCCCCCTGACCCTGGGCGGCGGCACCAAGGTGGAG
ATCAAG

SEQ.I.D.NO 34

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCCAGGT
GCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAGCCCGGCGCCAGCGTGAAGGTGAGCTGCA
AGGCCAGCGGCTACACCTTCATCGACTACGAGATCCACTGGGTGCGCCAGGCCCCCGGCCAG
GGCCTGGAGTGGATGGGCGCCATCGACCCCGAGACCGGCGGCACCGCCTACAACCAGAAGTT
CAAGGGCCGCGTGACCATGACCACCGACACCAGCACCAGCACCGCCTACATGGAGCTGCGCA
GCCTGCGCAGCGACGACACCGCCGTGTACTACTGCGCCCGCATCCTGCTGTACTACTACCCC
ATGGACTACTGGGGCCAGGGCACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATC
GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC
GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT

GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA
GCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCA
CCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAA
GGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACG
AAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA
AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCA
CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC
CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
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CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGA
CCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
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SEQ.I.D.NO 35

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCCAGGT
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GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC
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CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC
CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
CCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGA
CCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

SEQ.I.D.NO 36

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCCAGGT
GCAGCTGGTGCAGAGCGGCGCCGACGTGAAGAAGCCCGGCGCCAGCGTGAAGGTGAGCTGCA
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CAAGGGCCGCGTGACCATGACCACCGACACCAGCACCAGCACCGCCTACATGGAGCTGCGCA
GCCTGCGCAGCGACGACACCGCCGTGTACTACTGCACCCGCATCCTGCTGTACTACTACCCC
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GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC
GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
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AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCA
CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC
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CCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGA
CCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

SEQ.I.D.NO 37

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCCAGGT
GCAGCTGGTGCAGAGCGGCGCCGACGTGAAGAAGCCCGGCGCCAGCGTGAAGGTGAGCTGCA
AGGCCAGCGGCTACACCTTCATCGACTACGAGATCCACTGGGTGCGCCAGGCCCCCGGCCAG
GGCCTGGAGTGGATGGGCGCCATCGACCCCGAGACCGGCGGCACCGCCTACAACCAGAAGTT
CAAGGGCCGCGCCACCCTGACCGCCGACAAGAGCACCAGCACCGCCTACATGGAGCTGCGCA
GCCTGCGCAGCGACGACACCGCCGTGTACTACTGCACCCGCATCCTGCTGTACTACTACCCC
ATGGACTACTGGGGCCAGGGCACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATC
GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC
GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA
GCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCA
CCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAA
GGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACG
AAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA
AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCA
CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC

CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
CCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGA
CCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

SEQ.I.D.NO 38

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCGAGAT
CGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTGAGCCCCGGCGAGCGCGCCACCCTGAGCT
GCCGCGCCAGCCAGAACATCAGCGACTACCTGCACTGGTACCAGCAGAAGCCCGGCCAGGCC
CCCCGCCTGCTGATCTACTACGCCAGCCAGAGCATCAGCGGCATCCCCGCCCGCTTCAGCGG
CAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGGAGCCCGAGGACTTCGCCG
TGTACTACTGCCAGAACGGCCACAGCTTCCCCCTGACCTTCGGCGGCGGCACCAAGGTGGAG
ATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTAC
AGTGGAAGGTGGACAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC
AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAA
ACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGTTAG

SEQ.I.D.NO 39

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCGAGAT
CGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTGAGCCCCGGCGAGCGCGCCACCCTGAGCT
GCCGCGCCAGCCAGAACATCAGCGACTACCTGCACTGGTACCAGCAGAAGCCCGGCCAGGCC
CCCCGCCTGCTGATCTACTACGCCAGCCAGAGCATCAGCGGCATCCCCGCCCGCTTCAGCGG
CAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGGAGCCCGAGGACTTCGCCG
TGTACTACTGCCAGAACGGCCACAGCTTCCCCCTGACCCTGGGCGGCGGCACCAAGGTGGAG
ATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTAC
AGTGGAAGGTGGACAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC
AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAA
ACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGTTAG

SEQ.I.D.NO 40

ATGGGCTGGAGCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGTGCACAGCGAGAT
CGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTGAGCCCCGGCGAGCGCGCCACCCTGAGCT
GCCGCGCCAGCCAGAACATCAGCGACTACCTGCACTGGTACCAGCAGAAGCCCGGCCAGGCC

CCCCGCCTGCTGATCTACTACGCCAGCCAGAGCATCAGCGGCATCCCCGCCCGCTTCAGCGG
CAGCGGCAGCGGCACCGACTTCACCCTGACCATCAACAGCCTGGAGCCCGAGGACTTCGCCG
TGTACTACTGCCAGAACGGCCACAGCTTCCCCCTGACCCTGGGCGGCGGCACCAAGGTGGAG
ATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTAC
AGTGGAAGGTGGACAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC
AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAA
ACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGTTAG

SEQ.I.D.NO:41

SPVPPSTALKELIEELVNITQNQKAPLCNGSMVWSINLTAGVYCAALESLINVSGCSAIEKT
QRMLNGFCPHKVSAGQFSSLRVRDTKIEVAQFVKDLLVHLKKLFREGQFN

SEQ.I.D.NO:42

AGCCCTGTGCCTCCCTCTACAGCCCTCAAGGAGCTCATTGAGGAGCTGGTCAACATCACCCA
GAACCAGAAGGCCCCGCTCTGCAATGGCAGCATGGTGTGGAGCATCAACCTGACAGCTGGCG
TGTACTGTGCAGCCCTGGAATCCCTGATCAACGTGTCAGGCTGCAGTGCCATCGAGAAGACC
CAGAGGATGCTGAACGGATTCTGCCCGCACAAGGTCTCAGCTGGGCAGTTTTCCAGCTTGCG
TGTCCGAGACACCAAAATCGAGGTGGCCCAGTTTGTAAAGGACCTGCTCGTACATTTAAAGA
AACTTTTTCGCGAGGGACAGTTCAACTGA

SEQ.I.D.NO:43

MGWSCIILFLVATATGVHS

SEQ.I.D.NO:44

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQ
KLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARXXXXXXXXXXWGQGTLVTVSS

SEQ.I.D.NO:45

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCXXXXXXXXXFGGGTKVEIK

SEQ.I.D.NO:46

SGSGPSTALRELIEELVNIT

SEQ.I.D.NO:47

SGSGLRELIEELVNITQNQK

SEQ.I.D.NO:48

SGSGIEELVNITQNQKAPLC

SEQ.I.D.NO:49

SGSGVNITQNQKAPLCNGSM

SEQ.I.D.NO:50

SGSGQNQKAPLCNGSMVWSI

SEQ.I.D.NO:51

SGSGAPLCNGSMVWSINLTA

SEQ.I.D.NO:52

SGSGNGSMVWSINLTAGMYC

SEQ.I.D.NO:53

SGSGVWSINLTAGMYCAALE

SEQ.I.D.NO:54

SGSGNLTAGMYCAALESLIN

SEQ.I.D.NO:55

SGSGGMYCAALESLINVSGC

SEQ.I.D.NO:56

SGSGAALESLINVSGCSAIE

SEQ.I.D.NO:57

SGSGSLINVSGCSAIEKTQR

SEQ.I.D.NO:58

SGSGVSGCSAIEKTQRMLSG

SEQ.I.D.NO:59

SGSGSAIEKTQRMLSGFCPH

SEQ.I.D.NO:60

SGSGKTQRMLSGFCPHKVSA

SEQ.I.D.NO:61

SGSGMLSGFCPHKVSAGQFS

SEQ.I.D.NO:62

SGSGFCPHKVSAGQFSSLHV

SEQ.I.D.NO:63

SGSGKVSAGQFSSLHVRDTK

SEQ.I.D.NO:64

SGSGGQFSSLHVRDTKIEVA

SEQ.I.D.NO:65

SGSGSLHVRDTKIEVAQFVK

SEQ.I.D.NO:66

SGSGRDTKIEVAQFVKDLLL

SEQ.I.D.NO:67

SGSGIEVAQFVKDLLLHLKK

SEQ.I.D.NO:68

SGSGQFVKDLLLHLKKLFRE

SEQ.I.D.NO:69

SGSGDLLLHLKKLFREGRFN

SEQ.I.D.NO:70

SGSGPSTALKELIEELVNIT

SEQ.I.D.NO:71

SGSGLKELIEELVNITQNQK

SEQ.I.D.NO:72

SGSGNGSMVWSINLTAGVYC

SEQ.I.D.NO:73

SGSGVWSINLTAGVYCAALE

SEQ.I.D.NO:74

SGSGNLTAGVYCAALESLIN

SEQ.I.D.NO:75

SGSGGVYCAALESLINVSGC

SEQ.I.D.NO:76

SGSGVSGCSAIEKTQRMLNG

SEQ.I.D.NO:77

SGSGSAIEKTQRMLNGFCPH

SEQ.I.D.NO:78

SGSGKTQRMLNGFCPHKVSA

SEQ.I.D.NO:79

SGSGMLNGFCPHKVSAGQFS

SEQ.I.D.NO:80

SGSGFCPHKVSAGQFSSLRV

SEQ.I.D.NO:81

SGSGKVSAGQFSSLRVRDTK

SEQ.I.D.NO:82

SGSGGQFSSLRVRDTKIEVA

SEQ.I.D.NO:83

SGSGSLRVRDTKIEVAQFVK

SEQ.I.D.NO:84

SGSGRDTKIEVAQFVKDLLV

SEQ.I.D.NO:85

SGSGIEVAQFVKDLLVHLKK

SEQ.I.D.NO:86

SGSGQFVKDLLVHLKKLFRE

SEQ.I.D.NO:87

SGSGDLLVHLKKLFREGQFN

SEQ.I.D.NO:88

DLLLHLKKLFREGRFN

SEQ.I.D.NO:89

DLLVHLKKLFREGQFN

SEQ.I.D.NO:90

QFVKDLLLHLKKLFREGRFN

SEQ.I.D.NO:91

LLHLKKLFREG

SEQ.I.D.NO:92

LKKLFRE

SEQ ID No. 93

CAGGTGCAGCTGGTGCAGAGCGGAGCCGAGGTGAAGAAGCCTGGCGCCAGCGTCAAGGTG
TCCTGCAAGGCCAGCGGCTACACCTTCATCGACTACGAGATCCACTGGGTGCGGCAGGCT
CCTGGACAGGGCCTGGAATGGATGGGCGCCATCGACCCCGAGACAGGCGGCACCGCCTAC
AACCAGAAGTTCAAGGGCCGGGTCACCATGACCACCGACACCAGCACCAGCACCGCCTAT
ATGGAACTGCGGAGCCTGAGAAGCGACGACACCGCCGTGTACTACTGCACCCGGATCCTG
CTGTACTACTACCCCATGGACTACTGGGGCCAGGGCACACTAGTCACCGTGAGCAGC

SEQ ID No. 94

GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTGAGCCCTGGCGAGCGGGCCACC
CTGTCCTGCCGGGCCAGCCAGAACATCAGCGACTACCTGCACTGGTATCAGCAGAAGCCC
GGCCAGGCCCCCAGGCTGCTGATCTACTACGCCAGCCAGTCCATCTCCGGCATCCCCGCC
AGGTTCAGCGGCAGCGGCTCCGGCACCGACTTCACCCTGACCATCAGCTCTCTGGAACCC
GAGGACTTCGCCGTGTATTATTGCCAGAACGGCCACAGCTTCCCCCTGACCTTTGGCGGC
GGAACAAAGGTGGAGATCAAG

SEQ ID No. 95

ATGGGATGGAGCTGCATCATCCTCTTCCTGGTGGCCACGGCTACCGGCGTGCATAGCCAGGT
GCAGCTCGTCCAGTCTGGGGCCGAGGTGAAGAAGCCCGGAGCTTCTGTGAAGGTGTCCTGCA
AGGCCAGCGGCTATACCTTCATCGACTACGAGATCCATTGGGTGAGGCAGGCTCCCGGGCAG
GGCCTGGAGTGGATGGGCGCCATCGACCCAGAGACCGGAGGCACGGCGTACAACCAGAAGTT
CAAGGGACGGGTCACCATGACAACCGATACCAGCACCTCCACCGCTTACATGGAGCTGCGCA
GCCTGAGAAGCGACGACACCGCGGTGTACTACTGTACGCGCATCCTGCTCTACTACTACCCC
ATGGATTACTGGGGCCAGGGCACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATC

GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC
GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA
GCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCA
CCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAA
GGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACG
AAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA
AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCA
CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC
CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
CCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGA
CCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

SEQ ID No. 96

ATGGGATGGAGCTGCATCATCCTCTTCCTGGTGGCCACGGCTACCGGCGTGCATAGCCAGGT
GCAGCTCGTCCAGTCTGGGGCCGACGTGAAGAAGCCCGGAGCTTCTGTGAAGGTGTCCTGCA
AGGCCAGCGGCTATACCTTCATCGACTACGAGATCCATTGGGTGAGGCAGGCTCCCGGGCAG
GGCCTGGAGTGGATGGGCGCCATCGACCCAGAGACCGGAGGCACGGCGTACAACCAGAAGTT
CAAGGGACGGGTCACCATGACAACCGATACCAGCACCTCCACCGCTTACATGGAGCTGCGCA
GCCTGAGAAGCGACGACACCGCGGTGTACTACTGTACGCGCATCCTGCTCTACTACTACCCC
ATGGATTACTGGGGCCAGGGCACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATC
GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC
GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA
GCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCA
CCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAA
GGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACG
AAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA
AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCA
CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC
CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
CCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGA
CCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

SEQ ID No. 97

ATGGGATGGTCTTGTATCATCCTGTTCCTGGTGGCGACCGCCACCGGCGTGCACTCCGAGAT
CGTGCTGACCCAGAGTCCAGCCACCCTCAGCCTGAGCCCTGGGGAACGCGCCACCCTGTCCT
GCCGGGCGAGTCAGAACATCTCCGACTACCTGCATTGGTACCAGCAGAAGCCCGGCCAGGCC
CCTCGCCTGCTGATCTACTACGCCTCCCAGAGCATCAGCGGAATCCCCGCCCGGTTCTCCGG
AAGTGGGTCCGGAACCGACTTTACCCTGACCATCAGCTCTCTCGAGCCAGAGGACTTCGCGG
TGTACTACTGCCAGAACGGGCATAGTTTCCCACTGACCTTCGGAGGGGGCACAAAGGTGGAG
ATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTAC
AGTGGAAGGTGGACAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC
AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAA
ACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGTTAG

SEQ ID NO:98

LLVHLKKLFREG

## Claims

1. An antibody or antigen binding fragment thereof which specifically binds hIL-13 and comprises the CDRH3 as defined in SEQ.I.D.NO:3 or variants thereof in which one or two amino acid residues within said CDRH3 differ from the amino acid residues in the corresponding position in SEQ ID NO:3.

2. An antibody or antigen binding fragment thereof according to claim 1 wherein the antibody or antigen binding fragment neutralises human IL-13.

3. An antibody or antigen binding fragment thereof according to claim 1 or 2 wherein the antibody or antigen binding fragment modulates the binding of human IL-13 to its receptor.

4. An antibody or antigen binding fragment thereof according to any one of claims 1 to 3 wherein the CDRH3 comprises the sequence of SEQ ID NO:3.

5. An antibody or antigen binding fragment thereof according to any one of claims 1 to 4 wherein the antibody or antigen binding fragment thereof further comprises one or more of the following sequences CDRH2: SEQ.I.D.NO:2, CDRH1: SEQ.I.D.NO:1, CDRL1: SEQ.I.D.NO:4, CDRL2: SEQ.I.D.NO:5 and CDRL3: SEQ.I.D.NO:6 or a variant thereof in which one or two amino acid residues within said CDR differ from the amino acid residues in the corresponding position in the SEQ ID NO.

6. An antibody or antigen binding fragment thereof according to any preceding claim wherein the antibody or antigen binding fragment thereof comprises the following CDRs:
CDRH1: SEQ.I.D.NO:1
CDRH2: SEQ.I.D.NO:2
CDRH3: SEQ.I.D.NO:3
CDRL1: SEQ.I.D.NO:4
CDRL2: SEQ.I.D.NO:5
CDRL3: SEQ.I.D.NO:6

7. An antibody or antigen binding fragment thereof which binds to the peptides set out in SEQ ID NO:90, 99, 102, 103, 105, 106, 107, 108, 109, 110, 111, 112 and 114 but does not bind the peptides set out in SEQ ID NO:100, 101, 104 and 113.

8. An antibody or antigen binding fragment thereof according to any preceding claim which specifically binds the epitope set forth in SEQ.I.D.NO:91.

9. An antibody or antigen binding fragment thereof of any preceding claim wherein the antibody is an intact antibody.

10. An antibody or antigen binding fragment thereof of claim 9 wherein the antibody is a rat, mouse, primate (e.g. cynomolgus, Old World monkey or Great Ape) or human.

11. An antibody of any one of claims 1 to 9 wherein the antibody is a humanised or chimaeric antibody.

12. The antibody of any one of claims 9 to 11 wherein the antibody comprises a human constant region.

13. The antibody of claim 9 to 12 wherein the antibody comprises a constant region of IgG isotype.

14. The antibody of claim 13 wherein the antibody is IgG1, IgG2 or IgG4.

15. The antibody of claim 6 comprising a VH domain of SEQ.I.D.NO:7 and a VL domain of SEQ.I.D.NO:8.

16. A humanised antibody of claim 6 comprising a VH domain of SEQ.I.D.NO:12 and a VL domain of SEQ.I.D.NO:15.

17. A humanised antibody of claim 6 comprising a VH domain of SEQ.I.D.NO:13 and a VL domain of SEQ.I.D.NO:15.

18. A humanised antibody of claim 16 or 17 further comprising a human constant region of a IgG isotype (e.g. IgG1 or IgG4).

19. A humanised antibody comprising a heavy chain of SEQ.I.D.NO:19 and a light chain of SEQ.I.D.NO:22.

20. A humanised antibody comprising a heavy chain of SEQ.I.D.NO:20 and a light chain of SEQ.I.D.NO:22.

21. An antigen binding fragment of any one of claims 1-8 wherein the fragment is a Fab, Fab', $F(ab')_2$, Fv, diabody, triabody, tetrabody, miniantibody, minibody, isolated VH, isolated VL.

22. An antigen binding fragment according to claim 21 wherein the fragment comprises or consists of a ScFv.

23. An antibody according to any one of claims 1 to 20 comprising a mutated Fc region such that said antibody has reduced ADCC and/or complement activation.

24. A recombinant transformed or transfected host cell comprising a first and second vector, said first vector comprising a polynucleotide encoding a heavy chain of an antibody according to any preceding claim and said second vector comprising a polynucleotide encoding a light chain of any preceding claim.

25. The host cell of claim 24 wherein the cell is eukaryotic.

26. The host cell of claim 25 wherein the cell is mammalian.

27. The host cell of claim 26 wherein the cell is a CHO cell or a NS0 cell.

28. A method for the production of an antibody of any one of claims 1 to 20 or claim 23 which method comprises the step of culturing a host cell of any one of claims 24 to 27 in a serum-free culture media.

29. The method of claim 28 wherein said antibody is secreted by said host cell into said culture media.

30. The method of claim 29 wherein said antibody is further purified to at least 95% or greater (e.g. 98% or greater) with respect to said antibody containing culture media.

31. A pharmaceutical composition comprising an antibody or antigen fragment binding thereof of any one of claims 1 to 23 and a pharmaceutically acceptable carrier.

32. A kit-of-parts comprising the composition of claim 31 together with instructions for use.

33. A method of treating a human patient afflicted with asthma which method comprises the step of administering a therapeutically effective amount of the antibody or antigen binding fragment of any one of claims 1 to 23.

34. The method of claim 33 wherein said patient is afflicted with asthma selected from allergic asthma, severe asthma, difficult asthma, brittle asthma nocturnal asthma, premenstrual asthma, steroid resistant asthma, steroid dependent asthma, aspirin induced asthma, adult-onset asthma, paediatric asthma.

35. A method of treating a human patient afflicted with an asthmatic condition which is refractory to treatment with corticosteroids which method comprises the step of administering to said patient a therapeutically effective amount of the antibody or antigen binding fragment of any one of claims 1 to 23.

36. A method of preventing acute asthmatic attacks in a human patient which method comprises the step of administering to said patient a therapeutically effective amount of an antibody of claims 1 to 23.

37. A method of reducing the frequency of and/or mitigating the effects of acute asthmatic attacks in a human patient which method comprises the step of administering to said patient a therapeutically effective amount of an antibody of any one of claims 1 to 23.

38. A method of treating a human patient afflicted with a disease or disorder selected from the group consisting of atopic dermatitis, allergic rhinitis, Crohn's disease, COPD, fibrotic diseases or disorders such as idiopathic pulmonary fibrosis, progressive systemic sclerosis, hepatic fibrosis, hepatic granulomas, schistosomiasis, leishmaniasis, diseases of cell cycle regulation such as Hodgkins disease, B cell chronic lymphocytic leukaemia which method comprises administering to a human patient a therapeutically effective amount of an antibody of any one of claims 1 to 23.

39. Use of an antibody or antigen binding fragment thereof of any one of claims 1 to 23 in the manufacture of a medicament for the treatment of a disease or disorder selected from the group consisting of; Allergic asthma, severe asthma, difficult asthma, brittle asthma, nocturnal asthma, premenstrual asthma, steroid resistant asthma, steroid dependent asthma, aspirin induced asthma, adult-onset asthma, paediatric asthma, atopic dermatitis, allergic rhinitis, Crohn's disease, COPD, fibrotic diseases or disorders such as idiopathic pulmonary fibrosis, progressive systemic sclerosis, hepatic fibrosis, hepatic granulomas, schistosomiasis, leishmaniasis, diseases of cell cycle regulation such as Hodgkins disease, B cell chronic lymphocytic leukaemia.

40. A method of treating a human patient afflicted with a disease or disorder selected from the group consisting of; Allergic asthma, severe asthma, difficult asthma, brittle asthma, nocturnal asthma, premenstrual asthma, steroid resistant asthma, steroid dependent asthma, aspirin induced asthma, adult-onset asthma, paediatric asthma, atopic dermatitis, allergic rhinitis, Crohn's disease, COPD, fibrotic diseases or disorders such as idiopathic pulmonary fibrosis, progressive systemic sclerosis, hepatic fibrosis, hepatic granulomas, schistosomiasis, leishmaniasis, diseases of cell cycle regulation such as Hodgkins disease, B cell chronic lymphocytic leukaemia; which method comprises administering a therapeutically effective amount of an antibody of any one of claims 1 to 23 and a therapeutically effective amount of an anti-IL-4 monoclonal antibody.

41. The method of claim 40 wherein the anti-IL-4 monoclonal antibody is administered simultaneously, sequentially or separately with the antibody of any one of claims 1 to 23.

42. The method of claim 40 or 41 wherein the anti-IL-4 antibody is pascolizumab.

43. Use of an antibody of any one of claims 1 to 23 and an anti-IL-4 monoclonal antibody such as pascolizumab in the manufacture of a medicament for the treatment of a disease or disorder selected from the group consisting of; Allergic asthma, severe asthma, difficult asthma, brittle asthma, nocturnal asthma, premenstrual asthma, steroid resistant asthma, steroid dependent asthma, aspirin induced asthma, adult-onset asthma, paediatric asthma, atopic dermatitis, allergic rhinitis, Crohn's disease, COPD, fibrotic diseases or disorders such as idiopathic pulmonary fibrosis, progressive systemic sclerosis, hepatic fibrosis, hepatic granulomas, schistosomiasis, leishmaniasis, diseases of cell cycle regulation such as Hodgkins disease, B cell chronic lymphocytic leukaemia.

44. Use of an antibody of any one of claims 1 to 23 and an anti-IL-4 monoclonal antibody such as pascolizumab in the manufacture of a kit-of-parts for the treatment of a disease or disorder selected from the group consisting of; Allergic asthma, severe asthma, difficult asthma, brittle asthma, nocturnal asthma, premenstrual asthma, steroid resistant asthma, steroid dependent asthma, aspirin induced asthma, adult-onset asthma, paediatric asthma, atopic dermatitis, allergic rhinitis, Crohn's disease, COPD, fibrotic diseases or disorders such as idiopathic pulmonary fibrosis, progressive systemic sclerosis, hepatic fibrosis, hepatic granulomas, schistosomiasis, leishmaniasis, diseases of cell cycle regulation such as Hodgkins disease, B cell chronic lymphocytic leukaemia.

45. A kit-of-parts comprising a first pharmaceutical composition comprising an antibody of any one of claims 1 to 23 and a pharmaceutically acceptable carrier and a second pharmaceutical composition comprising an anti-IL-4 monoclonal antibody such as pascolizumab and a pharmaceutically acceptable carrier optionally together with instructions for use.

46. A pharmaceutical composition comprising a first antibody of any one of claims 1 to 23 and a second antibody wherein said second antibody is an anti-IL-4 antibody such as pascolizumab and a pharmaceutically acceptable carrier.