ANTIBODIES

The present invention relates to an anti-CSF-1R antibody and binding fragments thereof, DNA encoding the same, host cells comprising said DNA and methods of expressing the antibody or binding fragment in a host cell. The invention also extends to pharmaceutical compositions comprising the antibody or a binding fragment thereof and use of the antibody, binding fragment and compositions comprising the same in treatment.

The colony stimulating factor 1 (CSF-1), also known as macrophage colony stimulating factor (M-CSF) is a cytokine produced by a variety of cells, including macrophages, endothelial cells and fibroblasts. CSF-1 is composed of two "monomer" polypeptides, which form a biologically active dimeric CSF-1 protein. CSF-1 exists in at least three mature forms due to alternative RNA splicing (see, Cerretti *et al.,* 1988, Molecular Immunology, 25:761). The three forms of CSF-1 are translated from different mRNA precursors, which encode polypeptide monomers of 256 to 554 amino acids, having a 32 amino acid signal sequence at the amino terminus and a putative transmembrane region of approximately 23 amino acids near the carboxyl terminus. The precursor peptides are subsequently processed by amino terminal and carboxyl terminal proteolytic cleavages to release mature CSF-1. Residues 1-149 of all three mature forms of CSF-1 are identical and are believed to contain sequences essential for biological activity of CSF-1. *In vivo* CSF-1 monomers are glycosylated and dimerized via disulfide-linkage. CSF-1 belongs to a group of biological agonists that promote the production of blood cells. Specifically, it acts as a growth and differentiation factor for bone marrow progenitor cells of the mononuclear phagocyte lineage. Further, CSF-1 stimulates the survival, proliferation and function of macrophages via a specific receptor on responding cells.

The CSF-1 receptor (CSF-1R) is also referred to as the c-fms gene product or CD115. CSF-1R is a 165kDa type 1 TM glycoprotein belonging to the type III receptor tyrosine kinase family. In addition to CSF-1, the structurally similar but sequence unrelated molecule IL-34 has also been shown to be a ligand for CSF-1R (Lin, *et al.,* 2008, Science 320:807-811). Expression of CSF-1R is restricted to cells of the monocyte-macrophage lineage, both circulating and resident tissue populations, and osteoclasts. In addition, it is expressed in a number of cells of the female reproductive system including oocytes, decidual cells and trophoblasts.

Binding of the ligand CSF-1 to the CSF-1 receptor results in the phosphorylation of the receptor on one or more tyrosine residues, through the action of the tyrosine kinase domain. This

phosphorylation can be detected because antibodies are available that bind to the receptor only after phosphorylation (for example Phospho-M-CSF-Receptor (Tyr546) antibody #3083 from Cell Signaling Technology).

Expression of CSF-1 and CSF-1R correlates with tumour progression and poor diagnosis in many cancer types. Tumour-associated macrophages (TAMs) can be the major component of tumour stroma and high levels of CSF-1 and CSF-1R are associated with high TAM infiltrations and poor prognosis in a number of tumour types.

Antibodies to CSF-1R are known in the art. Sherr, C.J. *et al.,* 1989, Blood 73:1786-1793 describes antibodies against CSF-1R that inhibit the CSF-1 activity (Sherr, C.J. *et al.*,1989, Blood 73:1786-1793). WO2009/026303 discloses anti-CSF-1R antibodies which bind to human CSF-1R and *in vivo* mouse tumour models using an anti-murine CSF-1R antibody. WO2011/123381 discloses anti-CSF-1R antibodies which internalize CSF-1R and have ADCC activity. WO2011/123381 also discloses *in vivo* mouse tumour models using an anti-murine CSF-1R antibody. WO2011/140249 discloses anti-CSF-1R antibodies which block binding of CSF-1 to CSF-1R and are stated to be useful in the treatment of cancer. WO2009/112245 discloses an anti-CSF-1R IgG1 antibody which inhibits CSF-1 binding to CSF-1R and is stated to be useful in the treatment of cancer, inflammatory bowel disease and rheumatoid arthritis. WO2011/131407 discloses an anti-CSF-1R antibody which inhibits CSF-1 binding to CSF-1R and is stated to be useful in the treatment of bone loss and cancer. WO2011/107553 discloses an anti-CSF-1R antibody which inhibits CSF-1 binding to CSF-1R thought to be useful in the treatment of bone loss and cancer. WO2011/070024 discloses anti-CSF-1R antibodies which bind to human CSF-1R fragment delD4.

There is a need in the art to provide new anti-CSF-1R antibodies suitable for therapeutic applications. Whilst the therapeutic application of anti-CSF-1R antibodies in treating certain cancers has been previously described, there is still a need to provide new therapeutic applications for such antibodies.

The term 'fibrotic disease' refers to an aberrant wound healing response wherein excess fibrous connective tissue is formed in an organ or tissue. The deposition and accumulation of excess extracellular matrix components, such as collagen and fibronectrin, results in the hardening and scarring of tissues that ultimately can lead to organ failure.

Examples of fibrotic diseases include pulmonary fibrosis, such as idiopathic pulmonary fibrosis and cystic fibrosis, renal fibrosis, liver fibrosis, liver cirrhosis, primary sclerosing cholangitis, primary biliary cirrhosis, endomyocardial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, progressive massive fibrosis, nephrogenic systemic fibrosis, Crohn's disease, keloid, myocardial infarction, systemic sclerosis, scleroderma and arthofibrosis.

Wounding results in an immediate coagulation and clotting response with the development of a provisional extracellular matrix (ECM). The platelet aggregation and activation helps promote an inflammatory response characterised by vasodilation and an increase in blood vessel permeability allowing the recruitment of a variety of immune cells including neutrophils, macrophages, eosinophils and lymphocytes. Neutrophils and macrophages debride the wound thereby reducing the risk of infection and together with activated lymphocytes secrete a variety of growth factors and cytokines that serve to further amplify the inflammatory response. Molecules such as TGFβ, PDGF, IL-13 activate macrophages and lead to the recruitment, proliferation and activation of fibroblasts at the wound site. Activated fibroblasts or myofibroblasts, are characterised by the expression of α-smooth muscle actin and secrete collagen and other ECM components. The activated fibroblasts, contract the collagen lattice, drawing the edges of the wound to the centre. Epithelial and endothelial cells proliferate and migrate over the temporary matrix to regenerate the damaged tissue completing the wound repair.

Persistent tissue insult or injury or a disregulation of the repair pathway leads to an inappropriate wound response. Excess deposition and hyper cross-linking of the collagen and ECM occurs resulting in excessive formation and hardening of the scar tissue in place of the normal tissue architecture.

The cause of fibrotic disease can be dependent upon the organ or tissue involved and is unknown in some diseases such as idiopathic pulmonary fibrosis (IPF). Liver fibrosis and ultimately cirrhosis results from chronic liver damage sustained through exposure to a variety of factors including environmental and dietary factors or infectious agents. Long-term hepatitis B and C infections can cause liver fibrosis. Sustained over consumption of alcohol or a high fat/sugar diet can also lead to cirrhosis of the liver. Similarly, diabetes can damage and scar the kidneys leading to loss of function.

IPF is one of seven interstitial lung diseases the cause of which is unknown. Environmental factors such as radiation exposure or particles may play a role. Individuals who smoke are also at higher risk of this disease. Once diagnosed, the life-span of patients is very short with the average survival rate being 2-5 years.

Treatment of fibrotic disease typically includes anti-inflammatory and immunosuppressive agents but these are of little benefit for the patient. The lack of efficacy with these treatments contributed to the reconsideration of IPF and fibrotic disease in general, as an aberrant response to wound healing and not an inflammatory condition. Pirfenidone is a small molecule drug that was approved for use in the treatment of IPF in Japan in 2008 and Europe in 2011, which is likely to work via multiple mechanisms of action. To date, no targeted therapies and no antibody therapies have been approved for fibrotic indications.

Therefore, there is currently an unmet medical need for improved treatment of fibrotic disease. For example, for IPF there is a 3 year survival rate of 50% and a 5 year survival rate of only 20% and transplantation is required in about 20% of cases.

Summary of the Disclosure

In one aspect there is provided an anti-CSF-1R antibody or binding fragment thereof comprising a heavy chain, wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO:4 for CDR-H1, a CDR having the sequence given in SEQ ID NO:5 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:6 for CDR-H3, for example wherein CDR-H1 is SEQ ID NO: 4, CDR-H2 is SEQ ID NO: 5 and CDR-H3 is SEQ ID NO: 6.

In one aspect the antibodies or binding fragments according to the present disclosure comprise a light chain wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:1 for CDR-L1, a CDR having the sequence given in SEQ ID NO:2 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:3 for CDR-L3, for example wherein CDR-L1 is SEQ ID NO: 1, CDR-L2 is SEQ ID NO: 2 and CDR-L3 is SEQ ID NO: 3.

The antibodies of the disclosure have a high affinity for CSF-1R, are able to block binding of ligand to CSF-1R, are non-activating to CSF-1R and do not cause internalization of CSF-1R.

The disclosure also extends to a polynucleotide, such as DNA, encoding an antibody or fragment as described herein.

Also provided is a host cell comprising said polynucleotide.

Methods of expressing an antibody or binding fragment thereof are provided herein.

The present disclosure also relates to pharmaceutical compositions comprising said antibodies or binding fragments thereof.

In one embodiment there is provided a method of treatment comprising administering a therapeutically effective amount of an antibody, fragment or composition as described herein.

The present disclosure also extends to an antibody, binding fragment or composition according to the present disclosure for use in treatment, particularly in the treatment of cancer and/or fibrotic disease.

Details of the Disclosure

In one embodiment the antibodies provided by the present invention are capable of blocking ligand binding to CSF-1R. Blocking as employed herein refers to physically blocking such as occluding the receptor but will also include where the antibody or fragments binds an epitope that causes, for example a conformational change which means that the natural ligand to the receptor no longer binds (referred to herein as allosteric blocking or allosteric inhibition). In one embodiment the anitbodies of the present disclosure bind all isotypes of CSF-1R, for example those with variations in the ECD domain, such as V23G, A245S, H247P, V279M and combinations of two, three or four of said variations.

Assays suitable for determining the ability of an antibody to block CSF-1R are described in the Examples herein. CSF-1 and IL-34 are both ligands for CSF-1R and the antibodies of the invention preferably inhibit the activity both CSF-1 and IL-34 in a functional cellular screen. The antibodies according to the present invention also preferably do not cause CSF-1R activation and/or CSF-1R internalisation. The antibodies according to the present invention also preferably selectively deplete the non-classical population of monocytes *in vivo*.

Non-classic monocytes generally refers to monocytes with low expression of CD14 and high expression of CD16. This population of monocytes are thought to be pre-cursors of tumor associated macrophages.

The antibody molecules of the present invention suitably have a high binding affinity. Affinity may be measured using any suitable method known in the art, including techniques such

as surface plasmon resonance, for example BIAcore, as described in the Examples herein, using isolated natural or recombinant CSF-1R or a suitable fusion protein/polypeptide. In one example affinity is measured using recombinant human CSF-1R extracellular domain as described in the Examples herein. In one example the recombinant human CSF-1R extracellular domain used is a monomer. Suitably the antibody molecules of the present invention have a binding affinity for isolated human CSF-1R of about 1nM or less than 1nM. In one embodiment the antibody molecule of the present invention has a binding affinity of about 500pM or lower. In one embodiment the antibody molecule of the present invention has a binding affinity of about 250pM or lower. In one embodiment the antibody molecule of the present invention has a binding affinity of about 200pM or lower. In one embodiment the present invention provides an anti-CSF-1R antibody with a binding affinity of about 100pM or lower. In one embodiment the present invention provides a humanised anti-CSF-1R antibody with a binding affinity of about 100pM or lower, preferably about 10pM or lower, more preferably about 5pM or lower. In another embodiment the present invention provides a humanised anti-CSF-1R antibody with a binding affinity of about 100pM or lower, preferably about 10pM or lower, more preferably about 5pM or lower

The lower the numerical value of the affinity the higher the affinity of the antibody or fragment for the antigen.

Human CSF-1R as employed herein refers to the human protein name CSF-1R or a biological active fragment thereof, for example as given in SEQ ID NO: 39 or registered in UniProt under the number P07333. Of course the expressed mature protein does not comprise the signal sequence because the latter is cleaved post translation.

The present inventors have provided new anti-CSF-1R antibodies, including humanised antibodies. The antibodies were generated from immunisation of rats with rat fibroblasts that were transfected with a vector expressing CSF-1R extracellular domain. Primary screening of supernatants for human CSF-1R binding of antibody identified approximately 1000 wells containing antibody with anti-CSF-1R activity. Secondary screening for antibodies capable of preventing human CSF-1 binding to human CSF-1R identified 88 positive wells. Tertiary screening for antibodies capable of preventing CSF-1 dependent survival of primary human monocytes identified 18 positive wells. The variable regions of these 18 positive wells were cloned, which lead to successful cloning of 14 antibodies and subsequent expression provided 9

chimeric anti-CSF-1R antibodies which expressed at sufficient levels and were capable of inhibiting CSF-1 binding. These 9 antibodies were sequenced and found to all have unique sequences and were used for further study.

The 9 anti-CSF-1R chimeric antibodies were assessed for ligand-blocking activity and ability to inhibit CSF-1 and IL-34 mediated monocyte survival. Four of the antibodies were prioritised for further investigation because they demonstrated complete inhibition of CSF-1 binding and high levels of inhibition of monocyte survival. These 4 anti-CSF-1R chimeric antibodies were tested for their activity in a number of *in vitro* assays to assess affinity, inhibition of CSF-1 binding, cross-reactivity with rhesus monkey, cynomolgus monkey and canine CSF-1R, CSF-1R internalization and CSF-1R activation. The four anti-CSF-1R antibodies were also humanised and affinity of the humanised grafts was measured. The humanisation of two of the anti-CSF-1R antibodies generated fully humanised antibodies (no rat donor residues present) with affinity (K_D) equivalent to the parental chimeric antibody and a Tm that indicating the antibody having suitable thermal stability. In contrast, the other two humanised anti-CSF-1R antibodies had a reduced affinity for CSF-1R, relative to the chimeric antibody, and the Tm was lower. For these reasons, only the fully humanised grafts of two of the antibodies which retained affinity were expressed at a larger scale for further analysis.

Further analysis of the fully humanised grafts of these two antibodies was carried out and an MCP-1 inhibition assay, where the inhibition of CSF-1R signalling by antibody that blocks CSF-1 binding caused a reduction in the levels of MCP-1 secretion. This assay surprisingly revealed that fully humanised grafts exhibited reduced activity compared to the chimeric antibody. A series of experiments on one of the preferred antibodies, termed Ab969, was carried out to reveal why the fully humanised graft exhibited this reduced activity. A number of intermediate humanised grafts of Ab969 were generated and tested in the MCP-1 inhibition assay. It was found that grafts which contained the variable light chain donor residue Y71 generally showed activity in the MCP-1 inhibition assay comparable to that of the chimeric Ab969. It was hypothesised that this difference in activity of the various antibody grafts in the MCP-1 inhibition assay was due to a change in the antibody on-rate (decreased K_a) compared to the chimeric Ab969.

Comparing the thermal stability analysis of Ab969 with other anti-CSF-1R antibodies e.g. anti-CSF-1R antibody Ab970, suggest that Ab969 may be more stable.

Further biophysical analysis of humanised grafts of Ab969 revealed that some grafts precipitated when the antibody was concentrated. It was shown that a substitution of the lysine residue at position 38 in the light chain, for example glutamine, lead to an improved physical stability.

Accordingly, one antibody graft of Ab969, Ab969.g2 (also referred to as Ab969, was selected for further *in vitro* characterisation studies. In addition to the advantageous high binding affinity to CSF-1R, high thermal stability and high physical stability the antibody demonstrated good inhibition of IL-34 dependent monocyte activation and was also found to be capable of binding to SNP variants of CSF-1R. Ab969.g2 was also used for pharmacodynamics marker analysis in a cynomolgus monkey where it was shown to bind CSF-1R, block CSF-1 binding and selectively deplete the non-classical population of cynomolgus monkey monocytes *in vivo*, which are precursor cells of tumour-associated macrophages.

Accordingly, in one embodiment, the present invention provides an antibody comprising a heavy chain and/or a light chain, wherein the heavy chain and/or light chain comprise at least one CDR derived from the anti-CSF-1R antibody 969.2.

Ab969.2 is a full-length humanised IgG4 molecule; the light chain comprises a human kappa chain constant region (Km3 allotype) and the heavy chain comprises a human gamma-4 heavy chain constant region with the hinge stabilising mutation S241P (Angal *et al.*, 1993). A potential DG isomerisation motif is present within the light chain variable region at the junction of CDR-L2 and the framework. The sequences of Ab969.2 full antibody heavy and light chains are shown in SEQ ID NOs: 27 and 19.

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat *et al.*, 1987. This system is set forth in Kabat *et al.*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat *et al.* (*supra*)"). This numbering system is used in the present specification except where otherwise indicated.

The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues

may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. However, according to Chothia (Chothia, C. and Lesk, A.M., J. Mol. Biol., 196, 901-917 (1987)), the loop equivalent to CDR-H1 extends from residue 26 to residue 32. Thus unless indicated otherwise 'CDR-H1' as employed herein is intended to refer to residues 26 to 35, as described by a combination of the Kabat numbering system and Chothia's topological loop definition.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system.

Antibodies for use in the present disclosure may be obtained using any suitable method known in the art. The CSF-1R polypeptide/protein including fusion proteins, cells (recombinantly or naturally) expressing the polypeptide can be used to produce antibodies which specifically recognise CSF-1R. The polypeptide may be the 'mature' polypeptide or a biologically active fragment or derivative thereof. The human protein is registered in UniProt under the number P07333.

Polypeptides, for use to immunize a host, may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems or they may be recovered from natural biological sources. In the present application, the term "polypeptides" includes peptides, polypeptides and proteins. These are used interchangeably unless otherwise specified. The CSF-1R polypeptide may in some instances be part of a larger protein such as a fusion protein for example fused to an affinity tag or similar.

Antibodies generated against the CSF-1R polypeptide may be obtained, where immunisation of an animal is necessary, by administering the polypeptides to an animal, preferably a non-human animal, using well-known and routine protocols, see for example Handbook of Experimental Immunology, D. M. Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986). Many warm-blooded animals, such as rabbits, mice, rats, sheep, cows, camels or pigs may be immunized. However, mice, rabbits, pigs and rats are generally most suitable.

Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.,* 1983, Immunology Today, 4:72) and the EBV-hybridoma technique (Cole *et al.,* 1985, Monoclonal Antibodies and Cancer Therapy, pp77-96, Alan R Liss, Inc.).

Antibodies may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by, for example, the methods described by Babcook, J. *et al.*, 1996, Proc. Natl. Acad. Sci. USA 93:7843-7848; WO92/02551; WO04/051268 and International Patent Application number WO04/106377.

Screening for antibodies can be performed using assays to measure binding to human CSF-1R and/or assays to measure the ability to block ligand binding to the receptor. Examples of suitable assays are described in the Examples herein.

Specific as employed herein is intended to refer to an antibody that only recognises the antigen to which it is specific or an antibody that has significantly higher binding affinity to the antigen to which it is specific compared to binding to antigens to which it is non-specific, for example at least 5, 6, 7, 8, 9, 10 times higher binding affinity.

The amino acid sequences and the polynucleotide sequences of certain antibodies according to the present disclosure are provided in Figures 1 and 2.

In one aspect of the invention the antibody is an anti-CSF-1R antibody or binding fragment thereof comprising a heavy chain, wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO: 4 for CDR-H1, a CDR having the sequence given in SEQ ID NO:5 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:6 for CDR-H3. Preferably the variable domain of the heavy chain comprises the sequence given in SEQ ID NO: 4 for CDR-H1, the sequence given in SEQ ID NO:5 for CDR-H2 and the sequence given in SEQ ID NO:6 for CDR-H3.

In a second aspect of the invention the antibody is an anti-CSF-1R antibody or binding fragment thereof, comprising a light chain, wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO: 1 for CDR-L1, a CDR having the sequence given in SEQ ID NO:2 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:3 for CDR-L3. Preferably the variable domain of the light chain comprises

the sequence given in SEQ ID NO:1 for CDR-H1, the sequence given in SEQ ID NO:2 for CDR-H2 and the sequence given in SEQ ID NO:3 for CDR-H3.

In one embodiment the antibody of the invention is an anti-CSF-1R antibody or binding fragment thereof comprising a heavy chain as defined above and additionally comprising a light chain wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO: 1 for CDR-L1, a CDR having the sequence given in SEQ ID NO:2 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:3 for CDR-L3. The variable domain of the light chain preferably comprises the sequence given in SEQ ID NO:1 for CDR-L1, the sequence given in SEQ ID NO:2 for CDR-L2 and the sequence given in SEQ ID NO:3 for CDR-L3.

In one embodiment, at least one amino acid is replaced with a conservative substitution in one or more CDRs selected from the group consisting independently of:

any one of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, CDR-L3;

any one of the combinations CDR-H1 and H2, CDR-H1 and H3, CDR-H1 and L1, CDR-H1 and L2, CDR-H1 and L3, CDR-H2 and H3, CDR-H2 and L1, CDR-H2 and L2, CDR-H2 and L3, CDR-H3 and L1, CDR-H3 and L2, CDR-H3 and L3, CDR-L1 and L2, CDR-L1 and L3, CDR-L2 and L3;

CDR-H1, H2 and H3, CDR-H1, H2 and L1, CDR-H1, H2 and L2, CDR-H1, H2 and L3, CDR-H2, H3 and L1, CDR-H2, H3 and L2, CDR-H2, H3 and L3, CDR-H3, L1 and L2, CDR-H3, L1 and L3, CDR-L1, L2, L3;

any one of the combinations CDR-H1, H2, H3 and L1, CDR-H1, H2, H3 and L2, CDR-H1, H2, H3 and L3, CDR-H2, H3, L1 and L2, CDR-H2, H3, L2 and L3, CDR-H3, L1, L2 and L3, CDR-L1, L2, L3 and H1, CDR-L1, L2, L3 and H2, CDR-L1, L2, L3 and H3, CDR-L2, L3, H1 and H2,

CDR-H1, H2, H3, L1 and L2, CDR-H1, H2, H3, L1 and L3, CDR-H1, H2, H3, L2 and L3, CDR-L1, L2, L3, H1 and H2, CDR-L1, L2, L3, H1 and H3, CDR-L1, L2, L3, H2 and H3; and

the combination CDR-H1, H2, H3, L1, L2 and L3.

In one embodiment, a domain of the heavy chain disclosed herein includes the sequence with 1, 2, 3 or 4 conservative amino acid substitutions, for example wherein the substitutions are in the framework.

In one embodiment, the framework of the heavy chain variable region comprises 1, 2, 3, or 4 amino acids which have been inserted, deleted, substituted or a combination thereof. In one embodiment, the substituted amino acid is a corresponding amino acid from the donor antibody.

In one embodiment, a light variable region disclosed herein includes the sequence with 1, 2, 3 or 4 conservative amino acid substitutions, for example wherein the substitutions are in the framework.

In one embodiment, the framework of the light chain variable region comprises 1, 2, 3 or 4 amino acid which have been inserted, deleted substituted or a combination thereof. In one embodiment the substituted amino is a corresponding amino acid form a donor antibody.

In one aspect of the present invention, there is provided an anti-CSF-1R antibody or binding fragment thereof, wherein the variable domain of the heavy chain comprises three CDRs and the sequence of CDR-H1 has at least 60%, 70%, 80%, 90% or 95% identity or similarity to the sequence given in SEQ ID NO:4, the sequence of CDR-H2 has at least 60%, 70%, 80%, 90% or 95% identity or similarity to the sequence given in SEQ ID NO:5 and the sequence of CDR-H-3 has at least 60%, 70%, 80%, 90% or 95% identity or similarity to the sequence given in SEQ ID NO:6. Preferably, the anti-CSF-1R antibody or binding fragment thereof, additionally comprising a light chain, wherein the variable domain of the light chain comprises three CDRs and the sequence of CDR-L1 has at least 60%, 70%, 80%, 90% or 95% identity or similarity to the sequence given in SEQ ID NO:1, the sequence of CDR-L2 has at least 60%, 70%, 80%, 90% or 95% identity or similarity to the sequence given in SEQ ID NO:2 and the sequence of CDR-L3 has at least 60% identity or similarity to the sequence given in SEQ ID NO:3.

In one embodiment a variable regions is provided with at least 60%, 70%, 80%, 90% or 95% identity or similarity to a variable region sequence disclosed herein. In another embodiment there is provided an anti-CSF-1R antibody which competes with the binding of an antibody or fragment of the invention for binding to the CSF-1R receptor, preferably the extracellular domain of the CSF-1R receptor, more specifically to the CSF-1R receptor of SEQ ID NO:35, 36, 37, 38 and/or 39 or the sequence in the UniProt database entry P07333, in particular to the extracellular domain of the CSF-1R receptor of SEQ ID NO: 36 or the 498 amino acids of the extracellular domain disclosed in the UniProt database entry P07333 (amino acid 20 to 517 of P07333).

In one embodiment there is provided an anti-CSF-1R antibody which cross-blocks the binding of an antibody comprising a the 6 CDRs given in sequence SEQ ID NO:1 for CDR-L1, SEQ ID NO:2 for CDR-L2, SEQ ID NO:3 for CDR-L3, SEQ ID NO:4 for CDR-H1, SEQ ID NO:5 for CDR-H2 and SEQ ID NO:6 for CDR-H3, for example with affinity of 100pM or less, in particular wherein the cross blocking is allosteric.

In another embodiment, there is provided an anti-CSF-1R-antibody or binding fragment thereof which inhibits or overlaps with the binding of CSF-1 and/or IL-34 to the extracellular domain of the CSF-1R receptor.

In one embodiment there is provided an anti-CSF-1R antibody which cross-blocks the binding of an antibody comprising a the 6 CDRs given in sequence SEQ ID NO:1 for CDR-L1, SEQ ID NO:2 for CDR-L2, SEQ ID NO:3 for CDR-L3, SEQ ID NO:4 for CDR-H1, SEQ ID NO:5 for CDR-H2 and SEQ ID NO:6 for CDR-H3, for example with affinity of 100pM or less, in particular wherein the antibody cross-blocks the binding by binding the same epitope as the antibody which it blocks.

In one embodiment the antibody or binding fragment thereof is provided wherein a C-terminal residue of the antibody sequence is cleaved, for example the C-terminal residue of a heavy chain sequence, for example a terminal lysine. In one embodiment the amino acid is cleaved from a sequence disclosed herein. Generally the cleavage results from post-translation modifications of the expressed antibody or binding fragment.

In another embodiment the anti-CSF-1R antibody of any of the embodiments supra or infra is provided wherein the C-terminal lysine of the heavy chain sequence given in SEQ ID NO: 27 or SEQ ID NO: 29 is missing or deleted. Missing or deleted C-terminal lysine e.g. position 453 of SEQ ID NO: 27 or position 472 of SEQ ID NO: 30 can be achieved, for example by expression of the anti-CSF-1R antibody in an expression system without coding the terminal lysine. Alternatively the deletion of a C-terminal residue, such as lysine, may be effected as a post translational modification.

In one embodiment the antibody or binding fragments according to the invention is humanised.

As used herein, the term 'humanised antibody refers to an antibody or antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a murine monoclonal antibody) grafted into a

heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody) (see, *e.g.* US 5,585,089; WO91/09967). For a review, see Vaughan *et al*, Nature Biotechnology, 16, 535-539, 1998. In one embodiment rather than the entire CDR being transferred, only one or more of the specificity determining residues from any one of the CDRs described herein above are transferred to the human antibody framework (see for example, Kashmiri *et al.*, 2005, Methods, 36:25-34). In one embodiment only the specificity determining residues from one or more of the CDRs described herein above are transferred to the human antibody framework. In another embodiment only the specificity determining residues from each of the CDRs described herein above are transferred to the human antibody framework. When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions.

Suitably, the humanised antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs provided specifically herein. Thus, provided in one embodiment is a humanised antibody which binds human CSF-1R wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat *et al., supra*). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available at: http://vbase.mrc-cpe.cam.ac.uk/

In a humanised antibody of the present invention, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

In one embodiment a human framework comprises 1, 2, 3, or 4 amino acid substitutions, additions or deletions, for example 1, 2, 3 or 4 conservative substitutions or substitutions of donor residues.

In one embodiment the sequence employed as a human framework is 80%, 85%, 90%, 95% or more similar or identical to a sequence disclosed herein.

One such suitable framework region for the heavy chain of the humanised antibody of the present invention is derived from the human sub-group VH2 sequence 3-1 2-70 together with JH3 J-region (SEQ ID NO: 33).

Accordingly, in one example there is provided a humanised antibody comprising the sequence given in SEQ ID NO: 4 for CDR-H1, the sequence given in SEQ ID NO: 5 for CDR-H2 and the sequence given in SEQ ID NO: 6 for CDR-H3, wherein the heavy chain framework region is derived from the human subgroup VH3 sequence 1-3 3-07 together with JH4.

In one example the heavy chain variable domain of the antibody comprises the sequence given in SEQ ID NO: 23.

A suitable framework region for the light chain of the humanised antibody of the present invention is derived from the human germline sub-group VK1 2-1-(1) O12 together with JK4 J-region (SEQ ID NO: 31).

Accordingly, in one example there is provided a humanised antibody comprising the sequence given in SEQ ID NO: 1 for CDR-L1, the sequence given in SEQ ID NO: 2 for CDR-L2 and the sequence given in SEQ ID NO: 3 for CDR-L3, wherein the light chain framework region is derived from the human subgroup VK1 2-1-(1) O12 plus JK4 J-region.

In one example the light chain variable domain of the antibody comprises the sequence given in SEQ ID NO: 15.

In a humanised antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann *et al.*, 1998, Nature, 332:323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO91/09967.

Accordingly, in one example there is provided a humanised antibody, wherein at least the residue at position 78 of the variable domain of the heavy chain (Kabat numbering) is a donor residue. In one embodiment residue 78 of the heavy chain variable domain is replaced with alanine.

Donor residue as employed herein refers to a residue form the non-human antibody (e.g. murine antibody) which donated the CDRs.

In one embodiment there is provided a humanised antibody wherein the heavy chain variable domain does not contain any donor residues.

Accordingly, in one example there is provided a humanised antibody, wherein at least one of the residues at positions 38, 71 and 87 of the variable domain of the light chain (Kabat numbering) are donor residues. In one embodiment one of the residues selected from the residues at positions 38, 71 and 87 of the variable domain of the light chain (Kabat numbering) is a donor residue. In one embodiment two of the residues selected from the residues at positions 38, 71 and 87, for example 38 and 71; or 38 and 87; or 71 and 87, of the variable domain of the light chain (Kabat numbering) are donor residues. In one embodiment the three residues at positions 38, 71 and 87 of the variable domain of the light chain (Kabat numbering) are donor residues.

In one embodiment residue 38 of the light chain variable domain is replaced with lysine. In an alternative embodiment residue 38 of the light chain variable domain is replaced with glutamine.

In one embodiment residue 71 of the light chain variable domain is replaced with tyrosine.

In one embodiment residue 87 of the light chain variable domain is replaced with phenylalanine.

In one embodiment there is provided a humanised antibody wherein only residue 71 of the light chain variable region is a donor residue, preferably tyrosine.

In a particular embodiment, the present invention provides an anti-CSF-1R antibody or binding fragment thereof having a heavy chain comprising the heavy chain variable domain sequence given in SEQ ID NO: 23 and a light chain comprising the light chain variable domain sequence given in SEQ ID NO: 15.

In a further aspect of the present invention, an anti-CSF-1R antibody or binding fragment thereof is provided which binds CSF-1R preferably the extracellular domain of CSF-1R, most preferably the extracellular domain of human CSF-1R, wherein the antibody or binding fragment comprises the light chain variable domain of SEQ ID NO: 15 and the heavy chain variable domain of SEQ ID NO: 23, preferably wherein the antibody or binding fragment thereof is a

monoclonal antibody, is an antibody of the IgG1- IgG2-, IgG4-type, is a Fab, modified Fab, Fab', modified Fab', $F(ab')_2$, Fv, single domain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibody, Bis-scFv, diabody, triabody or tetrabody.

In one embodiment the disclosure provides an antibody sequence which is 80% similar or identical to a sequence disclosed herein, for example 85%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% over part or whole of the relevant sequence. In one embodiment the relevant sequence is SEQ ID NO: 15. In one embodiment the relevant sequence is SEQ ID NO: 23.

"Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains). Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987, Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991, the BLAST™ software available from NCBI (Altschul, S.F. *et al.,* 1990, J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. 1993, Nature Genet. 3:266-272. Madden, T.L. *et al.,* 1996, Meth. Enzymol. 266:131-141; Altschul, S.F. *et al.,* 1997, Nucleic Acids Res. 25:3389-3402; Zhang, J. & Madden, T.L. 1997, Genome Res. 7:649-656).

The antibody molecules of the present invention may comprise a complete antibody molecule having full length heavy and light chains or a binding fragment thereof and may be, but

are not limited to Fab, modified Fab, Fab', modified Fab', $F(ab')_2$, Fv, single domain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma *et al.*, 1998, Journal of Immunological Methods, 216:165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO05/003169, WO05/003170 and WO05/003171. Multi-valent antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO92/22853, WO05/113605, WO2009/040562 and WO2010/035012).

Binding fragment of an antibody as employed herein refers to a fragment capable of binding an antigen with affinity to characterise the fragment as specific for the antigen.

In one embodiment the antibody according to the present disclosure is provided as CSF-1R binding antibody fusion protein which comprises an immunoglobulin moiety, for example a Fab or Fab' fragment, and one or two single domain antibodies (dAb) linked directly or indirectly thereto, for example as described in WO2009/040562, WO2010/035012, WO2011/030107, WO2011/061492 and WO2011/086091 all incorporated herein by reference.

In one embodiment the fusion protein comprises two domain antibodies, for example as a variable heavy (VH) and variable light (VL) pairing, optionally linked by a disulphide bond.

In one embodiment the Fab or Fab' element of the fusion protein has the same or similar specificity to the single domain antibody or antibodies. In one embodiment the Fab or Fab' has a different specificity to the single domain antibody or antibodies, that is to say the fusion protein is multivalent. In one embodiment a multivalent fusion protein according to the present invention has an albumin binding site, for example a VH/VL pair therein provides an albumin binding site.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody

molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required.

In a specific embodiment, the antibody of the present invention is an IgG2 or IgG4 antibody.

It will be appreciated that sequence variants of these constant region domains may also be used. For example IgG4 molecules in which the serine at position 241 has been changed to proline as described in Angal *et al.*, 1993, Molecular Immunology, 1993, 30:105-108 may be used. Accordingly, in the embodiment where the antibody is an IgG4 antibody, the antibody may include the mutation S241P.

It will also be understood by one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. *Journal of Chromatography* 705:129-134, 1995). Accordingly, the C-terminal lysine of the antibody heavy chain may be absent.

In one embodiment the antibody heavy chain comprises a CH1 domain and the antibody light chain comprises a CL domain, either kappa or lambda.

In one embodiment the antibody heavy chain comprises a CH1 domain, a CH2 domain and a CH3 domain and the antibody light chain comprises a CL domain, either kappa or lambda.

An antibody provided by the present invention has a heavy chain comprising the sequence given in SEQ ID NO: 27 and a light chain comprising the sequence given in SEQ ID NO: 19. Also provided is an anti-CSF-1R antibody or binding fragment thereof, in which the heavy and light chains are at least 80% (preferably 85%, 90%, 95% or 98%) identical or similar to a heavy chain comprising the sequence given in SEQ ID NO: 27 and a light chain comprising the sequence given in SEQ ID NO: 19. In one embodiment, the light chain has or consists of the sequence given in SEQ ID NO: 19 and the heavy chain has or consists of the sequence given in SEQ ID NO: 27. In another embodiment, the light chain has or consists of the sequence of SEQ

ID NO: 19 and the heavy chain has or consists of the sequence of SEQ ID NO: 27, wherein the amino acid lysine at position 453 of SEQ ID NO: 27 is missing or deleted.

Also provided by the present invention is a specific region or epitope of human CSF-1R which is bound by an antibody provided by the present invention, in particular an antibody 969.g2 comprising the heavy chain sequence gH2 (SEQ ID NO: 27) and/or the light chain sequence gL7 (SEQ ID NO: 19).

This specific region or epitope of the human CSF-1R polypeptide can be identified by any suitable epitope mapping method known in the art in combination with any one of the antibodies provided by the present invention. Examples of such methods include screening peptides of varying lengths derived from CSF-1R for binding to the antibody of the present invention with the smallest fragment that can specifically bind to the antibody containing the sequence of the epitope recognised by the antibody (for example a peptide in the region of about 5 to 20, preferably about 7 amino acids in length). The CSF-1R peptides may be produced synthetically or by proteolytic digestion of the CSF-1R polypeptide. Peptides that bind the antibody can be identified by, for example, mass spectrometric analysis. In another example, NMR spectroscopy or X-ray crystallography can be used to identify the epitope bound by an antibody of the present invention. Once identified, the epitopic fragment which binds an antibody of the present invention can be used, if required, as an immunogen to obtain additional antibodies which bind the same epitope.

Biological molecules, such as antibodies or fragments, contain acidic and/or basic functional groups, thereby giving the molecule a net positive or negative charge. The amount of overall "observed" charge will depend on the absolute amino acid sequence of the entity, the local environment of the charged groups in the 3D structure and the environmental conditions of the molecule. The isoelectric point (pI) is the pH at which a particular molecule or solvent accessible surface thereof carries no net electrical charge. In one example, the CSF-1R antibody and fragments of the invention may be engineered to have an appropriate isoelectric point. This may lead to antibodies and/or fragments with more robust properties, in particular suitable solubility and/or stability profiles and/or improved purification characteristics.

Thus in one aspect the invention provides a humanised CSF-1R antibody engineered to have an isoelectric point different to that of the originally identified antibody. The antibody may, for example be engineered by replacing an amino acid residue such as replacing an acidic amino acid residue with one or more basic amino acid residues. Alternatively, basic amino acid

residues may be introduced or acidic amino acid residues can be removed. Alternatively, if the molecule has an unacceptably high pI value acidic residues may be introduced to lower the pI, as required. It is important that when manipulating the pI care must be taken to retain the desirable activity of the antibody or fragment. Thus in one embodiment the engineered antibody or fragment has the same or substantially the same activity as the "unmodified" antibody or fragment.

Programs such as ** ExPASY http://www.expasy.ch/tools/pi_tool.html, and http://www.iut-arles.up.univ-mrs.fr/w3bb/d_abim/compo-p.html, may be used to predict the isoelectric point of the antibody or fragment.

It will be appreciated that the affinity of antibodies provided by the present invention may be altered using any suitable method known in the art. The present invention therefore also relates to variants of the antibody molecules of the present invention, which have an improved affinity for CSF-1R. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang *et al.*, 1995, J. Mol. Biol., 254:392-403), chain shuffling (Marks *et al.*, 1992, Bio/Technology, 10:779-783), use of mutator strains of *E. coli* (Low *et al.*, 1996, J. Mol. Biol., 250:359-368), DNA shuffling (Patten *et al.*, 1997,Curr. Opin. Biotechnol., 8:724-733), phage display (Thompson *et al.*, J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Crameri *et al.*, 1998, Nature, 391:288-291). Vaughan *et al.* (*supra*) discusses these methods of affinity maturation.

If desired an antibody for use in the present invention may be conjugated to one or more effector molecule(s). It will be appreciated that the effector molecule may comprise a single effector molecule or two or more such molecules so linked as to form a single moiety that can be attached to the antibodies of the present invention. Where it is desired to obtain an antibody fragment linked to an effector molecule, this may be prepared by standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector molecule. Techniques for conjugating such effector molecules to antibodies are well known in the art (see, Hellstrom *et al.*, Controlled Drug Delivery, 2nd Ed., Robinson *et al.*, eds., 1987, pp. 623-53; Thorpe *et al.*, 1982, Immunol. Rev., 62:119-58 and Dubowchik *et al.*, 1999, Pharmacology and Therapeutics, 83, 67-123). Particular chemical procedures include, for example, those described in WO93/06231, WO92/22583, WO89/00195, WO89/01476 and WO03/031581. Alternatively, where the effector molecule is a protein or

polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO86/01533 and EP0392745.

The term effector molecule as used herein includes, for example, antineoplastic agents, drugs, toxins, biologically active proteins, for example enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups, such as fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.

Examples of effector molecules may include cytotoxins or cytotoxic agents including any agent that is detrimental to (*e.g.* kills) cells. Examples, include combrestatins, dolastatins, epothilones, staurosporin, maytansinoids, spongistatins, rhizoxin, halichondrins, roridins, hemiasterlins, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Effector molecules also include, but are not limited to, antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.* mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.* daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.* dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-mitotic agents (*e.g.* vincristine and vinblastine).

Other effector molecules may include chelated radionuclides such as ^{111}In and ^{90}Y, Lu^{177}, Bismuth^{213}, Californium^{252}, Iridium^{192} and Tungsten^{188}/Rhenium^{188}; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

Other effector molecules include proteins, peptides and enzymes. Enzymes of interest include, but are not limited to, proteolytic enzymes, hydrolases, lyases, isomerases, transferases. Proteins, polypeptides and peptides of interest include, but are not limited to, immunoglobulins, toxins such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, a protein such as

insulin, tumour necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), nerve growth factor (NGF) or other growth factor and immunoglobulins.

Other effector molecules may include detectable substances useful, for example in diagnosis. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and acquorin; and suitable radioactive nuclides include ^{125}I, ^{131}I, ^{111}In and ^{99}Tc.

In another example the effector molecule may increase the half-life of the antibody *in vivo*, and/or reduce immunogenicity of the antibody and/or enhance the delivery of an antibody across an epithelial barrier to the immune system. Examples of suitable effector molecules of this type include polymers, albumin, albumin binding proteins or albumin binding compounds such as those described in WO05/117984.

In one embodiment a half-life provided by an effector molecule which is independent of CSF-1R is advantageous.

Where the effector molecule is a polymer it may, in general, be a synthetic or a naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, e.g. a homo- or hetero- polysaccharide.

Specific optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups.

Specific examples of synthetic polymers include optionally substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol), such as methoxypoly(ethyleneglycol) or derivatives thereof.

Specific naturally occurring polymers include lactose, amylose, dextran, glycogen or derivatives thereof.

In one embodiment the polymer is albumin or a fragment thereof, such as human serum albumin or a fragment thereof.

"Derivatives" as used herein is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be appreciated that the residue of such a group will in some instances form part of the product as the linking group between the antibody fragment and the polymer.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, for example from 5000 to 40000Da, such as from 20000 to 40000Da. The polymer size may in particular be selected on the basis of the intended use of the product, for example ability to localize to certain tissues such as tumors or extend circulating half-life (for review see Chapman, 2002, Advanced Drug Delivery Reviews, 54, 531-545). Thus, for example, where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use a small molecular weight polymer, for example with a molecular weight of around 5000Da. For applications where the product remains in the circulation, it may be advantageous to use a higher molecular weight polymer, for example having a molecular weight in the range from 20000Da to 40000Da.

Suitable polymers include a polyalkylene polymer, such as a poly(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 15000Da to about 40000Da.

In one example antibodies for use in the present invention are attached to poly(ethyleneglycol) (PEG) moieties. In one particular example the antibody is an antibody fragment and the PEG molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free

amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids may occur naturally in the antibody fragment or may be engineered into the fragment using recombinant DNA methods (see for example US 5,219,996; US 5,667,425; WO98/25971, WO2008/038024). In one example the antibody molecule of the present invention is a modified Fab fragment wherein the modification is the addition to the C-terminal end of its heavy chain one or more amino acids to allow the attachment of an effector molecule. Suitably, the additional amino acids form a modified hinge region containing one or more cysteine residues to which the effector molecule may be attached. Multiple sites can be used to attach two or more PEG molecules.

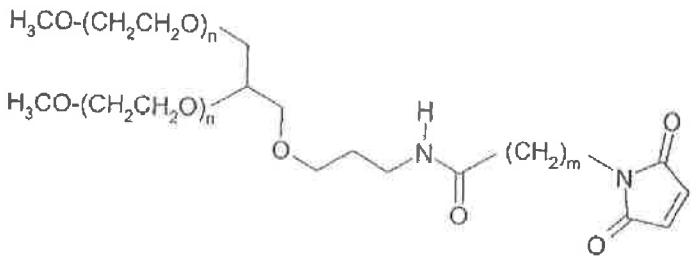
Suitably PEG molecules are covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond. Where a thiol group is used as the point of attachment appropriately activated effector molecules, for example thiol selective derivatives such as maleimides and cysteine derivatives may be used. An activated polymer may be used as the starting material in the preparation of polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an α-halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Nektar, formerly Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures. Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

In one embodiment, the antibody is a modified Fab fragment, Fab' fragment or diFab which is PEGylated, *i.e.* has PEG (poly(ethyleneglycol)) covalently attached thereto, *e.g.* according to the method disclosed in EP 0948544 or EP1090037 [see also "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam

and A. Dent, Grove Publishers, New York; Chapman, A. 2002, Advanced Drug Delivery Reviews 2002, 54:531-545]. In one example PEG is attached to a cysteine in the hinge region. In one example, a PEG modified Fab fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue may be covalently linked to the maleimide group and to each of the amine groups on the lysine residue may be attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000Da. The total molecular weight of the PEG attached to the Fab fragment may therefore be approximately 40,000Da.

Particular PEG molecules include 2-[3-(N-maleimido)propionamido]ethyl amide of N,N'-bis(methoxypoly(ethylene glycol) MW 20,000) modified lysine, also known as PEG2MAL40K (obtainable from Nektar, formerly Shearwater).

Alternative sources of PEG linkers include NOF who supply GL2-400MA3 (wherein m in the structure below is 5) and GL2-400MA (where m is 2) and n is approximately 450:

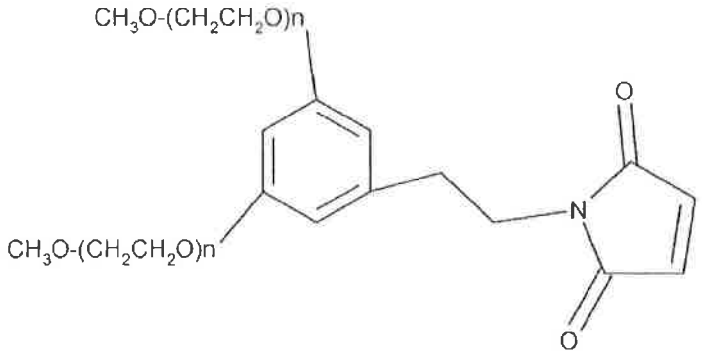


m is 2 or 5

That is to say each PEG is about 20,000Da.

Thus in one embodiment the PEG is 2,3-Bis(methylpolyoxyethylene-oxy)-1-{[3-(6-maleimido-1-oxohexyl)amino]propyloxy} hexane (the 2 arm branched PEG, $-CH_2)_3NHCO(CH_2)_5$-MAL, Mw 40,000 known as SUNBRIGHT GL2-400MA3.

Further alternative PEG effector molecules of the following type:



In one embodiment there is provided an antibody, such as a full length antibody, which is PEGylated (for example with a PEG described herein), attached through a cysteine amino acid residue at or about amino acid 226 in the chain, for example amino acid 226 of the heavy chain (by sequential numbering).

In one embodiment the present disclosure provides a Fab'PEG molecule comprising one or more PEG polymers, for example 1 or 2 polymers such as a 40kDa polymer or polymers.

Fab-PEG molecules according to the present disclosure may be particularly advantageous in that they have a half-life independent of the Fc fragment.

In one embodiment there is provided a scFv conjugated to a polymer, such as a PEG molecule, a starch molecule or an albumin molecule.

In one embodiment the antibody or fragment is conjugated to a starch molecule, for example to increase the half-life. Methods of conjugating start to a protein as described in US 8,017,739 incorporated herein by reference.

A reporter molecule as employed herein is a molecule which is capable of being detected, for example a fluorescent dye, radiolabel or other detectable entity.

The present invention also provides an isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody molecule of the present invention. Suitably, the DNA sequence encodes the heavy or the light chain of an antibody molecule of the present invention. The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

DNA sequences which encode an antibody molecule of the present invention can be obtained by methods well known to those skilled in the art. For example, DNA sequences

coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

DNA coding for acceptor framework sequences is widely available to those skilled in the art and can be readily synthesised on the basis of their known amino acid sequences.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibody molecule of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

Examples of suitable DNA sequences are provided in Figure 1.

The present invention also relates to a cloning or expression vector comprising one or more DNA sequences of the present invention. Accordingly, provided is a cloning or expression vector comprising one or more DNA sequences encoding an antibody of the present invention. In one embodiment the vector comprises the sequences given in SEQ ID NO: 28 and/or SEQ ID NO: 20. Suitably, the cloning or expression vector comprises two DNA sequences, encoding the light chain and the heavy chain of the antibody molecule of the present invention, preferably SEQ ID NO: 28 and SEQ ID NO: 20, respectively and suitable signal sequences. In one example the vector comprises an intergenic sequence between the heavy and the light chains (see WO03/048208).

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

Also provided is a host cell comprising one or more cloning or expression vectors comprising one or more DNA sequences encoding an antibody of the present invention. Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used or eukaryotic, for example mammalian, host cell expression systems may also be used. Suitable mammalian host cells include CHO, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell containing a vector of the

present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

The antibodies and fragments according to the present disclosure are expressed at good levels from host cells. Thus the properties of the antibodies and/or binding fragments are suitable for expression on a commercial scale.

Thus there is a provided a process for culturing a host cell and expressing an antibody or fragment thereof, isolating the latter and optionally purifying the same to provide an isolated antibody or fragment. In one embodiment the process further comprises the step of conjugating an effector molecule to the isolated antibody or fragment, for example conjugating to a PEG polymer in particular as described herein.

In one embodiment there is provided a process for purifying an antibody (in particular an antibody or fragment according to the invention) comprising performing anion exchange chromatography in non-binding mode such that the impurities are retained on the column and the antibody is eluted.

In one embodiment the purification employs affinity capture on a CSF-1R column.

In one embodiment the purification employs cibacron blue or similar for purification of albumin fusion or conjugate molecules.

Suitable ion exchange resins for use in the process include Q.FF resin (supplied by GE-Healthcare). The step may, for example be performed at a pH about 8.

The process may further comprise an initial capture step employing cation exchange chromatography, performed for example at a pH of about 4 to 5, such as 4.5. The cation exchange chromatography may, for example employ a resin such as CaptoS resin or SP sepharose FF (supplied by GE-Healthcare). The antibody or fragment can then be eluted from the resin employing an ionic salt solution such as sodium chloride, for example at a concentration of 200mM.

Thus the chromatograph step or steps may include one or more washing steps, as appropriate.

The purification process may also comprise one or more filtration steps, such as a diafiltration step.

Thus in one embodiment there is provided a purified anti-CSF-1R antibody or fragment, for example a humanised antibody or fragment, in particular an antibody or fragment according to the invention, in substantially purified from, in particular free or substantially free of endotoxin and/or host cell protein or DNA.

Purified form as used *supra* is intended to refer to at least 90% purity, such as 91, 92, 93, 94, 95, 96, 97, 98, 99% w/w or more pure.

Substantially free of endotoxin is generally intended to refer to an endotoxin content of 1 EU per mg antibody product or less such as 0.5 or 0.1 EU per mg product.

Substantially free of host cell protein or DNA is generally intended to refer to host cell protein and/or DNA content 400μg per mg of antibody product or less such as 100μg per mg or less, in particular 20μg per mg, as appropriate.

The present invention also provides an anti-CSF-1R antibody (or pharmaceutical compositions comprising same) according to the disclosure for use as a medicament.

The present invention also provides an anti-CSF-1R antibody (or pharmaceutical compositions comprising same) according to the disclosure, for the treatment of cancer.

The present invention also provides the use of an anti-CSF-1R antibody (or pharmaceutical composition comprising same) according to the disclosure in the manufacture of a medicament for the treatment or prophylaxis of cancer.

The present invention also provides a method for the treatment of a human subject suffering from or at risk of cancer, the method comprising administering to the subject an effective amount of an anti-CSF-1R antibody according to the disclosure.

The antibody according to the disclosure may be used to treat cancer that is selected from the group consisting of breast cancer, prostate cancer, bone cancer, myeloma, colorectal cancer, leukaemia, lymphoma, skin cancer such as melanoma, oesophageal cancer, gastric cancer, astrocytic cancer, endometrial cancer, cervical cancer, bladder cancer, renal cancer, lung cancer, liver cancer, thyroid cancer, head & neck cancer, pancreatic cancer and ovarian cancer.

In one embodiment the cancer is metastasised cancer from any of the above-listed original cancers, in particular bone cancer.

Surprisingly we have been able to demonstrate that an anti-CSF-1R antibody that inhibits CSF-1R activity is active in the treatment of fibrotic disease. Specifically, we have been able to demonstrate that an anti-CSF-1R antibody is active in *in vivo* animal models of pulmonary fibrosis.

The present invention also provides an anti-CSF-1R antibody (or pharmaceutical compositions comprising same) according to the disclosure, for the treatment of fibrotic disease.

The present invention also provides the use of an anti-CSF-1R antibody (or pharmaceutical composition comprising same) according to the disclosure in the manufacture of a medicament for the treatment or prophylaxis of fibrotic disease.

The present invention also provides a method for the treatment of a human subject suffering from or at risk of fibrotic disease, the method comprising administering to the subject an effective amount of an anti-CSF-1R antibody according to the disclosure.

In the present application, the term "fibrotic disease" includes diseases that are characterised by an aberrant response to wound healing wherein excess fibrous connective tissue is formed in an organ or tissue. Illustrative fibrotic diseases include but are not limited to pulmonary fibrosis such as idiopathic pulmonary fibrosis and cystic fibrosis, renal fibrosis including tubular atrophy and interstitial fibrosis, liver fibrosis, liver cirrhosis, primary sclerosing cholangitis, primary biliary cirrhosis, endomyocardial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, progressive massive fibrosis, nephrogenic systemic fibrosis, Crohn's disease, keloid, myocardial infarction, scleroderma, systemic sclerosis and arthofibrosis.

In one embodiment the antibodies or fragments according to the disclosure are employed in the treatment or prophylaxis of cancer or fibrotic disease.

The antibody according to the disclosure may be used in the treatment of inflammatory diseases, like, for example, inflammatory arthritis, atherosclerosis, multiple sclerosis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, rheumatoid spondylitis, ankylosing spondylitis, arthritis, psoriatic arthritis, rheumatoid arthritis, osteoarthritis, eczema, contact dermatitis, psoriasis, toxic shock syndrome, sepsis, septic shock, endotoxic shock, asthma, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, osteoporosis,

restenosis, cardiac and renal reperfusion injury, thrombosis, glomerulonephritis, diabetes, graft vs. host reaction, allograft rejection, multiple sclerosis, muscle degeneration, muscular dystrophy, Alzheimer's disease and stroke.

The antibodies and fragments according to the present disclosure may be employed in treatment or prophylaxis.

The antibody molecule of the present invention may also be used in diagnosis, for example in the *in vivo* diagnosis and imaging of disease states involving CSF-1R.

As the antibodies of the present invention are useful in the treatment and/or prophylaxis of a pathological condition, the present invention also provides a pharmaceutical or diagnostic composition comprising an antibody molecule of the present invention in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier. Accordingly, provided is the use of an antibody of the invention for the manufacture of a medicament. The composition will usually be supplied as part of a sterile, pharmaceutical composition that will normally include a pharmaceutically acceptable carrier. A pharmaceutical composition of the present invention may additionally comprise a pharmaceutically-acceptable adjuvant.

The present invention also provides a process for preparation of a pharmaceutical or diagnostic composition comprising adding and mixing the antibody molecule of the present invention together with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

The antibody molecule may be the sole active ingredient in the pharmaceutical or diagnostic composition. Alternatively, the antibody may be administered in combination, e.g. simultaneously, sequentially or separately, with one or more other therapeutically active ingredients. According the antibody molecule in the pharmaceutical or diagnostic composition may be accompanied by other active ingredients including other antibody ingredients, for example epidermal growth factor receptor family (EGFR, HER-2), vascular endothelial growth factor receptors (VEGFR), platelet derived growth factor receptor (PDGFR) antibodies, or non-antibody ingredients such as imatinib, dasatinib, nioltinib, basutinib, gefitinib, erlotinib, temsirolimus, vandetanib, vemurafenib, crizotinib, vorinostat, romidepsin, bortezomib, sorafenib, sunitinib, pazopanib, regorafenib, cabozantinib, Perfenidone, steroids or other drug molecules, in particular drug molecules whose half-life is independent of CSF-1R binding.

Active ingredient as employed herein refers to an ingredient with a pharmacological effect, such as a therapeutic effect, at a relevant dose.

The pharmaceutical compositions suitably comprise a therapeutically effective amount of the antibody of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic, pharmacologial or preventative effect. For any antibody, the therapeutically effective amount can be estimated initially either in cell culture assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, a therapeutically effective amount will be from 0.01 mg/kg to 500 mg/kg, for example 0.1 mg/kg to 200 mg/kg, such as 100mg/Kg.

Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose.

Therapeutic doses of the antibodies according the present disclosure show no apparent or limited toxicology effects *in vivo*.

Compositions may be administered individually to a patient or may be administered in combination (*e.g.* simultaneously, sequentially or separately) with other agents, drugs or hormones.

In one embodiment the antibodies or binding fragments according to the present disclosure are employed with one or more other cancer treatment options, such as, for example, chemotherapy, radiation therapy or surgery. If administered with a chemotherapeutic, the antibody can be administered before or after the chemotherapeutic agent or at the same time. Chemotherapy treatments that can be used in combination with the antigen binding proteins that are provided include, but are not limited to alkylating/DNA-damaging agents (e.g. carboplatin,

cisplatin), antimetabolites (e.g. capecitabine, gemcitabine, 5-fluorouracil), mitotic inhibitors (e.g. paclitaxel, vincristine).

The antibodies to be used to treat various inflammatory diseases can be used alone or combined with various other anti-inflammatory agents.

The antibodies to be used to treat various fibrotic diseases can be used alone or combined with various other anti-fibrotic agents. An examples of such an agent is Perfedidone.

The dose at which the antibody molecule of the present invention is administered depends on the nature of the condition to be treated, the severity of the condition present and on whether the antibody molecule is being used prophylactically or to treat an existing condition.

The frequency of dose will depend on the half-life of the antibody molecule and the duration of its effect. If the antibody molecule has a short half-life (e.g. 2 to 10 hours) it may be necessary to give one or more doses per day. Alternatively, if the antibody molecule has a long half-life (e.g. 2 to 15 days) and/or long lasting pharmacodynamics (PD) profile it may only be necessary to give a dosage once per day, once per week or even once every 1 or 2 months.

Half-life as employed herein is intended to refer the duration of the molecule in circulation, for example in serum/plasma.

Pharmacodynamics as employed herein refers to the profile and in particular duration of the biological action of the molecule according the present disclosure.

The pharmaceutically acceptable carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets,

pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Suitable forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, in one or more embodiments the compositions are adapted for administration to human subjects.

Suitably in formulations according to the present disclosure, the pH of the final formulation is not similar to the value of the isoelectric point of the antibody or fragment, for example if the pH of the formulation is 7 then a pI of from 8-9 or above may be appropriate. Whilst not wishing to be bound by theory it is thought that this may ultimately provide a final formulation with improved stability, for example the antibody or fragment remains in solution.

In one example the pharmaceutical formulation at a pH in the range of 4.0 to 7.0 comprises: 1 to 200mg/mL of an antibody according to the present disclosure, 1 to 100mM of a buffer, 0.001 to 1% of a surfactant, a) 10 to 500mM of a stabiliser, b) 10 to 500mM of a stabiliser and 5 to 500 mM of a tonicity agent, or c) 5 to 500 mM of a tonicity agent.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial

space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

It will be appreciated that the active ingredient in the composition will be an antibody molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

In one embodiment the formulation is provided as a formulation for topical administrations including inhalation.

Suitable inhalable preparations include inhalable powders, metering aerosols containing propellant gases or inhalable solutions free from propellant gases. Inhalable powders according to the disclosure containing the active substance may consist solely of the abovementioned active substances or of a mixture of the abovementioned active substances with physiologically acceptable excipient.

These inhalable powders may include monosaccharides (e.g. glucose or arabinose), disaccharides (e.g. lactose, saccharose, maltose), oligo- and polysaccharides (e.g. dextranes), polyalcohols (e.g. sorbitol, mannitol, xylitol), salts (e.g. sodium chloride, calcium carbonate) or mixtures of these with one another. Mono- or disaccharides are suitably used, the use of lactose or glucose, particularly but not exclusively in the form of their hydrates.

Particles for deposition in the lung require a particle size less than 10 microns, such as 1-9 microns for example from 0.1 to 5 µm, in particular from 1 to 5 µm. The particle size of the active ingredient (such as the antibody or fragment) is of primary importance.

The propellent gases which can be used to prepare the inhalable aerosols are known in the art. Suitable propellent gases are selected from among hydrocarbons such as n-propane, n-butane or isobutane and halohydrocarbons such as chlorinated and/or fluorinated derivatives of methane, ethane, propane, butane, cyclopropane or cyclobutane. The abovementioned propellent gases may be used on their own or in mixtures thereof.

Particularly suitable propellent gases are halogenated alkane derivatives selected from among TG 11, TG 12, TG 134a and TG227. Of the abovementioned halogenated hydrocarbons,

TG134a (1,1,1,2-tetrafluoroethane) and TG227 (1,1,1,2,3,3,3-heptafluoropropane) and mixtures thereof are particularly suitable.

The propellent-gas-containing inhalable aerosols may also contain other ingredients such as cosolvents, stabilisers, surface-active agents (surfactants), antioxidants, lubricants and means for adjusting the pH. All these ingredients are known in the art.

The propellant-gas-containing inhalable aerosols according to the invention may contain up to 5 % by weight of active substance. Aerosols according to the invention contain, for example, 0.002 to 5 % by weight, 0.01 to 3 % by weight, 0.015 to 2 % by weight, 0.1 to 2 % by weight, 0.5 to 2 % by weight or 0.5 to 1 % by weight of active ingredient.

Alternatively topical administrations to the lung may also be by administration of a liquid solution or suspension formulation, for example employing a device such as a nebulizer, for example, a nebulizer connected to a compressor (e.g., the Pari LC-Jet Plus(R) nebulizer connected to a Pari Master(R) compressor manufactured by Pari Respiratory Equipment, Inc., Richmond, Va.).

The antibody of the invention can be delivered dispersed in a solvent, e.g., in the form of a solution or a suspension. It can be suspended in an appropriate physiological solution, e.g., saline or other pharmacologically acceptable solvent or a buffered solution. Buffered solutions known in the art may contain 0.05 mg to 0.15 mg disodium edetate, 8.0 mg to 9.0 mg NaCl, 0.15 mg to 0.25 mg polysorbate, 0.25 mg to 0.30 mg anhydrous citric acid, and 0.45 mg to 0.55 mg sodium citrate per 1 ml of water so as to achieve a pH of about 4.0 to 5.0. A suspension can employ, for example, lyophilised antibody.

The therapeutic suspensions or solution formulations can also contain one or more excipients. Excipients are well known in the art and include buffers (e.g., citrate buffer, phosphate buffer, acetate buffer and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (e.g., serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. Solutions or suspensions can be encapsulated in liposomes or biodegradable microspheres. The formulation will generally be provided in a substantially sterile form employing sterile manufacture processes.

This may include production and sterilization by filtration of the buffered solvent/solution used for the formulation, aseptic suspension of the antibody in the sterile

buffered solvent solution, and dispensing of the formulation into sterile receptacles by methods familiar to those of ordinary skill in the art.

Nebulizable formulation according to the present disclosure may be provided, for example, as single dose units (e.g., sealed plastic containers or vials) packed in foil envelopes. Each vial contains a unit dose in a volume, e.g., 2 mL, of solvent/solution buffer.

The antibodies disclosed herein may be suitable for delivery via nebulisation.

It is also envisaged that the antibody of the present invention may be administered by use of gene therapy. In order to achieve this, DNA sequences encoding the heavy and light chains of the antibody molecule under the control of appropriate DNA components are introduced into a patient such that the antibody chains are expressed from the DNA sequences and assembled *in situ*.

In one embodiment the present disclosure comprises use of antibodies or fragments thereof as a reagent or diagnosis, for example conjugated to a reporter molecule. Thus there is provided antibody or fragment according to the disclosure which is labelled. In one aspect there is provided a column comprising an antibody or fragment according to the disclosure.

Thus there is provided an anti-CSF-1R antibody or fragment for use as a reagent for such uses as:

1) purification of CSF-1R protein (or binding fragment thereof) – being conjugated to a matrix and used as an affinity column, or (as a modified form of anti-CSF-1R) as a precipitating agent (e.g. as a form modified with a domain recognised by another molecule, which may be modified), which is optionally precipitated by an anti-Fc reagent)

2) detection and/or quantification of CSF-1R on cells or in cells, live or fixed (cells *in vitro* or in tissue or cell sections). Uses for this may include quantification of CSF-1R as a biomarker, to follow the effect of anti-CSF-1R treatment. For these purposes, the candidate might be used in a modified form (e.g. by addition another moiety, as a genetic fusion protein or chemical conjugate, such as addition of a reporter molecule, for example a fluorescent tag used for the purposes of detection).

3) purification or sorting of CSF-1R-bearing cells labeled by binding to candidate modified by ways exemplified in (1) and (2).

Comprising in the context of the present specification is intended to meaning including.

Where technically appropriate, embodiments of the invention may be combined.

Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

Technical references such as patents and applications are incorporated herein by reference.

Any embodiments specifically and explicitly recited herein may form the basis of a disclaimer either alone or in combination with one or more further embodiments.

The present invention is further described by way of illustration only in the following examples, which refer to the following Figures:

Figure 1A to 1F shows certain amino acid and polynucleotide sequences.

Figure 2A and 2B shows alignments of certain sequences.

Figure 3 shows the sequence of human CSF-1R extracellular domain encoded by a polynucleotide employed to transfect cells and expressing the protein on the surface of the cell. These cells were then employed to immunize host animals.

Figure 4 shows the inhibition of CSF-1 binding to THP-1 cells by antibody Ab969.

Figure 5a shows the inhibition of CSF-1 driven survival and proliferation by primary human monocytes by antibody Ab969.

Figure 5b shows the inhibition of IL-34 driven survival and proliferation by primary human monocytes by antibody Ab969.

Figure 6 shows the inhibition of CSF-1 driven survival and proliferation by primary murine monocytes by antibody Ab535.

Figure 7 shows the levels of cell-surface CSF-1R on THP-1 cells incubated with Ab969, human CSF-1 and an isotype control.

Figure 8 shows the relative level of cell-surface CSF-1R on THP-1 cells treated with Ab969 compared to an isotype control.

Figure 9 shows the levels of cell-surface CSF-1R on RAW264.7 cells incubated with Ab 535, CSF-1 and an isotype control.

Figure 10 shows the relative level of cell-surface CSF-1R on RAW264.7 cells treated with Ab535 compared to an isotype control.

Figure 11 shows the level of CSF-1R phosphorylation on HEK293F cells transfected with CSF-R upon stimulation with CSF-1 or treatment with Ab969.

Figure 12 shows the level of MCP-1 secretion from primary human monocytes when incubated with Ab969 and with CSF-1.

Figure 13 shows the effect on inhibition of CSF-1-mediated monocyte survival by humanised antibody 969.g5 compared to chimeric Ab969.g0.

Figure 14 shows the effect on inhibition of CSF-1-mediated monocyte survival by various humanised antibody grafts of Ab969.

Figure 15a shows the concentration of CSF-1 in serum samples taken from cynomolgus monkeys treated with a single intravenous dose of 7 mg/kg Ab969.g2.

Figure 15b shows the concentration of CSF-1 in serum samples taken from cynomolgus monkeys treated with a single intravenous dose of 1.5 mg/kg Ab969.g2.

Figure 15c shows the effect of administering antibody Ab969.g2 to cynomolgus monkeys at dosages of 7 mg/kg and 1.5 mg/kg on circulating non-classical monocytes at different timepoints.

Figure 16 shows the antitumor effect of antibody Ab535, vs. a control antibody, a positive control and the vehicle control in immunodeficient nude mice bearing subcutaneous transplants of the human breast cancer xenograft MCF-7.

Figure 17 shows the antitumoral efficacy of antibody Ab535 vs. a control antibody, a positive control and the vehicle control in an orthotopic prostate cancer model PC-3.

Figure 18a shows the effect of treatment of bleomycin-induced lung fibrosis with Ab535 on BALF collagen concentration compared to an isotype control.

Figure 18b shows the effect of treatment of bleomycin-induced lung fibrosis with Ab535 on the fibrotic pathology of lung samples measured by the Ashcroft score compared to an isotype control.

Figure 18c shows the effect of treatment of bleomycin-induced lung fibrosis with Ab535 on the concentration of albumin in the serum compared to an isotype control.

Figure 18d the number of macrophages in the BAL fluid of mice from a bleomycin-induced lung fibrosis study and shows that treatment of bleomycin-induced lung fibrosis with Ab535 reduced the number of macrophages in BAL fluid compared to isotype control treated animals. Data shown as means $\pm$ SEM; * Denotes significant difference from saline isotype; # denotes significant difference from bleomycin treated mice dosed with isotype control antibody ($p \leq 0.05$)

Figure 19 representative images of the histopathological analysis of lungs from saline control, bleomycin plus isotype control and bleomycin plus Ab535 treated animals.

Figure 20a shows the effect of treatment of ADA-deficient mice with induced lung fibrosis with Ab535 on BALF collagen concentration compared to treatment with the isotype control.

Figure 20b shows the effect of treatment of ADA-deficient mice with induced lung fibrosis with Ab535 on the fibrotic pathology of lung samples measured by the Ashcroft score compared to treatment with the isotype control.

Figure 20c shows the effect of treatment of ADA-deficient mice with induced lung fibrosis with Ab535 on the concentration of albumin in the serum compared to treatment with the isotype control.

Figure 20d shows the number of macrophages in the BAL fluid of mice from an ADA deficient model of pulmonary fibrosis and shows that treatment of ADA-deficient mice with induced lung fibrosis with Ab535 reduced the number of macrophages in the BAL fluid.

Figure 21 shows representative images of the histopathological analysis of lungs from normal mice (ADA+) and ADA-deficient mice with induced lung fibrosis (ADA-) both treated with isotype control or Ab535.

EXAMPLES

Example 1 – Generation of an anti-CSF-1R antibody

Immunisation:

Female Sprague Dawley rats were immunised with syngeneic RFL6 rat fibroblasts that had been transiently transfected with a vector expressing human CSF-1R extracellular domain linked to a glycosylphosphatidyl-inositol (GPI) anchor. Figure 3 shows SEQ ID NO: 39 which is the Human CSF-1R extracellular domain sequence used in the immunisation of rats.

Rats received five subcutaneous immunisations at three-weekly intervals of 3-9x10^6 transfected cells per animal. Freund's Complete Adjuvant (50% in PBS) was injected at an adjacent site with the first cell immunisation. Two weeks post-final immunisation, peripheral blood mononuclear cells (PBMCs) and spleens were harvested.

Primary Screening of Antibody Supernatants

Antibody supernatants were initially screened for their ability to bind human CSF-1R expressed on transfected HEK293 cells by fluorescence microvolume assay technology (FMAT).

Approximately 1000 wells with anti-CSF-1R reactivity were identified in a primary FMAT screen of 600 x 96-well plates. In total, approximately $3x10^8$ B cells (rat splenocytes) were screened for the production of CSF-1R binding antibodies by FMAT.

Secondary Screening of Antibody Supernatants

A medium-throughput assay was devised to identify FMAT-positive wells that contained neutralising anti-CSF-1R activity, i.e. antibodies that have the ability to prevent human CSF-1 binding to the human CSF-1R receptor. Antibody supernatants were incubated with CSF-1R-expressing THP-1 cells prior to incubation with human CSF-1. The level of CSF-1 binding to THP-1 cells was measured by flow cytometry using an anti-CSF-1 polyclonal antibody. Irrelevant antibody supernatants were used as negative controls.

The secondary screen was applied to supernatants from 779 antibody wells and 88 of these wells demonstrated detectable CSF-1 blocking activity.

Tertiary Screening of Antibody Supernatants

Antibody supernatants that had shown neutralising activity in the secondary screen were tested for their ability to prevent CSF-1-dependent survival of primary human monocytes. Monocytes were purified from human blood and $1x10^4$ cells incubated with each antibody supernatant in the presence of 20ng/ml human CSF-1. After 72-hour incubation, the number of viable monocytes was measured by CellTiter Glo assay.

The tertiary screen was applied to supernatants from 59 of the antibody wells identified in the secondary screen and 18 wells demonstrated an ability to reduce CSF-1-mediated monocyte survival.

Cloning of antibody variable regions and reanalysis of blocking activity

Variable region (V-region) cloning was attempted from the 18 antibody wells demonstrating CSF-1-neutralising activity. The heavy and light immunoglobulin V-regions were amplified by RT-PCR using primers specific to the rat antibody constant regions and a redundant primer set that anneals to sequences encoding rat immunoglobulin leader peptides. The V-regions genes were recovered from 14 antibodies and cloned into heavy- and light-chain human IgG4 expression vectors.

Antibody vector pairs were transiently transfected into HEK293F cells and the conditioned medium tested for CSF-1 neutralising activity on THP-1 cells (as described above).

Nine of the antibodies had the capacity to partially or fully inhibit CSF-1 binding compared to control conditioned medium.

Sequence analysis of nine neutralising anti-CSF-1R antibodies

The nine neutralising antibodies were sequenced to assess sequence diversity. All nine antibodies were unique. These antibodies were cloned and expressed for further profiling studies.

Example 2 – *In Vitro* Properties of Anti-Human CSF-1R Chimeric Ab969 and Anti-Murine CSF-1R Ab535

i) Expressing Chimeric Ab969

Variable regions of the nine neutralising antibodies identified in Example 1 were cloned into separate heavy- and light-chain expression vectors and were expressed as full-length human IgG4 antibodies.

The VH genes were cloned into vector pVhg4FL(V19H), which contains DNA encoding a natural leader sequence and the human gamma-4 heavy chain constant region with the hinge stabilising mutation S241P. The VL genes (kappa) were cloned into vector pKH10.1(V4L), which contains DNA encoding a natural leader sequence and the human kappa chain constant region (Km3 allotype).

Antibodies were expressed by transient co-transfection of matching heavy- and light-chain vector pairs into CHO-K1 cells. Purification of the antibodies into PBS pH 7.4 was performed so that the level of aggregate in the final preparation was less than 1%.

The panel of nine antibodies included an antibody designated antibody 969. The chimeric antibody comprising the rat variable regions and the human gamma-4 heavy chain constant region and human kappa chain constant region is referred to as antibody 969 or antibody 969cHcL or antibody 969.g0 in the following examples.

ii) Ligand-Blocking Assay

The capacity of each of the nine antibodies to inhibit CSF-1 binding to THP-1 cells was assessed by flow cytometry. THP-1 cells were incubated with each antibody at 0.5, 0.125, 0.031, 0.0078 and 0.00195µg/ml for 30 minutes. An irrelevant IgG4 antibody served as an isotype control. After washing, cells were incubated with 0.5µg/ml human CSF-1 for 30 minutes. After further washing, bound CSF-1 was detected by sequential incubation with biotinylated anti-CSF-

1 antibody and Alexa488-conjugated streptavidin. Receptor-bound ligand was measured by flow cytometry and median fluorescence intensity (MFI) plotted.

This assay identified four antibodies, including Ab969, that were superior to the remaining antibodies tested, demonstrating complete inhibition of CSF-1 binding at a concentration 31.3ng/ml. The results for Ab969 are shown in Figure 4.

iii) Inhibition of CSF-1- & IL-34-mediated monocyte survival

Anti-human CSF-1R:

The purified anti-human CSF-1R antibodies were tested for their capacity to inhibit the CSF-1 and IL-34 driven survival and proliferation of primary human monocytes. In each assay, the mitogen (CSF-1 or IL-34) was used at a concentration that gave maximal stimulation of the monocytes.

Human PBMCs were prepared from fresh human whole blood on a Ficoll gradient and monocytes purified by negative selection. Monocytes were incubated with 0.25µg/ml antibody and 20ng/ml CSF-1 for 72-hours and the relative number of viable cells determined using CellTiter Glo analysis. The luminescence readout correlates with the number of viable cells. Results shown in Figure 5a.

Human PBMCs were prepared from fresh human whole blood on a Ficoll gradient and monocytes purified by negative selection. Monocytes were incubated with 0.25µg/ml antibody and 20ng/ml IL-34 for 72-hours and the relative number of viable cells determined using CellTiter Glo analysis. The luminescence readout correlates with the number of viable cells. Results shown in Figure 5b.

Four of the antibodies including Ab969 demonstrated superior inhibition of monocyte survival compared to the remaining antibodies for both CSF-1 (Figure 5a) and IL-34 (Figure 5b) stimulation, in line with their ligand-blocking activities.

Anti-mouse CSF-1R:

Primary murine $CD11b^+$ monocytes were purified from mouse spleens. The monocytes were then incubated with a titration of murine CSF-1 (mCSF-1) for 24 hours. The release of MCP-1 into the cell medium was measured by ELISA and a dose-dependent release of MCP-1 by mCSF-1 demonstrated. In a separate arm of the study, 10µg/ml anti-murine CSF-1R Ab535 was added along with the mCSF-1 titration. The release of MCP-1 was completely inhibited by Ab535 at all concentrations of CSF-1 tested (Figure 6).

The MCP-1 release assay was performed using primary murine monocytes with a constant CSF-1 concentration of 100 ng/ml and a titration of Ab535. A dose-dependent inhibition of MCP-1 release was demonstrated and an IC_{50} calculated. The mean IC_{50} of Ab535 from two independent experiments was determined to be 8.08 ng/ml.

Conclusion: treatment of murine monocytes with CSF-1 caused a dose-dependent release of MCP-1, which was completely inhibited by treatment with 10 µg/ml Ab535. Ab535 inhibits CSF-1-driven MCP-1 release from murine monocytes in a dose-dependent manner, with a mean IC_{50} of 8.08 ng/ml (n=2). This IC_{50} value is similar to that exhibited by the anti-human-CSF-1R antibodies.

iv) Affinity

Antibodies were tested for their ability to bind CSF-1R in a BIAcore assay by measurement of binding kinetics to a purified recombinant CSF-1R/Fc fusion protein.

The assay format was capture of the anti-CSF-1R antibodies by immobilised anti-human IgG,$F(ab')_2$, then a titration of hCSF-1R/Fc over the captured surface. BIA (Biamolecular Interaction Analysis) was performed using a BIAcore 3000 (GE Healthcare Bio-Sciences AB). All experiments were performed at 25°C. Affinipure $F(ab')_2$ fragment goat anti-human IgG, $F(ab')_2$ fragment specific (Jackson ImmunoResearch) was immobilised on a CM5 Sensor Chip (GE Healthcare Bio-Sciences AB) via amine coupling chemistry to a level of ~5000 response units (RU). HBS-EP buffer (10mM HEPES pH7.4, 0.15M NaCl, 3mM EDTA, 0.005% Surfactant P20, GE Healthcare Bio-Sciences AB) was used as the running buffer with a flow rate of 10µl/min. An injection of an anti-CSF-1R antibody was performed to give a capture level of approximately 100 RU on the immobilised anti-human IgG,$F(ab)_2$.

Recombinant human CSF-1R/Fc (R&D Systems) was passed over the captured anti-CSF-1R antibody at 5nM at a flow rate of 30ul/min for 5min then the flow rate was increased to 100ul/min for 30min for the dissociation phase. The injection at 5nM was performed twice along with a corresponding buffer control. These sensorgrams were used to generate the dissociation rate. Recombinant human CSF-1R/Fc was titrated over the captured anti-CSF-1R antibody from 2.5nM at a flow rate of 30ul/min for 5min followed by a 10min dissociation phase. These sensorgrams were used to generate the association rate. The surface was regenerated at a flow rate of 10ul/min by a 10ul injection of 40mM HCl followed by a 5ul injection of 10mM NaOH. Double referenced background subtracted binding curves were analysed using the BIAevaluation

software (version 4.1) following standard procedures. Kinetic parameters were determined from the fitting algorithm.

Three of the four tested antibodies exhibited affinities (K_D) of less than 10pM. This compares favourably with the anti-murine-CSF-1R parallel reagent, Ab535. The results for Ab969 and Ab553 are shown in **Table 1**.

Table 1:

Antibody	On-rate K_a ($M^{-1}s^{-1}$)	Off-rate K_d (s^{-1})	Affinity K_D (M)	Affinity K_D (pM)
Ab969	$2.58x10^{6}$	$2.49x10^{-5}$	$9.65x10^{-12}$	9.6
Ab535	$2.24x10^{6}$	$1.06x10^{-5}$	$4.73x10^{-12}$	4.7

The affinity of the antibodies was also measured by a cell-based assay using THP-1 cells. The antibodies were directly labelled with Alexa-488 fluorescent dye and affinity measured by quantitative flow cytometry. An additional BIAcore analysis confirmed that fluorescent conjugation of the antibodies did not to alter the affinity towards recombinant CSF-1R protein. The results are shown in **Table 2**.

The absolute affinity values derived by the two methodologies are different, and this difference is usually observed when the two systems are compared. However, the cell-based method demonstrated that the antibodies bind CSF-1R with high affinity.

Table 2

Antibody	Cell-based affinity K_D ± S.D. (nM)	BIAcore affinity K_D (pM)
Ab969	2.20 ± 0.19	4.80

v) Inhibition of CSF-1 binding (IC_{50})

Four antibodies were analysed by measuring their relative potency at inhibiting CSF-1 binding to THP-1 cells. All four antibodies exhibited potent inhibition of CSF-1 binding with IC_{50} values of less than 5ng/ml (~30pM) in this assay format.

Table 3

Antibody	Mean IC_{50} ± S.D. (ng/ml)
Ab969	2.89 ± 1.22

vi) Antibody cross-reactivity

Four antibodies were tested for cross-reactivity with rhesus monkey, cynomolgus monkey and canine full-length CSF-1R. All four antibodies bound rhesus and cynomolgus CSF-1R in addition to human CSF-1R, demonstrating clear binding significantly above the level of the isotype control.

Table 4

Antibody	Cross-reactivity		
	Cynomolgus	Rhesus	Canine
Ab969	Yes	Yes	No

vii) CSF-1R Internalization

Ab969:

THP-1 cells were incubated with Ab969 for 0, 0.5, 2, 4, 24 and 48 hours. Cells were also treated with human CSF-1 and an isotype control that served as positive and negative controls respectively. At each time-point, the level of cell-surface CSF-1R was measured by flow cytometry (**Figure 7**). The relative level of cell-surface CSF-1R on cells treated with Ab969 compared to the isotype control was calculated and is shown in Figure 8.

Treatment of THP-1 cells with recombinant CSF-1 caused a rapid and sustained decrease in the level of cell-surface CSF-1R; the natural ligand binds to its cognate receptor and drives internalisation of the ligand-receptor complex.

THP-1 cells treated with Ab969 exhibited higher levels of cell-surface CSF-1R expression compared to the untreated and isotype control treated THP-1 cells throughout the whole 48 hour time course. This data strongly suggests that treatment of THP-1 cells with Ab969 does not cause internalisation of cell surface expressed hCSF-1R up to 48 hours post-treatment.

As the time course progressed there was a notable increase in cell-surface expressed CSF-1R on the untreated and treated THP-1 cells, with the exception of cells treated with CSF-1. This might be due to expression changes during the period of cell growth over the 48 hour time period of the experiment and could potentially be a stress response.

In order to provide further evidence that Ab969 does not potentiate receptor internalisation, and also preclude the possibility that the THP-1 cell line is not physiologically relevant, primary human monocytes were also used in an internalisation assay. The data from this assay also demonstrated that Ab969 does not cause rapid internalisation of cell-surface CSF-1R on healthy primary human monocytes.

Ab535:

The mouse leukaemic monocyte/macrophage cell line RAW264.7 expresses high levels of murine CSF-1R (mCSF-1R) and represents a suitable cell-based system for testing whether the anti-murine CSF-1R antibody Ab535 can elicit receptor internalisation.

RAW264.7 cells were incubated with Ab535 for 0, 0.5, 2, 4, 24 and 48 hours. Cells were also treated with human CSF-1 and an isotype control that served as a positive and negative control respectively. At each time-point, the level of cell-surface CSF-1R was measured by flow cytometry (**Figure 9**). The relative level of cell-surface CSF-1R compared to the isotype control was calculated and is shown in **Figure 10**.

The data show, that treatment of THP-1 cells with recombinant CSF-1 caused a rapid and sustained decrease in the level of cell-surface CSF-1R.

A clear reduction of cell-surface mCSF-1R levels was observed on RAW264.7 cells treated with human CSF-1 for 2 hours relative to the untreated and isotype control treated cells. This reduction is maintained throughout the 48 hour study. This demonstrates that human CSF-1 elicits internalisation of mCSF-1R and validates the experimental system for monitoring receptor internalisation.

RAW264.7 cells treated with Ab535 exhibit similar levels of cell-surface mCSF-1R compared to untreated and isotype control treated cells throughout the whole 48 hour time course. As antibody-mediated receptor internalisation would be expected to occur within this timeframe, this data strongly suggests that Ab535 does not trigger mCSF-1R internalisation.

In order to provide further evidence that Ab535 does not potentiate receptor internalisation, primary murine $CD11b^{+}$ monocytes-macrophages were used in an internalisation assay. These results also demonstrated that treatment of mouse monocyte-macrophages with Ab535 does not cause internalisation of cell surface expressed mCSF-1R up to 24 hours post treatment.

viii) CSF1-R Activation

CSF-1 binds to CSF-1R, causing the formation of receptor dimers, which triggers rapid receptor phosphorylation by bringing the kinase domains together in close proximity. This subsequently leads to receptor internalisation and the activation of several well characterised signal transduction pathways including the Ras-MAPK pathway. It is possible that an anti-CSF-1R antibody could elicit receptor clustering and trigger downstream signalling cascade. This may

be an undesired property for an antibody that should inhibit receptor signalling, so receptor agonism by Ab969 was tested using two independent *in vitro* assay formats.

In the first assay format, antibodies were incubated with cells transfected with human full-length CSF-1R and phosphorylation of the receptor and downstream signal transduction molecules monitored by Western blotting.

The THP-1 cell line represented a starting point for monitoring the activation status of CSF-1R. However, the expression level of CSF-1R in this cell line is relatively low making it difficult to perform biochemical analysis. Therefore an experimental system was devised so that the level of CSF-1R phosphorylation could be robustly detected. Phosphorylation of CSF-1R can be detected at two tyrosine residues, Y723 and Y809. In addition, phosphorylation of p44/42 MAPK (Erk1/2) at T202 and Y204 was measured as an independent readout of CSF-1R activity. Stimulation with CSF-1 was included as a positive control in all experiments.

HEK293F cells were transfected with a plasmid vector expressing full-length CSF-1R. After 24-hours incubation in serum-free conditions, cells were stimulated with a 100μg/ml to 0.001μg/ml titration of Ab969.g0 for 5 minutes. Cells were also treated with 500ng/ml recombinant CSF-1 to provide a positive control. Untreated cells were included as a negative control. Protein lysates from treated and untreated cells were separated by SDS-polyacrylamide electrophoresis and blotted onto nitrocellulose. Western immunoblotting was performed using antibodies to phospho-Y723 CSF-1R (Cell Signaling Technology #3151), phospho-Y809 (Cell Signaling Technology #3154), total CSF-1R (Cell Signaling Technology #3152) and phospho-ERK1/2 (p44/42 MAPK) (Cell Signaling Technology #5301).

Unstimulated CSF-1R-transfected HEK293F cells exhibited a low basal level of CSF-1R phosphorylation. Stimulation of cells with CSF-1 resulted in a level of CSF-1R phosphorylation at residues Y723 and Y809 readily observed by Western blotting analysis (Figure 11). There was also clear stimulation of ERK1/2 phosphorylation upon CSF-1 treatment. Treatment of the transfected HEK293F cells with antibody Ab969.g0 did not stimulate phosphorylation of CSF-1R or potentiate ERK1/2 activation.

In a second assay, antibody Ab969 (monomeric and cross-linked) was incubated with primary human monocytes and MCP-1 secretion used as a marker of CSF-1R activation. CSF-1 treatment of human monocytes causes them to release monocyte chemoattractant protein-1

(MCP-1). If anti-CSF-1R antibodies have an ability to activate the CSF-1R receptor on monocytes, a release of MCP-1 would be expected to occur.

The biochemical assays described previously used only monomeric anti-CSF-1R antibody. However, it is possible that a cross-linked IgG1 would have an enhanced capacity to bring CSF-1R molecules into close proximity and trigger tyrosine phosphorylation and downstream signalling. To assess this, the MCP-1 assay was also performed using Ab969.g0 that had been cross-linked with an anti-human-Fc antibody.

Human monocytes were prepared from human whole blood as follows: 60-100 ml human whole blood was collected in BD Vacutainer 10 ml Lithium Heparin 171 IU Tubes. Blood was split into 3-4 Leucosep Ficoll tubes (Greiner Bio-One) and topped up with PBS. Tubes were centrifuged at 1000g, 20°C for 10 minutes with no brake, and the PBMC layer was collected. Cells were pelleted, and monocytes were then isolated using positive selection human CD14 beads (Miltenyi Biotec 130-050-201) according to the manufacturer's protocol. Antibody Ab969.g0 was cross-linked by adding goat anti-human IgG Fc antibody (R&D Systems G-102-C) at a ratio of 2:1 Ab969.g0:Fc. Monocytes were seeded at 20,000 cells per well in media in the presence of a dose titration of antibody Ab969.g0 or cross-linked Ab969.g0 (half-log dilution series comprising 16 concentrations, maximum 10μg/ml). Control wells contained no antibody, in the presence and absence of 100ng/ml CSF-1, and in the presence and absence of anti-human-Fc antibody (at the same concentration as present in the top concentration of antibody Ab969.g0 (5μg/ml)). Cells were incubated for 24 hours, plates were spun to pellet cells, and supernatant collected. Secreted MCP-1 was measured by MSD (K151AYB-2) according to the manufacturer's protocol.

The result from the experiment is shown in Figure 12. No increase in MCP-1 levels was detected for any of the Ab969.g0 treatments, either in monomeric or cross-linked format; the concentration of MCP-1 in the medium of treated cells was identical to untreated cells. As expected, cells treated with CSF-1 gave a significant and reproducible increase in MCP-1 levels.

Example 3 – Humanisation of Antibody 969 & selection of humanised graft

Four anti-CSF-1R antibodies were selected for humanisation based on their affinity and properties measured in Example 2.

i) Generation of humanised grafts

Antibody 969 and 970 was humanised by grafting the CDRs from the rat antibody V-regions onto human germline antibody V-region frameworks.

The CDRs grafted from the donor to the acceptor sequence are as defined by Kabat (Kabat *et al.*, 1987), with the exception of CDR-H1 where the combined Chothia/Kabat definition is used (see Adair *et al.*, 1991 Humanised antibodies. WO91/09967).

Human V-region VK1 2-1-(1) O12 plus JK4 J-region (V BASE, http://vbase.mrc-cpe.cam.ac.uk/) was chosen as the acceptor for the light chain CDRs. Human V-region VH2 3-1 2-70 plus JH3 J-region (V BASE, http://vbase.mrc-cpe.cam.ac.uk/) was chosen as the acceptor for the heavy chain CDRs.

A number of framework residues from the rat V-regions were retained in the humanised sequences, as shown in Table 1.

Genes encoding initial humanised light and heavy chain V-region sequences, named gL1 and gH1, respectively, were designed and constructed by an automated synthesis approach. Further variants of both the light and heavy chain V-regions were created by modifying the gL1 and gH1 genes by oligonucleotide-directed mutagenesis.

The VK genes (gL1 to gL9) were cloned into the human light chain expression vector pKH10.1, which contains DNA encoding the human Kappa chain constant region (Km3 allotype). The VH genes (gH1 and gH2) were cloned into the human gamma-4 heavy chain expression vector pVhγ4P FL, which contains DNA encoding the human gamma-4 heavy chain constant region with the hinge stabilising mutation S241P (Angal *et al.*, 1993, Mol Immunol. 30:105-8). Different combinations of plasmids encoding the variant light and heavy chains were co-transfected into HEK293F, resulting in the expression of the humanised, recombinant 969 antibodies.

The other three anti-CSF-1R antibodies were also humanised by providing a conservative graft containing a number of donor residues in the heavy and light chains predicted to be of importance and a fully humanised graft containing no donor residues.

ii) Affinity of humanised antibodies

Each humanised graft was assessed for (i) binding affinity to human CSF-1R by BIAcore and (ii) melting temperature (Tm) measured by ThermoFluor analysis, both relative to the parental chimeric antibody. Melting temperature is believed to provide an early indication of the

stability of an antibody molecule, with unstable antibodies typically exhibiting a Tm less than 75.0°C.

The grafts representing the stages of humanisation of Ab969 are shown in **Table 5**. Table 5 also shows the chimeric antibody of Ab696 (969cHcL). The conservative graft (969gH1gL1) exhibited an affinity constant (K_D) of 2.4pM, so there was no apparent loss of affinity compared to the chimeric rat antibody (969cHcL). The Tm of the 969gH1gL1 conservative graft was 78.8°C and therefore above a threshold value of 75.0°C. Substitution of the A78 donor residue for V78 in the heavy chain to produce 969gH2gL1 did not reduce antibody affinity (K_D=2.3pM). Upon stepwise substitution of K38, Y71 and F87 donor residues, to Q38, F71 and Y87 respectively, no changes in affinity were observed. The final humanised graft 969gH2gL8 containing no donor residues exhibited an affinity similar to the parental chimeric antibody (4.1pM).

Table 5

Antibody Graft	VK donor residues	VH donor residues	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)
969cHcL	N/A	N/A	2.31E+06	7.99E-06	3.5
969gH1gL1	K38, Y71, F87	A78	2.04E+06	5.00E-06	2.4
969gH1gL2	Y71, F87	A78	1.46E+06	5.00E-06	3.4
969gH1gL3	K38, F87	A78	1.80E+06	5.00E-06	2.8
969gH1gL4	K38, Y71	A78	2.39E+06	5.00E-06	2.1
969gH1gL5	F87	A78	9.26E+05	6.11E-06	6.6
969gH1gL6	K38	A78	1.15E+06	5.00E-06	4.4
969gH1gL7	Y71	A78	1.50E+06	5.00E-06	3.3
969gH1gL8	-	A78	1.17E+06	5.00E-06	4.3
969gH2gL1	K38, Y71, F87	-	2.27E+06	5.23E-06	2.3
969gH2gL2	Y71, F87	-	1.78E+06	6.11E-06	3.4
969gH2gL3	K38, F87	-	2.12E+06	7.29E-06	3.4
969gH2gL4	K38, Y71	-	2.20E+06	9.64E-06	4.4
969gH2gL5	F87	-	7.68E+05	5.00E-06	6.5
969gH2gL6	K38	-	1.40E+06	6.23E-06	4.4
969gH2gL7	Y71	-	1.40E+06	5.00E-06	3.6
969gH2gL8	-	-	1.23E+06	5.00E-06	4.1

Ab969 possesses a potential DG isomerisation motif at the junction of CDR-L2 and the framework. This DG site aspartic acid residue in the 969gH2gL7 and 969gH2gL8 grafts was mutated to serine to give an inert SG sequence. Affinity towards CSF-1R was measured by

BIAcore and no apparent loss of affinity detected (**Table 6**). Furthermore, the final 969H2gL7(SG) and 969gH2gL8(SG) grafts retained a high Tm of 80.5°C and 79.9°C respectively.

Table 6

Antibody Graft	VK donor residues	VH donor residues	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)
969cHcL	N/A	N/A	4.35E+06	8.53E-06	2.0
969gH2gL7 DG	Y71	-	2.56E+06	7.16E-06	2.8
969gH2gL7 SG	Y71	-	2.54E+06	5.00E-06	2.0
969gH2gL8 DG	-	-	2.18E+06	5.00E-06	2.3
969gH2gL8 SG	-	-	3.22E+06	5.00E-06	1.6

The humanisation of Ab969 and one other anti-CSF-1R antibody Ab970 generated fully humanised antibodies (no rat donor residues present) with affinity (K_D) equivalent to the parental chimeric antibody and a Tm that gave an initial prediction of molecule stability. The fully humanised version of Ab969 (969gH2gL8 SG)) was renamed Ab969.g5. From hereon, the chimeric version of Ab969 will be referred to as Ab969.g0.

The affinity of purified chimeric and humanised grafts of Ab969 antibodies towards recombinant CSF-1R was measured again using by BIAcore analysis. Previous BIAcore experiments performed during the humanisation process were carried out using crude cell supernatants rather than purified antibody. A slight reduction in the affinity was detected, with K_D values being increased from approximately 4pM to 5pM.

Table 7

Antibody	Experiment	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)
969.g0	1	2.16E+06	9.49E-06	4.4
	2	2.16E+06	9.10E-06	4.2
	Mean	**2.16E+06**	**9.30E-06**	**4.3**
969.g5	1	1.63E+06	8.13E-06	5.0
	2	1.50E+06	7.96E-06	5.3
	Mean	**1.57E+06**	**8.05E-06**	**5.1**

iii) Inhibition of CSF-1-mediated monocyte survival by Ab969.g0 and Ab969.g5

CSF-1 is required for the activation and survival of monocytes in culture; if CSF-1 is removed then monocytes rapidly undergo apoptosis. An assay was devised that used MCP-1 (monocyte chemotactic protein-1; also known as CCL2, chemokine C-C motif ligand 2) as a

read-out. Upon CSF-1 stimulation, human monocytes secrete MCP-1, which can be detected in cell supernatants by an ELISA, typically 24-hours after stimulation. The inhibition of CSF-1R signalling by antibodies that block CSF-1-binding causes a reduction in MCP-1 secretion.

Human PBMCs were prepared from fresh human whole blood on a Ficoll gradient and $CD14^+$ monocytes purified by positive selection. A total of $2x10^4$ monocytes were incubated with a half-log dilution series of 10μg/ml to 0.35pg/ml antibody in the presence of 100ng/ml human CSF-1 for 24-hours. Controls comprising 'no antibody, no CSF-1' and 'no antibody, with CSF-1' were included to provide minimum and maximum MCP-1 release values. After the 24-hour incubation, cells were pelleted by centrifugation and the supernatant collected. The concentration of MCP-1 was measured using the Human CCL2/MCP-1 DuoSet ELISA (R&D Systems DY279) following the manufacturer's instructions.

From henceforth the assay is referred to as the 'MCP-1 inhibition assay'.

The MCP-1 inhibition assay was employed to compare the activity of humanised 969.g5 with chimeric Ab969.g0. Five independent assays were performed using four different donors of monocytes. In all assays, Ab969.g5 humanised graft exhibited an unexpected significantly lower activity compared to the parental chimeric antibody 969.g0. A single representative experiment is shown in **Figure 13**. The mean IC_{50} for 969.g0 in the monocyte assay was 24.6ng/ml, compared to 333.0ng/ml for 969.g5. This indicates a 13.5-fold decrease in antibody potency when both antibodies are compared using this assay format.

A series of experiments were performed using Ab969 in order to reveal why the humanised antibody exhibited a reduced activity in the MCP-1 inhibition assay. The data suggested that the activity loss of Ab969.g5 observed in the MCP-1 inhibition assay was due to the order in which antibody and ligand were added to the target cells. The activity of both Ab969.g0 and Ab969.g5 was reduced when a competitive assay format is applied, but more importantly, a larger differential in their blocking-activity was detected. To analyse whether a lower 'on-rate' of humanised Ab969 could be responsible for the reduction in potency a BIAcore analysis was performed. These data had identified that, while the K_D of Ab969.g0 and Ab969.g5 were similar, the Ka was lower for Ab969.g5, at $1.57x10^6$ $M^{-1}s^{-1}$ compared to $2.16x10^6$ $M^{-1}s^{-1}$ for 969.g0 (see **Table 5**). In a competitive assay, where CSF-1 and the anti-CSF-1R antibody are competing for binding to the same receptor, a slower antibody on-rate could result in reduced blocking activity if the on-rate for the ligand is also high and the ligand is present at a high

concentration. It is known that human CSF-1 has a particularly high on-rate at $2.19x10^6$ $M^{-1}s^{-1}$, similar to the antibodies, and the assay was performed with a high CSF-1 concentration (250ng/ml).

In order to provide further evidence that the reduction in blocking activity of 969.g5 was due to an innate property of the antibody, an ELISA that measures CSF-1 binding to CSF-1R was developed. This method is from here on referred to as the 'ELISA ligand-blocking assay'. This assay was performed using competitive binding, where CSF-1 and antibody were pre-mixed before application to plate-bound CSF-1R. The assay was also carried out to assess the influence of CSF-1 concentration on antibody activity.

When the IC_{50} of 969.g0 and 969.g5 was measured in the ligand-blocking ELISA using a CSF-1 concentration of 1ng/ml, both antibodies appeared to have a similar activity with an IC_{50} of 12.83ng/ml versus 19.65ng/ml respectively. When a concentration of 10ng/ml CSF-1 was used in the assay, the IC_{50} of both Ab969.g0 and Ab969.g5 increased, but more importantly, the differential between them increased further to 79.29ng/ml versus 268.10ng/ml respectively. The trend continued in an assay using 100ng/ml CSF-1, where Ab969.g0 and Ab969.g5 gave IC_{50} values of 828.70ng/ml and 3947.00ng/ml respectively. A competitive assay demonstrates that Ab969.g5 is less active than Ab969.g0 at blocking CSF-1 binding to CSF-1R. This reduction in potency becomes more pronounced as the concentration of CSF-1 is increased.

iv) Identification of humanised grafts of Ab 969 with activity equivalent to chimeric Ab 969 in MCP-1 inhibition assay

A panel of Ab969 humanised intermediate grafts were prepared by transient expression so that *in vitro* activity could be compared (**Table 8**). The corresponding chimeric antibody (Ab969.g0) and fully humanised graft (Ab969.g5) were also included in the transient expression so that a direct comparison of antibody characteristics could be made within the same batch.

Table 8:

Antibody	Graft	VK donor residues	VH donor residues
969.g0	969cHcL	N/A	N/A
969.g7	969gH2gL4	K38, Y71	-
969.g9	969gH2gL6	K38	-
969.g2	969gH2gL7	Y71	-
969.g5	969gH2gL8(SG)	-	-

The *in vitro* activity of each antibody was tested in the MCP-1 inhibition assay. The assay format that uses a CSF-1 titration with a single concentration of antibody was chosen, as the format enables rapid screening of a large antibody panel and highlights any differential CSF-1R blocking activity. In this assay, a dose-dependent release of MCP-1 from the primary human monocytes was detected and the relative ability of each antibody to block CSF-1R activity measured by the concentration of CSF-1 where MCP-1 was released. Monocytes were seeded at 20,000 cells per well in media in the presence of a titration of recombinant human CSF-1 (2-fold dilution series comprising 18 concentrations, maximum 500ng/ml). A single dose of 1μg/ml antibody was added. Cells were incubated for 24h and supernatant collected. Secreted MCP-1 was measured by ELISA. The MCP-1 inhibition assay revealed that antibodies Ab969.g2 and Ab969.g7 retained high CSF-1R blocking activity, with both entities capable of completely inhibiting MCP-1 secretion when CSF-1 was added to the monocytes at a concentration greater of 100ng/ml (**Figure 14**). In this assay, a clear loss of activity was detected for Ab969.g5, which could only inhibit MCP-1 secretion up to a CSF-1 concentration of 10ng/ml. Similarly, antibody Ab969.g9 exhibited reduced CSF-1R blocking activity compared to Ab969.g2 and Ab969.g7.

The IC_{50} of selected Ab969 humanised grafts was measured in the MCP-1 assay to provide a more thorough assessment of their relative ability to inhibit CSF-1-mediated monocyte activation. It was considered important to assess antibody activity using several different donors with no pooling of monocytes from mixed sources. **Table 9** shows IC_{50} values of the antibodies accumulated from assays performed on Ab969 using 6 different donors.

Both the Ab969.g2 and Ab969.g7 humanised grafts exhibit a relative IC_{50} that is comparable with the chimeric Ab969.g0 antibody. In stark contrast, Ab969.g5, exhibits much less potency in the assay (mean chimeric/graft IC_{50} ratio of a 19.6).

Table 9:

Antibody	VK donor residues	VH donor residues	IC_{50} (ng/ml)							
			Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Mean ± S.E.	Mean chimeric/graft
969.g0	N/A	N/A	31.0	5.7	59.4	41.7	20.9	7.1	27.6 +/- 8.5	N/A
969.g7	K38, Y71	-	46.3 (1.5)	6.1 (1.1)	N/A	N/A	N/A	N/A	26.2 +/- 14.2	1.3
969.g2	Y71	-	N/A	N/A	80.6 (1.4)	42.2 (1.0)	22.3 (1.1)	14.4 (2.0)	39.9 +/- 14.8	1.3
969.g5	- (SG)	-	667.1 (21.5)	100.3 (17.6)	N/A	N/A	N/A	N/A	383.7 +/- 200.4	19.6

v) Affinity of humanised Ab696 antibody panel

The affinity of each Ab969 humanised antibody graft and the parental chimeric antibody was measured by BIAcore (**Table 10**) where three independent experiments were carried out and the mean values calculated. The data shows that affinity (K_D) does not appear to change during

the humanisation process from chimeric molecule (Ab969.g0) through to the 'fully humanised' antibody Ab969.g5. However, the antibody 'on-rate' decreases when humanisation proceeds beyond the Ab969.g2 graft; both Ab969.g4 and Ab969.g5 possess lower K_a values than the preceding grafts. Furthermore, the K_a for Ab969.g5 is lower than Ab969.g4, potentially indicating that the mutation of the DG isomerisation site to SG reduces the antibody on-rate further still.

Table 10:

Antibody Graft	Antibody Name	VK donor residues	VH donor residues	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)
969cHcL	969.0	N/A	N/A	2.83E+06	1.03E-05	3.6
				2.79E+06	1.10E-05	3.9
				2.68E+06	9.51E-06	3.6
				2.77E+06	**1.03E-05**	**3.7**
969gH1gL1	969.g1	K38, Y71, F87	A78	2.65E+06	8.47E-06	3.2
				2.57E+06	6.04E-06	2.4
				2.51E+06	9.88E-06	3.9
				2.58E+06	**8.13E-06**	**3.2**
969gH2gL1	969.g6	K38, Y71, F87	-	2.43E+06	8.84E-06	3.6
				2.44E+06	8.95E-06	3.7
				2.54E+06	6.63E-06	2.6
				2.47E+06	**8.14E-06**	**3.3**
969gH2gL4	969.g7	K38, Y71	-	2.57E+06	7.73E-06	3.0
				2.60E+06	9.41E-06	3.6
				2.52E+06	1.15E-05	4.6
				2.56E+06	**9.55E-06**	**3.7**
969gH2gL7	969.g2	Y71	-	2.46E+06	1.07E-05	4.3
				2.49E+06	8.39E-06	3.4
				2.44E+06	8.74E-06	3.6
				2.46E+06	**9.28E-06**	**3.8**
969gH2gL8	969.g4	-	-	1.99E+06	5.00E-06	2.5
				1.94E+06	6.65E-06	3.4
				1.97E+06	5.00E-06	2.5
				1.97E+06	**5.55E-06**	**2.8**
969gH2gL8(SG)	969.g5	-	-	1.71E+06	5.00E-06	2.9
				1.85E+06	7.03E-06	3.8
				1.84E+06	6.76E-06	3.7
				1.80E+06	**6.26E-06**	**3.5**

Conclusions:

The testing of a panel of Ab969 humanised grafts in several assays revealed that the tyrosine residue at position 71 (e.g. Y71) within the light chain improved activity of the antibody. Substitution of Y71 for e.g. a phenylalanine results in a reduction of antibody on-rate (decreased K_a) relative to the parental chimeric molecule. This results in a reduced ability of the antibody to block CSF-1 binding to CSF-1R, revealed in an assay monitoring the activity of CSF-1R in primary human monocytes (MCP-1 inhibition assay).

Example 4 – Molecular Stability of humanised Ab696 panel

i) Thermal Stability

Thermal stability (measured as melting temperature, Tm) was determined by two independent methods; one method monitors unfolding by binding of fluorescent dye to exposed hydrophobic surfaces (Thermofluor method), the other by calorimetry (DSC), an orthogonal technique.

T_m measured by Thermofluor for various grafts of antibody 969 (in PBS pH7.4) is summarised in Table 11.

Table 11

Sample	T_m1 Mean (°C)	T_m1 S.D. (°C)	T_m2 Mean (°C)	T_m2 S.D. (°C)
969.g2	80.5	0.6	65.6	0.4
969.g2	80.5	0.6	65.6	0.4
969.g5	79.7	0.6	64.7	0.2
969.g5 (pH 5.0)	82.7	0.1	59.9	0.5
969.g7	79.8	0.5	65.9	0.4
969.g9	79.4	0.5	65.8	0.5
IgG4 Control	66.4	0.8	ND	ND

Overall, the Fab' T_m as measured by Thermofluor suggests that most 969 grafts are thermally more stable than the IgG4 control.

ii) The effect of sample concentration on aggregation propensity

As a predictor of the stability of samples during storage, the effect of antibody concentration on stability in PBS pH 7.4 was studied.

Experiment 1:

Antibodies were concentrated to >10 mg/ml and incubated at room temperature for 5 days. Immediately after concentration (T_0) 969.g7 appeared cloudy and 969.g8 appeared slightly opalescent. In contrast, 969.g5 sample was judged to be clear by visual inspection. After 5 days incubation at room temperature 965.g5 sample remained clear, whereas the aggregation of 969.g7 and 969.g8 had progressed further, each exhibiting a heavy precipitate.

From this study, it was possible to rank the samples in order of resistance to aggregation in these conditions as follows: 969.g5 > 969.g8 > 969g7.

Experiment 2:

Prior to concentration, all three antibody samples were clear by visual inspection with the exception of 969.g6 (at 4.68 mg/ml) which appeared opalescent. Upon concentration to 16 mg/ml, precipitation of 969.g6 was noted after 24 hours storage at both room temperature and

4°C. However, both 969.g1 and 969.g4 remained visibly clear. After 5 days further incubation, large particles were evident for sample 969.g1 at both room temperature and 4°C. No aggregation was observed for sample 969.g4 at either room temperature or 4°C, as judged by visual inspection.

From this study it was possible to show that 969.g6 had some aggregation instability when stored at low concentration (4.68mg/ml) in PBS pH 7.4. Furthermore, this precipitation was exacerbated by further antibody concentration. Concentrated 969.g1 showed a slower rate of aggregation, whereas concentrated 969.g4 remained stable up to 5 days at either storage temperature. The order of stability to aggregation was therefore: 969.g4 > 969.g1 > 969.g6.

Experiment 3: Antibodies 969.g2, 969.g5, 969.g7 and 969.g9

Analysis was performed on the samples immediately after concentration (T_0), after an overnight incubation and 5 days after concentration. All samples were clear by visual inspection before concentration and there was no evidence of particle formation. Immediately after concentration to 23.07 mg/ml, the 969.g7 sample showed precipitation as had been observed previously in experiment 1. There was slight opalescence observed with 969.g9, which became more noticeable after 24hour storage at either room temperature or 4°C. All of the other 969 grafts appeared clear by visual inspection immediately post concentration.

After 21 days incubation there was no further visible change from that observed after overnight incubation at room temperature; both 969.g7 and 969.g9 grafts aggregated as a result of concentrating the samples while there was no obvious visible aggregation for the other samples.

Overall the 969.g2 sample showed the least tendency to aggregate as a result of increasing the concentration. This also correlated with the highest T_m as measured by Thermofluor.

Conclusion: During the expression and purification of the humanised Ab969 panel, it became apparent that increasing the antibody concentration above 10 mg/ml resulted in rapid precipitation of some humanised antibody grafts. Specifically, Ab969.g1, Ab969.g6 and Ab969.g7 formed precipitate, while Ab969.g2, Ab969.g4 and Ab969.g5 did not. This data indicates that a substitution of the lysine residue at position 38 (K38) within the light chain for glutamine improves antibody stability when concentrated above 10 mg/ml.

Example 5 *In vitro* Analysis of Ab969.g2

i) Sequence of Ab969.g2

Ab969.g2 contains the gL7 light chain graft and gH2 heavy chain graft. Alignments of the rat antibody (donor) V-region sequences with the human germline (acceptor) V-region sequences are shown in Figures 2A and 2B, together with the final humanised sequences for the light chain graft gL7 and heavy chain graft gH2.

The heavy chain framework residues in graft gH2 are all from the human germline gene. The Glutamine residue at position 1 of the human heavy chain framework was replaced with Glutamic acid (E1) to improve the expression and purification of a homogeneous product e.g. by the conversion of Glutamine to pyroGlutamate at the N-terminus of antibodies and antibody fragments.

The light chain framework residues in graft gL7 are all from the human germline gene, with the exception of residue 71 (Kabat numbering), where the donor residue Tyrosine was retained. Retention of this residue provided improved potency of the humanised antibody as shown in Example 3.

ii) Inhibition of IL-34 dependent human monocyte activation

The activity of Ab969.g2 compared to 969.g0 was assessed in the IL-34-dependent human monocyte assay. The experiment was performed using two separate monocyte donors and the mean IC_{50} for inhibition of IL-34-mediated monocyte stimulation calculated (**Table 12**). There was no significant difference in IC_{50} between Ab969.g2 and Ab969.g0.

Primary human monocytes were seeded at 20,000 cells per well in the presence of 100ng/ml recombinant human IL-34 and a dose titration of anti-CSF-1R antibody (half-log dilution series comprising 16 concentrations, maximum 10µg/ml). Cells were incubated for 24 hours and supernatant collected. Secreted MCP-1 was measured by ELISA (R&D systems DY279). Graph shows percentage inhibition of MCP-1 production compared to the CSF-1 only control.

Table 12

Antibody	IC_{50} (ng/ml)		
	Donor 1	Donor 2	Mean ± S.E.
969.g0	20.10	9.79	14.95 +/- 5.17
969.g2	20.58	9.59	15.09 +/- 5.51

iii) Binding of Ab969.g2 to SNPs of human CSF-1R

There are four non-synonymous single nucleotide polymorphisms (SNPs) located within the ligand-binding domain of the human CSF-1R gene that have been reported in the human population. These SNPs are V32G, A245S, H247P and V279M (**Figure 1F**). Each SNP variant of human CSF-1R was generated and stably expressed in cell lines. Binding of Ab969.g2 to each SNP was confirmed by flow cytometry.

Example 6 Pharmacodynamic marker analysis in cynomolgus monkey

A pharmacokinetic/pharmacodynamic (PK/PD) study with the humanised monoclonal anti-CSF-1R antibody 969.g2 was performed to demonstrate the pharmacological activity of the antibody in a non-human primate (cynomolgus monkey). Groups of three cynomolgus monkeys were dosed intravenously with a single dose of either 7 mg/kg (Group 1) or 1.5 mg/kg (Group 2) 969.g2 antibody. The antibody was well tolerated with no adverse clinical signs. Serum samples were taken at multiple timepoints throughout the 25-day study.

The serum concentration of 969.g2 was measured by ELISA and pharmacokinetic analysis performed (Table 13). PK parameters were calculated using WinNonlin software.

$t_{1/2}$ is half-life of antibody in serum.

C_{max} is peak concentration of antibody in serum.

AUC is the area under the curve (the integral of the concentration-time curve) and provides an indication of total exposure to the drug.

Clearance is the volume of plasma cleared of drug per unit time.

Vol. Dist. is the volume of distribution, the apparent volume in which a drug is distributed.

There was a good correlation between observed and predicted values (animal 2 being an exception and regarded as an outlier). C_{max} was proportional to the dose; AUC was greater than dose proportional, indicating slower clearance at the higher dose. The majority of 969.g2 was detected in the serum.

Table 13

Animal	Dose [mg/kg]	t½ [h]	Cmax [µg/mL]	AUC_{INF_obs} [h*µg/mL]	Clearance (Cl_{obs}) [mL/h/kg]	Vol. Distribution (V_{z_obs}) [mL/kg]
1	7	66.1	209.4	23184.5	0.30	28.8
2	7	30.3	117.6	6075.9	1.15	50.4

3	7	63.3	188.7	16761.0	0.42	38.1
4	1.5	27.9	36.1	1928.1	0.78	31.3
5	1.5	22.7	34.7	1668.6	0.90	29.5
6	1.5	27.1	36.6	1634.8	0.92	35.9

The primary route for clearance of CSF-1 is through binding to its cognate receptor, CSF-1R. Blockade of CSF-1 binding to CSF-1R is expected to increase the serum concentration of CSF-1 though prevention of receptor-mediated clearance; a phenomenon that has been observed in murine models. Therefore, an increase in serum CSF-1 concentration is a pharmacodynamic marker of CSF-1R engagement and inhibition.

Both doses of 969.g2 instigated a rapid and significant accumulation of serum CSF-1. The effect was dose dependent, with the 7 mg/kg dose giving a peak CSF-1 concentration of approximately 10-fold higher than the 1.5 mg/kg dose. A pharmacokinetic/pharmacodynamic relationship was observed with normalisation of CSF-1 levels following clearance of the antibody. CSF-1 levels returned to baseline by the end of the study for both treatment groups. Results are shown in **Figures 15a** and **15b**.

Figure 15a shows the concentration of CSF-1 in serum samples taken from cynomolgus monkeys treated with a single intravenous dose of 7 mg/kg Ab969.g2. The serum concentration of CSF-1 was measured in two independent assays. The graph shows the mean concentration of CSF-1 for the three animals in Group 1 (7 mg/kg) with standard error (squares). Also shown is the mean serum concentration of Ab969.g2 (circles).

Figure 15b shows the concentration of CSF-1 in serum samples taken from cynomolgus monkeys treated with a single intravenous dose of 1.5 mg/kg Ab969.g2. The serum concentration of CSF-1 was measured in two independent assays. The graph shows the mean concentration of CSF-1 for the three animals in Group 2 (1.5 mg/kg) with standard error (squares). Also shown is the mean serum concentration of Ab969.g2 (circles).

There are two major populations of circulating human and cynomolgus monkey monocytes; (i) CD14+ CD16- 'classical' monocytes and (ii) CD14+ CD16+ 'non-classical' or 'resident' monocytes. Murine models have demonstrated that resident tissue macrophages, including TAMs, are derived from the non-classical monocyte population. Furthermore, non-classical monocytes are derived from further differentiation of the classical monocyte population.

Circulating monocyte populations in cynomolgus monkeys dosed with 969.g2 were monitored by four-colour flow cytometry of whole blood. Flow cytometry was performed using anti-cyno-CD45-PerCP, anti-human-HLA-DR-APC, anti-human-CD14-FITC (My4 clone) and anti-human-CD16-PE (3G8 clone) antibodies. Gates were set using the appropriate isotype control for each antibody. Classical monocytes were defined as $CD45^+$ $HLA\text{-}DR^+$ $CD14^+$ $CD16^-$. Non-classical monocytes were defined as $CD45^+$ $HLA\text{-}DR^+$ $CD14^+$ $CD16^+$.

Both doses of 969.g2 instigated a gradual depletion of non-classical $CD14^+$ $CD16^+$ monocytes throughout the first week of the study. Almost total depletion of non-classical monocytes was caused by the 7 mg/kg dose. Non-classical monocyte reduction by both 7 mg/kg and 1.5 mg/kg doses appeared to peak at the day 4 timepoint, with numbers returning to normal by day 11. Results are shown in Figure 15c. The bar graphs show mean number of circulating non-classical $CD14^+$ $CD16^+$ monocytes at timepoints throughout the study (days (D) 0, 1, 4, 18 and 25) with standard error. The mean concentration of serum CSF-1 is also plotted with standard error.

This example confirmed that (i) antibody 969.g2 was capable of binding CSF-1R and blocking CSF-1 binding in the cynomolgus monkey, (ii) demonstrated pharmacological activity of the antibody 969.g2 in a non-human primate model, (iii) demonstrated that antibody 969.g2 selectively depleted the non-classical population of cynomolgus monkey monocytes *in vivo* – the monocyte population believed to be precursors of tumour-associated macrophages, and (iv) that serum CSF-1 concentration was suitable as a biomarker for measuring 969.g2 activity.

Example 7: Inhibition of Growth of MCF-7 Breast cancer xenograft

Antibody 969.g2 is not capable of binding to mouse CSF-1R. Accordingly, *in vivo* mouse studies were carried out using an anti-murine CSF-1R antibody Ab535 to show the utility of Ab969.g2 to treat cancer and fibrosis. Ab535 has been shown to have comparable properties and activity to Ab969.g2 in a number of *in vitro* experiments.

Ab535 has been shown to inhibit CSF-1-mediated monocyte survival in Example 2 iii), has a comparable affinity in Example 2 iv) and does not trigger CSF-1R internalization in Example 2 vii).

As *in vivo* study on Ab535 the *in vivo* MCF-7 breast cancer xenograft model was carried out as follows:

The study measured the therapeutic efficacy of the antibody Ab535, administered subcutaneously (s.c.), vs. a control antibody, a positive control and the vehicle control in immunodeficient nude mice bearing subcutaneous transplants of the human breast cancer xenograft MCF-7. Tumour growth inhibition was used as therapeutic parameter. The human breast cancer MCF-7 was used as subcutaneous xenotransplantation model in immunodeficient female NMRI:nu/nu mice. The MCF-7 cell line was obtained from the tumour bank of the National Cancer Institute (USA). For experimental use, cells were cultivated *in vitro* in RPMI 1640 medium + 10% FCS. Cells were taken from sub-confluent cultures and inoculated subcutaneously into mice. At palpable tumour size (4-10 mm) treatment started. The test compounds and the vehicle control were given s.c. three times per week. The positive control was administered intravenously once daily on days 24, 33 and 40. The injection volumes were individually adjusted to the body weight at time of injection. Tumour diameters were measured three times weekly with a calliper. Tumour volumes were calculated according to V = (length x (width)2)/2. For calculation of the relative tumour volume (RTV) the volumes at each measurement day were related to the day of first treatment. At each measurement day the median and mean tumour volumes per group and also the median treated to control (T/C) values in percent were calculated.

Results are shown in **Figure 16**. Ab535 showed a dose-dependent antitumour effect. Both dosages of the control mouse IgG antibody did not induce a tumour growth inhibition. Relative tumour volumes were comparable with the vehicle control and the positive control caused tumour growth inhibition.

Conclusion: the test antibody Ab535 induced a statically significant, antitumour effect in the human breast cancer xenograft MCF-7 at the highest dose (30 mg/kg/day).

Example 8: Inhibition of Growth of PC-3 orthotopic prostate cancer

The antitumoral and antimetastatic efficacy of antibody Ab535 was also tested using an orthotopic prostate cancer model PC-3 *in vivo*. The PC-3 cell line was genetically altered to continuously express luciferase allowing *in vivo* bioluminescence imaging analysis, which allowed to monitor tumor growth in vivo and to perform *ex vivo* metastasis analysis in selected organs.

The study consisted of 6 experimental groups, each containing either 11 (Group 5) or 12 (all other Groups) male NMRI nude mice after randomization. At day 0, PC-3 cells were

orthotopically implanted into the prostate of all participating male NMRI nude mice. On day 3, the onset of tumor growth was verified via *in vivo* bioluminescence imaging. On day 8, a second in vivo bioluminescence imaging was performed and tumor-bearing animals were randomized into six groups according to the imaging results such that mean bioluminescence intensity and thus the tumor size was similar in each Group. On the following day (day 9), therapy was initiated. Animals of Groups 2 and 3 received 30 and 10 mg/kg of Control Antibody, respectively, 3x weekly s.c. until day 42. Animals of Treatment Groups 4 and 5 received 30 and 10 mg/kg of antibody Ab535, respectively, 3x weekly s.c. until day 42. Animals of Group 1 represented the Vehicle Control and received Vehicle (PBS) 3x weekly s.c. until day 42. Animals of Group 6 represented the Positive Control and received 360 mg/kg control i.v. once weekly for four weeks (on days 10, 17, 24 and 31). During the course of the study, the growth of the orthotopically implanted PC-3 tumors was monitored *in vivo* on days 3, 8 (randomization), 15, 22, 29, 36 and 43 using bioluminescence imaging.

A necropsy was performed at study end. Primary tumor weight and volume were determined. Selected organs (liver, spleen and lung) were collected, a portion of each fixed in formalin and the remainder analyzed regarding the metastasis pattern via bioluminescence imaging using an *in vitro* luciferase assay. Additionally, the femur from one leg and the same portion of lumbar spine were collected for analyzing the metastases pattern in bones using the *in vitro* luciferase assay.

The *in vivo* bioluminescence signal was composed out of both primary tumor and metastases (whole body imaging). Based on these data, a tumor growth curve could be calculated for all groups (**Figure 17**). Tumor development was homogeneous within all study groups until randomization and start of therapy. In the following, Vehicle Control Group 1 as well as the two Control Antibody RTE11 Groups 2 and 3 showed a regular tumor growth. A highly significant reduction of tumor growth measured *in vivo* on day 43 could be observed for the positive control (Group 6). Antibody Ab535, administered at 30 or 10 mg/kg, respectively (Groups 4 and 5), led to a significant reduction of tumor growth when monitored using *in vivo* bioluminescence imaging.

Primary tumor volumes and wet weights were determined during necropsy on day 44. The Positive Control (Group 6), led to a highly significant reduction of both primary tumor volume and weight. Antibody Ab535 was administered at 30mg/kg (Group 4), a significant

reduction of both primary tumor volume and weight could be observed. When Ab535 was administered at 10mg/kg (Group 5), reduction of tumor volume was noticable but less than shown by Group 4. No significant antitumoral efficacy could be observed in case of the Control Antibody.

Using the bioluminescence imaging technique, primary tumor luciferase activities were measured post necropsy. The obtained results were comparable to the results of the necropsy findings and the *in vivo* growth curve. Positive control (Group 6) led to a highly significant reduction of the primary tumor luciferase activity. Antibody Ab535, administered at 30mg/kg (Group 4), led to a significant reduction of the primary tumor luciferase activity, whereas the reduction was less, when Ab535 was administered at 10mg/kg (Group 5). No significant reduction of the luciferase activity could be found for the Control Antibody.

Several additional organs (liver, spleen, lung, femur and a part of the lumbar spine) were analyzed using the ex vivo bioluminescence imaging technique. In case of the positive control, significant reductions of luciferase activity could be shown for femur and lumbar spine, whereas the signal reduction was just below significance in case of liver and spleen. In case of Antibody Ab535, administered at 30mg/kg (Group 4), the reduction of the luciferase activity was significant for liver and noticeable for all other organs. The Control Antibody (Groups 2 and 3) did not lead to any reduction of the luciferase activity in all organs tested.

In conclusion, a significant antitumoral efficacy could be demonstrated for the antibody Ab535 in this tumor model, combined with a noticable antimetastatic efficacy.

Example 9: Effect of anti-CSF-1R antibody in a Bleomycin induced *in vivo* model of pulmonary fibrosis

Bleomycin is an antibiotic first isolated from *Streptomyces verticillatus* and has been used as a chemotherapeutic for various cancers. The bleomycin model of lung fibrosis is a well-established model and was used essentially as described in Madtes, DK et al, 1999, Am J Respir Cell Mol Biol, 20, 924-34. The detailed protocol can be found in the following reference Morschl, E., Molina, J. G., Volmer, J., Mohsenin, A., Pero, R. S., Hong, J. S., Kheradmand, F., Lee, J. J. and Blackburn, M. R. (2008), A3 adenosine receptor signaling influences pulmonary inflammation and fibrosis. Am. J. Respir. Cell Mol. Biol.39: 697-705.

All mice used were wild type C57Blk6 female mice (20g) purchased from Harlan Labs. An intra-tracheal (IT) cut down instillation was used where mice were anaesthetised with avertin

and a tracheostomy performed in order to instil a 3.5 Unit dose of bleomycin in 50 µl of saline or 50 µl of saline alone as a control. Treatment in this manner leads to an inflammatory phase that peaks on day 7 after bleomycin exposure and a fibrotic phase that is maximum on day 21 post exposure. The effect of antibody Ab535 was investigated where antibody was dosed only in the fibrotic phase of the model and was administered subcutaneously at 30mg/kg, three times per week from day 9-21. Animals were sacrificed at day 21 and readouts included histopathological analysis of the lungs, BAL (bronchoalveolar lavage) fluid cellularity and the measurement of soluble collagen in BAL fluid. Histopathological analysis was conducted to score lung damage using a modified Ashcroft scoring system to determine the severity of lung fibrosis (Hubner RH et al, 2008, Biotechniques 44: 507-17). Excised lungs were inflated with 10% formalin to 25cm pressure and processed through a series of alcohols and xylene, embedded in paraffin and tissue sections de-paraffinised prior to processing and staining with Masson's Trichrome.

The amount of soluble collagen in BAL fluid was assessed using a commercially available Sircol assay kit following the manufacturer's instructions.

The effect on the macrophage population in the BAL fluid was determined using cytospin preparations. Aliquots of BAL cells were spun onto microscope slides, stained with Diff-Quick and macrophages counted.

It was found that therapeutic treatment with the anti-CSF-1R antibody, Ab535, with dosing started at day 9 resulted in greatly reduced bleomycin-induced lung fibrosis. Both the severity and extent of fibrosis was significantly reduced. The treated mice had reduced collagen production, improved fibrotic pathology and improved pulmonary barrier protection.

Figure 18a shows that treatment of bleomycin-induced lung fibrosis with Ab535 reduced BALF collagen concentration compared to treatment with the isotype control.

Figure 18b shows that treatment of bleomycin-induced lung fibrosis with Ab535 reduced the Ashcroft score of the samples compared to treatment with the isotype control which thus shows that the mice treated with Ab535 had improved fibrotic pathology.

Figure 18c shows that treatment of bleomycin-induced lung fibrosis with Ab535 reduced the concentration of albumin in the serum compared to treatment with the isotype control which thus shows that the vascular permeability of the mice treated with Ab535 was improved.

Figure 19 shows representative images of the histopathological analysis of lungs from saline control, bleomycin plus isotype control and bleomycin plus Ab535 treated animals.

Animals treated with Ab535 had a greatly reduced fibrosis of the lungs compared to the isotype control, bleomycin treated animals.

Example 10. Effect of anti-CSF-1R antibody in an adenosine deaminase-deficient mouse model of pulmonary fibrosis

Adenosine is a potent signalling nucleoside, the levels of which increase when cells suffer stress or are damaged and a wide variety of responses are produced when adenosine engages its specific G protein coupled receptors. Adenosine deaminase (ADA) is a purine catabolism enzyme that converts adenosine to inosine. ADA knockout mice have been generated and shown to have increased levels of adenosine in serum as well as in tissues such as kidney, liver and lung (Blackburn, MR et al, 1998, J Biol Chem, 273(9): 5093-5100). These mice exhibit features of chronic lung disease such as alveolar destruction, airway inflammation and excessive mucus production which are associated with increased levels of adenosine in the lung (Blackburn MR et al, 2000, J Exp Med, 192: 159-70). The effects are such that the mice die by three weeks of age due to respiratory distress. Administration of exogenous ADA using a low-dose regimen reduces adenosine levels and extends the life-span of these mice allowing a model of pulmonary fibrosis to be developed (Chunn JL et al, 2005, J Immunol 175: 1937-46, Pedrosa M et al, 2011, PLoS One, 6(7): e22667). In this model, chronic elevation of adenosine levels is associated with an increase in pro-fibrotic mediators including TGF □ in the lungs, increased collagen deposition in lung tissue and increased fibrotic lung pathology. To investigate the effect of molecules with anti-fibrotic potential, ADA-deficient mice are maintained on a low dose exogenous ADA regimen for several weeks, the ADA treatment is then stopped and the potential anti-fibrotic agent administered.

The effect of the anti-CSF-1R antibody Ab535 was investigated in the ADA knockout mouse model of pulmonary fibrosis. In this model, the enzyme deficient mice were maintained on ADA enzyme therapy from day 1 to day 21 post-birth. An ADA-polyethylene glycol (PEG) conjugate was prepared (Young HW et al, 2004, J Immunol 173: 1380-89) and intramuscular injections administered on postnatal days 1, 5, 9, 13 and 17 (0.625, 1.25, 2.5, 2.5 and 2.5 units respectively), followed by 5 units injected intraperitoneally on day 21. No further enzyme was administered after day 21. Ab535 was dosed subcutaneously at 30mg/kg, in a volume of 100 µl three times/week from day 25 (post-natally) until the animals were sacrificed on day 42.

Animals were sacrificed at day 42 and readouts included histopathological analysis of the lungs, BAL fluid cellularity and quantitation of soluble collagen in BAL fluid. Histopathological analysis was conducted to score lung damage using a modified Ashcroft scoring system to determine the severity of lung fibrosis (Hubner et al, 2008, Biotechniques 44: 507-17). Excised lungs were inflated with 10% formalin to 25cm pressure and processed through a series of alcohols and xylene, embedded in paraffin and tissue sections de-paraffinised prior to processing and staining with Masson's Trichrome.

The amount of soluble collagen in BAL fluid was also assessed using a commercially available Sircol assay kit following the manufacturer's instructions.

The effect on the macrophage population in the BAL fluid was determined using cytospin preparations. Aliquots of BAL cells were spun onto microscope slides, stained with Diff-Quick and macrophages counted.

It was found that therapeutic treatment with anti-CSF-1R antibody Ab535 significantly reduced lung fibrosis in ADA-deficient mice. The treated mice had reduced collagen production, improved fibrotic pathology and improved pulmonary barrier function and protection.

Figure 20a shows that treatment of ADA-deficient mice with induced lung fibrosis with Ab535 reduced BALF collagen concentration compared to treatment with the isotype control.

Figure 20b shows that treatment of ADA-deficient mice with induced lung fibrosis with Ab535 reduced the Ashcroft score of the samples compared to treatment with the isotype control which thus shows that the mice treated with Ab535 had improved fibrotic pathology.

Figure 20c shows that treatment of ADA-deficient mice with induced lung fibrosis with Ab535 reduced the concentration of albumin in the serum compared to treatment with the isotype control which thus shows that the vascular permeability of the mice treated with Ab535 was improved.

Figure 20d shows that treatment of ADA-deficient mice with induced lung fibrosis receiving Ab535 had reduced numbers of macrophages in BAL fluid

Figure 21 shows representative images of the histopathological analysis of lungs from normal mice (ADA+) and ADA-deficient mice with induced lung fibrosis (ADA-), both treated with isotype control or Ab535. In the ADA- mice, animals treated with Ab535 had a greatly reduced fibrosis of the lungs compared to the isotype control.

Accordingly, it has been shown in two mouse models of lung fibrosis that treatment with an anti-CSF-1R antibody can effectively treat fibrotic disease.

Claims:

1. An anti-CSF-1R antibody comprising: a heavy chain, wherein the heavy chain comprises the sequence given in SEQ ID NO:23; and a light chain, wherein the light chain comprises the sequence given in SEQ ID NO:15.

2. The anti-CSF-1R antibody according to claim 1, wherein the antibody molecule is selected from the group consisting of: a complete antibody molecule having full length heavy and light chains or a fragment thereof, for example selected from the group comprising a Fab, modified Fab', Fab', $F(ab')_2$, Fv, and scFv fragment.

3. The anti-CSF-1R antibody according to claims 1 or 2 having a heavy chain comprising the sequence given in SEQ ID NO:27 and a light chain comprising the sequence given in SEQ ID NO:19.

4. The anti-CSF-1R antibody according to any one of claims 1 to 3, having an effector or a reporter molecule attached to it.

5. The anti-CSF-1R antibody according to any one of claims 1 to 4, having a binding affinity for human CSF-1R of 10pM or less than 10pM.

6. An anti-CSF-1R antibody comprising a light chain, wherein the variable domain of the light chain comprises SEQ ID NO:1 for CDR-L1, SEQ ID NO:2 for CDR-L2, and SEQ ID NO:3 for CDR-L3, and a heavy chain, wherein the variable domain of the heavy chain comprises SEQ ID NO:4 for CDR-H1, SEQ ID NO:5 for CDR-H2 and SEQ ID NO:6 for CDR-H3.

7. An isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody according to any one of claims 1 to 5.

8. A cloning or expression vector comprising one or more DNA sequences according to claim 7.

9. A vector according to claim 8, wherein the vector comprises the sequences given in SEQ ID NO:28 and/or SEQ ID NO:20.

10. A host cell comprising one or more cloning or expression vectors according to claim 9.

11. A process for the production of the antibody of any one of claims 1 to 5, comprising culturing the host cell of claim 10 and isolating the antibody.

12. A pharmaceutical composition comprising an antibody according to any one of claims 1 to 5, in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

13. A pharmaceutical composition according to claim 12, additionally comprising other active ingredients.

14. An antibody according to any one of claims 1 to 5 or a composition according to claim 12 for use as a medicament.

15. An antibody according to any one of claims 1 to 5 or a composition according to claim 12 for use in the treatment of cancer or a fibrotic disease.

16. The antibody or the composition for use according to claim 15, wherein the cancer is selected from the group consisting of breast cancer, prostate cancer, bone cancer, colorectal cancer, leukaemia, lymphoma, skin cancer, esophageal cancer, gastric cancer, astrocytic cancer, endometrial cancer, cervical cancer, bladder cancer, renal cancer, lung cancer, liver cancer, thyroid cancer, head and neck cancer, pancreatic cancer and ovarian cancer.

17. The antibody or the composition for use according to claim 15, wherein the fibrotic disease is selected from the group consisting of pulmonary fibrosis such as idiopathic pulmonary fibrosis and cystic fibrosis, renal fibrosis, liver cirrhosis, endomyocardial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, progressive massive fibrosis, nephrogenic systemic fibrosis, Crohn's disease, keloid, myocardial infarction, scleroderma and arthrofibrosis.

18. Use of the antibody according to any one of claims 1 to 5 or the composition according to claim 12 in the manufacture of a medicament for the treatment of cancer or a fibrotic disease.

19. The use according to claim 18, wherein the cancer is selected from the group consisting of breast cancer, prostate cancer, bone cancer, colorectal cancer, leukaemia, lymphoma, skin cancer, esophageal cancer,

gastric cancer, astrocytic cancer, endometrial cancer, cervical cancer, bladder cancer, renal cancer, lung cancer, liver cancer, thyroid cancer, head and neck cancer, pancreatic cancer and ovarian cancer.

20. The use according to claim 18, wherein the fibrotic disease is selected from the group consisting of pulmonary fibrosis such as idiopathic pulmonary fibrosis and cystic fibrosis, renal fibrosis, liver cirrhosis, endomyocardial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, progressive massive fibrosis, nephrogenic systemic fibrosis, Crohn's disease, keloid, myocardial infarction, scleroderma and arthrofibrosis.