ANTIBODY FORMULATION

Technical Field

The present invention relates to a pharmaceutical formulation of an antibody against CD40, a process for the preparation thereof and uses of the formulation.

Background

Despite the availability of several immunosuppressive treatments for autoimmune diseases, there remains a large unmet need for more efficacious and safer drugs in a large fraction of the patient population. For example, despite the reported efficacy of B cell depleting/inhibiting therapies like Rituximab and Belimumab in rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, and multiple sclerosis, these therapies are only effective in a portion of diseased individuals, and with Rituximab, with an accompanying risk of progressive multifocal leukoencephalopathy. Further, multiple other leukocyte cell types are often involved in the pathology of these autoimmune diseases such as macrophages, dendritic cells and T cells, therefore therapeutic intervention targeting additional cell types or key immunological pathways that would inhibit their function could provide benefit. Given the multiple immunologically relevant roles of CD40-CD154 in the activation and function or these cell types, it is likely that an anti-CD40 antibody would confer therapeutic benefit to patients suffering autoimmune diseases outlined above beyond that currently provided by current therapies. Further, the central role for CD40-CD154 interactions in intestinal inflammatory disorders such as Crohn's disease and ulcerative colitis, and mechanistic links of the CD40 pathway to pathology in more rare disorders such as autoimmune vasculitis, pemphigus vulgaris, and idiopathic thrombocytopenic purpura (ITP) also highlights the potential of anti-CD40 antibodies in these indications.

The currently available immunosuppressants used after solid organ transplantation provide excellent short-tem efficacy. Acute rejections within the de novo period are observed in 5%-20% of the recipients (depending on organ, patient population, and regimen) and the proportion of grafts lost to acute rejection within the de novo period is below 5% for any setting. Currently the key unmet need is the tolerability of immunosuppression with patient and graft survival in the long term. After renal transplant, 33% patients die and/or lose their graft within 5 years; the average age of death of transplant recipient is 58 years. Calcineurin inhibitors (CNI) remain the mainstay of immunosuppressive therapy for the vast majority of transplant patients. While nephrotoxicity and cardiovascular morbidity associated with CNIs is one of the drivers of chronic allograft nephropathy as well as patient death with a functioning graft, alternative

primary immunosuppression have not been able to replace CNIs. Overall, there is still room for improvement in long-term transplant immunosuppression. B-cell mediated immunological damage of transplanted kidneys may contribute to poor long-term outcomes and the need for new agents to target B-cell rejection is increasingly recognised by the medical community.

Antibodies against CD40 are known in the art. Chir12.12 is a fully humanised, non-agonist anti-CD40 antibody (IgG1, kappa) that blocks CD154 (also known as CD40 ligand; CD40L) -mediated leukocyte activation and can mediate antibody-dependent cellular cytotoxicity (ADCC) of human leukocytes and B cell lymphomas *in vitro* (see WO 2006/073443). WO 2005/044306 describes anti-CD40 antagonist antibodies, including Chir12.12 for use in particular in the treatment of autoimmune and inflammatory disorders. Further Chir12.12 is effective in delaying kidney allograft rejection when dosed as a monotherapy in *Macaca fascicularis* (Cynomolgus monkeys) [Li *et al.* (2008) Transplantation; 86 (1):10-15]. However, Chir12.12 can also mediate depletion of peripheral B cells in non human primates (NHPs).

Anti-CD40 mAbs with silenced ADCC activity are predicted to have an improved safety profile relative to the parental anti-CD40 antibodies, and in particular may be more suitable for non-oncologic indications, such as autoimmune diseases and use in a transplant setting. The applicant has developed three silent anti-CD40 antibodies based on the Chir12.12 antibody. These antibodies, hereinafter designated mAb1, mAb2 and mAb3 are characterised by certain amino acid mutations in the Fc region which silence ADCC activity.

mAb1 comprises an N297A mutation in the antibody heavy chain amino acid sequence. The antibody comprises the heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8, respectively. mAb1 comprises a V_H domain with the amino acid sequence of SEQ ID NO: 1 and a V_L domain with the amino acid sequence of SEQ ID NO: 2. mAb1 comprises the full length heavy chain amino acid sequence of SEQ ID NO: 9 and the full length light chain amino acid sequence of SEQ ID NO: 10.

mAb2 comprises a D265A mutation in the antibody heavy chain amino acid sequence. The antibody comprises the heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8, respectively. mAb2 comprises a V_H domain with the amino acid sequence of SEQ ID NO: 1 and a V_L domain with the amino acid sequence of SEQ ID NO: 2. mAb2 comprises the full length heavy chain amino acid sequence of SEQ ID NO: 13 and the full length light chain amino acid sequence of SEQ ID NO: 14.

mAb3 comprises a L234A, L235A mutation (LALA) in the antibody heavy chain amino acid sequence. The antibody comprises the heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8, respectively. mAb3 comprises a V_H domain with the amino acid sequence of SEQ ID NO: 1 and a V_L domain with the amino acid sequence of SEQ ID NO: 2. mAb3 comprises the full length heavy chain amino acid sequence of SEQ ID NO: 17 and the full length light chain amino acid sequence of SEQ ID NO: 18.

Formulations with high concentration of antibody may have short shelf lives and the formulated antibodies may lose biological activity resulting from chemical and physical instabilities during the storage. Among those, aggregation, deamidation and oxidation are known to be the most common causes of antibody degradation. In particular, aggregation can potentially lead to increased immune response in patients, leading to safety concerns. Thus it must be minimised or prevented.

It is an object of the invention to provide further and improved formulations of anti-CD40 antibodies, and in particular formulations with high concentration of anti-CD40 antibodies and low levels of antibody aggregation.

Disclosure of the invention

Therapeutic antibodies are typically formulated either in aqueous form ready for parenteral administration or as lyophilisates for reconstitution with a suitable diluent prior to administration. According to the invention, an anti-CD40 antibody may be formulated either as a lyophilisate, or as an aqueous composition, for example in pre-filled syringes. Suitable formulation can provide an aqueous pharmaceutical composition or a lyophilisate which can be reconstituted to give a solution with a high concentration of the antibody active ingredient and a low level of antibody aggregation for delivery to a patient. High concentrations of antibody are useful as they reduce the amount of material which must be delivered to a patient. Reduced dosing volumes minimise the time taken to deliver a fixed dose to the patient. The aqueous compositions of the invention with high concentration of anti-CD40 antibodies are particularly suitable for subcutaneous administration.

Thus the invention provides an aqueous pharmaceutical composition, suitable for parenteral administration in a subject, e.g., for subcutaneous administration, comprising an anti-CD40 antibody.

The following specific embodiments of the invention are described as numbered hereafter:

1. An aqueous pharmaceutical composition, suitable for subcutaneous administration in a subject, comprising an anti-CD40 antibody in which the antibody has a concentration of at least 50mg/ml, and wherein said anti-CD40 antibody includes: (i) one or more heavy chain CDRs selected from the group consisting of SEQ ID NOs: 3, 4 and 5; and/or (ii) one or more light chain CDRs selected from the group consisting of SEQ ID NOs: 6, 7 and 8.

2. The aqueous pharmaceutical composition, suitable for subcutaneous administration in a subject, according to Embodiment 1, wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8.

3. The aqueous pharmaceutical composition of Embodiment 1 or 2, wherein the anti-CD40 antibody comprises a V_H domain with amino acid SEQ ID NO: 1 and a V_L domain with amino acid SEQ ID NO: 2.

4. The aqueous pharmaceutical composition of Embodiment 1, 2 or 3, wherein the anti-CD40 antibody comprises a heavy chain region of SEQ ID NO: 9 and a light chain region of SEQ ID NO: 10, or a heavy chain region of SEQ ID NO: 13 and a light chain region of SEQ ID NO: 14, or a heavy chain region of SEQ ID NO: 17 and a light chain region of SEQ ID NO: 18.

5. The aqueous pharmaceutical composition of any one of Embodiments 1 to 4, wherein less than 5% of the anti-CD40 antibody is aggregated or degraded.

6. The aqueous pharmaceutical composition of any one of Embodiments 1 to 5, comprising one or more of the following components selected among the group consisting of: a stabiliser, a buffering agent; and a surfactant.

7. The aqueous pharmaceutical composition of Embodiment 6, wherein the stabiliser is a sugar.

8. The aqueous pharmaceutical composition of Embodiment 6 or 7, comprising: a sugar, a buffering agent, and a surfactant.

9. The aqueous pharmaceutical composition of Embodiment 6 or 7, further comprising a free amino acid.

10. The aqueous pharmaceutical composition of Embodiment 7 to 9, comprising sucrose as a sugar.

11. The aqueous pharmaceutical composition of Embodiment 10, comprising 200-300 mM sucrose.

12. The aqueous pharmaceutical composition of Embodiments 6-11, comprising a histidine buffer as the buffering agent.

13. The aqueous pharmaceutical composition of Embodiment 12, comprising 25-35 mM histidine buffer.

14. The aqueous pharmaceutical composition of Embodiments 6 to 13, comprising polysorbate 20 as a surfactant.

15. The aqueous pharmaceutical composition of Embodiment 14, comprising 0.01 to 0.2% polysorbate 20.

16. The aqueous pharmaceutical composition of Embodiment 9, further comprising arginine and/or methionine as free amino acid.

17. The aqueous pharmaceutical composition of Embodiment 16, comprising 40-80 mM arginine.

18. The aqueous pharmaceutical composition of any preceding Embodiment, comprising sucrose, a histidine buffer, polysorbate 20 and arginine.

19. The aqueous pharmaceutical composition of any preceding Embodiment, comprising sucrose, a histidine buffer, polysorbate 20 and methionine.

20. The aqueous pharmaceutical composition of any preceding Embodiment, comprising sucrose, a histidine buffer, polysorbate 20, arginine and methionine.

21. A lyophilisate suitable for preparing the aqueous pharmaceutical composition of any preceding Embodiments.

22. A lyophilisate according to Embodiment 21, comprising sucrose, a histidine buffer, and polysorbate 20.

23. A method for preparing a lyophilisate, comprising the steps of: (i) preparing an aqueous solution comprising an anti-CD40 antibody, a sugar, a buffering agent, a surfactant and optionally a free amino acid; and (ii) lyophilising the aqueous solution.

24. A delivery device including the aqueous pharmaceutical composition of any one of Embodiments 1-20.

25. A pre-filled syringe including the aqueous pharmaceutical composition of any one of Embodiments 1-20.

26. A method for delivering an anti-CD40 monoclonal antibody to a mammal, comprising a step of administering to the patient a pharmaceutical composition of any one of Embodiments 1-20.

27. The composition of any one of Embodiments 1-20, for use in treating a disease or disorder that is mediated by CD40.

28. The composition of Embodiment 27, for the treatment of autoimmune diseases.

29. The composition of Embodiment 28, for the treatment of rheumatoid arthritis, systemic lupus erythematosus, or Pemphigus vulgaris.

30. The aqueous pharmaceutical composition of any one of Embodiments 1-20 in which the antibody has a concentration of at least at least 50mg/ml, at least 100mg/ml, at least 150mg/ml, at least 200mg/ml, at least 250mg/ml, or at least 300mg/ml.

The invention also provides an aqueous pharmaceutical composition comprising: an anti-CD40 monoclonal antibody as described above, for example mAb1, mAb2 or mAb3, especially mAb1; a stabiliser; a buffering agent; and a surfactant. The composition preferably also includes a free amino acid.

The invention also provides a lyophilisate comprising: an anti-CD40 monoclonal antibody as described above, for example mAb1, mAb2 or mAb3, especially mAb1; a sugar; a buffering agent; and a surfactant. The lyophilisate preferably also includes a free amino acid.

The invention also provides a lyophilisate comprising an anti-CD40 monoclonal antibody as described above, for example mAb1, mAb2 or mAb3, especially mAb1; wherein the lyophilisate can be reconstituted with an aqueous reconstituent to provide an aqueous composition in which the antibody has a concentration of at least 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml, or 300mg/ml, after reconstitution in an aqueous solution.

The invention also provides an aqueous pharmaceutical composition comprising high concentration of an anti-CD40 monoclonal antibody as described above, for example mAb1, mAb2 or mAb3, especially mAb1; wherein less than 5%, 4%, 3%, 2% or 1% of the anti-CD40 antibody is aggregated or degraded.

The invention also provides a process for preparing a lyophilisate, comprising steps of: (i) preparing an aqueous solution comprising an anti-CD40 monoclonal antibody, a sugar, a buffering agent, a surfactant, and optionally a free amino acid; and (ii) lyophilising the aqueous solution.

The invention also provides a process for preparing a pharmaceutical composition, comprising a step of mixing a lyophilisate with an aqueous reconstituent, wherein the lyophilisate comprises an anti-CD40 monoclonal antibody, a sugar, a buffering agent, a surfactant, and optionally a free amino acid.

More specifically the invention provides a lyophilised formulation prepared by lyophilising an aqueous formulation having a pH of 5.0-7.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 20-150 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) a stabiliser,

(iii) a buffering agent,

(iv) a surfactant, and optionally

(v) an amino acid.

In one embodiment said formulation is prepared from an aqueous formulation having a pH of 5.0-7.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 20-150mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) sucrose or trehalose as a stabiliser,

(iii) histidine as a buffering agent,

(iv) polysorbate 20 as a surfactant, and optionally

(v) an amino acid selected from arginine, methionine and glycine.

In one embodiment said lyophilised formulation is prepared from an aqueous formulation having a pH of 5.0-7.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 20-150 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 3-300 mM sucrose or trehalose as a stabiliser,

(iii) 1-60 mM histidine as a buffering agent,

(iv) up to 0.2% polysorbate 20 as a surfactant, and optionally

(v) 2-80 mM arginine, methionine or glycine.

In one embodiment said lyophilised formulation is prepared from an aqueous formulation having a pH of 6.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 90 mM sucrose as a stabiliser,

(iii) 10 mM histidine as a buffering agent,

(iv) 0.02% polysorbate 20 as a surfactant.

In one embodiment said lyophilised formulation is prepared from an aqueous formulation having a pH of 6.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 90 mM sucrose as a stabiliser,

(iii) 10 mM histidine as a buffering agent,

(iv) 0.02% polysorbate 20 as a surfactant, and

(v) 17 mM arginine.

In one embodiment said lyophilised formulation is prepared from an aqueous formulation having a pH of 6.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 150 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 270 mM sucrose as a stabiliser,

(iii) 30 mM histidine as a buffering agent, and

(iv) 0.06% polysorbate 20 as a surfactant.

The invention also provides an aqueous pharmaceutical composition obtained by reconstituting a lyophilised formulation as described above, wherein the reconstitution factor is between 1:0.5 to 1:6.

In one embodiment the reconstitution factor is 1:3.

The invention also provides an aqueous pharmaceutical composition having a pH of 5.0 to 7.0 comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) a stabiliser,

(iii) a buffering agent,

(iv) a surfactant, and optionally

(v) an amino acid.

In one embodiment the aqueous pharmaceutical composition having a pH of 5.0 to 7.0 comprises

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) sucrose or trehalose as a stabiliser,

(iii) histidine as a buffering agent,

(iv) polysorbate 20 as a surfactant, and optionally

(v) an amino acid selected from arginine, methionine or glycine.

In one embodiment the aqueous pharmaceutical composition having a pH of 5.0 to 7.0 comprises

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 200-300 mM sucrose as a stabiliser,

(iii) 25-35 mM histidine as a buffering agent,

(iv) up to 0.2% polysorbate 20 as a surfactant, and optionally

(v) 10-80 mM arginine, methionine or glycine.

In one embodiment the aqueous pharmaceutical composition has a pH of 6.0 and comprises

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 270 mM sucrose as a stabiliser,

(iii) 30 mM histidine as a buffering agent, and

(iv) 0.06% polysorbate 20 as a surfactant.

In one embodiment the aqueous pharmaceutical composition has a pH of 6.0 and comprises

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 270 mM sucrose as a stabiliser,

(iii) 30 mM histidine as a buffering agent,

(iv) 0.06% polysorbate 20 as a surfactant, and

(v) 51 mM arginine.

In one embodiment the aqueous pharmaceutical composition of the invention has an anti-CD40 antibody concentration of 150 mg/ml.

In one embodiment the lyophilised formulation or the aqueous pharmaceutical composition of the invention comprises a VH domain having the amino acid sequence of SEQ ID NO: 1 and a VL domain having the amino acid sequence of SEQ ID NO: 2.

In one embodiment the lyophilised formulation or the aqueous pharmaceutical composition of the invention comprises an anti-CD40 antibody comprising a heavy chain region of SEQ ID NO: 9 and a light chain region of SEQ ID NO: 10.

In one embodiment the lyophilised formulation or the aqueous pharmaceutical composition of the invention comprises the anti-CD40 antibody Chir12.12 having an N297A mutation.

In one embodiment the lyophilised formulation or the aqueous pharmaceutical composition of the invention comprises an anti-CD40 antibody comprising a heavy chain region of SEQ ID NO: 13 and a light chain region of SEQ ID NO: 14.

In one embodiment the lyophilised formulation or the aqueous pharmaceutical composition of the invention comprises the anti-CD40 antibody Chir12.12 having a D265A mutation.

In one embodiment the lyophilised formulation or the aqueous pharmaceutical composition of the invention comprises an anti-CD40 antibody comprising a heavy chain region of SEQ ID NO: 17 and a light chain region of SEQ ID NO: 18.

In one embodiment the lyophilised formulation or the aqueous pharmaceutical composition of the invention comprises the anti-CD40 antibody Chir12.12 having an L234A L235A mutation.

The invention also comprises a delivery device including the aqueous pharmaceutical composition of the invention.

The invention also comprises a pre-filled syringe including the aqueous pharmaceutical composition of the invention.

The invention also comprises a method for delivering an anti-CD40 antibody to a mammal, comprising a step of administering to the patient an aqueous pharmaceutical composition of the invention.

The invention also comprises a lyophilised formulation or an aqueous pharmaceutical composition according to the invention for use in treating a disease or disorder that is mediated by CD40.

The invention also comprises a lyophilised formulation or aqueous pharmaceutical composition according to the invention for use in the treatment of autoimmune diseases.

The invention also provides a lyophilised formulation or aqueous pharmaceutical composition according to the invention Multiple Sclerosis, Systemic Lupus Erythematosus, Sjögren's syndrome, Rheumatoid Arthritis, transplant rejection; graft-versus-host disease, Pemphigus vulgaris.

Aqueous pharmaceutical compositions with high concentration of anti-CD40 antibodies

The invention relies, at least partly, in the formulation properties of antibodies such as mAb1, which retain remarkable stability and bioactive properties when formulated in a high concentration either as a liquid (aqueous) or lyophilisate composition.

As used herein, an “aqueous” pharmaceutical composition is a composition suitable for pharmaceutical use, wherein the aqueous carrier is distilled water. A composition suitable for pharmaceutical use may be sterile, homogeneous and/or isotonic. Aqueous pharmaceutical compositions may be prepared either directly in an aqueous form, for example in pre-filled syringe ready for use (the “liquid formulations”) or as lyophilisate to be reconstituted shortly before use. As used herein, the term “aqueous pharmaceutical composition” refers to the liquid formulation or reconstituted lyophilised formulation. In certain embodiments, the aqueous pharmaceutical compositions of the invention are suitable for parenteral administration to a human subject. In a specific embodiment, the aqueous pharmaceutical compositions of the invention are suitable for subcutaneous administration.

As used herein, the phrase “parenteral administration” means mode of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal injection and infusion.

The use of antibodies as the active ingredient of pharmaceuticals is now widespread, including the products HERCEPTIN™ (trastuzumab), RITUXAN™ (rituximab), SYNAGIS™ (palivizumab), *etc.* Techniques for purification of therapeutic antibodies to a pharmaceutical grade are well known in the art.

The composition will usually be non-pyrogenic *e.g.* containing <1 EU (endotoxin unit, a standard measure) per dose, and preferably <0.1 EU per dose. The composition is preferably gluten-free.

In specific embodiments, the aqueous pharmaceutical compositions of the invention exhibit low to undetectable levels of antibody aggregation or degradation, with very little to no loss of the biological activities during manufacture, preparation, transportation and long periods of storage, the concentration of the anti-CD40 antibody being at least about 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml, or 300mg/ml.

In one aspect, the invention relates to an aqueous pharmaceutical composition with high concentration of anti-CD40 antibodies.

It is known in the art that such high concentration aqueous pharmaceutical compositions can be diluted prior to injection, for example, if lower antibody concentrations are required for specific therapeutic interventions or when treating patients of lower body weight including children. Suitable concentrations can be 25mg/ml or 10mg/ml. Alternatively, the original formulation may be produced with such a lower concentration.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (i. e., "antigen-binding portion") or single chains thereof. A naturally occurring "antibody" is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three or four domains, depending on the isotype, C_H1, C_H2, C_H3 and C_H4. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody (or simply "antigen portion"), as used herein, refers to full length or one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., a portion of CD40). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 domains; a $F(ab)_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the V_H and C_H1 domains; a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward et al., 1989 Nature 341:544-546), which consists of a V_H domain; and an isolated complementarity determining region (CDR).

Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al., 1988 Science 242:423-426; and Huston et al., 1988 Proc. Natl. Acad. Sci. 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding region" of an antibody. These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

An "isolated antibody", as used herein, refers to an antibody that is substantially free of other antibodies having different antigenic specificities, e.g., an isolated antibody that specifically binds human CD40 is substantially free of antibodies that specifically bind antigens other than CD40. An isolated antibody that specifically binds CD40 may, however, have cross-reactivity to other antigens, such as CD40 molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

The term "human antibody", as used herein, includes antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences, e.g., human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik, et al. (2000. J Mol Biol 296, 57-86).

The structures and locations of immunoglobulin variable domains, e.g., CDRs, may be defined using well known numbering schemes, e.g., the Kabat numbering scheme, the Chothia numbering scheme, a combination of Kabat and Chothia (AbM), etc. (see, e.g., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services (1991), eds. Kabat et al.; Al Lazikani et al. (1997) J. Mol. Bio. 273:927 948). Throughout this specification, the complementarity determining region (“CDR”) is defined according to the Kabat definition with the exception of CDRH1 which is the stretch of amino acids defined by a combination of both Kabat and Chothia definitions for this CDR.

The human antibodies of the invention may include amino acid residues not encoded by human sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human sequences.

The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the human antibody, e.g., from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire in vivo.

As used herein, "isotype" refers to the antibody class (e.g., IgM, IgA, IgD, IgE and IgG such as IgG1, IgG2, IgG3 or IgG4) that is provided by the heavy chain constant region genes.

The phrases "an antibody recognising an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen".

As used herein, an antibody that "specifically binds to CD40 polypeptide" or an "anti-CD40 antibody" refers to an antibody that binds to human CD40 polypeptide of SEQ ID NO: 21 with a K_D of 100nM or less, 10nM or less, 1nM or less. An antibody that "cross-reacts with an antigen other than CD40" refers to an antibody that binds that antigen with a K_D of 0.5 x 10^{-8} M or less, 5 x 10^{-9} M or less, or 2 x 10^{-9} M or less. An antibody that "does not cross-react with a particular antigen" is intended to refer to an antibody that binds to that antigen, with a K_D of 1.5 x 10^{-8} M or greater, or a K_D of 5-10 x 10^{-8} M or 1 x 10^{-7} M or greater. In certain embodiments, such antibodies that do not cross-react with the antigen exhibit essentially undetectable binding against these proteins in standard binding assays.

In one embodiment, a high concentration of an anti-CD40 antibody in the aqueous pharmaceutical composition of the invention is at least 50mg/ml. In one embodiment, a high concentration is at least 100mg/ml. In one embodiment, a high concentration is at least 150mg/ml. In one embodiment, a high concentration is at least 200mg/ml. In one

embodiment, a high concentration is at least 250mg/ml. In one embodiment, a high concentration is at least 300mg/ml.

In one embodiment, the aqueous pharmaceutical composition of the invention comprises between 50mg/ml and 300mg/ml of an anti-CD40 antibody, for example, mAb1.

In one embodiment, the aqueous pharmaceutical composition of the invention comprises between 75mg/ml and 250mg/ml of an anti-CD40 antibody, for example, mAb1.

In one embodiment, the aqueous pharmaceutical composition of the invention comprises between 100mg/ml and 250mg/ml of an anti-CD40 antibody, for example, mAb1.

In one embodiment, the aqueous pharmaceutical composition of the invention comprises between 100mg/ml and 200mg/ml of an anti-CD40 antibody, for example, mAb1.

In one embodiment, the aqueous pharmaceutical composition of the invention comprises 150mg/ml of an anti-CD40 antibody, for example, mAb1.

In one embodiment, the aqueous pharmaceutical composition of the invention comprises about 50mg/ml, about 60mg/ml, about 70mg/ml, about 80mg/ml, about 90mg/ml, about 100mg/ml, about 110mg/ml, about 120mg/ml, about 130mg/ml, about 140mg/ml, about 150mg/ml, about 160mg/ml, about 170mg/ml, about 180mg/ml, about 190mg/ml, about 200mg/ml, about 210mg/ml, about 220mg/ml, about 230mg/ml, about 240mg/ml, about 250mg/ml or about 300mg/ml of an anti-CD40 antibody, for example, mAb1.

Furthermore, the aqueous pharmaceutical compositions are stable such that, even after storage for 4 weeks at 2-8°C, less than 5%, 4%, 3%, 2%, 1%, 0.05% or 0.01% of the total anti-CD40 antibody is aggregated as measured by SEC-HPLC.

The aqueous pharmaceutical compositions may include, in addition to the anti-CD40 antibody, further components such as one or more of the following: (i) a stabiliser; (ii) a buffering agent; (iii) a surfactant; and (iv) a free amino acid. Inclusion of each of such additional components can give compositions with low aggregation of the anti-CD40 antibody.

Suitable stabilisers for use with the invention can act, e.g., as viscosity enhancing agents, bulking agents, solubilising agents, and/or the like. The stabiliser can be ionic or non-ionic (e.g. sugars). As sugars they include, but are not limited to, monosaccharides, e.g., fructose, maltose, galactose, glucose, D-mannose, sorbose and the like; disaccharides, e.g. lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, e.g. raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and the like. For example, the sugar may be sucrose, trehalose, raffinose, maltose, sorbitol or mannitol. The sugar may be a sugar alcohol or an amino sugar. Sucrose is particularly useful. As

ionic stabiliser they include salts such as NaCl or amino acid components such as arginine-HCl.

Suitable buffering agents for use with the invention include, but are not limited to, organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid or phtalic acid; Tris, thomethamine hydrochloride, or phosphate buffer. In addition, amino acid components can also be used as buffering agent. Such amino acid component includes without limitation glycine and histidine. A histidine buffer is particularly useful.

The aqueous pharmaceutical compositions include such buffering agent or pH adjusting agent to provide improved pH control. In one embodiment, an aqueous pharmaceutical composition of the invention has a pH between 5.0 and 8.0, between 5.5 and 7.5, between 5.0 and 7.0, between 6.0 and 8.0, or between 6.0 and 7.0. In a specific embodiment, an aqueous pharmaceutical composition of the invention has a pH of about 6.0.

As used herein, the term “surfactant” herein refers to organic substances having amphipathic structures; i.e., they are composed of groups of opposing solubility tendencies, typically an oil-soluble hydrocarbon chain and a water-soluble ionic group. Surfactants can be classified, depending on the charge of the surface-active moiety, into anionic, cationic and dispersing agents for various pharmaceutical compositions and preparations of biological materials.

Suitable surfactants for use with the invention include, but are not limited to, non-ionic surfactants, ionic surfactants and zwitterionic surfactants. Typical surfactants for use with the invention include, but are not limited to, sorbitan fatty acid esters (*e.g.* sorbitan monocaprylate, sorbitan monolaurate, sorbitan monopalmitate), sorbitan trioleate, glycerine fatty acid esters (*e.g.* glycerine monocaprylate, glycerine monomyristate, glycerine monostearate), polyglycerine fatty acid esters (*e.g.* decaglyceryl monostearate, decaglyceryl distearate, decaglyceryl monolinoleate), polyoxyethylene sorbitan fatty acid esters (*e.g.* polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan trioleate, polyoxyethylene sorbitan tristearate), polyoxyethylene sorbitol fatty acid esters (*e.g.* polyoxyethylene sorbitol tetrastearate, polyoxyethylene sorbitol tetraoleate), polyoxyethylene glycerine fatty acid esters (*e.g.* polyoxyethylene glyceryl monostearate), polyethylene glycol fatty acid esters (*e.g.* polyethylene glycol distearate), polyoxyethylene alkyl ethers (*e.g.* polyoxyethylene lauryl ether), polyoxyethylene polyoxypropylene alkyl ethers (*e.g.* polyoxyethylene polyoxypropylene glycol, polyoxyethylene polyoxypropylene propyl ether, polyoxyethylene polyoxypropylene cetyl ether), polyoxyethylene alkylphenyl ethers (*e.g.*

polyoxyethylene nonylphenyl ether), polyoxyethylene hydrogenated castor oils (*e.g.* polyoxyethylene castor oil, polyoxyethylene hydrogenated castor oil), polyoxyethylene beeswax derivatives (*e.g.* polyoxyethylene sorbitol beeswax), polyoxyethylene lanolin derivatives (*e.g.* polyoxyethylene lanolin), and polyoxyethylene fatty acid amides (*e.g.* polyoxyethylene stearic acid amide); C_{10}-C_{18} alkyl sulfates (*e.g.* sodium cetyl sulfate, sodium lauryl sulfate, sodium oleyl sulfate), polyoxyethylene C_{10}-C_{18} alkyl ether sulfate with an average of 2 to 4 moles of ethylene oxide units added (*e.g.* sodium polyoxyethylene lauryl sulfate), and C_1-C_{18} alkyl sulfosuccinate ester salts (*e.g.* sodium lauryl sulfosuccinate ester); and natural surfactants such as lecithin, glycerophospholipid, sphingophospholipids (*e.g.* sphingomyelin), and sucrose esters of C_{12}-C_{18} fatty acids. A composition may include one or more of these surfactants. Preferred surfactants are polyoxyethylene sorbitan fatty acid esters *e.g.* polysorbate 20, 40, 60 or 80. Polysorbate 80 (Tween 80) is particularly useful.

Suitable free amino acids for use with the invention include, but are not limited to, arginine, lysine, histidine, methionine, ornithine, isoleucine, leucine, alanine, glycine, glutamic acid or aspartic acid. The inclusion of a basic amino acid is preferred *i.e.* arginine, lysine and/or histidine. If a composition includes histidine then this may act both as a buffering agent and a free amino acid, but when a histidine buffer is used it is typical to include a non-histidine free amino acid *e.g.* to include histidine buffer and lysine. An amino acid may be present in its D- and/or L- form, but the L-form is typical. The amino acid may be present as any suitable salt *e.g.* a hydrochloride salt, such as arginine-HCl.

When present, components (i) to (iv) will be at a concentration sufficient to maintain the anti-CD40 antibody in a form which is active and soluble after either

(i) lyophilisation and storage and reconstitution (for lyophilisates), or

(ii) conditioning in dosing units and storage (for liquid formulations).

Thus a sugar may be present in the aqueous pharmaceutical composition of the invention, e.g. after reconstitution of a lyophilisate in water, at a concentration of between 3 and 400mM *e.g.* 50-380mM, 100-350mM, 200-300mM. A concentration of 270 mM sucrose is useful.

A buffering agent may be present in the aqueous pharmaceutical composition of the invention, e.g. after reconstitution of a lyophilisate in water, at a concentration of between 1 and 60 mM *e.g.* 10-50 mM, 20-40 mM, 25-35 mM. A concentration of 30 mM histidine buffer is useful.

A surfactant may be present in the aqueous pharmaceutical composition of the invention, e.g. after reconstitution of a lyophilisate in water, at a concentration of up to 0.2% (by

volume) *e.g.* 0.01-0.1%, 0.03-0.08%, 0.04-0.08%. A concentration of 0.06% polysorbate 20 is useful. In some embodiments polysorbate 80 may be used.

A free amino acid may be present in the aqueous pharmaceutical composition of the invention, e.g. after reconstitution of a lyophilisate in water, at a concentration of between 2 and 100 mM *e.g.* 10-80 mM, 20-70 mM, 30-60 mM, 40-60 mM. A concentration of 51mM arginine (e.g. arginine-HCl) or 60 mM methionine or glycine (e.g. glycine-HCl) is useful.

A formulation containing histidine buffer, sucrose and polysorbate 20 has been shown to be suitable for lyophilisation of antibody mAb1 at a concentration of at least 150mg/ml after reconstitution.

In one embodiment the aqueous pharmaceutical composition consists of 150 mg/ml mAb1, 30 mM histidine, 270 mM sucrose and 0.06% polysorbate 20.

In one embodiment the aqueous pharmaceutical composition consists of 150 mg/ml mAb1, 30 mM histidine, 270 mM sucrose, 0.06% polysorbate 20 and 51 mM arginine-HCl.

In one embodiment the aqueous pharmaceutical composition consists of 150 mg/ml mAb1, 30 mM histidine, 270 mM sucrose, 0.06% polysorbate 20 and 60 mM glycine-HCl.

In one embodiment the aqueous pharmaceutical composition consists of 200 mg/ml mAb1, 30 mM histidine, 270 mM sucrose, and 0.06% polysorbate 20.

In one embodiment the aqueous pharmaceutical composition consists of 200 mg/ml mAb1, 30 mM histidine, 270 mM sucrose, 0.06% polysorbate 20 and 51 mM arginine-HCl.

In one embodiment the aqueous pharmaceutical composition consists of 75 mg/ml mAb1, 30 mM histidine, 270 mM sucrose, and 0.06% polysorbate 20.

In one embodiment the aqueous pharmaceutical composition consists of 75 mg/ml mAb1, 30 mM histidine, 270 mM sucrose, 0.06% polysorbate 20 and 51 mM arginine-HCl.

Other contemplated excipients, which may be utilised in the aqueous pharmaceutical compositions of the invention include, for example, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, lipids such as phospholipids or fatty acids, steroids such as cholesterol, protein excipients such as serum albumin (human serum albumin), recombinant human albumin, gelatin, casein, salt-forming counterions such sodium and the like. These and additional known pharmaceutical excipients and/or additives suitable for use in the formulations of the invention are known in the art, e.g., as

listed in "The Handbook of Pharmaceutical Excipients, 4th edition, Rowe et al., Eds., American Pharmaceuticals Association (2003); and Remington: the Science and Practice of Pharmacy, 21th edition, Gennaro, Ed., Lippincott Williams & Wilkins (2005).

The aqueous pharmaceutical compositions of the invention may include further active ingredients in addition to the anti-CD40 antibody. Further pharmacological agents may include, for instance, chemotherapeutic compounds.

Lyophilisates

Techniques for lyophilisation of antibodies are well known in the art *e.g.* see John F. Carpenter and Michael J. Pikal, 1997 (*Pharm. Res.* 14, 969-975); Xialin (Charlie) Tang and Michael J. Pikal, 2004 (*Pharm. Res.* 21, 191-200). For example, the monoclonal antibody products SYNAGIS™, REMICADE™, RAPTIVA™, SIMULECT™, XOLAIR™ and HERCEPTIN™ are supplied as lyophilisates. These antibodies are reconstituted to various final concentrations *e.g.* SIMULECT™ is reconstituted to a concentration of 4 mg/ml antibody, REMICADE™ is reconstituted to a concentration of 10 mg/ml, HERCEPTIN™ to 21 mg/ml, SYNAGIS™ and RAPTIVA™ to 100 mg/ml, and XOLAIR™ to 125 mg/ml.

Pre-lyophilisates, lyophilisates and aqueous reconstitution

Before a lyophilisate can be administered to a patient it should be reconstituted with an aqueous reconstituent. This step permits antibody and other components in the lyophilisate to re-dissolve to give a solution which is suitable for injection to a patient.

The volume of aqueous material used for reconstitution dictates the concentration of the antibody in a resulting pharmaceutical composition. Reconstitution with a smaller volume of reconstituent than the pre-lyophilisation volume provides a composition which is more concentrated than before lyophilisation. The reconstitution factor (volume of formulation after lyophilisation:volume of formulation before) may be from 1:0.5 to 1:6. A reconstitution factor of 1:3 is useful. As mentioned above, lyophilisates of the invention can be reconstituted to give aqueous compositions with an anti-CD40 antibody concentration of at least 50 mg/ml, 100 mg/ml, 150 mg/ml. 200 mg/ml, 250 mg/ml or 300 mg/ml, and the volume of reconstituent will be selected accordingly. If required, the reconstituted formulation can be diluted prior to administration to a patient as appropriate to deliver the intended dose.

Typical reconstituents for lyophilised antibodies include sterile water or buffer, optionally containing a preservative. If the lyophilisate includes a buffering agent then the reconstituent may include further buffering agent (which may be the same as or different from the lyophilisate's buffering agent) or it may instead include no buffering agent (*e.g.* WFI (water for injection), or physiological saline).

When present, components (i) to (iv) will be at a pre-lyophilisation concentration sufficient to maintain the anti-CD40 antibody in a form which is active and soluble after storage (under normal conditions) and reconstitution. The components will also be present after reconstitution.

Thus a sugar, such as sucrose or trehalose, may be present before lyophilisation at a concentration of between 3 and 300 mM *e.g.* 15-200 mM, 30-150 mM, 80-100 mM. A concentration of 90 mM sucrose is useful. A buffering agent, such as histidine, may be present before lyophilisation at a concentration of between 1 and 60 mM *e.g.* 3-30 mM, 5-20 mM, 5-15 mM. A concentration of 10 mM histidine buffer is useful. A surfactant, such as polysorbate 80 or polysorbate 20 may be present before lyophilisation at a concentration of up to 0.2% (by volume) *e.g.* 0.01-0.1%, 0.01-0.08%, 0.01-0.04%. A concentration of 0.02% polysorbate 80 or polysorbate 20 is useful. A free amino acid, such as arginine, methionine or glycine, may be present before lyophilisation at a concentration of between 2 and 80 mM *e.g.* 3-60mM, 3-50 mM, 6-30 mM, 10-25 mM, 15-20 mM. A concentration of 17 mM arginine-HCl or 20 mM glycine-HCl or 60mM methionine is useful. The anti-CD40 antibody is present before lyophilisation at a concentration of between 20mg/ml and 120mg/ml, e.g. 20mg/ml, 30 mg/ml, 40mg/ml, 50mg/ml, 60mg/ml, 66.6mg/ml, 70mg/ml, 80mg/ml, 90mg/ml, 100mg/ml, 110mg/ml, or 120mg/ml. A concentration of 50 mg/ml is useful.

The pre-lyophilisate of the invention has a pH between 5.0 and 8.0, between 5.0 and 7.0, between 5.5 and 6.5. In a specific embodiment, the pre-lyophilisate of the invention has a pH of about 6.0.

In one embodiment the pre-lyophilisate of the invention has a molar ratio of sucrose:antibody of 90:1 and a molar ratio of histidine:antibody of 10:1.

In one embodiment the pre-lyophilisate of the invention has a molar ratio of sucrose:antibody of 90:1, a molar ratio of histidine:antibody of 10:1, and a molar ratio of arginine-HCl:antibody of 17:1.

In one embodiment the pre-lyophilisate of the invention has a molar ratio of sucrose:antibody of 90:1, a molar ratio of histidine:antibody of 10:1, and a molar ratio of glycine-HCl:antibody of 60:1.

A formulation containing histidine buffer, sucrose, polysorbate 20 and, optionally arginine, methionine or glycine has been shown to be suitable for lyophilisation of antibody mAb1. After reconstitution, the components of the lyophilisate may be present at a concentration of the aqueous pharmaceutical compositions as described hereinbefore.

Target diseases and disorders

The aqueous pharmaceutical compositions of the invention comprising anti-CD40 antibodies can be used to treat, ameliorate or prevent CD40-related autoimmune disorders, CD40-related inflammatory disorders and/or to prevent or reduce the risk of graft rejection in transplantation. For the purposes of the present invention, the term "inflammatory disorders" includes "autoimmune disorders".

As used herein, the term "autoimmunity" is generally understood to encompass inflammatory immune-mediated processes involving "self' antigens. In autoimmune diseases, self antigen(s) trigger host immune responses.

Pharmaceutical compositions comprising anti-CD40 antibodies are particularly useful to treat Multiple Sclerosis, Systemic Lupus Erythematosus, Sjögren's syndrome, Rheumatoid Arthritis, transplant rejection; graft-versus-host disease, Pemphigus vulgaris; and B cell neoplasms such as acute lymphoblastic leukemia (ALL) and B-cell chronic lymphocytic leukemia (CLL).

Also, the present invention includes treatment of inflammation associated with tissue transplant rejection. "Transplant rejection" or "graft rejection" refers to any host-mounted immune response against a graft including but not limited to HLA antigens, blood group antigens, and the like.

The invention can also be used to treat graft versus host disease, such as that associated with bone marrow transplantation, for example. In such graft versus host disease, the donor bone marrow includes lymphocytes and cells that mature into lymphocytes. The donor's lymphocytes recognise the recipient's antigens as non-self and mount an inflammatory immune response. Hence, as used herein, "graft versus host disease" or "graft versus host reaction" refers to any T cell mediated immune response in which donor lymphocytes react to the host's antigens.

The antagonist anti-CD40 antibodies or proteins described herein, for example mAb1, mAb2 or mAb3, can be used in accordance with the methods of the invention to treat autoimmune and/or inflammatory disorders including, but not limited to, systemic lupus erythematosus (SLE), discoid lupus, lupus nephritis, sarcoidosis, inflammatory arthritis, including juvenile arthritis, rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, and gouty arthritis, rejection of an organ or tissue transplant, hyperacute, acute, or chronic rejection and/or graft versus host disease, multiple sclerosis, hyper IgE syndrome, polyarteritis nodosa, primary biliary cirrhosis, inflammatory bowel disease, Crohn's disease, celiac's disease (gluten-sensitive enteropathy), primary Sjögren's syndrome (pSS), autoimmune hepatitis, pernicious anemia, autoimmune hemolytic anemia, psoriasis, scleroderma, myasthenia gravis,

autoimmune thrombocytopenic purpura, autoimmune thyroiditis, Grave's disease, Hasimoto's thyroiditis, immune complex disease, chronic fatigue, immune dysfunction syndrome (CFIDS), polymyositis and dermatomyositis, cryoglobulinemia, thrombolysis, cardiomyopathy, pemphigus vulgaris, pulmonary interstitial fibrosis, Type I and Type II diabetes mellitus, type 1,2, 3, and 4 delayed-type hypersensitivity, allergy or allergic disorders, unwanted/unintended immune responses to therapeutic proteins (see for example, U. S. Patent Application No. US 2002/0119151 and Koren, *et al.* (2002) Curr. Pharm. Biotechnol. 3: 349-60), asthma, Churg-Strauss syndrome (allergic granulomatosis), atopic dermatitis, allergic and irritant contact dermatitis, urtecaria, IgE-mediated allergy, atherosclerosis, ANCA-associated Vasculitides, vasculitis, idiopathic inflammatory myopathies, hemolytic disease, Alzheimer's disease, chronic inflammatory demyelinating polyneuropathy, and the like.

Genetic ablation or pharmacological inhibition of the CD40-CD154 pathway has previously demonstrated therapeutic benefit in either the clinic or in preclinical models of SLE, pSS, ITP, MS, Crohn's disease, Pemphigus vulgaris, autoimmune vasculitis and RA (Law CL, Grewal IS. (2009). Adv. Exp. Med. Biol. 2009;647:8-36); the medical need of which is detailed below.

In preferred embodiments the anti-CD40 antibodies or proteins of the invention are useful in treating: (i) systemic lupus erythematosus (lupus nephritis), preferably in providing effective steroid-sparing therapies for induction and maintenance of remission, and prevention of end-stage renal disease; (ii) primary Sjögren's syndrome, preferably in prevention of salivary and lacrimary gland destruction, and induction and maintenance of remission of extraglandular manifestations; (iii) autoimmune thrombocytopenic purpura, preferably treatment of patients refractory to standard of care; (iv) ANCA-associated Vasculitides, preferably inducing and maintaining remission in patients refractory to corticosteroids, and steroid-sparing treatment; (v) Pemphigus Vulgaris, preferably in induction and maintenance of remission in patients refractory to corticosteroids, and steroid-sparing treatment; (vi) Multiple Sclerosis, preferably in providing more effective treatments for prevention of relapses and disability progression, and achieving disease-free status; and (vii) Crohn's disease, preferably in providing more effective therapies for maintenance of remission, and treatment of patients refractory to anti-TNF.

In some other embodiments, the anti-CD40 antibodies or proteins of the invention are useful in treating pulmonary inflammation including but not limited to lung graft rejection, asthma, sarcoidosis, emphysema, cystic fibrosis, idiopathic pulmonary fibrosis, chronic bronchitis, allergic rhinitis and allergic diseases of the lung such as hypersensitivity pneumonitis, eosinophilic pneumonia, bronchiolitis obliterans due to bone marrow and/or lung transplantation or other causes, graft atherosclerosis/graft phlebosclerosis, as well

as pulmonary fibrosis resulting from collagen, vascular, and autoimmune diseases such as rheumatoid arthritis, scleroderma and lupus erythematosus.

"Treatment" is herein defined as the application or administration of an anti-CD40 antibody or protein according to the invention, for example, mAb1, mAb2 or mAb3 antibody, to a subject, or application or administration a pharmaceutical composition comprising said anti-CD40 antibody or protein of the invention to an isolated tissue or cell line from a subject, where the subject has an autoimmune disease and/or inflammatory disease, a symptom associated with an autoimmune disease and/or inflammatory disease, or a predisposition toward development of an autoimmune disease and/or inflammatory disease, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the autoimmune disease and/or inflammatory disease, any associated symptoms of the autoimmune disease and/or inflammatory disease, or the predisposition toward the development of the autoimmune disease and/or inflammatory disease.

By "treatment" is also intended the application or administration of a pharmaceutical composition comprising an anti-CD40 antibodies or protein of the invention, for example, mAb1, mAb2 or mAb3 antibody, to a subject, or application or administration of a pharmaceutical composition comprising said anti-CD40 antibody or protein of the invention to an isolated tissue or cell line from a subject, where the subject has an autoimmune disease and/or inflammatory disease, a symptom associated with an autoimmune disease and/or inflammatory disease, or a predisposition toward development of an autoimmune disease and/or inflammatory disease, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the autoimmune disease and/or inflammatory disease, any associated symptoms of the autoimmune disease and/or inflammatory disease, or the predisposition toward the development of the autoimmune disease and/or inflammatory disease.

By "anti-inflammatory activity" is intended a reduction or prevention of inflammation. Therapy with at least one anti-CD40 antibody or protein according to the invention causes a physiological response that is beneficial with respect to treatment of an autoimmune disease and/or inflammatory disease, where the disease involves cells expressing the CD40 antigen. It is recognised that the methods of the invention may be useful in preventing phenotypic change in cells such as proliferation, activation, and the like.

Patient administration

A pharmaceutical composition of the invention can be administered to a patient. Administration will typically be via a syringe. Thus the invention provides a delivery device (*e.g.* a syringe) including a pharmaceutical composition of the invention (e.g., pre-

filled syringe). Patients will receive an effective amount of the anti-CD40 antibody as the principal active ingredient *i.e.* an amount that is sufficient to treat, ameliorate, or prevent the disease or disorder in question. Therapeutic effects may also include reduction in physical symptoms. The optimum effective amount and concentration of antibody for any particular subject will depend upon various factors, including the patient's age size health and/or gender, the nature and extent of the condition, the activity of the particular antibody, the rate of its clearance by the body, and also on any possible further therapeutic(s) administered in combination with the antibody. The effective amount delivered for a given situation can be determined within the judgment of a clinician. For purposes of the present invention, an effective dose may be from about 0.005 mg/kg to about 50 mg/kg, or about 0.05 mg/kg to about 10 mg/kg. Known antibody-based pharmaceuticals provide guidance in this respect *e.g.* HERCEPTIN™ is administered with an initial loading dose of 4 mg/kg body weight and a weekly maintenance dose of 2 mg/kg body weight; RITUXAN™ is administered weekly at 375 mg/m^2; SYNAGIS™ is administered intramuscularly at 15 mg/kg body weight; *etc.*

The invention provides a method for delivering a monoclonal antibody to a mammal, comprising a step of administering to the patient a pharmaceutical composition of the invention.

The invention also provides a method for delivering a monoclonal antibody to a mammal, comprising steps of: (i) reconstituting a lyophilisate of the invention to give an aqueous formulation, and (ii) administering the aqueous formulation to the patient. Step (ii) ideally takes place within 24 hours of step (i) *e.g.* within 12 hours, within 6 hours, within 3 hours, or within 1 hour.

The invention also provides formulations of the invention for use as medicaments *e.g.* for use in delivering an antibody to a mammal, or for use in treating, preventing or ameliorating one or more of the diseases and disorders described above.

The mammal is preferably a human but may also be, for example, a horse or a cow or a dog or a cat. The antibodies will ideally be chosen to match the target species *e.g.* a human antibody for human administration, an equine antibody for horses, a canine antibody for dogs, *etc.* If native host antibodies are not available then transfer of antibody specificity from one species to another can be achieved by transfer of CDR residues (and typically, in addition, one or more framework residues) from a donor antibody into a recipient framework from the host species *e.g.* as in humanisation. Equinised, bovinised, caninised and felinised antibodies are known in the art. The antibody will bind to CD40 from the target species, but it may also cross-react with CD40 from other species.

Dosage can be by a single dose schedule or a multiple dose schedule.

Ingredients for forming compositions of the invention (*e.g.* lyophilisates and reconstituents) may be supplied in hermetically-sealed containers.

The anti-CD40 antibody

The invention concerns the formulation of anti-CD40 antibodies and more specifically the antibodies designated mAb1, mAb2, and mAb3, especially mAb1.

One suitable antibody that can be comprised in the pharmaceutical compositions of the invention is the human recombinant antibody mAb1, structurally characterised as further described below. The V_H amino acid sequence of such isolated anti-CD40 antibody is shown in SEQ ID NO: 1. The V_L amino acid sequence of such isolated anti-CD40 antibody is shown in SEQ ID NO: 2. The full length heavy chain amino acid sequence of such isolated anti-CD40 antibody is shown in SEQ ID NO: 9. The full-length light chain amino acid sequence of such isolated anti-CD40 antibody is shown in SEQ ID NO: 10. Another example of heavy and light chain amino acid sequences of such isolated anti-CD40 antibodies are those encoded by the nucleotide sequences of SEQ ID NO: 11 and SEQ ID NO: 12 respectively.

Other anti-CD40 antibodies that can be used for preparing the pharmaceutical compositions of the invention include anti-CD40 antibodies with amino acids that have been mutated by amino acid deletion, insertion or substitution, yet have no more than 1, 2, 3, 4 or 5 amino acid deletions, insertions or substitutions in either the heavy or light chain regions described above. In a specific embodiment, such amino acid changes appear only within the framework and/or constant regions and the CDR regions are 100% identical to the heavy chain CDR1, CDR2 and CDR3 regions of SEQ ID NO: 3, 4 and 5 and to the light chain CDR1, CDR2 and CDR3 regions of SEQ ID NO: 6, 7, and 8 respectively. In one more specific embodiment, the changes that have been made are only conservative amino acid substitutions outside of the CDR regions.

Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues outside of the CDR regions of an anti-CD40 antibody, can be replaced with other amino acid residues from the same side chain family, and the altered antibody can be tested for retained function, in particular the same binding properties to CD40.

Antibodies may typically be glycosylated. N-linked glycans attached to the C_H2 domain of a heavy chain, for instance, can influence C1q and FcR binding, and aglycosylated antibodies (for example comprising an N297A mutation) may have lower or different affinity for these receptors. The glycan structure can also affect activity *e.g.* differences in complement-mediated cell death may be seen depending on the number of galactose sugars (0, 1 or 2) at the terminus of a glycan's biantennary chain. An antibody's glycans preferably do not lead to a human immunogenic response after administration.

Another modification of the anti-CD40 antibodies herein that is contemplated by the invention is pegylation. An antibody can be pegylated to, for example, increase the biological (e.g., serum) half-life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically may be reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. The pegylation can be carried out by an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer).

As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatise other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the invention. See for example, EP 0 154 316 by Nishimura et al. and EP 0 401 384 by Ishikawa et al.

Any other natural or non-natural post-translational modification of anti-CD40 antibodies (e.g. mAb1) is further contemplated as specific embodiments of anti-CD40 antibodies that could be used for preparing the pharmaceutical compositions of the invention.

Antibodies can be prepared in a form free from products with which they would naturally be associated. Contaminant components of an antibody's natural environment include materials such as enzymes, hormones, or other host cell proteins.

The various features and embodiments of the present invention, referred to in individual sections and embodiments above apply, as appropriate, to other sections and embodiments, mutatis mutandis. Consequently features specified in one section or embodiment may be combined with features specified in other sections or embodiments, as appropriate.

EXAMPLES

Preparing anti-CD40 antibodies

CHIR-12.12, and mAb1 (N297A CHIR-12.12), mAb2 (D265A CHIR-12.12), and mAb3 (CHIR-12.12 LALA) bind specifically to CD40. Tables 1 and 2 below summarise the

sequence characteristics of these antibodies. These antibodies may be produced in mammalian host cells, such as, a CHO cell line transfected with expression vectors carrying heavy and light chain coding sequences under suitable expression promoters.

Table 1: Brief description of the sequences listed in the sequence listing of Table 2

SEQ ID NO:	Description of the sequence
1	Amino acid sequence of the variable region (V_H) of the heavy chain of CHIR-12.12, mAb1, mAb2 and mAb3
2	Amino acid sequence of the variable region (V_L) of the light chain of CHIR-12.12, mAb1, mAb2 and mAb3
3	Amino acid sequence of HCDR1 of CHIR-12.12, mAb1, mAb2 and mAb3
4	Amino acid sequence of HCDR2 of CHIR-12.12, mAb1, mAb2 and mAb3
5	Amino acid sequence of HCDR3 of CHIR-12.12, mAb1, mAb2 and mAb3
6	Amino acid sequence of LCDR1 of CHIR-12.12, mAb1, mAb2 and mAb3
7	Amino acid sequence of LCDR2 of CHIR-12.12, mAb1, mAb2 and mAb3
8	Amino acid sequence of LCDR3 of CHIR-12.12, mAb1, mAb2 and mAb3
9	Amino acid sequence of the full length heavy chain of mAb1
10	Amino acid sequence of the full length light chain of mAb1
11	Nucleotide sequence encoding full length heavy chain of mAb1 (SEQ ID NO:9)
12	Nucleotide sequence encoding full length light chain of mAb1 (SEQ ID NO:10)
13	Amino acid sequence of the full length heavy chain of mAb2
14	Amino acid sequence of the full length light chain of mAb2
15	Nucleotide sequence encoding full length heavy chain of mAb2 (SEQ ID NO: 13)
16	Nucleotide sequence encoding full length light chain of mAb2 (SEQ ID NO: 14)
17	Amino acid sequence of the full length heavy chain of mAb3
18	Amino acid sequence of the full length light chain of mAb3
19	Nucleotide sequence encoding full length heavy chain of mAb3 (SEQ ID NO:17)
20	Nucleotide sequence encoding full length light chain of mAb3 (SEQ ID NO:18)
21	Amino acid sequence of human CD40

5

Table 2: Sequence listing

SEQ ID NO:	Amino acid or Nucleotide Sequence
1	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAV ISYEESNRYHADSVKGRFTISRDNSKITLYLQMNSLRTEDTAVYYCARDGGIA APGPDYWGQGTLVTVSS
2	DIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLI SLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFG PGTKVDIR
3	SYGMH
4	VISYEESNRYHADSVKG
5	DGGIAAPGPDY
6	RSSQSLLYSNGYNYLD
7	LGSNRAS
8	MQARQTPFT
9	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAV ISYEESNRYHADSVKGRFTISRDNSKITLYLQMNSLRTEDTAVYYCARDGGIA APGPDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVL T VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQVYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
10	DIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLI SLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFG PGTKVDIRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
11	CAGGTGCAGCTGGTGGAATCTGGCGGCGGAGTGGTGCAGCCTGGCCGG TCCCTGAGACTGTCTTGCGCCGCCTCCGGCTTCACCTTCTCCAGCTACGG CATGCACTGGGTGCGACAGGCCCCTGGCAAGGGACTGGAATGGGTGGC CGTGATCTCCTACGAGGAATCCAACAGATACCACGCTGACTCCGTGAAGG GCCGGTTCACAATCTCCCGGGACAACTCCAAGATCACCCTGTACCTGCAG ATGAACTCCCTGCGGACCGAGGACACCGCCGTGTACTACTGCGCCAGGG ACGGAGGAATCGCCGCTCCTGGACCTGATTATTGGGGCCAGGGCACCCT GGTGACAGTGTCCTCCGCTAGCACCAAGGGCCCCTCCGTGTTCCCTCTG GCCCCCTCCAGCAAGTCCACCTCTGGCGGCACCGCCGCTCTGGGCTGCC TGGTGAAAGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAACTCTGG CGCCCTGACCTCCGGCGTGCACACCTTTCCAGCCGTGCTGCAGTCCTCC GGCCTGTACTCCCTGTCCTCCGTGGTGACCGTGCCCTCTAGCTCTCTGG GCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAG GTGGACAAGCGGGTGGAACCCAAGTCCTGCGACAAGACCCACACCTGTC CCCCCTGCCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTT CCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTG ACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAAGTGAAGTTCA ATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCAG AGAGGAACAGTACGCCTCCACCTACCGGGTGGTGTCTGTGCTGACCGTG CTGCACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCA ACAAGGCCCTGCCTGCCCCCATCGAAAAGACCATCTCCAAGGCCAAGGG CCAGCCCCGCGAGCCACAGGTGTACACACTGCCCCCCAGCCGGGAAGA GATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAAGGCTTCTACC CCTCCGATATCGCCGTGGAGTGGGAGTCCAACGGACAGCCCGAGAACAA CTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGT

	ACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTT CTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAG TCCCTGTCCCTGAGCCCCGGCAAG
12	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGACCGTGACACCTGGCG AGCCTGCCTCTATCTCCTGCAGATCCTCCCAGTCCCTGCTGTACTCCAAC GGCTACAACTACCTGGACTGGTATCTGCAGAAGCCCGGCCAGTCCCCAC AGGTGCTGATCTCCCTGGGCTCCAACAGAGCCTCTGGCGTGCCCGACCG GTTCTCCGGCTCTGGCTCTGGCACCGACTTCACACTGAAGATCTCACGGG TGGAAGCCGAGGACGTGGGCGTGTACTACTGCATGCAGGCCCGGCAGA CCCCCTTCACCTTCGGCCCTGGCACCAAGGTGGACATCCGGCGTACGGT GGCCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAG AGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCTACCCCCGGG AGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACA GCCAGGAGAGCGTCACCGAGCAGGACAGCAAGGACTCCACCTACAGCCT GAGCAGCACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAGGTG TACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGA GCTTCAACAGGGGCGAGTGC
13	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAV ISYEESNRYHADSVKGRFTISRDNSKITLYLQMNSLRTEDTAVYYCARDGGIA APGPDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
14	DIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLI SLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFG PGTKVDIRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PV TKSFNRGEC
15	CAGGTGCAGCTGGTGGAATCTGGCGGCGGAGTGGTGCAGCCTGGCCGG TCCCTGAGACTGTCTTGCGCCGCCTCCGGCTTCACCTTCTCCAGCTACGG CATGCACTGGGTGCGACAGGCCCCTGGCAAGGGACTGGAATGGGTGGC CGTGATCTCCTACGAGGAATCCAACAGATACCACGCTGACTCCGTGAAGG GCCGGTTCACAATCTCCCGGGACAACTCCAAGATCACCCTGTACCTGCAG ATGAACTCCCTGCGGACCGAGGACACCGCCGTGTACTACTGCGCCAGGG ACGGAGGAATCGCCGCTCCTGGACCTGATTATTGGGGCCAGGGCACCCT GGTGACAGTGTCCTCCGCTAGCACCAAGGGCCCCTCCGTGTTCCCTCTG GCCCCCTCCAGCAAGTCCACCTCTGGCGGCACCGCCGCTCTGGGCTGCC TGGTGAAAGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAACTCTGG CGCCCTGACCTCCGGCGTGCACACCTTTCCAGCCGTGCTGCAGTCCTCC GGCCTGTACTCCCTGTCCTCCGTGGTGACCGTGCCCTCTAGCTCTCTGG GCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAG GTGGACAAGCGGGTGGAACCCAAGTCCTGCGACAAGACCCACACCTGTC CCCCCTGCCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTT CCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTG ACCTGCGTGGTGGTGGCCGTGTCCCACGAGGACCCTGAAGTGAAGTTCA ATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCAG AGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCTGTGCTGACCGTG CTGCACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCA ACAAGGCCCTGCCTGCCCCCATCGAAAAGACCATCTCCAAGGCCAAGGG CCAGCCCCGCGAGCCACAGGTGTACACACTGCCCCCCAGCCGGGAAGA GATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAAGGCTTCTACC CCTCCGATATCGCCGTGGAGTGGGAGTCCAACGGACAGCCCGAGAACAA

	CTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGT ACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTT CTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAG TCCCTGTCCCTGAGCCCCGGCAAG
16	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGACCGTGACACCTGGCG AGCCTGCCTCTATCTCCTGCAGATCCTCCCAGTCCCTGCTGTACTCCAAC GGCTACAACTACCTGGACTGGTATCTGCAGAAGCCCGGCCAGTCCCCAC AGGTGCTGATCTCCCTGGGCTCCAACAGAGCCTCTGGCGTGCCCGACCG GTTCTCCGGCTCTGGCTCTGGCACCGACTTCACACTGAAGATCTCACGGG TGGAAGCCGAGGACGTGGGCGTGTACTACTGCATGCAGGCCCGGCAGA CCCCCTTCACCTTCGGCCCTGGCACCAAGGTGGACATCCGGCGTACGGT GGCCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAG AGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCTACCCCCGGG AGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACA GCCAGGAGAGCGTCACCGAGCAGGACAGCAAGGACTCCACCTACAGCCT GAGCAGCACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAGGTG TACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGA GCTTCAACAGGGGCGAGTGC
17	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAV ISYEESNRYHADSVKGRFTISRDNSKITLYLQMNSLRTEDTAVYYCARDGGIA APGPDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
18	DIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLI SLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFG PGTKVDIRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
19	CAGGTGCAGCTGGTGGAATCTGGCGGCGGAGTGGTGCAGCCTGGCCGG TCCCTGAGACTGTCTTGCGCCGCCTCCGGCTTCACCTTCTCCAGCTACGG CATGCACTGGGTGCGACAGGCCCCTGGCAAGGGACTGGAATGGGTGGC CGTGATCTCCTACGAGGAATCCAACAGATACCACGCTGACTCCGTGAAGG GCCGGTTCACAATCTCCCGGGACAACTCCAAGATCACCCTGTACCTGCAG ATGAACTCCCTGCGGACCGAGGACACCGCCGTGTACTACTGCGCCAGGG ACGGAGGAATCGCCGCTCCTGGACCTGATTATTGGGGCCAGGGCACCCT GGTGACAGTGTCCTCCGCTAGCACCAAGGGCCCCTCCGTGTTCCCTCTG GCCCCTTCCAGCAAGTCTACCTCCGGCGGCACAGCTGCTCTGGGCTGCC TGGTCAAGGACTACTTCCCTGAGCCTGTGACAGTGTCCTGGAACTCTGGC GCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCG GCCTGTACTCCCTGTCCTCCGTGGTCACAGTGCCTTCAAGCAGCCTGGG CACCCAGACCTATATCTGCAACGTGAACCACAAGCCTTCCAACACCAAGG TGGACAAGCGGGTGGAGCCTAAGTCCTGCGACAAGACCCACACCTGTCC TCCCTGCCCTGCTCCTGAAGCTGCTGGCGGCCCTTCTGTGTTCCTGTTCC CTCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGAC CTGCGTGGTGGTGGACGTGTCCCACGAGGATCCTGAAGTGAAGTTCAATT GGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGGG AGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCT GCACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAAGTCTCCAAC AAGGCCCTGCCTGCCCCTATCGAAAAGACAATCTCCAAGGCCAAGGGCC AGCCTAGGGAACCCCAGGTGTACACCCTGCCACCCAGCCGGGAGGAAAT GACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCTT

	CCGATATCGCCGTGGAGTGGGAGTCTAACGGCCAGCCTGAGAACAACTA CAAGACCACCCCTCCTGTGCTGGACTCCGACGGCTCCTTCTTCCTGTACT CCAAACTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTC CTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCC CTGTCCCTGTCTCCCGGCAAG
20	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGACCGTGACACCTGGCG AGCCTGCCTCTATCTCCTGCAGATCCTCCCAGTCCCTGCTGTACTCCAAC GGCTACAACTACCTGGACTGGTATCTGCAGAAGCCCGGCCAGTCCCCAC AGGTGCTGATCTCCCTGGGCTCCAACAGAGCCTCTGGCGTGCCCGACCG GTTCTCCGGCTCTGGCTCTGGCACCGACTTCACACTGAAGATCTCACGGG TGGAAGCCGAGGACGTGGGCGTGTACTACTGCATGCAGGCCCGGCAGA CCCCCTTCACCTTCGGCCCTGGCACCAAGGTGGACATCCGGCGTACGGT GGCCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAG AGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCTACCCCCGGG AGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACA GCCAGGAGAGCGTCACCGAGCAGGACAGCAAGGACTCCACCTACAGCCT GAGCAGCACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAGGTG TACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGA GCTTCAACAGGGGCGAGTGC
21	MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLCQPGQKLVSDC TEFTETECLPCGESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTIC TCEEGWHCTSEACESCVLHRSCSPGFGVKQIATGVSDTICEPCPVGFFSNVS SAFEKCHPWTSCETKDLVVQQAGTNKTDVVCGPQDRLRALVVIPIIFGILFAIL LVLVFIKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVT QEDGKESRISVQERQ

Examples of formulations

A high concentration lyophilised or liquid formulation of mAb1 was desired and so formulation studies were performed. A lyophilised formulation comprising a sugar, a buffering agent and a surfactant was stable and could maintain high antibody concentrations after reconstitution.

Five formulations (F1, F2, F3, F4 and F5) of mAb1 were evaluated for stability. F1 was a liquid formulation at 50 mg/mL mAb1 at pH 6.0. Formulations F2, F3, F4, and F5 had prior to lyophilisation, 50 mg/vial mAb1. Formulations F2, F3, F4 and F5 had, prior to lyophilisation, 50mg/ml mAb1 at pH 6.0. Formulations F2, F3, F4 and F5 had a fill volume of 3.6 ml. The five formulations included buffer, sugar, surfactant and free amino acid as listed in Table 3:

Table 3: Examples of formulations

	mAb1	Histidine buffer	Stabiliser	Surfactant	Amino acid
F1	50 mg/ml	10mM	190mM trehalose	0.02% polysorbate 20	40mM arginine-HCl

F2	50 mg/ml	10mM	90mM trehalose	0.02% polysorbate 20	17mM arginine-HCl
F3	50 mg/ml	10mM	90mM sucrose	0.02% polysorbate 20	17mM arginine-HCl
F4	50 mg/ml	10mM	90mM trehalose	0.02% polysorbate 20	-
F5	50 mg/ml	10mM	90mM sucrose	0.02% polysorbate 20	-

The F2, F3, F4, and F5 lyophilisates were reconstituted with WFI (1.0 ml) to give a reconstituted volume of 1.2 ml (1/3 the original aqueous volume). The reconstituted compositions were as listed in Table 4:

Table 4: Examples of formulations

	mAb1	Histidine buffer	Stabiliser	Surfactant	Amino acid
F2	150 mg/ml	30mM histidine	270mM trehalose	0.06% polysorbate 20	51mM arginine-HCl
F3	150 mg/ml	30mM histidine	270mM sucrose	0.06% polysorbate 20	51mM arginine-HCl
F4	150 mg/ml	30mM histidine	270mM trehalose	0.06% polysorbate 20	-
F5	150 mg/ml	30mM histidine	270mM sucrose	0.06% polysorbate 20	-

The lyophilisation cycle used is reported in Table 5.

Table 5: The lyophilisation cycle parameters

Step	**Operation**	**Time [hh:mm]**	**Shelf temp.**	**Chamber pressure**
1	Vial loading	As required	20°C	Ambient
2	5°C cooling	00:30	5°C	Ambient

3	5°C hold	03:00	5°C	Ambient
4	Freeze ramp	01:24	5°C to - 37°C	Ambient
5	Freeze hold	06:00	- 37°C	Ambient
6	Chamber Vacuum	as required	-37°C	0.2 mbar[a]
7	Primary drying ramp	16:00	-37°C to 25°C	0.2 mbar[a]
8	Secondary drying hold	24:00	25°C	0.2 mbar[a]
11	Vial stoppering		25°C	850 ± 50 mbar

[a] Chamber pressure was controlled using sterile filtered nitrogen. The pressure was determined by instruments based on capacitance measurements.

The five formulations were tested for stability at various time points after formulation/reconstitution as listed below.

Size exclusion–High Pressure Liquid Chromatography (SEC-HPLC) was used to assess the amount of mAb1 in each formulation at the time of formulation (T0), 4 weeks (25°C), 4 weeks (40°C), 6 months (2-8°C), 6 months (25°C) and 6 months (40°C). The amount of antibody is expressed as a percentage of the starting amount, as listed in Table 6. The F1 formulation remains close to 50 mg/ml ± 5 mg/ml (100% ± 10%), the F2, F3, F4 and F5 formulations remain close to 150 mg/ml ± 15 mg/ml (100% ± 10%).

Table 6: Amount of antibody from SEC-HPLC

	T0	4 weeks (25°C)	4 weeks (40°C)	6 months (2-8°C)	6 months (25°C)	6 months (40°C)
F1	100%±10%	100%±10%	100%±10%	100%±10%	100%±10%	100%±10%
F2	100%±10%	-	100%±10%	-	100%±10%	100%±10%
F3	100%±10%	-	100%±10%	-	-	100%±10%
F4	100%±10%	-	100%±10%	-	100%±10%	100%±10%
F5	100%±10%	-	100%±10%	-	100%±10%	100%±10%

The potency of the mAb1 antibody in each formulation was measured by ELISA assay at T0, 4 weeks (25°C), 4 weeks (40°C), 6 months (2-8°C), 6 months (25°C) and 6 months (40°C). The results, expressed as a percentage of the starting potency (100%) are listed in Table 7. The potency of the F1 formulation decreases with progression of time and increase of temperature; after 6m at 40°C potency was found to be 79%. The lowest value measured was 85% for F4 (6m at 25°C) and 86% for F3 (6m at 40°C)

Table 7: Potency of antibody as measured in ELISA

	T0	4 weeks (25°C)	4 weeks (40°C)	6 months (2-8°C)	6 months (25°C)	6 months (40°C)
F1	91.3	104.0	98.0	91.0	91.0	79.0
F2	93.2					
F3	95.7					86.0
F4	96.5				85.0	90.0
F5	97.8					

Stability of the formulations was evaluated by % impurities as measured by size exclusion–High Pressure Liquid Chromatography (SEC-HPLC) and Dynamic Light Scattering (DLS). The results are shown in Tables 8 and 9.

Table 8: Aggregation products results from SEC-HPLC

	T0	4 weeks (25°C)	4 weeks (40°C)	6 months (2-8°C)	6 months (25°C)	6 months (40°C)
F1	0.26%	0.26%	0.59%	0.38%	0.47%	2.76%
F2	0.38%		1.37%		1.40%	3.80%
F3	0.29%		0.96%			2.88%
F4	0.38%		1.94%		1.97%	5.59%
F5	0.33%		1.32%		1.40%	4.01%

Table 9: Small particles; results for PolyDispensity Index (PDI) from Dynamic Light Scattering

	T0	4 weeks (25°C)	4 weeks (40°C)	6 months (2-8°C)	6 months (25°C)	6 months (40°C)
F1	52.8%	30.2%	39.3%	41.3%	52.9%	59.9%
F2	48.8%		61.0%		44.4%	46.6%
F3	50.4%		57.0%			40.8%
F4	54.7%		61.8%		61.9%	59.2%
F5	47.2%		60.0%		60.5%	51.3%

The F1 formulation showed an increase of aggregation products with increasing temperature and time. For the F2 to F5 formulations addition of arginine (F2 and F3) slightly reduced the level of aggregation; and sucrose (F3 and F5) was superior to trehalose.

Aggregation products were also evaluated by measuring turbidity. The F1 formulation showed an increase of turbidity with increase in temperature and with increase in time. The F2 to F5 formulations showed slightly increased turbidity values for formulations containing arginine (F2 and F3).

Table 10: Turbidity measurements (absorbance units)

	T0	4 weeks (25°C)	4 weeks (40°C)	6 months (2-8°C)	6 months (25°C)	6 months (40°C)
F1	7.38	11.30	12.90	9.7	12.7	17.9
F2	9.36		14.90		12.1	11.3
F3	8.98		14.40			11.0
F4	6.34		10.40		9.2	7.6
F5	6.91		10.70		9.7	8.2

Stability of the formulations was evaluated by % degradation products as measured SEC-HPLC. With the F2 to F5 formulations the addition of arginine slightly reduced degradation. The results are shown in Table 11.

Table 11: Degradation products results from SEC-HPLC

	T0	4 weeks (25°C)	4 weeks (40°C)	6 months (2-8°C)	6 months (25°C)	6 months (40°C)
F1	0.11%	1.40%	2.76%	1.04%	2.10%	8.88%
F2	0.11%		0.14%		0.12%	0.05%
F3	0.15%		0.18%		0.00%	2.97%
F4	0.10%		0.19%		0.15%	5.70%
F5	0.11%		0.18%		0.14%	4.10%

The four lyophilised formulations F2, F3, F4, and F5 were assessed for visual clarity after reconstitution. The results are presented in Table 12.

Table 12: Visual clarity

	T0	4 weeks (25°C)	4 weeks (40°C)	6 months (2-8°C)	6 months (25°C)	6 months (40°C)
F2	clear without particles	clear without particles	clear without particles	clear without particles	clear without particles	clear without particles
F3	clear without particles	clear without particles	clear without particles	clear without particles	clear without particles	clear without particles
F4	clear without particles	clear without particles	clear without particles	clear without particles	clear without particles	clear without particles
F5	clear without particles	clear without particles	clear without particles	clear without particles	clear without particles	clear without particles

The physico-chemical properties of the formulations were assessed; the results are presented in Table 13. The viscosity values are the values of the formulations having 50 mg/ml antibody prior to lyophilisation. The osmolality values correspond to the reconstituted compositions having 150 mg/ml antibody.

Table 13: Physico-chemical properties

	Viscosity mPa.s	Osmolality mOsm/kg
F1	2.1	318
F2	1.7	580
F3	1.6	556
F4	1.6	461
F5	1.6	443

Conclusions

Overall, the formulations tested showed good results. The bioassays (SEC and ELISA) showed that the formulations are comparable. Soluble aggregates were slightly reduced in the presence of arginine and were slightly lower for the sucrose containing formulations compared to the trehalose containing formulations. Turbidity was slightly increased in the presence of arginine; there was no difference between trehalose or sucrose containing formulations. Degradation was reduced in the presence of arginine. With regard to physico-chemical properties the pH, viscosity, residual moisture content, and visual aspects were comparable for all formulations. Osmolality was >300 mOsm/kg for all formulations, and was even higher in the presence of arginine-HCl (F2 and F3). It is desirable to have a formulation which is isotonic to plasma (290 mOsm/kg), or as close to this value as possible. The formulations having sucrose (F3 and F5) were considered preferable to those comprising trehalose. Whilst some beneficial effects on degradation were seen with arginine (F3), the lower osmolality of the F5 formulation was considered to be a more important factor. The F5 formulation was deemed to be the most optimal formulation.

CLAIMS

1. A lyophilised formulation prepared by lyophilising an aqueous formulation having a pH of 5.0-7.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 20-150 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) a stabiliser,

(iii) a buffering agent,

(iv) a surfactant, and optionally

(v) an amino acid.

2. The lyophilised formulation of claim 1, wherein said formulation is prepared from an aqueous formulation having a pH of 5.0-7.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 20-150mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) sucrose or trehalose as a stabiliser,

(iii) histidine as a buffering agent,

(iv) polysorbate 20 as a surfactant, and optionally

(v) an amino acid selected from arginine, methionine and glycine.

3. The lyophilised formulation of claim 1 or claim 2, wherein said formulation is prepared from an aqueous formulation having a pH of 5.0-7.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 20-150 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 3-300 mM sucrose or trehalose as a stabiliser,

(iii) 1-60 mM histidine as a buffering agent,

(iv) up to 0.2% polysorbate 20 as a surfactant, and optionally

(v) 2-80 mM arginine, methionine or glycine.

4. The lyophilised formulation as claimed in any previous claim, wherein said formulation is prepared from an aqueous formulation having a pH of 6.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 90 mM sucrose as a stabiliser,

(iii) 10 mM histidine as a buffering agent,

(iv) 0.02% polysorbate 20 as a surfactant.

5. The lyophilised formulation as claimed in any previous claim, wherein said formulation is prepared from an aqueous formulation having a pH of 6.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 90 mM sucrose as a stabiliser,

(iii) 10 mM histidine as a buffering agent,

(iv) 0.02% polysorbate 20 as a surfactant, and

(v) 17 mM arginine.

6. The lyophilised formulation as claimed in any previous claim, wherein said formulation is prepared from an aqueous formulation having a pH of 6.0 and comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of 150 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 270 mM sucrose as a stabiliser,

(iii) 30 mM histidine as a buffering agent, and

(iv) 0.06% polysorbate 20 as a surfactant.

7. An aqueous pharmaceutical composition obtained by reconstituting a lyophilised formulation of any one of the preceding claims, wherein the reconstitution factor is between 1:0.5 to 1:6.

8. The aqueous pharmaceutical composition of claim 8, wherein the reconstitution factor is 1:3.

9. An aqueous pharmaceutical composition having a pH of 5.0 to 7.0 comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) a stabiliser,

(iii) a buffering agent,

(iv) a surfactant, and optionally

(v) an amino acid.

10. The aqueous pharmaceutical composition of claim 9 comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) sucrose or trehalose as a stabiliser,

(iii) histidine as a buffering agent,

(iv) polysorbate 20 as a surfactant, and optionally

(v) an amino acid selected from arginine, methionine or glycine.

11. The aqueous pharmaceutical composition of claim 9 or 10 comprising

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 200-300 mM sucrose as a stabiliser,

(iii) 25-35 mM histidine as a buffering agent,

(iv) up to 0.2% polysorbate 20 as a surfactant, and optionally

(v) 10-80 mM arginine, methionine or glycine.

12. The aqueous pharmaceutical composition of any one of claims 9 to 11, wherein the composition has a pH of 6.0 and comprises

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 270 mM sucrose as a stabiliser,

(iii) 30 mM histidine as a buffering agent, and

(iv) 0.06% polysorbate 20 as a surfactant.

13. The aqueous pharmaceutical composition of any one of claims 9 to 11, wherein the composition has a pH of 6.0 and comprises

(i) an anti-CD40 antibody wherein the antibody has a concentration of at least 50 mg/ml, and wherein said anti-CD40 antibody includes heavy chain CDR1, CDR2 and CDR3 of SEQ ID NOs 3, 4 and 5 respectively, and light chain CDR1, CDR2 and CDR3 of SEQ ID NOs: 6, 7 and 8,

(ii) 270 mM sucrose as a stabiliser,

(iii) 30 mM histidine as a buffering agent,

(iv) 0.06% polysorbate 20 as a surfactant, and

(v) 51 mM arginine.

14. The aqueous pharmaceutical composition of any one of claims 9 to 13, wherein the anti-CD40 antibody has a concentration of 150 mg/ml.

15. The lyophilised formulation of any one of claims 1 to 6 or the aqueous pharmaceutical composition of any one of claims 7 to 14, wherein the anti-CD40 antibody comprises a V_H domain having the amino acid sequence of SEQ ID NO: 1 and a V_L domain having the amino acid sequence of SEQ ID NO: 2.

16. The lyophilised formulation of any one of claims 1 to 6 or the aqueous pharmaceutical composition of any one of claims 7 to 14, wherein the anti-CD40 antibody comprises a heavy chain region of SEQ ID NO: 9 and a light chain region of SEQ ID NO: 10.

17. The lyophilised formulation of any one of claims 1 to 6 or the aqueous pharmaceutical composition of any one of claims 7 to 14, wherein the anti-CD40 antibody comprises a heavy chain region of SEQ ID NO: 13 and a light chain region of SEQ ID NO: 14.

18. The lyophilised formulation of any one of claims 1 to 6 or the aqueous pharmaceutical composition of any one of claims 7 to 14, wherein the anti-CD40 antibody comprises a heavy chain region of SEQ ID NO: 17 and a light chain region of SEQ ID NO: 18.

19. A delivery device including the aqueous pharmaceutical composition of any one of claims 7 to 18.

20. A pre-filled syringe including the aqueous pharmaceutical composition of any one of claims 7 to 18.

21. A method for delivering an anti-CD40 antibody to a mammal, comprising a step of administering to the mammal an aqueous pharmaceutical composition as claimed in any one of claims 7 to 18.

22. A lyophilised formulation of any one of claims 1 to 6 or an aqueous pharmaceutical composition of any one of claims 7 to 14 for use in treating a disease or disorder that is mediated by CD40.

23. The lyophilised formulation or aqueous pharmaceutical composition according to claim 22 for use in the treatment of autoimmune diseases.

24. The lyophilised formulation or aqueous pharmaceutical composition according to claim 23 for use in the treatment of rheumatoid arthritis, systemic lupus erythematosus, or Pemphigus vulgaris.