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(54) Title: NEW ANTIBODIES AGAINST PHOSPHORYLCHOLINE

(57) Abstract: The present invention relates to an antibody or antibody fragment capable of binding to phosphorylcholine and/or a phosphorylcholine conjugate, wherein the antibody or antibody fragment comprises a variable heavy chain (VH) domain and/or a variable light chain (VL) domain, and wherein - (a) the VH domain comprises an amino acid sequence that includes one, two or three complementarity determining regions (CDRs) selected from the group consisting of: a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17; a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18; and a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 19, 20, 21 or 22; and/or (b) the VL domain comprises an amino acid sequence that includes one, two or three complementarity determining regions (CDRs) selected from the group consisting of: a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 23 or 24; a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25; a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26.

NEW ANTIBODIES AGAINST PHOSPHORYLCHOLINE

FIELD OF THE INVENTION

The present invention relates to new antibodies with binding to phosphorylcholine (PC) and/or PC conjugates and having surprisingly effective *in vivo* properties.

BACKGROUND TO THE INVENTION

The listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

Despite the available treatment options available for cardiovascular disease, acute coronary syndrome (ACS) is the leading cause of death in the industrialized world. ACS occurs as a result of thrombus formation within the lumen of a coronary artery, which is associated with chronic inflammation within the wall of the artery. Arterial inflammation is initiated by the formation of a lipid core and infiltration of inflammatory cells leading to plaque formation. Unstable plaques contain a substantial necrotic core and apoptotic cells that disrupt the endothelium and can lead to plaque rupture exposing of underlying collagen, von Willebrand factor (vWF), tissue factor, lipids and smooth muscle allowing initiation of platelet adhesion, activation, and aggregation (Libby *et al.* 1996. *Macrophages and atherosclerotic plaque stability.* Curr Opin Lipidol **7**, 330-335). ACS is treated with a combination of anti-platelet therapies, cholesterol lowering medications (e.g. statins), anti-coagulants, as well as surgical recanalization through percutaneous coronary intervention (PCI) and implantation of stents.

Anti-platelet therapies such as COX-1 inhibitors (e.g. aspirin), ADP receptor antagonists (e.g. Ticlopedine and clopidogrel), and glycoprotein IIb/IIIa receptor antagonists have been shown to reduce the incidence of major adverse coronary events (MACE) in a number of different clinical trials (Dupont et al- 2009- *Antiplatelet therapies and the role of antiplatelet resistance in acute coronary syndrome.* Thromb Res **124**, 6-13). However, a proportion of patients on long-term anti-platelet therapy continue to have cardiovascular events. Moreover, chronic prevention therapy may take up to two years to show maximum beneficial effects, and many patients are then still at high risk for recurrent disease. There is a period

of up to 6-12 months after a myocardial infarction that the patient is susceptible to further MACE, frequently due to re-occlusion due to restenosis (Tabas. 2010. *Macrophage death and defective inflammation resolution in atherosclerosis.* Nat Rev Immunol **10**, 36-46).

Consequently, there is a significant need for treatments directed specifically at preventing further plaque progression and promoting plaque regression could substantially lower events during this period.

Phosphorylcholine, a polar head group on certain phospholipids, has been extensively implicated in cardiovascular disease. Reactive oxygen species generated during coronary inflammation causes the oxidation of low density lipoprotein (LDL) to generate oxidized LDL (oxLDL). In fact, cardiovascular diseases (CVD) such as atherosclerosis, unstabile angina, or acute coronary syndrome have been shown to be associated with elevated plasma levels of oxLDL (Itabe and Ueda. 2007. *Measurement of plasma oxidized low-density lipoprotein and its clinical implications.* J Atheroscler Thromb **14**, 1-11). LDL is a circulating lipoprotein particle that contains lipids with a PC polar head group and an apoB100 protein.

During oxidation of LDL PC containing neo-epitopes that are not present on unmodified LDL, are generated. Newly exposed PC on oxLDL is recognized by scavenger receptors on macrophages, such as CD36, and the resulting macrophage-engulfed oxLDL proceeds towards the formation of proinflammatory foam cells in the vessel wall. Oxidized LDL is also recognized by receptors on endothelial cell surfaces and has been reported to stimulate a range of responses including endothelial dysfunction, apoptosis, and the unfolded protein response (Gora et al. 2010. *Phospholipolyzed LDL induces an inflammatory response in endothelial cells through endoplasmic reticulum stress signaling.* FASEB J 24(9):3284-97). PC neo-epitopes are also exposed on LDL following modification with phospholipase A2 or amine reactive disease metabolites, such as aldehydes generated from the oxidation of glycated proteins. These alternately modified LDL particles are also pro-inflammatory factors in CVD.

Antibodies towards phosphorylcholine (PC) have been shown to bind oxidized, or otherwise modified, LDL and block the pro-inflammatory activity of oxLDL in in vivo models or in vitro studies (Shaw et al. 2000. *Natural antibodies with the T15 idiotype*

may act in atherosclerosis, apoptotic clearance, and protective immunity. J Clin Invest 105, 1731-1740; Shaw et al. 2001. *Human-derived anti-oxidized LDL autoantibody blocks uptake of oxidized LDL by macrophages and localizes to atherosclerotic lesions in vivo.* Arterioscler Thromb Vasc Biol 21, 1333-1339.

Furthermore, an examination of clinical data has demonstrated that low levels of natural IgM anti-PC antibodies are associated with an increased risk of MACE in ACS patients (Frostegard, J. 2010. *Low level natural antibodies against phosphorylcholine: a novel risk marker and potential mechanism in atherosclerosis and cardiovascular disease.* Clin Immunol 134, 47-54).

Accordingly, there is a need for anti-PC antibody molecules that can be effectively used in therapy, particularly fully human anti-PC antibodies suitable for human therapy. To the applicant's knowledge, to date the art has failed to provide therapeutically efficacious human anti-PC antibodies. The identification of such antibodies has been hampered by the fact that *in vitro* screening methods for human antibodies with anti-PC binding activity are poor predictors of *in vivo* therapeutic activity.

In view of this, there is a need in the art for human anti-PC antibody molecules that provide effective and advantageous properties when used in *in vivo* systems, in particular when administered to humans for therapy.

DESCRIPTION OF THE INVENTION

The present application describes the production and testing of new antibodies and antibody fragments comprising novel antigen-binding regions capable of binding to phosphorylcholine and/or phosphorylcholine conjugates.

In a first aspect, the present invention provides an antibody or antibody fragment capable of binding to phosphorylcholine and/or a phosphorylcholine conjugate, wherein the antibody or antibody fragment comprises a variable heavy chain (VH) domain and/or a variable light chain (VL) domain, and wherein -

(a) the VH domain comprises an amino acid sequence that includes one, two or preferably three complementarity determining regions (CDRs) selected from the group consisting of:

a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17;

a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18; and

a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 19, 20, 21 or 22; and/or

(b) the VL domain comprises an amino acid sequence that includes one, two or preferably three complementarity determining regions (CDRs) selected from the group consisting of:

a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 23 or 24;

a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25;

a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26.

In one embodiment according to the first aspect of the present invention, the antibody or antibody fragment comprises a VH domain that comprises an amino acid sequence that includes a CDR1 sequence, a CDR2 and a CDR3 sequence as defined above, and/or a VL domain that comprises an amino acid sequence that includes a CDR4 sequence, a CDR5 and a CDR6 sequence as defined above.

In a further embodiment of the first aspect of the present invention, the antibody or antibody fragment comprises –

a VH domain that comprises an amino acid sequence that includes all three of the CDR1, CDR2 and CDR3 sequences present in an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15 or an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to an amino acid sequence of any of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15; and/or

a VL domain that comprises an amino acid sequence that includes all three of the CDR4, CDR5 and CDR6 sequences present in an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16 or an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to an amino acid sequence of any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16.

In a further embodiment of the first aspect of the present invention, the antibody or antibody fragment comprises a variable heavy chain (VH) domain and/or a variable light chain (VL) domain, wherein -

the VH domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15 or an amino acid sequence having at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% sequence identity to an amino acid sequence of any of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15; and

the VL domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16 or an amino acid sequence having at least 50%, 60%, 70%, 80%, 85%, 90%, or 95% sequence identity to an amino acid sequence of any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16.

SEQ ID NO:1 is the variable heavy (VH) domain of the X19-A05 antibody as described in the following examples, and has the sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSGYWMHWVRQAPGKGLEWVS
YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRF
RSVCSNAVCRPTAYDAFDI WGQGTMVTVSS,

and includes the complementarity determining regions (CDRs):

VH CDR1: GYWM (SEQ ID NO: 17);
VH CDR2: YISPSGGGTHYADSVKG (SEQ ID NO: 18);
VH CDR3: VRFRSVCSNAVCRPTAYDAFDI (SEQ ID NO: 19);

SEQ ID NO:2 is the variable light (VL) domain of the X19-A05 antibody and has the sequence:

DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT
FGQGTKVEIK,

and includes the complementarity determining regions (CDRs):

VL CDR4: KSSQSVFYQSNKKNYLA (SEQ ID NO: 23);

VL CDR5: WASTRES (SEQ ID NO: 25);
VL CDR6: QQYFNAPRT (SEQ ID NO: 26),

SEQ ID NO:3 is the variable heavy (VH) domain of the M99-B05 antibody as described in the following examples, and has the sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI
SPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR
SVCSNGVCRPTAYDAFDIWGQGTAVTVSS,

and includes the complementarity determining regions (CDRs):

VH CDR1: GYWM (SEQ ID NO: 17);
VH CDR2: YISPSGGGTHYADSVKG (SEQ ID NO: 18);
VH CDR3: VRFRSVCSNGVCRPTAYDAFDI (SEQ ID NO: 20),

SEQ ID NO:4 is the variable light (VL) domain of the M99-B05 antibody and has the sequence:

QDIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPP
KLLIHWASTRESGVPDRFSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPR
TFGQGTKVEIK,

and includes the complementarity determining regions (CDRs):

VL CDR4: KSSQSVFYNSNKKNYLA (SEQ ID NO: 24);
VL CDR5: WASTRES (SEQ ID NO: 25);
VL CDR6: QQYFNAPRT (SEQ ID NO: 26),

SEQ ID NO:5 is the variable heavy (VH) domain of the X19-A01 antibody as described in the following examples, and has the sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI
SPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR
SVCSNGVCRPTAYDAFDIWGQGTAVTVSS,

and includes the complementarity determining regions (CDRs):

VH CDR1: GYWM (SEQ ID NO: 17);
VH CDR2: YISPSGGGTHYADSVKG (SEQ ID NO: 18);
VH CDR3: VRFRSVCSNGVCRPTAYDAFDI (SEQ ID NO: 20),

SEQ ID NO:6 is the variable light (VL) domain of the X19-A01 antibody and has the sequence:

DIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPK
LLIHWASTRESGVPDRFSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRT
FGQGTKVEIK,

and includes the complementarity determining regions (CDRs):

VL CDR4: KSSQSVFYNSNKKNYLA (SEQ ID NO: 24);
VL CDR5: WASTRES (SEQ ID NO: 25);
VL CDR6: QQYFNAPRT (SEQ ID NO: 26),

SEQ ID NO:7 is the variable heavy (VH) domain of the X19-A03 antibody as described in the following examples, and has the sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI
SPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR
SVCSNAVCRPTAYDAFDIWGQGTMVTVSS,

and includes the complementarity determining regions (CDRs):

VH CDR1: GYWM (SEQ ID NO: 17);
VH CDR2: YISPSGGGTHYADSVKG (SEQ ID NO: 18);
VH CDR3: VRFRSVCSNAVCRPTAYDAFDI (SEQ ID NO: 19),

SEQ ID NO:8 is the variable light (VL) domain of the X19-A03 antibody and has the sequence:

DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT
FGQGTKVEIK,

and includes the complementarity determining regions (CDRs):

VL CDR4: KSSQSVFYQSNKKNYLA (SEQ ID NO: 23);
VL CDR5: WASTRES (SEQ ID NO: 25);
VL CDR6: QQYFNAPRT (SEQ ID NO: 26),

SEQ ID NO:9 is the variable heavy (VH) domain of the X19-A07 antibody as described in the following examples, and has the sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI
SPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR
SVCSNGVCRPTAYDAFDIWGQGTMVTVSS,

and includes the complementarity determining regions (CDRs):

VH CDR1: GYWM (SEQ ID NO: 17);
VH CDR2: YISPSGGGTHYADSVKG (SEQ ID NO: 18);
VH CDR3: VRFRSVCSNGVCRPTAYDAFDI (SEQ ID NO: 20),

SEQ ID NO:10 is the variable light (VL) domain of the X19-A07 antibody and has the sequence:

DIVMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT
FGQGTKVEIK,

and includes the complementarity determining regions (CDRs):

VL CDR4: KSSQSVFYNSNKKNYLA (SEQ ID NO: 24);
VL CDR5: WASTRES (SEQ ID NO: 25);
VL CDR6: QQYFNAPRT (SEQ ID NO: 26),

SEQ ID NO:11 is the variable heavy (VH) domain of the X19-A09 antibody as described in the following examples, and has the sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSGYWMHWVRQAPGKGLEWVS
YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRF
RSVCSNGVCRPTAYDAFDIWGQGTMVTVSS,

and includes the complementarity determining regions (CDRs):

VH CDR1: GYWM (SEQ ID NO: 17);
VH CDR2: YISPSGGGTHYADSVKG (SEQ ID NO: 18);
VH CDR3: VRFRSVCSNGVCRPTAYDAFDI (SEQ ID NO: 20),

SEQ ID NO:12 is the variable light (VL) domain of the X19-A09 antibody and has the sequence:

DIVMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT
FGQGTKVEIK,

and includes the complementarity determining regions (CDRs):

VL CDR4: KSSQSVFYNSNKKNYLA (SEQ ID NO: 24);
VL CDR5: WASTRES (SEQ ID NO: 25);
VL CDR6: QQYFNAPRT (SEQ ID NO: 26),

SEQ ID NO:13 is the variable heavy (VH) domain of the X19-A11 antibody as described in the following examples, and has the sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI
SPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR
SVSSNGVSRPTAYDAFDIWGQGTAVTVSS,

and includes the complementarity determining regions (CDRs):

VH CDR1: GYWM (SEQ ID NO: 17);
VH CDR2: YISPSGGGTHYADSVKG (SEQ ID NO: 18);
VH CDR3: VRFRSVSSNGVSRPTAYDAFDI (SEQ ID NO: 21),

SEQ ID NO:14 is the variable light (VL) domain of the X19-A11 antibody and has the sequence:

DIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPK
LLIHWASTRESGVPDRFSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRT
FGQGTKVEIK,

and includes the complementarity determining regions (CDRs):

VL CDR4: KSSQSVFYNSNKKNYLA (SEQ ID NO: 24);
VL CDR5: WASTRES (SEQ ID NO: 25);
VL CDR6: QQYFNAPRT (SEQ ID NO: 26),

SEQ ID NO:15 is the variable heavy (VH) domain of the X19-C01 antibody as described in the following examples, and has the sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI
SPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR
SVSSNAVSRPTAYDAFDIWGQGTMVTVSS,

and includes the complementarity determining regions (CDRs):

VH CDR1: GYWM (SEQ ID NO: 17);
VH CDR2: YISPSGGGTHYADSVKG (SEQ ID NO: 18);
VH CDR3: VRFRSVSSNAVSRPTAYDAFDI (SEQ ID NO: 22),

SEQ ID NO:16 is the variable light (VL) domain of the X19-C01 antibody and has the sequence:

DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT
FGQGTKVEIK,

and includes the complementarity determining regions (CDRs):

VL CDR4: KSSQSVFYQSNKKNYLA (SEQ ID NO: 23);
VL CDR5: WASTRES (SEQ ID NO: 25);
VL CDR6: QQYFNAPRT (SEQ ID NO: 26).

A summary of the SEQ ID NOS, as defined above, is shown as follows:

	VH	VL	CDR1	CDR2	CDR3	CDR4	CDR5	CDR6
X19-A05	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 19	SEQ ID NO: 23	SEQ ID NO: 25	SEQ ID NO: 26
M99-B05	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 20	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-A01	SEQ ID NO: 5	SEQ ID NO: 6	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 20	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-A03	SEQ ID NO: 7	SEQ ID NO: 8	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 19	SEQ ID NO: 23	SEQ ID NO: 25	SEQ ID NO: 26
X19-A07	SEQ ID NO: 9	SEQ ID NO:10	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 20	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-A09	SEQ ID NO: 11	SEQ ID NO:12	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 20	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-A11	SEQ ID NO: 13	SEQ ID NO:14	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 21	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-C01	SEQ ID NO: 15	SEQ ID NO:16	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 22	SEQ ID NO: 23	SEQ ID NO: 25	SEQ ID NO: 26

In a further embodiment of the first aspect of the invention, the antibody or antibody fragment is based on the VH and/or VL domains of the X19-A05 antibody, and so –

the VH domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:1 and/or (ii) comprises a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17, a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18, and a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 19; and/or

the VL domain (iii) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 2 and/or (iv) comprises a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 23, a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25 and a CDR6 sequence

comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26. It may be preferred that the VH domain comprises the sequence of SEQ ID NO:1 and the VL domain comprises the sequence of SEQ ID NO: 2.

The antibody or antibody fragment of this embodiment may further comprise a heavy chain constant (CH) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320 or more amino acids of a CH region. The CH region or a fragment thereof may be joined to the VH domain. There is no particular limitation on the CH region although in one embodiment it is a human CH region. The art contains many examples of human CH regions. Exemplary human CH regions for use in this context include:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 27); and

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPG (SEQ ID NO: 28).

SEQ ID NO:27 is the CH region of M99-B05 and has the sequence of a CH region of Human IgG1 (UniProtKB/Swiss-Prot: P01857.1). SEQ ID NO: 28 is the CH region of X19-A05. SEQ ID NO: 28 differs from SEQ ID NO: 27 by the removal of the terminal K (Lys) in the CH region of SEQ ID NO: 28, which reduces or avoids the potential for peptidase degradation.

The antibody or antibody fragment of this embodiment may additionally, or alternatively further comprise a light chain constant (CL) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids of a CL region. The CL region or a fragment thereof may be joined to the VL domain. There is no particular limitation on the CL region although in one embodiment it is a human CL region. The art contains many examples of human CL regions. An exemplary human CL region for use in this context includes:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 29).

SEQ ID NO:29 is the CL region of both of M99-B05 and X19-A05, and possesses the sequence of the CL region of Human kappa (UniProtKB/Swiss-Prot: P01834.1).

According to this embodiment, it may be preferred that the VH domain comprises the sequence of SEQ ID NO:1, linked to the CH region of SEQ ID NO: 28 and the VL domain comprises the sequence of SEQ ID NO: 2 linked to the CL region of SEQ ID NO: 29.

In another embodiment of the first aspect of the invention, the antibody or antibody fragment is based on the VH and/or VL domains of the M99-B05 antibody, and so –

the VH domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:3 and/or (ii) comprises a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17, a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18, and a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 20; and/or

the VL domain (iii) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 4 and/or (iv) comprises a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100%

sequence identity to the sequence of SEQ ID NO: 24, a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25 and a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26. It may be preferred that the VH domain comprises the sequence of SEQ ID NO:3 and the VL domain comprises the sequence of SEQ ID NO: 4.

The antibody or antibody fragment of this embodiment may further comprise a heavy chain constant (CH) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320 or more amino acids of a CH region. The CH region or a fragment thereof may be joined to the VH domain. There is no particular limitation on the CH region although in one embodiment it is a human CH region. The art contains many examples of human CH regions. Exemplary human CH regions for use in this context include SEQ ID NO: 27 and SEQ ID NO: 28.

The antibody or antibody fragment of this embodiment may additionally, or alternatively further comprise a light chain constant (CL) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids of a CL region. The CL region or a fragment thereof may be joined to the VL domain. There is no particular limitation on the CL region although in one embodiment it is a human CL region. The art contains many examples of human CL regions. An exemplary human CL region for use in this context includes SEQ ID NO: 29.

According to this embodiment, it may be preferred that the VH domain comprises the sequence of SEQ ID NO:3, linked to the CH region of SEQ ID NO: 27 or 28 and the VL domain comprises the sequence of SEQ ID NO: 4 linked to the CL region of SEQ ID NO: 29.

In another embodiment of the first aspect of the invention, the antibody or antibody fragment is based on the VH and/or VL domains of the X19-A01 antibody, and so –

the VH domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:5 and/or (ii) comprises a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17, a CDR2

sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18, and a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 20; and/or

the VL domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 6 and/or (iv) comprises a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 24, a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25 and a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26. It may be preferred that the VH domain comprises the sequence of SEQ ID NO:5 and the VL domain comprises the sequence of SEQ ID NO: 6.

The antibody or antibody fragment of this embodiment may further comprise a heavy chain constant (CH) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320 or more amino acids of a CH region. The CH region or a fragment thereof may be joined to the VH domain. There is no particular limitation on the CH region although in one embodiment it is a human CH region. The art contains many examples of human CH regions. Exemplary human CH regions for use in this context include SEQ ID NO: 27 and SEQ ID NO: 28..

The antibody or antibody fragment of this embodiment may additionally, or alternatively further comprise a light chain constant (CL) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids of a CL region. The CL region or a fragment thereof may be joined to the VL domain. There is no particular limitation on the CL region although in one embodiment it is a human CL region. The art contains many examples of human CL regions. An exemplary human CL region for use in this context includes SEQ ID NO: 29.

According to this embodiment, it may be preferred that the VH domain comprises the sequence of SEQ ID NO:5, linked to the CH region of SEQ ID NO: 27 or 28 and the VL domain comprises the sequence of SEQ ID NO: 6 linked to the CL region of SEQ ID NO: 29.

In another embodiment of the first aspect of the invention, the antibody or antibody fragment is based on the VH and/or VL domains of the X19-A03 antibody, and so –

the VH domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:7 and/or (ii) comprises a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17, a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18, and a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 19; and/or

the VL domain (iii) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 8 and/or (iv) comprises a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 23, a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25 and a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26. It may be preferred that the VH domain comprises the sequence of SEQ ID NO:7 and the VL domain comprises the sequence of SEQ ID NO: 8.

The antibody or antibody fragment of this embodiment may further comprise a heavy chain constant (CH) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320 or more amino acids of a CH region. The CH region or a fragment thereof may be joined to the VH domain. There is no particular limitation on the CH region although in one embodiment it is a human CH region. The art contains many examples of human CH regions. Exemplary human CH regions for use in this context include SEQ ID NO: 27 and SEQ ID NO: 28.

The antibody or antibody fragment of this embodiment may additionally, or alternatively further comprise a light chain constant (CL) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids of a CL region. The CL region or a fragment thereof may be joined to the VL domain. There is no particular limitation on the CL region although in one embodiment it is a human CL region. The art contains many examples of human CL regions. An exemplary human CL region for use in this context includes SEQ ID NO: 29.

According to this embodiment, it may be preferred that the VH domain comprises the sequence of SEQ ID NO:7, linked to the CH region of SEQ ID NO: 27 or 28 and the VL domain comprises the sequence of SEQ ID NO: 8 linked to the CL region of SEQ ID NO: 29.

In another embodiment of the first aspect of the invention, the antibody or antibody fragment is based on the VH and/or VL domains of the X19-A07 antibody, and so –

the VH domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:9 and/or (ii) comprises a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17, a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18, and a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 20; and/or

the VL domain (iii) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 10 and/or (iv) comprises a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 24, a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25 and a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26. It

may be preferred that the VH domain comprises the sequence of SEQ ID NO:9 and the VL domain comprises the sequence of SEQ ID NO: 10.

The antibody or antibody fragment of this embodiment may further comprise a heavy chain constant (CH) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320 or more amino acids of a CH region. The CH region or a fragment thereof may be joined to the VH domain. There is no particular limitation on the CH region although in one embodiment it is a human CH region. The art contains many examples of human CH regions. Exemplary human CH regions for use in this context include SEQ ID NO: 27 and SEQ ID NO: 28..

The antibody or antibody fragment of this embodiment may additionally, or alternatively further comprise a light chain constant (CL) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids of a CL region. The CL region or a fragment thereof may be joined to the VL domain. There is no particular limitation on the CL region although in one embodiment it is a human CL region. The art contains many examples of human CL regions. An exemplary human CL region for use in this context includes SEQ ID NO: 29

According to this embodiment, it may be preferred that the VH domain comprises the sequence of SEQ ID NO:9, linked to the CH region of SEQ ID NO: 27 or 28 and the VL domain comprises the sequence of SEQ ID NO: 10 linked to the CL region of SEQ ID NO: 29.

In another embodiment of the first aspect of the invention, the antibody or antibody fragment is based on the VH and/or VL domains of the X19-A09 antibody, and so –

the VH domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:11 and/or (ii) comprises a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17, a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18, and a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%,

45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 20; and/or

the VL domain (iii) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 12 and/or (iv) comprises a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 24, a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25 and a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26. It may be preferred that the VH domain comprises the sequence of SEQ ID NO:11 and the VL domain comprises the sequence of SEQ ID NO: 12.

The antibody or antibody fragment of this embodiment may further comprise a heavy chain constant (CH) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320 or more amino acids of a CH region. The CH region or a fragment thereof may be joined to the VH domain. There is no particular limitation on the CH region although in one embodiment it is a human CH region. The art contains many examples of human CH regions. Exemplary human CH regions for use in this context include SEQ ID NO: 27 and SEQ ID NO: 28..

The antibody or antibody fragment of this embodiment may additionally, or alternatively further comprise a light chain constant (CL) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids of a CL region. The CL region or a fragment thereof may be joined to the VL domain. There is no particular limitation on the CL region although in one embodiment it is a human CL region. The art contains many examples of human CL regions. An exemplary human CL region for use in this context includes SEQ ID NO: 29.

According to this embodiment, it may be preferred that the VH domain comprises the sequence of SEQ ID NO:11, linked to the CH region of SEQ ID NO: 27 or 28 and the VL domain comprises the sequence of SEQ ID NO: 12 linked to the CL region of SEQ ID NO: 29.

In another embodiment of the first aspect of the invention, the antibody or antibody fragment is based on the VH and/or VL domains of the X19-A11 antibody, and so –

the VH domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:13 and/or (ii) comprises a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17, a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18, and a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 21; and/or

the VL domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 14 and/or (iv) comprises a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 24, a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25 and a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26. It may be preferred that the VH domain comprises the sequence of SEQ ID NO:13 and the VL domain comprises the sequence of SEQ ID NO: 14.

The antibody or antibody fragment of this embodiment may further comprise a heavy chain constant (CH) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320 or more amino acids of a CH region. The CH region or a fragment thereof may be joined to the VH domain. There is no particular limitation on the CH region although in one embodiment it is a human CH region. The art contains many examples of human CH regions. Exemplary human CH regions for use in this context include SEQ ID NO: 27 and SEQ ID NO: 28.

The antibody or antibody fragment of this embodiment may additionally, or alternatively further comprise a light chain constant (CL) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids of a CL region. The CL region or a fragment

thereof may be joined to the VL domain. There is no particular limitation on the CL region although in one embodiment it is a human CL region. The art contains many examples of human CL regions. An exemplary human CL region for use in this context includes SEQ ID NO: 29.

According to this embodiment, it may be preferred that the VH domain comprises the sequence of SEQ ID NO:13, linked to the CH region of SEQ ID NO: 27 or 28 and the VL domain comprises the sequence of SEQ ID NO: 14 linked to the CL region of SEQ ID NO: 29.

In another embodiment of the first aspect of the invention, the antibody or antibody fragment is based on the VH and/or VL domains of the X19-C01 antibody, and so –

the VH domain (i) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:15 and/or (ii) comprises a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17, a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18, and a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 22; and/or

the VL domain (iii) comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 16 and/or (iv) comprises a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 23, a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25 and a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26. It may be preferred that the VH domain comprises the sequence of SEQ ID NO:15 and the VL domain comprises the sequence of SEQ ID NO: 16.

The antibody or antibody fragment of this embodiment may further comprise a heavy chain constant (CH) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140,

160, 180, 200, 220, 240, 260, 280, 300, 320 or more amino acids of a CH region. The CH region or a fragment thereof may be joined to the VH domain. There is no particular limitation on the CH region although in one embodiment it is a human CH region. The art contains many examples of human CH regions. Exemplary human CH regions for use in this context include SEQ ID NO: 27 and SEQ ID NO: 28..

The antibody or antibody fragment of this embodiment may additionally, or alternatively further comprise a light chain constant (CL) region or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids of a CL region. The CL region or a fragment thereof may be joined to the VL domain. There is no particular limitation on the CL region although in one embodiment it is a human CL region. The art contains many examples of human CL regions. An exemplary human CL region for use in this context includes SEQ ID NO: 29

According to this embodiment, it may be preferred that the VH domain comprises the sequence of SEQ ID NO:15, linked to the CH region of SEQ ID NO: 27 or 28 and the VL domain comprises the sequence of SEQ ID NO: 16 linked to the CL region of SEQ ID NO: 29.

In the various foregoing embodiments, the discussion of CH regions and fragments thereof is also intended to include the option of using a variant of either. The variant comprises a sequence having less than 100% sequence identity to the stated CH region or fragment thereof, such as greater than 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity. Accordingly, variants of a CH region or a fragment thereof may posses one or more (such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70 80, 90, 100, 110, 120, 130, 140, 150 160 or more) sequence variations compared to the stated CH region or fragment thereof. Variations in sequence may be due to one or more amino acid additions, one or more amino acid deletions and/or one or more amino acid substitutions compared to the stated CH region or fragment thereof. Where there is more than one variation, then the variations may be in consecutive or non-consecutive positions.

Likewise, in the various foregoing embodiments, the discussion of CL regions and fragments thereof is also intended to include the option of using a variant of either. The variant comprises a sequence having less than 100% sequence identity to the stated CL region or fragment thereof, such as greater than 50%, 60%, 70%, 80%,

85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity. Accordingly, variants of a CL region or a fragment thereof may posses one or more (such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60 or more) sequence variations compared to the stated CL region or fragment thereof. Variations in sequence may be due to one or more amino acid additions, one or more amino acid deletions and/or one or more amino acid substitutions compared to the stated CL region or fragment thereof. Where there is more than one variation, then the variations may be in consecutive or non-consecutive positions.

In the antibody or antibody fragment according to the foregoing embodiments, it may be preferred that the VH domain, the VL domain, or preferably both of the VH and VL domains, comprise an amino acid sequence having 100% sequence identity to the, or in the case of stated SEQ ID NOs that correspond to individual CDR sequences then one or more (such as, two or three) of each, stated SEQ ID NO.

Alternatively, the VH domain, the VL domain, or both of the VH and VL domains, may comprise an amino acid sequence having less than 100% sequence identity to the, or in the case of stated SEQ ID NOs that correspond to individual CDR sequences then one or more (such as, two or three) of each, stated SEQ ID NO.

In accordance with the first aspect of the present invention, a sequence comprising an amino acid sequence having less than 100% to the stated SEQ ID NO may be a sequence possessing one or more (such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) sequence variations compared to the stated SEQ ID NO. Variations in sequence may be due to one or more (such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) amino acid additions, one or more (such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) amino acid deletions and/or one or more (such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) amino acid substitutions compared to the stated SEQ ID NO. Where there is more than one variation, then the variations may be in consecutive or non-consecutive positions.

The one or more variations in sequence in a variant antigen binding region that has less than 100%, but at least 80%, 85%, 90%, 95%, sequence identity to a stated SEQ ID NO selected from SEQ ID NOs: 1 to 16 may be present in, or exclusively in, the amino acid sequence that form one or more of the framework regions. Framework regions comprise the amino acid regions that do not form the CDRs as defined herein.

Additionally or alternatively, one or more variations in sequence in an antigen binding region that has less than 100%, but at least 80%, 85%, 90%, 95%, sequence identity to a stated SEQ ID NO selected from SEQ ID NOs: 1 to 16 may be present in, or exclusively in, the amino acid sequence that form one or more of the complementarity determining regions (CDRs). The CDRs in SEQ ID NOs: 1- 16 are as defined above and are also shown in Tables 2 and 3 below.

In all embodiments of the first aspect of the invention, in general higher levels of sequence modifications may be tolerated in the framework regions than in the CDRs without substantially altering the binding characteristics and/or *in vivo* efficacy of the antibody or antibody fragment.

Thus, for example, in a further embodiment, a, the, or each, CDR in an antibody or antibody fragment according to the first aspect of the present invention may comprise up to 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions, insertions and/or deletions compared to the 'parent' CDR sequence defined one of SEQ ID NOs 17 to 26 and preferably not more than 5, 4, 3, 2 or 1 amino acid substitutions, insertions and/or deletions; it may be preferred that the number of amino acid substitutions, insertions and/or deletions implemented in the CDR sequence to not reduce the level of sequence identity to less than 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% compared to the corresponding defined SEQ ID NO.

Additionally, and/or alternatively, a, the, or each, framework region in an antibody or antibody fragment according to the first aspect of the present invention may comprise up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acid substitutions, insertions and/or deletions compared to the corresponding framework sequence present in any of the VH or VL sequences defined SEQ ID NOs 1 to 16, and optionally not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions, insertions and/or deletions; it may be preferred that the number of amino acid substitutions, insertions and/or deletions implemented in any framework region to not reduce the level of sequence identity to less than 10%, 20%, 30%, 40% 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% compared to the corresponding defined SEQ ID NO.

Substitutions, whether in one or more of the framework or complementarity determining regions, may be conservative or non-conservative substitutions. By

"conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

Sequence variations may, for example, be introduced in order to render the sequence of the antigen binding region(s) closer to germline sequences, to improve the stability of the antibody or antibody fragment comprising the variant antigen binding region(s), to reduce the immunogenicity of the antibody or antibody fragment comprising the variant antigen binding region(s), and/or to avoid or reduce properties that could be disadvantageous in the manufacturing process. Non-limiting examples of suitable sequence variations are shown in the examples with reference to the variations introduced into the heavy and/or light chain sequences of M99-B05 in order to produce X19-A01, X19-A03, X19-A05, X19-A07, X19-A09, X19-A11, and/or X19-C01.

Such variants may be made using the methods of protein engineering and site-directed mutagenesis as described below or alternative methods that are well known in the art.

Where the VH domain, the VL domain, or both of the VH and VL domains, of the antibody or antibody fragment of the first aspect of the present invention comprise(s) one or more amino acid sequences having less than 100% sequence identity to the, or one or more of each, stated SEQ ID NO, then in one embodiment the ability of the antibody or antibody fragment to bind to phosphorylcholine and/or a phosphorylcholine conjugate may, for example, be substantially equivalent to (that is, at least 80%, 85%, 90% or 95%), or greater than, the ability of a corresponding 'parent' antibody or antibody fragment, wherein the VH domain and the VL domain of the corresponding 'parent' antibody or antibody fragment each comprise an amino acid sequence having 100% sequence identity to the, or each, stated SEQ ID NO.

Thus, for example, where the antibody or antibody fragment is based on the X19-A05 antibody, and the VH domain comprises an amino acid sequence having less than 100%, but at least 80%, 85%, 90%, or 95% sequence identity SEQ ID NO:1; and/or the VL domain comprises an amino acid sequence having less than 100%, but at least 80%, 85%, 90%, or 95% sequence identity SEQ ID NO: 2, then the ability of the antibody or antibody fragment to bind to phosphorylcholine and/or a phosphorylcholine conjugate may, for example, be equivalent to the binding ability of a corresponding 'parent' antibody or antibody fragment having a VH domain that

comprises the sequence of SEQ ID NO:1 and a VL domain that comprises the sequence of SEQ ID NO: 2. In this context, by "corresponding 'parent' antibody or antibody fragment" is meant that the only sequence difference between the "antibody or antibody fragment" in hand and the "corresponding 'parent' antibody or antibody fragment" is in one or both of the VH and/or VL domains. In one embodiment, the corresponding parent antibody is an antibody having the sequence of the VH, VL, CH and CL regions of X19-A05, that is, a VH domain of SEQ ID NO:1 linked to the CH region of SEQ ID NO: 28 and the VL domain of SEQ ID NO: 2 linked to the CL region of SEQ ID NO: 29.

The same applies, *mutatis mutandis*, to the other antibody or antibody fragment listed above wherein the VH and/or VL domains comprise(s) one or more amino acid sequences having less than 100% sequence identity to the, or one or more of each, stated SEQ ID No, and the "corresponding 'parent' antibody or antibody fragment" for the purposes of determining binding equivalence to phosphorylcholine and/or a phosphorylcholine conjugate differs only in the one or both of the sequences of the VH and/or VL domain and possess(es) the, or each, sequence comprising an amino acid sequence having 100% sequence identity to the, or each, stated SEQ ID No.

Accordingly, where the antibody or antibody fragment is based on the M99-B05 then, in one embodiment, the corresponding parent antibody is an antibody having the sequence of the VH, VL, CH and CL regions of M99-B05, that is, a VH domain of SEQ ID NO:3 linked to the CH region of SEQ ID NO: 27 and the VL domain of SEQ ID NO: 4 linked to the CL region of SEQ ID NO: 29.

In this regard, the ability of an antibody or antibody fragment to bind to phosphorylcholine and/or a phosphorylcholine conjugate may be determined by any suitable method, such as by Surface Plasmon Resonance (SPR) analysis, to measure the binding of the antibody or antibody fragment to phosphorylcholine immobilized (for example via an aminophenyl linker) to a solid surface such as the Biacore SPR biosensor.

In an additional embodiment, an antibody or antibody fragment according to the first aspect of the present invention competes with a 'comparator' antibody or antibody fragment for binding to PC or a PC conjugate as defined herein (e.g., as determined in an ELISA or SPR assay). In this context, a comparator antibody or antibody

fragment may comprise the VH and VL domains, and optionally also the CH and CL domains, of X19-A05 (as defined by SEQ ID NOs: 1, 2, 28 and 29, respectively), M99-B05 (as defined by SEQ ID NOs: 3, 4, 27 and 29), X19-A01 (as defined by SEQ ID NOs: 5, 6, 27 and 29, respectively), X19-A03 (as defined by SEQ ID NOs: 7, 8, 27 and 29, respectively), X19-A07 (as defined by SEQ ID NOs: 9, 10, 27 and 29, respectively), X19-A09 (as defined by SEQ ID NOs: 11, 12, 27 and 29, respectively), X19-A11 (as defined by SEQ ID NOs: 13, 14, 27 and 29, respectively) or X19-C01 (as defined by SEQ ID NOs: 15, 16, 27 and 29, respectively) and preferably differs from the antibody or antibody fragment being tested only by sequence variation in the VH and/or VL regions. By 'competes', we mean that inclusion of equimolar amounts of the antibody or antibody fragment according to the first aspect of the present invention and the 'comparator' antibody in an assay can reduce the detectable level of binding to PC or a PC conjugate of the comparator antibody by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more, such as substantially 100%, in comparison to the detectable level of binding to PC or a PC conjugate of the 'comparator' antibody in the same assay in the absence of the antibody or antibody fragment according to the first aspect of the present invention.

As discussed in the examples below, M99-B05 binds aminophenyl phosphorylcholine with an apparent Kd of about 150 nM. In one embodiment, an antibody or antibody fragment according to the present invention will bind to immobilized aminophenyl phosphorylcholine with an apparent Kd of no greater than about 500nM, about 400 nM, about 300nM, about 250nM, about 200 nM, about 190 nM, about 180 nM, about 170 nM, about 160nM, about 155 nM, about 150 nM, or less when tested under conditions (such as the SPR conditions used in the examples) that provide for binding of an antibody or antibody fragment having the VH and VL domains of M99-B05 (as defined by SEQ ID NOS 3 and 4, respectively) to immobilized aminophenyl phosphorylcholine with an apparent Kd of about 150 nM. In this context, the term "about" is used to mean a value that is within ±20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% of the stated value.

As also discussed in the examples below, M99-B05 can block the release of MCP-1 from monocytes in response to stimulation with oxLDL with an IC_{50} in the nM range. In another embodiment, an antibody or antibody fragment according to the present invention will block the release of MCP-1 from monocytes in response to stimulation with oxLDL with an IC_{50} of less than about 10 nM, about 5 nM, about 4 nM, about 3

nM, about 2.8 nM, about 2.6 nM, about 2.4 nM, about 2 nM, about 1.8 nM, about 1.6 nM, about 1.4 nM, about 1.3 nM, about 1.2 nM, about 1.1 nM, about 1.0 nM, about 0.9 nM, about 0.8 nM, about 0.7 nM or less when tested under conditions (such as described in the example below) that provide for an IC_{50} of an antibody or antibody fragment having the VH and VL domains of M99-B05 (as defined by SEQ ID NOS 3 and 4, respectively) in the 0.7-2.6 nM range. In this context, the term "about" is used to mean a value that is within ±20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% of the stated value.

The ability of an antibody or antibody fragment according to the present invention to bind to a phosphorylcholine conjugate may be determined by equivalent methods to those described above, replacing phosphorylcholine with the phosphorylcholine conjugate. Suitable phosphorylcholine conjugates include those discussed above, comprising a phosphorylcholine moiety linked to a carrier, optionally via a spacer, such as PC-BSA and PC-KLH conjugates. Preferably, where the ability of an antibody or antibody fragment to bind to the phosphorylcholine conjugate is determined, it is determined with respect to the ability of the antibody or antibody fragment to bind specifically to the phosphorylcholine moiety in the phosphorylcholine conjugate. This can be determined by art-known techniques such as by comparing the ability of the antibody or antibody fragment to bind to the phosphorylcholine conjugate and the corresponding molecule that does not contain a phosphorylcholine moiety.

In one embodiment, the antibody or antibody fragment of the present invention may be comprise the VH domain and the VL domain in a linear polypeptide sequence.

In another embodiment, the antibody or antibody fragment of the present invention may comprise the VH domain and the VL domain each in a separate polypeptide sequence. In this embodiment, it may be preferred that the separate polypeptide sequence are directly or indirectly bound together (such as by one or more disulphide bonds between the separate polypeptide sequence).

In another embodiment, the VH domain may be joined to a CH region, or a fragment thereof which fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320 or more amino acids of a CH region, or a variant of the CH region or a fragment thereof, as described above. The join may be a direct fusion via a peptide bond, such that the

VH domain and CH region are presented as a single polypeptide, or the join may be through a linker, such as a peptide or other linker, or via a direct chemical bond other than a peptide bond. There is no particular limitation on the CH region although in one embodiment it is a human CH region. The art contains many examples of human CH regions. Exemplary human CH regions for use in this context include SEQ ID NO: 27 and SEQ ID NO: 28. When using any CH regions, terminal amino acid modifications (including the deletion of, or masking by addition of another amino acid or other chemical moiety) may be introduced to reduce or avoid the potential for peptidase degradation.

In another embodiment, the VL domain may be joined to a CL region, or a fragment may comprise, for example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids of a CL region, or a variant of the CL region or a fragment thereof, as described above. The join may be a direct fusion via a peptide bond, such that the VL domain and CL region are presented as a single polypeptide, or the join may be through a linker, such as a peptide or other linker, or via a direct chemical bond other than a peptide bond. There is no particular limitation on the CL region although in one embodiment it is a human CL region. The art contains many examples of human CL regions. An exemplary human CL region for use in this context includes SEQ ID NO: 29. Terminal amino acid modifications (including the deletion of, or masking by addition of another amino acid or other chemical moiety) may be introduced to reduce or avoid the potential for peptidase degradation of any CL region that is used.

In another embodiment, the antibody or antibody fragment of the present invention may comprise a VH domain joined to a CH region in one polypeptide sequence, and a VL domain joined to a CL region in another separate polypeptide sequence. In this embodiment, it may be preferred that the separate polypeptide sequence are directly or indirectly bound together (such as by one or more disulphide bonds between the separate polypeptide sequence).

In a further embodiment, the antibody or antibody fragment of the present invention may comprise –

- a first heavy chain comprising a first VH domain joined to a first CH region,
- a first light chain comprising a first VL domain joined to a first CL region;
- a second heavy chain comprising a second VH domain joined to a second CH region,

- a second light chain comprising a second VL domain joined to a second CL region; and

wherein optionally, the first light and first heavy chains are directly or indirectly bound together (such as by one or more disulphide bonds between the separate polypeptide sequence) and the second light and second heavy chains are directly or indirectly bound together (such as by one or more disulphide bonds between the separate polypeptide sequence), and further optionally, wherein the first and second heavy chains directly or indirectly bound together (such as by one or more disulphide bonds between the separate polypeptide sequence).

In a further embodiment, the antibody or antibody fragment of the present invention may be a monoclonal antibody, more preferably a human monoclonal antibody.

The antibody or antibody fragment of the present invention may be a humanized antibody or a chimeric antibody.

In one preferred embodiment, the antibody or antibody fragment of the present invention is an isolated antibody or antibody fragment.

In another embodiment, the antibody or antibody fragment of the present invention may comprise one or more of the amino acid sequences comprising the VH, VL, CDR1, CDR2, CDR3, CDR4, CDR5 and/or CDR6 sequences described above grafted onto a protein scaffolds of immunoglobulins using standard protein engineering techniques. The skilled person will appreciate that various protein scaffolds are available for use and commonly known in the art. The end result is preserved antigen-binding activity in a new framework.

For example, the scaffolds of immunoglobulins can be derived from IgA, IgE, IgG1, IgG2a, IgG2b, IgG3, IgM. The scaffolds can be derived from an immunoglobulin from any mammal, such as mice, rats, rabbits, goats, camels, llamas, primates. It may be preferred that the immunoglobulin scaffold is derived from human immunoglobulins.

The antibody fragments according to the first aspect of the present invention can be generated by standard molecular biology techniques or by cleavage of purified antibodies using enzymes (*e.g.* pepsin or papain) that generates these fragments. Such antibody fragments according to the invention are exemplified, but not limited

to, single chain antibodies, Fv, scFv, Fab, F(ab')$_2$, Fab', Fd, dAb, CDR, or scFv-Fc fragments or nanobodies, and diabodies, or any fragment that may have been stabilized by *e.g.* PEGylation.

A second aspect of the present invention provides a pharmaceutical composition comprising an antibody or an antibody fragment according to the first aspect of the invention and a pharmaceutically acceptable carrier or excipient. Optionally, the only antibodies or antibody fragments present in the composition are those of the first aspect of the present invention. More preferably, there may be a single type of antibody or antibody fragment present in the composition, for example wherein type is determined with respect to amino acid sequence, molecular weight and/or binding specificity to phosphorylcholine. In this regard, the skilled person will appreciate that there may be some low levels of variation in the sequences of antibodies or antibody fragments in any population due, for example, to N-terminal variation and/or partial degradation; accordingly, in this context, a composition can be said to contain a single type of antibody or antibody fragment if, for example, at least about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or substantially 100% by weight of the detectable level of antibodies or antibody fragments in the composition are of a single type as determined with respect to amino acid sequence, molecular weight and/or binding specificity to phosphorylcholine.

A third aspect of the present invention provides an antibody or antibody fragment according to the first aspect of the present invention, or a pharmaceutical composition according to the second aspect of the present invention for use in medicine, such as for use in a method of therapy, surgery or diagnosis that is performed on the human or animal body or on an *ex vivo* sample therefrom.

For example, the third aspect of the present invention provides an antibody or antibody fragment according to the first aspect of the present invention, or a pharmaceutical composition according to the second aspect of the present invention, for use in the prevention, prophylaxis and/or treatment of mammals, including humans, against atherosclerosis, an atherosclerotic related disease or cardiovascular disease.

In other words, the third aspect of the present invention provides for the use of an antibody or antibody fragment according to the first aspect of the present invention,

or a pharmaceutical composition according to the second aspect of the present invention, in the manufacture of a medicament for the prevention, prophylaxis and/or treatment of mammals, including humans, against atherosclerosis, an atherosclerotic related disease or cardiovascular disease.

Also provided is a method for prevention, prophylaxis and/or treatment of a mammal, including a human, against atherosclerosis, an atherosclerotic related disease, or cardiovascular disease, the method comprising the step of administering to the mammal an antibody or antibody fragment according to the first aspect of the invention, or a pharmaceutical composition according to the second aspect of the invention.

The third aspect of the present invention also provides an antibody or antibody fragment according to the first aspect of the present invention, or a pharmaceutical composition according to the second aspect of the present invention, for use in the prophylaxis, prevention and/or treatment of Alzheimer's disease.

In other words, the third aspect of the present invention provides for the use of an antibody or antibody fragment according to the first aspect of the present invention, or a pharmaceutical composition according to the second aspect of the present invention, in the manufacture of a medicament for the prophylaxis, prevention and/or treatment of Alzheimer's disease.

Also provided is a method for immunization and prophylaxis, prevention and/or treatment of a subject against Alzheimer's disease, the method comprising the step of administering to the subject an antibody or antibody fragment according to the first aspect of the invention, or a pharmaceutical composition according to the second aspect of the invention.

The third aspect of the present invention also provides an antibody or antibody fragment according to the first aspect of the present invention, or a pharmaceutical composition according to the second aspect of the present invention, for use in the immunization or prophylaxis against, or the prevention or treatment of, metabolic disease in mammals, including humans.

In other words, the third aspect of the present invention provides for the use of an antibody or antibody fragment according to the first aspect of the present invention,

or a pharmaceutical composition according to the second aspect of the present invention, in the manufacture of a medicament for the prophylaxis, prevention or treatment of, metabolic disease in mammals, including humans.

Also provided is a method for the immunization or prophylaxis against, or the treatment of, metabolic diseases in a mammal, such as a human, the method comprising the step of administering to the mammal an antibody or antibody fragment according to the first aspect of the present invention, or a pharmaceutical composition according to the second aspect of the present invention.

The metabolic disease to be addressed and/or treated in accordance with the third aspect of the present invention may, for example, be a condition selected from the group consisting of metabolic syndrome, insulin resistance, glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

A fourth aspect of the present invention provides a nucleic acid molecule comprising a sequence encoding an antibody or an antibody fragment, or polypeptide chain forming part of the antibody or an antibody fragment, according to the first aspect of the invention. The nucleic acid molecule may, for example, be DNA or RNA. The nucleic acid molecule may comprise additional sequence 5' and/or 3' to the sequence encoding the, or part of, the antibody or an antibody fragment according to the first aspect of the invention. Such 5' and 3' sequences may include transcriptional and/or translational regulatory sequences, such as promoter and/or terminator sequences which are well known in the art and may, for example, be selected in order to be functional in a host cell of choice. Accordingly, the nucleic acid molecule may comprise an expression cassette that, following transformation into a host cell of choice, can be expressed by the transcriptional and/or translational systems of the host cell to result in the production of the encoded antibody or an antibody fragment, or polypeptide chain forming part of the antibody or an antibody fragment, according to the first aspect of the invention.

A fifth aspect of the present invention provides a vector or plasmid comprising one or more nucleic acid sequences according to the fourth aspect of the invention. Where the antibody or antibody fragment comprises more than one polypeptide chain, the vector or plasmid may, for example, comprise a nucleic acid coding

sequence encoding each polypeptide chain, such that a host cell transformed with the vector or plasmid can express all polypeptide chains present in the antibody or antibody fragment.

Accordingly, the fifth aspect also provides for the use of a vector or plasmid in the transformation of a host cell. Methods of transforming host cells with vectors or plasmids are well known in the art. To aid the selection of transformed host cells, the vector or plasmid may comprise a selectable marker.

A sixth aspect of the present invention provides a host cell comprising one or more vectors or plasmids according to the fifth aspect of the invention. The sixth aspect also provides for a culture of cells comprising the one or more vectors or plasmids according to the fifth aspect of the invention, such as monoculture in which all or substantially all cells comprise the same one or more vectors or plasmids according to the fifth aspect of the invention. Such monocultures can be obtained, for example, by selecting cells for the presence of one or more selectable markers on the one or more plasmids or vectors and optionally maintaining the selective pressure during the growth of the selected cell in culture.

Where the antibody or antibody fragment according to the first aspect of the present invention comprises more than one polypeptide chain, the host cell may be transformed with a single vector or plasmid that comprises a nucleic acid coding sequence encoding each polypeptide chain, such that a host cell transformed with the vector or plasmid can express all polypeptide chains present in the antibody or antibody fragment.

Alternatively, where the antibody or antibody fragment according to the first aspect of the present invention comprises more than one polypeptide chain, the host cell may be transformed with more than one vector or plasmid that each comprises a nucleic acid coding sequence encoding at least one of the polypeptide chains, such that a host cell transformed with the more than one vectors or plasmids can express all polypeptide chains present in the antibody or antibody fragment.

In a further alternative, where the antibody or antibody fragment according to the first aspect of the present invention comprises more than one polypeptide chain, multiple host cells may each be transformed with a vector or plasmid that each comprises a different nucleic acid coding sequence each encoding one or more

different members of the different polypeptide chains that form the antibody or antibody fragment, and each different host cell cultured separately to express each polypeptide chain. The recovered different polypeptide chains can then be combined to produce the antibody or antibody fragment.

Any suitable host cell can be used in the fifth and/or sixth aspects of the invention. For example, the host cell may be a prokaryotic cell, such as an *Escherichia coli* cell. The host cell may be an eukaryotic cell, such as animal cell, a plant cell, and a fungal cell. Suitable animal cells may include mammalian cells, avian cells, and insect cells. Suitable mammalian cells can include CHO cells, and COS cells. Suitable fungal cells can include yeast cells, such as a *Saccharomyces cerevisiae* cells. Mammalian cells may, or may not, include human cells, and may or may not include embryonic cells.

A seventh aspect of the present invention provides a method for producing an antibody or an antibody fragment antigen-binding sequence according to the first aspect of the present invention comprising culturing one or more transformed host cells as described above, and recovering therefrom an antibody or an antibody fragment according to the first aspect of the present invention.

An eighth aspect of the present invention provides a method of preparing a variant of the antibody or antibody fragments of the first aspect of the present invention, which variant retains the ability to bind to phosphorylcholine and/or a phosphorylcholine conjugate, the method comprising –

(i) providing a nucleic acid according to the fourth aspect of the present invention encoding a parent antibody or antibody fragment or polypeptide chain forming part thereof;

(ii) introducing one or more nucleotide mutations (optionally, up to 50, 40, 30, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleotide mutations), into the amino acid coding regions of the nucleic acid sequence, optionally within the regions encoding the VH and/or VL domain(s), such that the mutated nucleic acid encodes a variant antibody or antibody fragment having a different amino acid sequence compared to the parent antibody or antibody fragment;

(iii) expressing the variant antibody or antibody fragment, or polypeptide chain forming part thereof, that is encoded by the mutated nucleic acid sequence; and

(iv) comparing the ability of the variant antibody or antibody fragment and the parent antibody or antibody fragment to bind to phosphorylcholine and/or a phosphorylcholine conjugate.

In accordance with the eighth aspect of the present invention, nucleotide mutations may be introduced into the amino acid coding regions of the nucleic acid sequence randomly, or in a site-directed manner. Such mutations may result in the coding region encoding an amino acid sequence that contains one or more amino acid additions, one or more amino acid deletions and/or one or more amino acid substitutions compared to the amino acid sequence encoded by nucleic acid prior to mutation.

Such nucleotide mutations may, or may not, result in the coding region encoding an amino acid sequence that contains one or more variations in sequence in the antigen binding region. Such nucleotide mutations may, for example, result in amino acid sequence variation (that is, one or more amino acid additions, one or more amino acid deletions and/or one or more amino acid substitutions) present in, or exclusively in, the amino acid sequence that form one or more of the framework regions. Additionally or alternatively, such nucleotide mutations may, for example, result in amino acid sequence variation (that is, one or more amino acid additions, one or more amino acid deletions and/or one or more amino acid substitutions) present in, or exclusively in, the amino acid sequence that form one or more of the complementarity determining regions. Levels of amino acid variations/modifications tolerated in respect of framework regions, CDRs and/or VH or VL domains as whole are discussed above in respect of the first aspect of the present invention and may be applied, *mutatis mutandis*, to the level of variation/modification that can be introduced according to the method of the eighth aspect of the present invention.

Additionally or alternatively, such nucleotide mutations may, or may not, result in the coding region encoding an amino acid sequence that contains one or more variations in sequence in one or more parts of the antibody or antibody fragment other than the antigen binding region, such as in one or more of the CH1, CH2, CH3, CL regions or other regions.

Where one or more nucleotide mutations result in one or more amino acid substitutions in the encoded product, then the one or more substitutions may each, independently, be conservative or non-conservative substitutions. By "conservative

substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

Nucleotide mutations may, for example, be introduced in order to render the sequence of the encoded antibody or antibody fragments closer to germline sequences, to improve the stability of the antibody or antibody fragment comprising the variant antigen binding region(s), to reduce the immunogenicity of the antibody or antibody fragment comprising the variant antigen binding region(s), and/or to avoid or reduce properties that could be disadvantageous in the manufacturing process.

Such nucleotide mutations may be made using methods that are well known in the art.

In accordance with the eighth aspect of the present invention, the step of assessing the ability of the variant antibody or antibody fragment to bind to phosphorylcholine and/or a phosphorylcholine conjugate may further comprise selecting those variants that have substantially equal or enhanced ability to bind to phosphorylcholine and/or a phosphorylcholine conjugate compared to the parent.

The ability of variants and parents to bind phosphorylcholine and/or a phosphorylcholine conjugate can be assessed by methods such as those discussed above in respect of the first aspect of the present invention.

The method of the eighth aspect of the present invention may optionally further comprising recovering a nucleic acid molecule that comprises the mutated nucleic acid sequence that encodes the variant antibody or antibody fragment, and optionally transforming a host cell with a composition comprising the recovered nucleic acid molecule and further optionally expressing the variant antibody or antibody fragment from the host cell, and yet further optionally recovering the thus-expressed variant antibody or antibody fragment from the host cell, and yet further optionally, formulating the recovered variant antibody or antibody fragment into a pharmaceutically acceptable composition.

The eighth aspect of the present invention also provides a variant antibody or antibody fragment obtained or obtainable by the method of the eighth aspect of the

invention, or a pharmaceutically acceptable obtained or obtainable by the method of the eighth aspect of the invention, for use in medicine.

The eighth aspect of the present invention also provides a variant antibody or antibody fragment obtained or obtainable by the method of the eighth aspect of the invention, or a pharmaceutically acceptable obtained or obtainable by the method of the eighth aspect of the invention, for use in –

(i) the prevention, prophylaxis and/or treatment of mammals, including humans, against atherosclerosis, an atherosclerotic related disease or cardiovascular disease;

(ii) in the prophylaxis, prevention and/or treatment of Alzheimer's disease; and/or

(iii) in the immunization or prophylaxis against, or the prevention or treatment of, metabolic disease in mammals, including humans.

In other words, eighth aspect of the present invention also provides for the use of a variant antibody or antibody fragment obtained or obtainable by the method of the eighth aspect of the invention, or the use of a pharmaceutically acceptable obtained or obtainable by the method of the eighth aspect of the invention, in the manufacture of a medicament for –

(i) the prevention, prophylaxis and/or treatment of mammals, including humans, against atherosclerosis, an atherosclerotic related disease or cardiovascular disease;

(ii) in the prophylaxis, prevention and/or treatment of Alzheimer's disease; and/or

(iii) in the immunization or prophylaxis against, or the prevention or treatment of, metabolic disease in mammals, including humans.

Accordingly, also provided by the eighth aspect of the present invention is a method for –

(i) prevention, prophylaxis and/or treatment of a mammal, including a human, against atherosclerosis, an atherosclerotic related disease, or cardiovascular disease,

(ii) immunization and prophylaxis, prevention and/or treatment of a subject against Alzheimer's disease; and/or

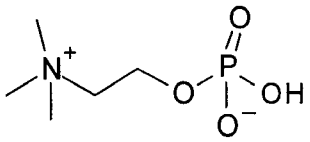
(iii) immunization or prophylaxis against, or the treatment of, metabolic diseases in a mammal, such as a human,

the method comprising the step of administering to the mammal or subject a variant antibody or antibody fragment obtained or obtainable by the method of the eighth aspect of the invention, or the use of a pharmaceutically acceptable obtained or obtainable by the method of the eighth aspect of the invention.

The metabolic disease to be addressed and/or treated in accordance with the eighth aspect of the present invention may, for example, be a condition selected from the group consisting of metabolic syndrome, insulin resistance, glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

Phosphorylcholine

By phosphorylcholine (PC) is meant phosphorylcholine according to the formula.



By a phosphorylcholine conjugate is meant a phosphorylcholine moiety linked to a carrier, preferably via a spacer. The phosphorylcholine moiety can be covalently or non-covalently linked to the carrier. Preferably the phosphorylcholine moiety is linked to the carrier via the phosphate group.

The carrier can be, for example, a protein, a carbohydrate, a polymer, latex beads, or colloid metal.

The phosphorylcholine conjugate may for example be a protein-PC conjugate, such as a human serum albumin (HSA)-PC conjugate, a transferrin–PC conjugate, a keyhole limpet hemocyanin (KLH)-PC conjugate or a bovine serum albumin (BSA)-PC conjugate.

Where the PC conjugate comprises PC linked to a carrier via a spacer, then any suitable spacer may be used. Non-limiting examples of spacers include coupling agents (typically, bi-functional compounds), such as a di-carboxylic acids like succinic and glutaric acid, the corresponding di-aldehydes, di-amines such as 1,6

diaminohexane, di-substituted phenols such as p-amino-phenol, p-diazo-phenol, p-phenylenediamine, p-benzoquinone, and the like.

Cardiovascular disease

The term cardiovascular diseases, is intended to include but is not limited to atherosclerosis, acute coronary syndrome, acute myocardial infarction, myocardial infarction (heart attack), stable and unstable angina pectoris, aneurysms, coronary artery disease (CAD), ischemic heart disease, ischemic myocardium, cardiac and sudden cardiac death, cardiomyopathy, congestive heart failure, heart failure, stenosis, peripheral arterial disease (PAD), intermittent claudication, critical limb ischemia, and stroke.

The treatment or prevention of cardiovascular diseases using antibodies with reactivity to phosphorylcholine and phosphorylcholine conjugates is discussed, for example, in WO 2005/100405 and US 2007-0286868, the contents of both of which are incorporated herein by reference.

Alzheimer's disease

In accordance with the present invention, antibody or antibody fragments according to the first aspect may be used to treat or prevent Alzheimer's disease in individuals in need or risk thereof.

WO 2010/003602 and US Patent Application No. 61/078677 describe the treatment or prevention of Alzheimer's disease using antibodies with reactivity to phosphorylcholine and phosphorylcholine conjugates, and the contents of both of which are incorporated herein by reference as further disclosure of ways in which antibody or antibody fragments according to the first aspect may be used to treat or prevent Alzheimer's disease.

Metabolic diseases

The term metabolic diseases, is intended to include but is not limited to metabolic syndrome X, insulin resistance (IRS), glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia, hypertriglyceridemia,

hypercholesterolemia, dyslipidemia polycystic ovary syndrome (PCOS) and related diseases.

Further discussion of metabolic diseases to be treated with antibodies with reactivity to phosphorylcholine and phosphorylcholine conjugates are discussed in WO 2012/010291, the contents of which are also incorporated herein by reference for further disclosure of ways in which antibody or antibody fragments according to the first aspect may be used to treat or prevent metabolic diseases.

Amino acid sequence identity

The percent identity between two amino acid sequences is determined as follows. First, an amino acid sequence is compared to, for example, SEQ ID NO:1 using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from the U.S. government's National Center for Biotechnology Information web site at ncbi.nlm.nih.gov. Instructions explaining how to use the Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq –i c:\seq1.txt –j c:\seq2.txt –p blastp –o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences. Once aligned, the number of matches is determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences.

The percent identity is determined by dividing the number of matches by the length of the sequence set forth in an identified sequence followed by multiplying the resulting value by 100. For example, if a sequence is compared to the sequence set forth in SEQ ID NO:A (the length of the sequence set forth in SEQ ID NO:A being

10) and the number of matches is 9, then the sequence has a percent identity of 90 % (i.e., 9 ÷ 10 * 100 = 90) to the sequence set forth in SEQ ID NO:A.

Antibodies

The term "antibody or antibody fragment" as referred to herein in the context of the present invention includes whole antibodies and any antigen binding fragment referred to as "antigen-binding region" or single chains thereof.

An "antibody" may refer to a protein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL.

The VH and VL 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 VH typically comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Likewise, each VL typically comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR5, CDR4, FR6, CDR5, FR7, CDR6, FR8. 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 (C1q) of the classical complement system.

The term "antigen-binding region", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. 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 region" of an antibody include –

(i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains;

(ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region;

(iii) a Fab' fragment, which is essentially an Fab with part of the hinge region;

(iv) a Fd fragment consisting of the VH and CH1 domains;

(v) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody,

(vi) a dAb fragment which consists of a VH domain;

(vii) an isolated complementarity determining region (CDR); and

(viii) a nanobody, a heavy chain variable region containing a single variable domain and two constant domains.

Furthermore, although the two domains of the Fv fragment, VL and VH, 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 VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv)). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody.

Diabodies consists of two polypeptides each comprising a heavy (VH) chain variable domain connected to a light chain variable domain (VL) on the same polypeptide chain (VH-VL) connected by a peptide linker. These antibody fragments are obtained using conventional techniques known to those with 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, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds phosphorylcholine is substantially free of antibodies that specifically bind antigens other than phosphorylcholine). 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 "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

Pharmaceutical compositions

A pharmaceutical composition according to the invention may comprise a binding protein according to the invention in admixture with a pharmaceutically acceptable carrier and/or excipient, which will typically be selected with regard to the intended route of administration and standard pharmaceutical practice. The composition may be in the form of immediate-, delayed- or controlled-release applications. Preferably, the formulation is a unit dosage containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of the active ingredient.

The pharmaceutical composition according to the invention may, or may not, be intended for, and, thus formulated in a manner suitable for, parenteral, intravenous, intra-arterial, intraperitoneal, intra-muscular, *intra-cerebroventricular,* or subcutaneous administration, or they may be administered by infusion techniques. They may be best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood or cerebral spinal fluid (CSF). The aqueous solutions may be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable pharmaceutical formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

Such formulations may include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood or CSF of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

A therapeutically effective amount of an antibody or an antibody fragment according to the invention for administration to a patient, such as a human patient, on the basis of a daily dosage level may be from 0.01 to 1000 mg of antibody or antibody fragment per adult (for example, from about 0.001 to 20 mg per kg of the patient's body weight, such as 0.01 to 10 mg/kg, for example greater than 0.1 mg/kg and less than 20, 10, 5, 4, 3 or 2 mg/kg, such as about 1 mg/kg), administered in single or divided doses.

The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention

DESCRIPTION OF THE DRAWINGS

Figure 1. Estimates of binding affinity from an equilibrium binding analysis by Biacore.

(◆) M99-B05 (lot W21573) (Kd = 160 ± 32 nM), (O) M99-B05 (lot W22595) (Kd = 148 ± 8 nM). The panel compares these two different preparations of the antibody.

Figure 2. Purified IgGs binding to PC-BSA as measured by ELISA.

(●) M4-G02 (EC_{50} = 0.14 nM), (O) M73-G03 (EC_{50} = 0.91 nM), (△) M99-B05 (EC_{50} = 0.11 nM). The data were fit to a 4 parameter logistic equation with a global B_{max} to obtain EC50 value estimates.

Figure 3. Inhibition of CD45 positive leukocyte influx into medial in femoral artery cuffed mice.

Transgenic male ApoE*3 Leiden mice were fed a high-cholesterol and high-fat diet containing 1% cholesterol and 0.05% cholate to induce hypercholesterolemia. After three weeks of the high fat diet, mice were anesthetized and the femoral artery was dissected from its surroundings and loosely sheathed with a non-constrictive polyethylene cuff (Portex, 0.40 mm inner diameter, 0.80 mm outer diameter and 2.0 mm length). Mice were treated with either 10 mg/kg recombinant anti-PC IgG antibodies dissolved in PBS, 10 mg/kg anti-streptavidin A2 IgG antibodies dissolved in PBS or PBS only through IP injection on day 0. Mice were sacrificed three days after surgery and cuffed femoral arteries were harvested and paraffin-embedded. Serial cross-sections (5 µm) were taken from the entire length of the cuffed femoral artery segment for histochemical analysis. * $p < 0.01$, n=15.

Figure 4. Inhibition of intimal thickening in femoral artery cuffed mice.

Transgenic male ApoE*3 Leiden mice were fed a high-cholesterol and high-fat diet containing 1% cholesterol and 0.05% cholate to induce hypercholesterolemia. After three weeks of the high fat diet, mice were anesthetized and the femoral artery was dissected from its surroundings and loosely sheathed with a non-constrictive polyethylene cuff (Portex, 0.40 mm inner diameter, 0.80 mm outer diameter and 2.0 mm length). Mice were treated with either 10 mg/kg recombinant anti-PC IgG antibodies dissolved in PBS, 10 mg/kg anti-streptavidin A2 IgG antibodies dissolved in PBS or PBS only through IP injection on day 0, 3, 7, and 10 after surgery. Mice were sacrificed 14 days after surgery and cuffed femoral arteries were harvested and paraffin-embedded. Serial cross-sections (5 µm) were taken from the entire length of the cuffed femoral artery segment for histochemical analysis.

A. Comparison of the intimal area (indicated by the arrow) in the 3 panels indicates that the antibodies M99-B05 reduced the intimal thickening that was observed 14 days after cuff-induced vascular injury.

B. Intimal thickening in $(\mu m)^2$, n = 10, * $p < 0.05$

Figure 5. PC binding activity of M99-B05 mutants measured using ELISA

(●) M99-B05 (EC_{50} = 0.28 nM), (O) X19-A01 (EC_{50} = 0.42 nM), (▼) X19-A03 (EC_{50} = 0.54 nM), (△) X19-A05 (EC_{50} = 0.52 nM), (■) X19-A07 (EC_{50} = 0.62 nM), (□) X19-A09 (EC_{50}= 0.58 nM), (◆) X19-A11 (EC_{50} = 0.97 nM), (◇) X19-C01(EC_{50} = 1.4 nM).

Figure 6. Immunohistochemistry staining of frozen human atherosclerotic lesion tissue with an anti-phosphorylcholine antibody.

Human atherosclerotic lesion tissue, along with a normal tissue control was obtained commercially from Biochain Human frozen tissues. The tissue was incubated with 0.1 µg/mL biotinylated M99-B05 anti-phosphorylcholine IgG overnight at 4°C. Antibody binding to tissue was visualized following the addition of streptavidin-horse radish peroxidase (HRP) and HRP substrate. The presence of antibody binding is show by the color that is generated from the HRP substrate. No binding was observed with an isotype control (data not shown).

Figure 7. Inhibition of intimal thickening in femoral artery cuffed mice.

Transgenic male ApoE*3 Leiden mice were fed a high-cholesterol and high-fat diet containing 1% cholesterol and 0.05% cholate to induce hypercholesterolemia. After three weeks of the high fat diet, mice were anesthetized and the femoral artery was dissected from its surroundings and loosely sheathed with a non-constrictive polyethylene cuff (Portex, 0.40 mm inner diameter, 0.80 mm outer diameter and 2.0 mm length). Mice were treated with either the indicated antibody and amount dissolved in PBS by IP injection on day 0, 3, 7, and 10 after surgery. Mice were sacrificed 14 days after surgery and cuffed femoral arteries were harvested and paraffin-embedded. Serial cross-sections (5 µm) were taken from the entire length of the cuffed femoral artery segment for histochemical analysis and the intimal thickening in $(\mu m)^2$ calculated, n = 10, * $p < 0.05$.

EXAMPLES

The following examples are included to further illustrate various aspects of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques and/or compositions discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Screening of phage display antibody library

A phage display selection and screening campaign to identify human antibodies that bind PC and neutralize the pro-inflammatory activity of PC that becomes exposed on oxLDL or apoptotic endothelial cells in cardiovascular disease was performed.

The selection of anti-PC antibodies was directed using PC conjugated to bovine serum albumin (BSA) and alternated between rounds with PC conjugated to ferritin.

The phage display selection output was screened as individual phage for binding to PC-BSA by ELISA and the hits were DNA sequenced to identify the exact number of unique antibodies; all of which were recombinantly converted to IgG. In total, after performing selections on two different phage display libraries 41 fully human IgGs we identified and produced. These antibodies were identified after screening a total of 10,660 different phage clones by ELISA, from which there were 1,511 ELISA positive hits.

An ELISA hit was defined as have a signal on immobilized target (i.e. PC-BSA) that was at least 3-fold greater than the background signal (streptavidin-coated plate).

After sequencing the 1,511 ELISA positives and converting the antibodies from Fab fragments displayed on phage to fully human IgGs, 56 different antibody sequences that bind PC, 26 from the first phagemid library and 30 from the second phage library were recovered.

IgG reformatting, Expression and Purification

Here we describe the results of recovery of 40 of the 56 antibodies after recombinant reformatting from Fab displayed on phage to full length IgG.

DNA for each IgG was prepared and transfected into human kidney 293T cells to transiently generate IgG after a 10 day media harvest. The IgGs used for *in vitro* studies were purified using protein A Sepharose (MabSelect) and buffer exchanged into PBS.

IgGs intended for *in vivo* testing were purified by protein A Sepharose, followed by cation ion exchange (Poros HS) with gradient elution. IgG antibodies intended for *in*

vivo testing were buffer exchanged into Antibody Formulation Buffer (0.1 M citrate-phosphate, 50 mM NaCl, 0.01% Tween-80, 2% Trehalose, pH 6.0). Antibody concentrations were determined on purified samples by absorbance at 280 nm (1 mg/mL = 1.4 O.D.).

In vitro assays

The 40 IgGs were tested in a battery of *in vitro* tests to identify the antibodies with the desired properties. Table 1 summarizes binding properties for a selection of fully human IgG Anti-Phosphorylcholine antibodies.

The second column (column A) in Table 1 shows the ELISA signal obtained using only 15.6 ng/mL IgG added to PC-BSA immobilized on a 96 well plate surface. Antibodies with ELISA signals > 1 are expected to be higher affinity antibodies.

The third column (column B) in Table 1 shows the signal obtained when the antibodies were injected over aminophenyl phosphorylcholine covalently immobilized on a biosensor chip and binding was detected by surface plasmon resonance using a Biacore 3000 instrument. The higher the Biacore signal, the more binding was observed.

The fourth column (column C) in Table 1 shows the results of test to determine specificity of the antibodies towards phosphorylcholine, by testing for binding to covalently immobilized aminophenol, which is the linker used to covalently couple phosphorylcholine to BSA or the biosensor chip. Several of the antibodies bind the linker molecule as well as, or better than, aminophenyl phosphorylcholine. These antibodies are not likely to be effective therapeutic anti-phosphorylcholine antibodies.

The fifth column (column D) in Table 1 summarizes the results of testing the ability of the antibodies to inhibit the uptake of oxLDL by macrophages, which is an early event in cardiovascular inflammation and leads to the formation of foam cells. The macrophage uptake was monitored by flow cytometry using fluorescently modified oxLDL in the presence or absence of 80 µg/mL tested antibody. In each experiment, 100 µg/mL of affinity purified IgM anti-PC polyclonal antibodies was used as a positive control. The amount of oxLDL taken up in the presence of the tested monoclonal antibodies, as monitored by fluorescence, was divided by the

fluorescence observed in the presence of the polyclonal antibody, and then multiplied by 100. Thus, a value below 100 indicate that the antibody in a concentration of 80 µg/mL was more effective in inhibiting oxLDL uptake than the polyclonal anti-PC extracted from human serum in a concentration of 100 µg/mL. A value above 100 similarly indicate that the antibody was less effective than the polyclonal anti-PC.

It was observed that several of the antibodies inhibited the uptake similarly, or better than, the polyclonal anti-PC control. In addition, it was observed that several antibodies stimulated macrophage uptake of oxLDL, a property that excludes these antibodies from lead selection.

The last column (column E) of Table 1 shows ELISA data obtained by adding the IgGs to wells of a 96 well plate that contain either oxLDL or native LDL. The ratio of the ELISA signal observed for binding to oxLDL divided by that observed with LDL is listed in Table 1 for each tested antibody. It is evident that certain antibodies are better binders of oxLDL as compared to LDL.

Table 1. Summary of Binding Properties for Fully Human IgG Anti-PC Antibodies

Full Column Headings:

A) Binding to PC conjugated to BSA by ELISA at 15.6 ng/ml Ab (OD)

B) Binding to aminophenyl PC by Biacore (RU)

C) Binding to aminophenol linker by Biacore (RU)

D) Percent oxLDL Uptake by Macro-phages in presence of 80 µg/ml Ab (a)

E) Binding to oxLDL versus LDL by ELISA (oxLDL signal/LDL signal) (b)

Sample ID	A	B	C	D	E
M0004-B02	1.24	366.4	38.6	233.3	6.7
M0004-C02	0.11	44.8	0.2	93	1.2
M0004-G02	1.23	1028.5	15.7	nd	8.4
M0007-H10	0.49	415.8	2.7	105	0.6

M0009-A06	0.48	912.1	2.5	80.5	2.8
M0011-F05	1.56	4473.6	155.6	547.5	10.3
M0024-B01	0.26	nd	nd	nd	11.1
M0026-H05	0.03	1.6	17.8	73.7	1.4
M0027-H05	0.03	-3.3	1.4	79.3	1.1
M0028-H05	0.03	1.8	5	86	0.6
M0029-H05	0.08	nd	nd	370	0.9
M0030-H05	0.02	19.1	32.8	nd	nd
M0031-H05	0.03	-4.1	0.2	81	1
M0034-G12	0.84	462.3	14.6	78	nd
M0035-E11	0.14	41.5	2.1	68	0.5
M0039-H05	2.73	-6.4	2.1	80.4	0.7
M0042-G07	nd	-2.9	2.3	93.7	0.8
M0043-D09	1.24	172.7	2.1	1310	16.8
M0050-H09	0.22	279.1	7	71.5	nd
M0073-G03	0.18	46.3	19.9	51.1	1.2
M0077-A11	0.26	836.3	1.3	78.4	0.7
M0086-F02	0.99	1.4	12.6	315	nd
M0086-H01	0.41	51.2	4.9	85	1
M0086-H11	1	-1.1	0.9	74	nd
M0097-B04	0.22	109.5	-0.5	98	1.3
M0097-B05	1.01	699.6	-3.2	80	1.1
M0099-B05	1.03	5219.3	23.3	71	1.5
M0099-D11	0.03	170.7	8.6	560	2.1
M0100-A01	1.53	7532.8	3934.7	nd	1.1
M0102-E11	0.02	1.6	-1.3	83	nd
M0108-H03	nd	532.7	4.5	nd	1.1
M0126-A04	0.03	34.2	-8	nd	2.8
M0126-F10	nd	32.9	-8.3	nd	nd
M0126-H08	0.03	114.3	566.1	98	nd
M0127-A09	0.03	18.2	-8.7	160	1.6
M0127-B07	0.05	16.3	-7	67	nd
M0127-E06	nd	21.9	-4.2	nd	nd
M0127-E07	nd	15.4	-6.2	nd	1.8
M0127-F01	0.02	9.6	3.6	77	nd
X0009-A01	0.23	198.1	2	95	1.5

a) OxLDL uptake by macrophages The uptake of DiI-labelled (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) Cu-oxidized LDL (oxLDL, Intracel Corp, US) was investigated in macrophages that were derived from human THP-1 monocytes (ATCC, US). Differentiation was induced by incubation with 100nM PMA (Sigma-Aldrich) in RPMI and 10 % FCS for 24h, after which medium was replaced and cells left for another 48 hours. Cells were then incubated with antibodies as indicated at 37°C for 50-60 min. Thereafter, 20 µg/ml oxLDL was added and incubation continued for 5 hours. At the end of the incubation period, cells were washed two times with ice-cold PBS/0.2% BSA and once with PBS. The cells were harvested in PBS containing 2% PFA. For data acquisition and analysis, FACS Calibur with Cell Quest software was used. For each sample, a minimum of 10.000 cells were analyzed.

b) OxLDL ELISA. hLDL (Kalen Biomedical #770200-4), oxLDL (Kalen Biomedical # 770252-7) (as these data are not shown) were coated at a concentration of 10 µg/ml and a volume of 100 µl/well on an ELISA plate (Immulon 2HB) overnight at 4°C. Plates were blocked with a 1% BSA solution (300 µl/well) for 2 hours at room temperature. After washing, the plate was incubated with the indicated antibodies (100 µl/well; 25 - 100 nM) for 1 hour at room temperature. AP-conjugated goat anti-human secondary antibody (ThermoScientific #31316) at a 1:5000 dilution was added to the washed plate at 100 µl/well and incubated for 1 hour at room temperature. Detection reagent (ThermoScientific #37621) was added (100 µl/well) and the plate was immediately read in kinetic mode at 405 nm with the temperature at 30°C. Results are shown as OD_{oxLDL}/OD_{LDL}.

Analysis of Anti-PC IgG Affinity to PC by SPR.

The IgGs were screened for binding to PC using the Biacore surface plasmon resonance (SPR) biosensor. Aminophenyl phosphorylcholine (Biosearch Technologies) was coupled through the free amine group to one flow cell of a CM5 chip to a density of 120 RU. The aminophenol linker was coupled to another flow cell of the same CM5 chip to a density of approximately 120 RU. PC-KLH and PC-BSA were also coupled to separate flow cells of a CM5 chip.

Using these surfaces with PC immobilized in different contexts, the antibodies were injected at 100 nM at 50 µL/min and binding sensorgrams were obtained. The affinity of M99-B05 was investigated by flowing different concentrations of antibody

over the surface at 50 µL/min. Towards this immobilized antigen the antibodies display a fast on rate and a fast off rate, which prevented us from obtaining reliable k_{on} and k_{off} estimates from the kinetic sensorgrams.

The observed signal for each antibody concentration near the end of the injection was plotted versus the antibody concentration and fit the data to a standard hyperbolic equilibrium binding equation (Figure 1). As seen in Figure 1, M99-B05 appears to bind aminophenyl PC with an apparent Kd of approximately 150 nM. Both tested preparations having equivalent Kd values but the Rmax (the maximum response) differs. The apparent Kd values observed for each antibody on this surface may or may not represent the affinity observed on more physiological substrates.

ELISA Screening of Purified Anti-PC IgGs

The purified IgGs were also screened for binding to PC using an ELISA with PC-BSA. This data was fitted to provide estimated EC50 values (Figure 2).

Inhibition of oxLDL induced MCP-1 release from monocytes

Several of the antibodies were tested for their ability to block the release of the chemokine MCP-1 from monocytes in response to stimulation with oxLDL. As shown in Table 2, M99-B05 was very effective in blocking oxLDL-induced MCP-1 release. This antibody potently inhibited MCP-1 release with an IC_{50} in the nM range.

MCP-1 is a potent pro-inflammatory chemokine that promotes the influx of leukocytes at the site of an atherosclerotic lesion (Reape and Groot. 1999. *Chemokines and atherosclerosis.* Atherosclerosis **147**, 213-225). Control IgG anti streptavidin A2 as negative control showed no inhibition of oxLDL induced MCP-1 release from monocytes (data not shown).

Table 2. Anti-PC inhibition of oxLDL-induced MCP-1 secretion from human monocytes.

	IC_{50} of M99-B05
Donor 1	1.8 ± 0.74 nM
Donor 2	1.3 ± 0.7 nM

Moncytes were isolated from human blood and stimulated with 2 µg/mL copper-oxidized oxLDL in the presence or absence of 10 pM to 40 nM anti-PC IgG. MCP-1 levels in the cell media were quantified using a commercially available MCP-1 specific ELISA kit

The antibody (M99-B05) was also shown to bind human atherosclerotic lesion tissue (Figure 6).

In vivo assays

Based on a combination of favorable *in vitro* binding properties and functionality in *in vitro* assays antibodies M4-G2, M73-G03, and M99-B05 were further tested in an *in vivo* model of coronary inflammation.

This mouse model measured inflammatory cell influx into the sub-endothelial tissue (i.e. the media) in response to vascular injury induced by placing a restrictive cuff around the exposed femoral artery (Figure 3). It is evident from Figure 3 that M99-B05 reduced leukocyte influx into the sub-endothelial layer, the reduction seen being statistically significant. By contrast, and despite their favorable *in vitro* binding properties and functionality in *in vitro* assays, neither of M4-G2 or M73-G03 showed any statistically significant reduction compared to the control antibody (the anti-strepavidin A2 IgG termed "HuIgG1 a-A2").

The very distinctive effect of M99-B05 in this assay, compared to M4-G2 and M73-G03, could not have been predicted and was a surprise to the inventors. This demonstrates that *in vivo* efficacy of anti-PC antibodies may not be predictable from positive *in vitro* data.

Consequently, M99-B05 was tested in a vascular restenosis model in mice, in which injury was again induced by positioning a cuff around the femoral artery but was

allowed to progress for 14 days instead of 3 days. The amount of stenosis, observed as a thickening of the vessel neotima in the affected arteries, was then analyzed by histochemistry (Figure 4). From Figure 4 it is evident that M99-B05 significantly inhibited vessel wall thickening after cuff-induced vascular injury. This further demonstrates that M99-B05 is highly effective *in vivo*.

Construction of Germline and Stability Mutants

An amino acid sequence analysis of the variable domains of both the heavy and the light chains of the M99-B05 antibody, identified amino acid substitutions to construct with the intention of reducing potential immunogenicity and avoiding susceptible amino acid modification that may occur during antibody expression and purification.

The following tables show the alignment of the amino acid sequence of the variable domain with its most closely related germline antibody sequence using the Kabat database. Also highlighted in the tables are the amino acid substitutions that were made in the antibody to make it closer to germline, in addition to mutants that removed potential deamidation sites, an HCDR3 disulfide bond, all of which may raise concerns for manufacturability (so called "Stability Mutants").

Mutants of M99-B05

The X19-A01 mutant has the same heavy and light amino acid sequences as wild type M99-B05, except that first amino acid of the light chain in M99-B05 (a glutamine) is deleted in X19-A01 to better match the germline sequence.

The sequence of the X19-A03 mutant encodes the fully germlined antibody, relative to the VH3-23, JH3 heavy chain and VK4-B3, JK1 light chain germline sequences, without an inserted phenylalanine (F) in framework 1 of the heavy variable region (HV-FR1), plus amino acid substitutions (stability mutations) to potentially reduce protein amino acid modification during expression and purification. The M99-B05 antibody was found to have a deleted F amino acid at the tail of HV-FR1 relative to the germline sequence. Inserting the F at this position makes the antibody closer to the germline sequence and possibly less likely to be immunogenic. The X19-A03 mutant was constructed to contain all other germline substitutions except the F insertion in the event that this insertion affected PC binding. The stability mutants contain a G to A mutation in the HV-CDR3 that was performed to disrupt a potential

deamidation site (NG) and an N to Q substitution in LV-CDR1 to remove another potential deamidation site.

The sequence of the X19-A05 mutant contains all the germline substitutions, including the inserted F in HV-FR1, and the stability mutations. The X19-A05 antibody is the only mutant antibody generated in this example that contains all the germline substitutions and stability mutations.

The X19-A11 mutant has the same sequence as X19-A01 but has two C to S substitutions in the HV-CDR3 to remove the disulfide that is expected to be formed in this region.

The X19-C01 is germlined, without the F insertion, and with stability mutants with the C to S substitutions to remove the disulfide. The comparable antibody (pre-disulfide removal) is X19-A03.

Table 3. Heavy chain sequence optimization of M99-B05.

M99-B05	EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
X19-A01	EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWM***H***WVRQAPGKGLEWVS
X19-A03	EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
X19-A05	EVQLLESGGGLVQPGGSLRLSCAASGFT**F**SGYWMHWVRQAPGKGLEWVS
X19-A07	EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
X19-A09	EVQLLESGGGLVQPGGSLRLSCAASGFT**F**SGYWMHWVRQAPGKGLEWVS
X19-A11	EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
X19-C01	EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
	********** **********
M99-B05	YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A01	YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A03	YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A05	YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A07	YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A09	YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A11	YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-C01	YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
	**********

M99-B05	VRFRSVCSNGVCRPTAYDAFDIWGQGTAVTVSS	SEQ ID NO: 3
X19-A01	VRFRSVCSNGVCRPTAYDAFDIWGQGTAVTVSS	SEQ ID NO: 5
X19-A03	VRFRSVCSNAVCRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 7
X19-A05	VRFRSVCSNAVCRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 1
X19-A07	VRFRSVCSNGVCRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 9
X19-A09	VRFRSVCSNGVCRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 11
X19-A11	VRFRSVSSNGVSRPTAYDAFDIWGQGTAVTVSS	SEQ ID NO: 13
X19-C01	VRFRSVSSNAVSRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 15
	******.**.*.*************** *****	

Germlined sequence mutations are shown in **bold**. Residue mutations that may alleviate possible manufacturing issues are underscored. CDR regions are boxed.

Table 4. Light chain sequence optimization of M99-B05

```
M99-B05     QDIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPKL
X19-A01     -DIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPKL
X19-A03     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPKL
X19-A05     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPKL
X19-A07     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKPGQPPKL
X19-A09     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKPGQPPKL
X19-A11     -DIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPKL
X19-C01     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPKL
             ** ****************************:*************.******

M99-B05      LIHWASTRESGVPDRFSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRTF
X19-A01      LIHWASTRESGVPDRFSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRTF
X19-A03      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
X19-A05      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
X19-A07      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
X19-A09      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
X19-A11      LIHWASTRESGVPDRFSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRTF
X19-C01      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
             **:****************************.*******:*************

M99-B05      GQGTKVEIK          SEQ ID NO:  4
X19-A01      GQGTKVEIK          SEQ ID NO:  6
X19-A03      GQGTKVEIK          SEQ ID NO:  8
X19-A05      GQGTKVEIK          SEQ ID NO:  2
X19-A07      GQGTKVEIK          SEQ ID NO: 10
X19-A09      GQGTKVEIK          SEQ ID NO: 12
X19-A11      GQGTKVEIK          SEQ ID NO: 14
X19-C01      GQGTKVEIK          SEQ ID NO: 16
             *********
```

Germlined sequence mutations are shown in **bold**. Residue mutations that may alleviate possible manufacturing issues are underscored. CDR regions are boxed.

For the avoidance of doubt, in the event of any inadvertent disparity between the presentation of sequences within this application, the sequences provided for the VH and VL domains and the various CDR sequences in Tables 3 and 4 are the definitive sequences.

PC binding of the mutants of M99-B05.

PC binding of the mutants of M99-B05 was assessed by ELISA (Figure 5). From the ELISA data (Figure 5) it is evident that many of the mutations in M99-B05 did not significantly affect the binding to PC. However, replacing the cysteine residues in the Hv-CDR3 with serine (X19-A11 and X19-C01) did reduce the affinity. These cysteine residues are expected to form a disulfide and are present in the germline antibody sequence encoded by VK4-B3. The differences in the observed binding signals may be attributed to differences in the amount of active antibody in each preparation and/or slight errors in the concentration measurements. The antibody mutant of M99-B05 that contained the maximum number of permissive sequence optimized substitutions was X19-A05. This antibody contained all the stability and germline substitutions but retains the Hv-CDR3 disulfide.

Comparison of the *in vivo* effect of M99-B05 and X19-A05

M99-B05 and X19-A05 were tested in the vascular restenosis model in mice, in which injury was again induced by positioning a cuff around the femoral artery but was allowed to progress for 14 days instead of 3 days. The amount of stenosis, observed as a thickening of the vessel neotima in the affected arteries, was then analyzed by histochemistry and the intimal thickening calculated (Figure 7). From Figure 7 it is evident that X19-A05 significantly inhibited vessel wall thickening after cuff-induced vascular injury to a similar extent as compared to M99-B05. The effect of X19-A05 also showed a clear dose-response relation.

Claims

1. An antibody or antibody fragment capable of binding to phosphorylcholine and/or a phosphorylcholine conjugate, wherein the antibody or antibody fragment comprises a variable heavy chain (VH) domain and/or a variable light chain (VL) domain, and wherein -

(a) the VH domain comprises an amino acid sequence that includes one, two or three complementarity determining regions (CDRs) selected from the group consisting of:

a CDR1 sequence comprising an amino acid sequence having at least 25%, 50%, 75% or 100% sequence identity to the sequence of SEQ ID NO: 17;

a CDR2 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 18; and

a CDR3 sequence comprising an amino acid sequence having at least 4%, 9%, 13%, 18%, 22%, 27%, 31%, 36%, 40%, 45%, 50%, 54%, 59%, 63%, 68%, 72%, 77%, 81%, 86%, 90%, 95% or 100% sequence identity to the sequence of SEQ ID NO: 19, 20, 21 or 22; and/or

(b) the VL domain comprises an amino acid sequence that includes one, two or three complementarity determining regions (CDRs) selected from the group consisting of:

a CDR4 sequence comprising an amino acid sequence having at least 5%, 11%, 17%, 23%, 29%, 35%, 47%, 52%, 58%, 64%, 70%, 76%, 82%, 94% or 100% sequence identity to the sequence of SEQ ID NO: 23 or 24;

a CDR5 sequence comprising an amino acid sequence having at least 14%, 28%, 42%, 57%, 71%, 85% or 100% sequence identity to the sequence of SEQ ID NO: 25;

a CDR6 sequence comprising an amino acid sequence having at least 11%, 22%, 33%, 44%, 55%, 66%, 77%, 88% or 100% sequence identity to the sequence of SEQ ID NO: 26.

2. The antibody or antibody fragment of Claim 1 wherein the VH domain comprises an amino acid sequence that includes a CDR1 sequence, a CDR2 and a

CDR3 sequence as defined by Claim 1, and/or the VL domain comprises an amino acid sequence that includes a CDR4 sequence, a CDR5 and a CDR6 sequence as defined by Claim 1.

3. The antibody or antibody fragment of Claim 1 or 2 wherein –

the VH domain comprises an amino acid sequence that includes the CDR1, CDR2 and CDR3 sequences present in an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15 or an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to an amino acid sequence of any of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15; and/or

the VL domain comprises an amino acid sequence that includes the CDR4, CDR5 and CDR6 sequences present in an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16 or an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to an amino acid sequence of any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16.

4. An antibody or antibody fragment according to any preceding claim, wherein -

the VH domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15 or an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to an amino acid sequence of any of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15; and

the VL domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16 or an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to an amino acid sequence of any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16.

5. The antibody or antibody fragment according to any of the preceding claims, wherein -

the VH domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:1; and

the VL domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 2.

6. The antibody or antibody fragment according to any of Claims 1 to 4, wherein -

the VH domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:3; and

the VL domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 4.

7. The antibody or antibody fragment according to any of Claims 1 to 4, wherein -

the VH domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:5; and

the VL domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 6.

8. The antibody or antibody fragment according to any of Claims 1 to 4, wherein -

the VH domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:7; and

the VL domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 8.

9. The antibody or antibody fragment according to any of Claims 1 to 4, wherein -

the VH domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:9; and

the VL domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 10.

10. The antibody or antibody fragment according to any one of Claims 1 to 4, wherein -

the VH domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:11; and

the VL domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 12.

11. The antibody or antibody fragment according to any one of Claims 1 to 4, wherein -

the VH domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:13; and

the VL domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 14.

12. The antibody or antibody fragment according to any one of Claims 1 to 4, wherein -

the VH domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO:15; and

the VL domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% sequence identity SEQ ID NO: 16.

13. The antibody or antibody fragment according to any preceding claim,

wherein the VH domain, the VL domain, or preferably both of the VH and VL domains, comprise an amino acid sequence having 100% sequence identity to the, or one or more (such as all) of each, stated SEQ ID NO.

14. The antibody or antibody fragment according to any preceding claim,

wherein the VH domain, the VL domain, or both of the VH and VL domains, comprise an amino acid sequence having less than 100%, but at least 80%, 85%, 90%, 95%, sequence identity to the, or one or more (such as all) of each, stated SEQ ID NO.

15. The antibody or antibody fragment according to any of preceding claim,

wherein the VH domain, the VL domain, or both of the VH and VL domains, comprise an amino acid sequence having less than 100%, but at least 80%, 85%, 90%, 95%, sequence identity to the, or one or more (such as all) of each, stated SEQ ID NO, and wherein

the ability of the antibody or antibody fragment to bind to phosphorylcholine and/or a phosphorylcholine conjugate is equivalent to (that is, at least 80%, 85%, 90% or 95%, of), or greater than, the ability of a corresponding antibody or antibody fragment, wherein the VH domain and the VL domain of the corresponding antibody or antibody fragment each comprise an antigen-binding sequence comprising an amino acid sequence having 100% sequence identity to the, or each, stated SEQ ID NO.

16. The antibody or antibody fragment according to any preceding claim wherein the VH domain and the VL domain are present in a linear polypeptide sequence.

17. The antibody or antibody fragment according to any preceding claim wherein the VH domain and the VL domain are each present in a separate polypeptide sequence, and preferably wherein the separate polypeptide sequence are directly or indirectly bound together (such as by one or more disulphide bonds between the separate polypeptide sequence).

18. The antibody according to any of Claims 1-15 or 17 wherein the antibody is a monoclonal antibody.

19. The antibody fragment according to any of Claims 1 to 15 wherein the antibody fragment is a single chain antibody, Fv, scFv, Fab, $F(ab')_2$, Fab', Fd, dAb, CDR, or scFv-Fc fragment, a nanobody, and diabody, or any such fragment that has been stabilized such as by PEGylation.

20. The antibody or antibody fragment according to any preceding claim which is a human or humanized antibody or antibody fragment, such as a human or humanized monoclonal antibody.

21. The antibody or antibody fragment according to any preceding claim which is capable of binding to a phosphorylcholine conjugate.

22. The antibody or antibody fragment according to Claim 21 wherein the phosphorylcholine conjugate is a phosphorylcholine moiety linked to a carrier, optionally via a spacer, and preferably the antibody or antibody fragment binds specifically to the phosphorylcholine moiety in the phosphorylcholine conjugate.

23. A pharmaceutical composition comprising or consisting essentially of an antibody or an antibody fragment according to any of the preceding claims and a pharmaceutically acceptable carrier or excipient, optionally wherein the only antibodies or antibody fragments present in the composition are those defined by any of the preceding claims.

24. An antibody or antibody fragment according to any of Claims 1 to 22, or a pharmaceutical composition according to Claim 23 for use in medicine.

25. An antibody or antibody fragment according to any of Claims 1 to 22, or a pharmaceutical composition according to Claim 23 for use in the prevention, prophylaxis and/or treatment of mammals, including humans, against atherosclerosis, an atherosclerotic related disease or ischemic cardiovascular disease.

26. An antibody or antibody fragment according to any of Claims 1 to 22, or a pharmaceutical composition according to Claim 23 for use in the prophylaxis, prevention and/or treatment of Alzheimer's disease.

27. An antibody or antibody fragment according to any of Claims 1 to 22, or a pharmaceutical composition according to Claim 23 for use in the immunization or prophylaxis against, or the prevention or treatment of, metabolic diseases in mammals, including humans.

28. A method for prevention, prophylaxis and/or treatment of a mammal, including a human, against atherosclerosis, an atherosclerotic related disease, or ischemic cardiovascular disease, the method comprising the step of administering to the mammal an antibody or antibody fragment according to any of Claims 1 to 22, or a pharmaceutical composition according to Claim 23.

29. A method for immunization and prophylaxis, prevention and/or treatment of a subject against Alzheimer's disease, the method comprising the step of administering to the subject an antibody or antibody fragment according to any of Claims 1 to 22, or a pharmaceutical composition according to Claim 23.

30. A method for the immunization or prophylaxis against, or the treatment of, metabolic diseases in a mammal, such as a human, the method comprising the step of administering to the mammal an antibody or antibody fragment according to any of Claims 1 to 22, or a pharmaceutical composition according to Claim 23.

31. The antibody or antibody fragment according to any of Claims 1 to 22, or a pharmaceutical composition according to Claim 23 for use according to Claim 27, or the method according to Claim 30, wherein the metabolic disease is a condition selected from the group consisting of metabolic syndrome, insulin resistance, glucose intolerance, hyperglycemia, type I diabetes, type II diabetes, hyperlipidemia,

hypertriglyceridemia, hypercholesterolemia, dyslipidemia, and polycystic ovary syndrome (PCOS).

32. A nucleic acid sequences encoding an antibody or an antibody fragment according to any of Claims 1 to 22.

33. A vector or plasmid comprising the nucleic acid sequence of Claim 32.

34. A host cell comprising the nucleic acid sequence of Claim 32 and/or a vector or plasmid according to Claim 33.

35. The host cell of Claim 34 wherein the cell is a prokaryotic cell, such as an *Escherichia coli* cell, or a eukaryotic cell, such as animal, plant, or fungal cell.

36. The host cell of Claims 34 or 35 which expresses the nucleic acid sequence of Claim 32 and thereby produces an antibody or an antibody fragment according to any of Claims 1 to 22.

37. A method of producing an antibody or an antibody fragment according to any of Claims 1 to 22 comprising culturing a host cell according to Claim 36, and recovering therefrom an antibody or an antibody fragment according to any of Claims 1 to 22.

38. A method of preparing a variant of the antibody or antibody fragments of according to any of Claims 1 to 22, which variant retains the ability to bind to phosphorylcholine and/or a phosphorylcholine conjugate, the method comprising –

(i) providing a nucleic acid according to Claim 32 encoding a parent antibody or antibody fragment;

(ii) introducing one ore more nucleotide mutations into the amino acid coding regions of the nucleic acid sequence, optionally within the regions encoding the VH and/or VL domain(s), such that the mutated nucleic acid encodes a variant antibody or antibody fragment having a different amino acid sequence compared to the parent antibody or antibody fragment;

(iii) expressing the variant antibody or antibody fragment that is encoded by the mutated nucleic acid sequence; and

(iv) comparing the ability of the variant antibody or antibody fragment and the parent antibody or antibody fragment to bind to phosphorylcholine and/or a phosphorylcholine conjugate.

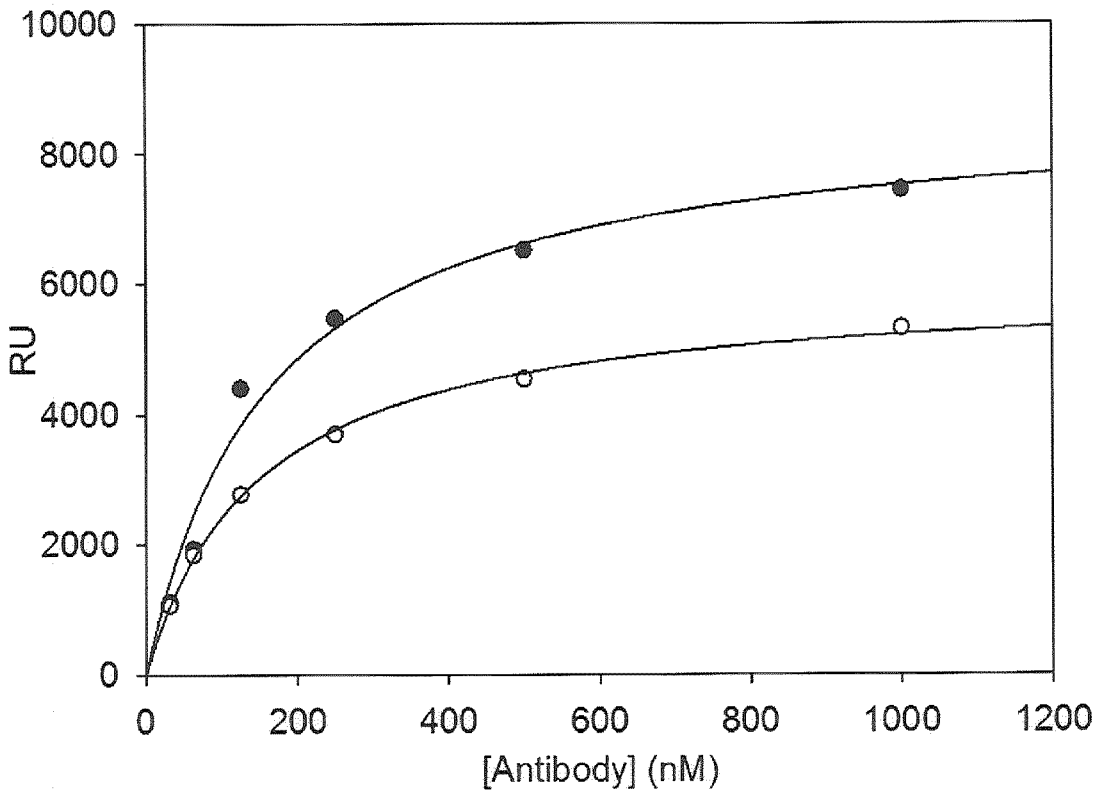
39. The method of Claim 38 wherein the step of assessing the ability of the variant antibody or antibody fragment to bind to phosphorylcholine and/or a phosphorylcholine conjugate further comprises selecting those variants that have substantially equal or enhanced ability to bind to phosphorylcholine and/or a phosphorylcholine conjugate compared to the parent.

40. The method of Claim 38 or 39 further comprising recovering a nucleic acid molecule that comprises the mutated nucleic acid sequence that encodes the variant antibody or antibody fragment, and optionally transforming a host cell with a composition comprising the recovered nucleic acid molecule and further optionally expressing the variant antibody or antibody fragment from the host cell, and yet further optionally recovering the thus-expressed variant antibody or antibody fragment from the host cell.

41. The method of Claim 40 that comprises the step of recovering the thus-expressed variant antibody or antibody fragment from the host cell, and further comprises the step of formulating the recovered variant antibody or antibody fragment into a pharmaceutically acceptable composition.

42. A variant antibody or antibody fragment obtained or obtainable by the method of Claim 38-40, or a pharmaceutically acceptable obtained or obtainable by the method of Claim 41 for use in medicine and/or for use in accordance with Claims 25, 26 and/or 27 or for use in a method according to any of Claims 29, 30 and/or 31.

Figure 1


10000
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6000
4000
2000
0
RU
0
200
400
600
800
1000
1200
[Antibody] (nM)

Figure 2

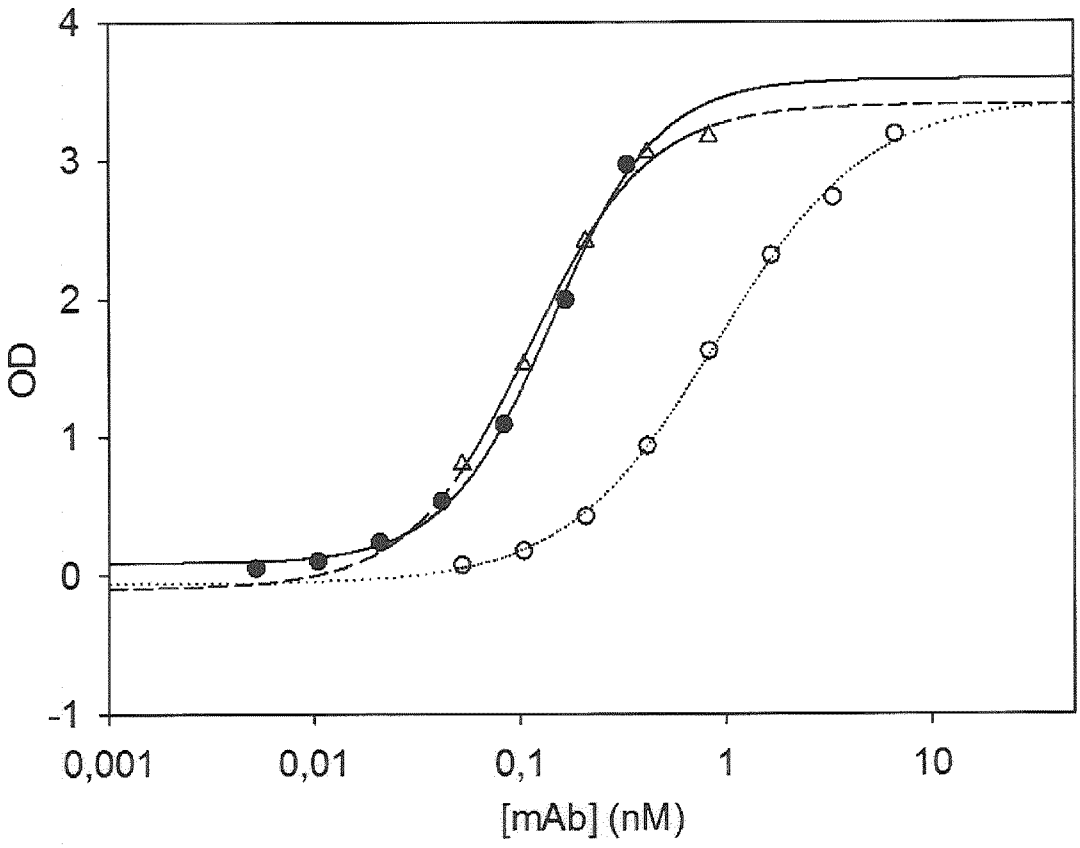


Figure 3

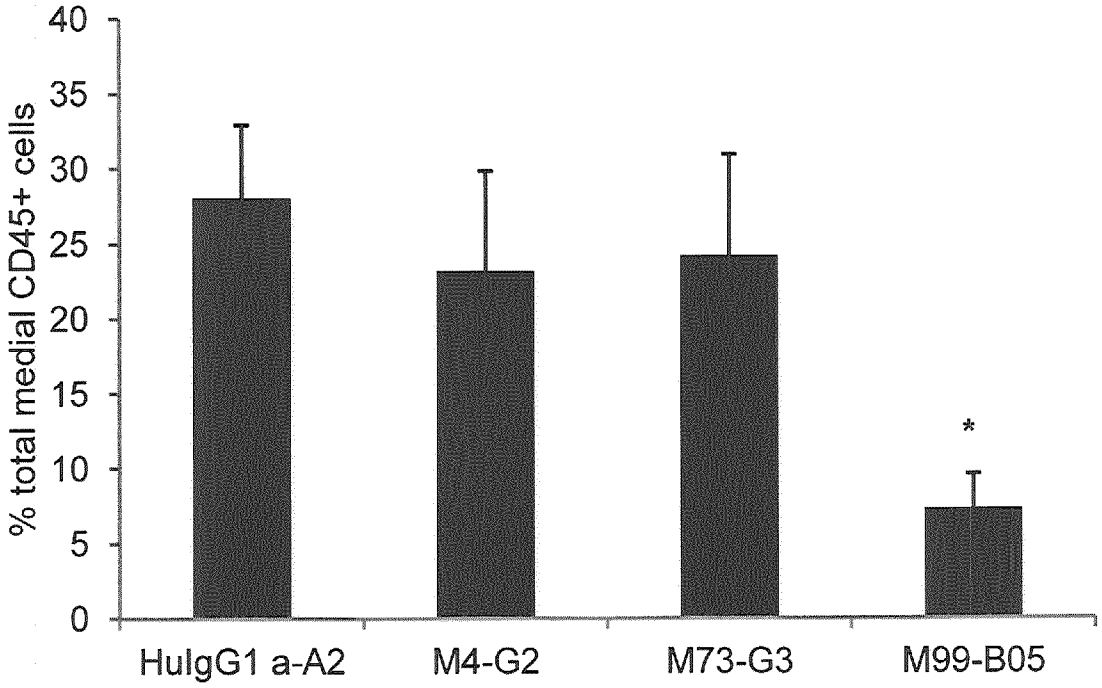
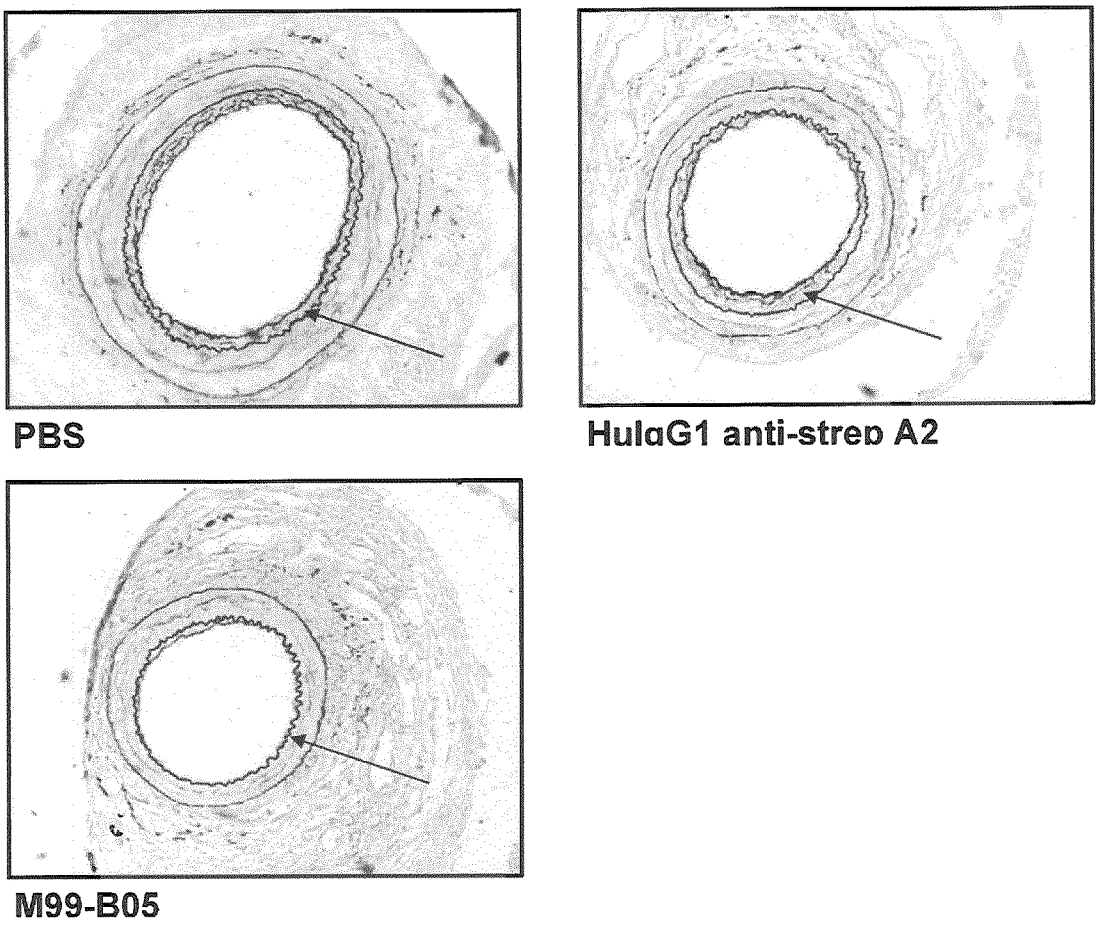

% total medial CD45+ cells
40
35
30
25
20
15
10
5
0
*
HuIgG1 a-A2
M4-G2
M73-G3
M99-B05

Figure 4

A


PBS
HulαG1 anti-strep A2
M99-B05

B

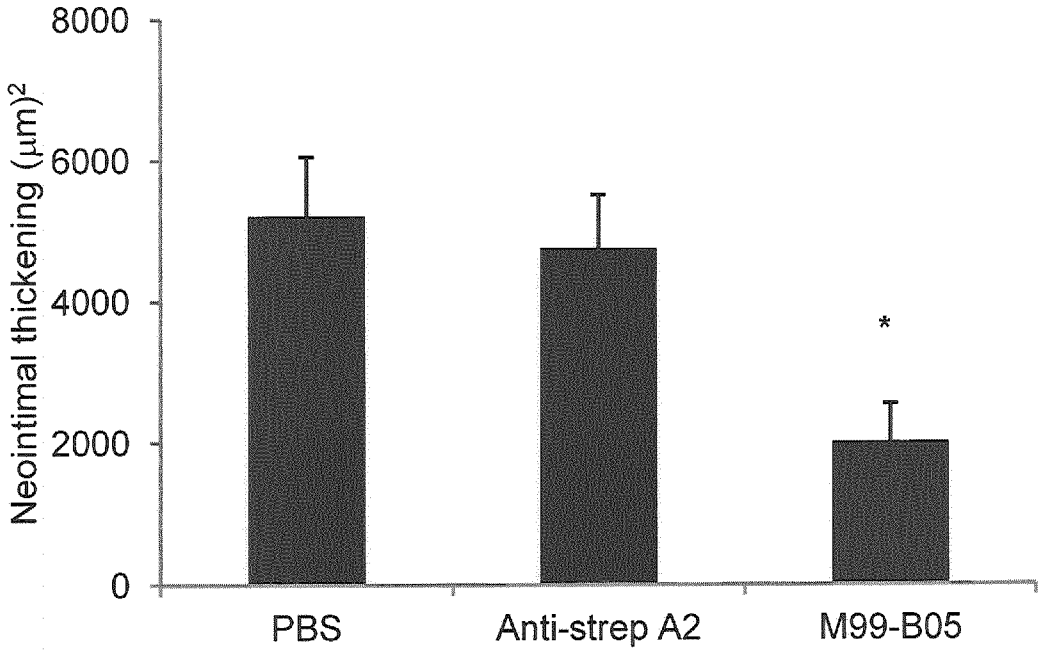

8000
6000
4000
2000
0
Neointimal thickening (μm)²
*
PBS
Anti-strep A2
M99-B05

Figure 5

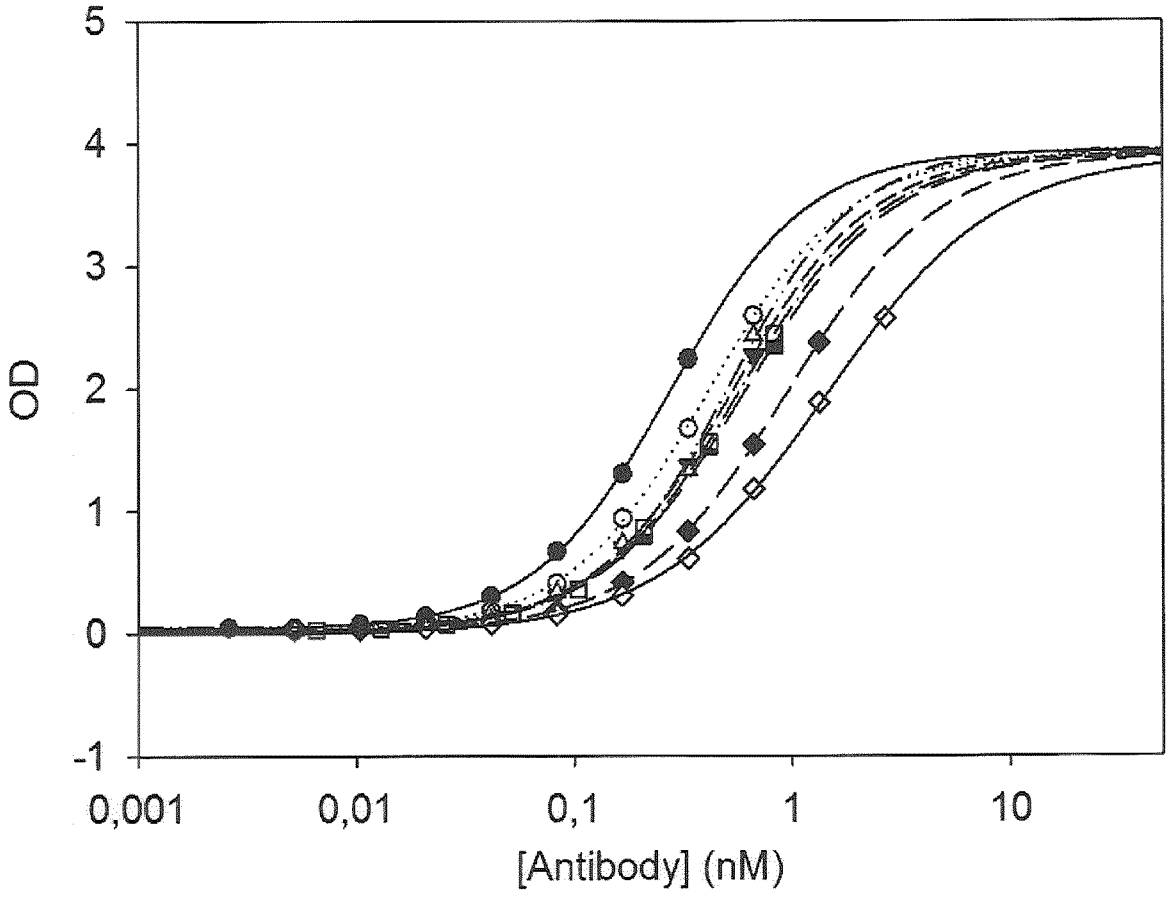


Figure 6

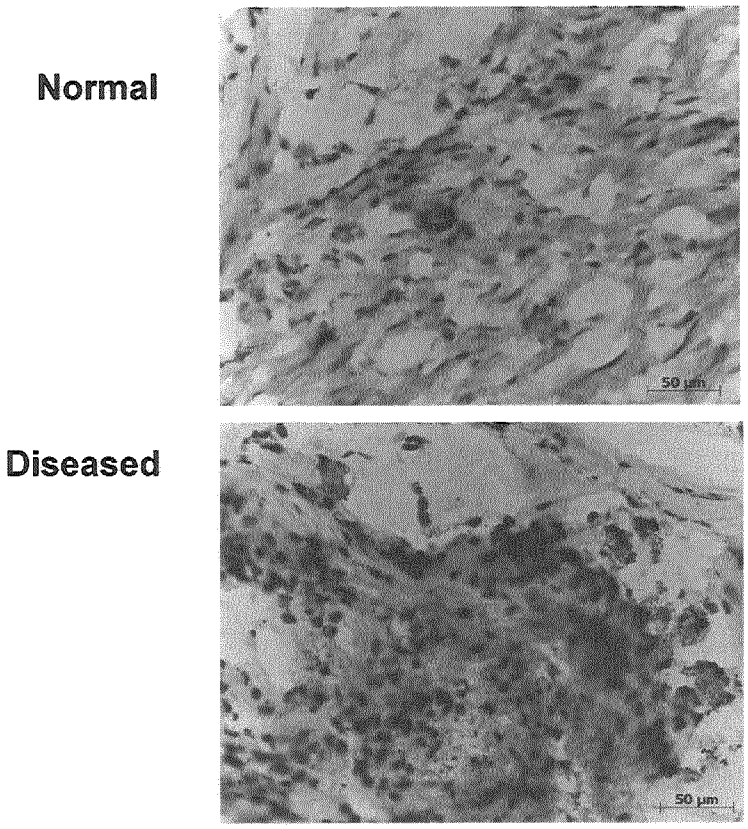
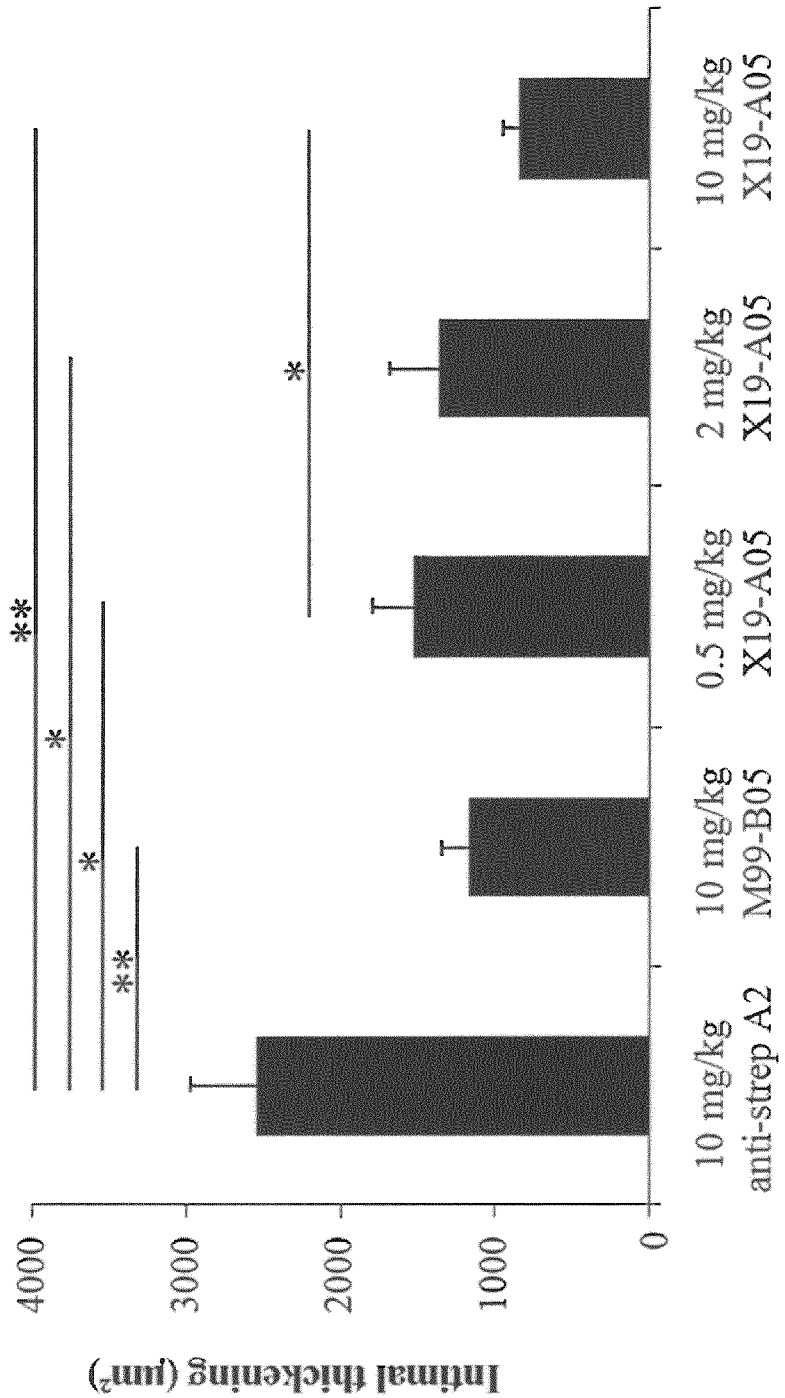


Figure 7


Intimal thickening (µm²)
4000
3000
2000
1000
0
**
*
*
**
*
10 mg/kg anti-strep A2
10 mg/kg M99-B05
0.5 mg/kg X19-A05
2 mg/kg X19-A05
10 mg/kg X19-A05

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/065505

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/44 A61K39/395 A61P37/06 A61P9/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EWING MARK ET AL: "Anti-Phosphorylcholine IgG Antibodies Reduce Restenosis and Vascular Inflammation by Inhibition of the Unfolded Protein Response in a Mouse Model of Accelerated Atherosclerosis", CIRCULATION, LIPPINCOTT WILLIAMS & WILKINS, US , vol. 122, no. 21, Suppl. S 23 November 2010 (2010-11-23), page A14320, XP008157426, ISSN: 0009-7322 Retrieved from the Internet: URL:http://circ.ahajournals.org/ [retrieved on 2012-10-22] the whole document ----- -/--	1,2, 4-12,18, 21-25, 27,28, 30,32-42

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 October 2012	07/11/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bumb, Peter

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PETER X SHAW ET AL: "Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity", JOURNAL OF CLINICAL INVESTIGATION, AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, US, vol. 105, no. 12, 15 June 2000 (2000-06-15), pages 1731-1740, XP008145278, ISSN: 0021-9738, DOI: 10.1172/JCI8472 figure 2	1,2, 4-12,18, 21,22, 38-42
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A	WO 2010/003602 A1 (ATHERA BIOTECHNOLOGIES AB [SE]; FROSTEGAARD JOHAN [SE]; DE FAIRE ULF [) 14 January 2010 (2010-01-14) claim 24	26,29
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(54)发明名称

抗磷酰胆碱的新抗体

(57)摘要

本发明涉及能够结合磷酰胆碱和/或磷酰胆碱结合物的抗体或抗体片段，其中抗体或抗体片段包括重链可变(VH)区域和/或轻链可变(VL)区域，并且其中(a)VH区域包括包含1、2或3个选自下列的互补决定区(CDR)的氨基酸序列：CDR1序列，其包括与SEQ ID NO:17的序列具有至少25%、50%、75%或100%序列同一性的氨基酸序列；CDR2序列，其包括与SEQ ID NO:18的序列具有至少5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94%或100%序列同一性的氨基酸序列；和CDR3序列，其包括与SEQ ID NO:19、20、21或22的序列具有至少4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95%或100%序列同一性的氨基酸序列；和/或(b)VL区域包括包含1、2或3个选自下列的互补决定区(CDR)的氨基酸序列：CDR4序列，其包括与SEQ ID NO:23或24的序列具有至少5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94%或100%序列同一性的氨基酸序列；CDR5序列，其包括与SEQ ID NO:25的序列具有至少14%、28%、42%、57%、71%、85%或100%序列同一性的氨基酸序列；CDR6序列，其包括与SEQ ID NO:26的序列至少具有11%、22%、33%、44%、55%、66%、77%、88%或100%序列同一性的氨基酸序列。

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1. 一种能够结合磷酰胆碱和 / 或磷酰胆碱结合物的抗体或抗体片段，其中抗体或抗体片段包括重链可变（VH）区域和 / 或轻链可变（VL）区域，且其中：

（a）VH 区域包括氨基酸序列，该氨基酸序列包含 1、2 或 3 个选自下列的互补决定区（CDR）：

CDR1 序列，其包括与 SEQ ID NO：17 的序列具有至少 25%、50%、75% 或 100% 序列同一性的氨基酸序列；

CDR2 序列，其包括与 SEQ ID NO：18 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列；和

CDR3 序列，其包括与 SEQ ID NO：19、20、21 或 22 的序列具有至少 4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95% 或 100% 序列同一性的氨基酸序列；和 / 或

（b）VL 区域包括氨基酸序列，该氨基酸序列包含 1、2 或 3 个选自下列的互补决定区（CDR）：

CDR4 序列，其包括与 SEQ ID NO：23 或 24 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列；

CDR5 序列，其包括与 SEQ ID NO：25 的序列具有至少 14%、28%、42%、57%、71%、85% 或 100% 序列同一性的氨基酸序列；

CDR6 序列，其包括与 SEQ ID NO：26 的序列具有至少 11%、22%、33%、44%、55%、66%、77%、88% 或 100% 序列同一性的氨基酸序列。

2. 如权利要求 1 所述的抗体或抗体片段，其中 VH 区域包括包含如权利要求 1 所定义的 CDR1 序列、CDR2 及 CDR3 序列的氨基酸序列，和 / 或 VL 区域包括包含如权利要求 1 所定义的 CDR4 序列、CDR5 及 CDR6 序列的氨基酸序列。

3. 如权利要求 1 或 2 所述的抗体或抗体片段，其中：

VH 区域包括包含 CDR1、CDR2 及 CDR3 序列的氨基酸序列，其中 CDR1、CDR2 及 CDR3 序列存在于选自 SEQ ID NO：1、3、5、7、9、11、13 或 15 的氨基酸序列中或与 SEQID NO：1、3、5、7、9、11、13 或 15 任一个的氨基酸序列具有至少 80%、85%、90% 或 95% 序列同一性的氨基酸序列中；和 / 或

VL 区域包括包含 CDR4、CDR5 及 CDR6 序列的氨基酸序列，其中 CDR4、CDR5 及 CDR6 序列存在于选自 SEQ ID NO：2、4、6、8、10、12、14 或 16 的氨基酸序列中或与 SEQ ID NO：2、4、6、8、10、12、14 或 16 任一个的氨基酸序列具有至少 80%、85%、90% 或 95% 序列同一性的氨基酸序列中。

4. 如上述权利要求任一项所述的抗体或抗体片段，其中：

VH 区域包括选自 SEQ ID NO：1、3、5、7、9、11、13 或 15 的氨基酸序列或与 SEQ IDNO：1、3、5、7、9、11、13 或 15 任一个的氨基酸序列具有至少 80%、85%、90% 或 95% 序列同一性的氨基酸序列；和

VL 区域包括选自 SEQ ID NO：2、4、6、8、10、12、14 或 16 的氨基酸序列或与 SEQID NO：2、4、6、8、10、12、14 或 16 任一个的氨基酸序列具有至少 80%、85%、90% 或 95% 序列同一性的氨基酸序列。

5. 如上述权利要求任一项所述的抗体或抗体片段，其中：

VH 区域包括与 SEQ ID NO :1 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和

VL 区域包括与 SEQ ID NO :2 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列。

6. 如权利要求 1 至 4 任一项所述的抗体或抗体片段,其中 :

VH 区域包括与 SEQ ID NO :3 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和

VL 区域包括与 SEQ ID NO :4 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列。

7. 如权利要求 1 至 4 任一项所述的抗体或抗体片段,其中 :

VH 区域包括与 SEQ ID NO :5 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和

VL 区域包括与 SEQ ID NO :6 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列。

8. 如权利要求 1 至 4 任一项所述的抗体或抗体片段,其中 :

VH 区域包括与 SEQ ID NO :7 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和

VL 区域包括与 SEQ ID NO :8 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列。

9. 如权利要求 1 至 4 任一项所述的抗体或抗体片段,其中 :

VH 区域包括与 SEQ ID NO :9 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和

VL 区域包括与 SEQ ID NO :10 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列。

10. 如权利要求 1 至 4 任一项所述的抗体或抗体片段,其中 :

VH 区域包括与 SEQ ID NO :11 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和

VL 区域包括与 SEQ ID NO :12 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列。

11. 如权利要求 1 至 4 任一项所述的抗体或抗体片段,其中 :

VH 区域包括与 SEQ ID NO :13 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和

VL 区域包括与 SEQ ID NO :14 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列。

12. 如权利要求 1 至 4 任一项所述的抗体或抗体片段,其中 :

VH 区域包括与 SEQ ID NO :15 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和

VL 区域包括与 SEQ ID NO :16 具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列。

13. 如上述权利要求任一项所述的抗体或抗体片段，

其中 VH 区域、VL 区域或优选地 VH 及 VL 两个区域包括与所述 SEQ ID NO 或各个所述 SEQ ID NO 的一种或多种(例如全部)具有 100% 序列同一性的氨基酸序列。

14. 如上述权利要求任一项所述的抗体或抗体片段，

其中 VH 区域、VL 区域或 VH 及 VL 两个区域包括与所述 SEQ ID NO 或各个所述 SEQID NO 的一种或多种(例如全部)具有少于 100% 但至少 80%、85%、90%、95% 序列同一性的氨基酸序列。

15. 如上述权利要求任一项所述的抗体或抗体片段，

其中 VH 区域、VL 区域或 VH 及 VL 两个区域包括与所述 SEQ ID NO 或各个所述 SEQID NO 的一种或多种(例如全部)具有少于 100% 但至少 80%、85%、90%、95% 序列同一性的氨基酸序列，并且其中

抗体或抗体片段结合磷酰胆碱和 / 或磷酰胆碱结合物的能力等于(即，至少 80%、85%、90% 或 95%)或大于相应抗体或抗体片段的能力，其中相应抗体或抗体片段的 VH 区域及 VL 区域每一个均包括包含与所述 SEQ ID NO 或所述 SEQ ID NO 每一个具有 100% 序列同一性的氨基酸序列的抗原结合序列。

16. 如上述权利要求任一项所述的抗体或抗体片段，其中 VH 区域和 VL 区域以线性多肽序列存在。

17. 如上述权利要求任一项所述的抗体或抗体片段，其中 VH 区域和 VL 区域每一个均以独立的多肽序列存在，并优选其中独立的多肽序列直接或间接结合在一起(例如通过介于独立的多肽序列间的一个或多个二硫键)。

18. 如权利要求 1 至 15 或 17 任一项所述的抗体，其中抗体为单克隆抗体。

19. 如权利要求 1 至 15 任一项所述的抗体片段，其中抗体片段为单链抗体、Fv、scFv、Fab、F (ab')$_2$、Fab'、Fd、dAb、CDR 或 scFv-Fc 片段、纳米抗体及双特异抗体，或任何稳定的此类片段，例如利用聚乙二醇化稳定的此类片段。

20. 如上述权利要求任一项所述的抗体或抗体片段，其为人类抗体或人源化抗体或抗体片段，例如人类单克隆抗体或人源化单克隆抗体。

21. 如上述权利要求任一项所述的抗体或抗体片段，其能够结合磷酰胆碱结合物。

22. 如权利要求 21 所述的抗体或抗体片段，其中磷酰胆碱结合物为任选地经由间隔物连接至载体的磷酰胆碱部分，且优选地，抗体或抗体片段特异性地结合磷酰胆碱结合物中的磷酰胆碱部分。

23. 一种药物组合物，其包括上述权利要求任一项所述的抗体或抗体片段以及药学上可接受的载体或赋形剂或基本上由上述权利要求任一项所述的抗体或抗体片段以及药学上可接受的载体或赋形剂组成，任选地，其中存在于组合物中的仅有的抗体或抗体片段为上述权利要求任一项所述的抗体或抗体片段。

24. 如权利要求 1 至 22 任一项所述的抗体或抗体片段，或如权利要求 23 所述的药物组合物，其用于医药中。

25. 如权利要求 1 至 22 任一项所述的抗体或抗体片段，或如权利要求 23 所述的药物组合物，其用于防止、预防和 / 或治疗哺乳动物包括人的动脉粥样硬化、动脉粥样硬化相关疾病或缺血性心血管疾病。

26. 如权利要求 1 至 22 任一项所述的抗体或抗体片段，或如权利要求 23 所述的药物组合物，其用于预防、防止和 / 或治疗阿尔茨海默病。

27. 如权利要求 1 至 22 任一项所述的抗体或抗体片段，或如权利要求 23 所述的药物组合物，其用于免疫或预防，或防止或治疗哺乳动物包括人的代谢疾病。

28. 一种防止、预防和 / 或治疗哺乳动物包括人的动脉粥样硬化、动脉粥样硬化相关疾病或缺血性心血管疾病的方法，该方法包括对哺乳动物施用如权利要求 1 至 22 任一项所述的抗体或抗体片段或如权利要求 23 所述的药物组合物的步骤。

29. 一种免疫及预防、防止和 / 或治疗受试者的阿尔茨海默病的方法，该方法包括对受试者施用如权利要求 1 至 22 任一项所述的抗体或抗体片段或如权利要求 23 所述的药物组合物的步骤。

30. 一种免疫、预防或治疗哺乳动物包括人的代谢疾病的方法，该方法包括对哺乳动物施用如权利要求 1 至 22 任一项所述的抗体或抗体片段或如权利要求 23 所述的药物组合物的步骤。

31. 如权利要求 1 至 22 任一项所述的抗体或抗体片段，或如权利要求 23 所述的药物组合物，如权利要求 27 所述的用途或如权利要求 30 所述的方法，其中代谢疾病选自下列的症状：代谢综合征、胰岛素抗药性、葡糖耐受不良、高血糖症、I 型糖尿病、II 型糖尿病、高脂血症、高甘油三脂血症、高胆固醇血症、血脂异常及多囊性卵巢综合征（PCOS）。

32. 一种编码权利要求 1 至 22 任一项所述的抗体或抗体片段的核酸序列。

33. 一种包括权利要求 32 所述的核酸序列的载体或质粒。

34. 一种包括权利要求 32 所述的核酸序列和 / 或权利要求 33 所述的载体或质粒的宿主细胞。

35. 如权利要求 34 所述的宿主细胞，其中细胞为原核细胞，例如大肠杆菌（Escherichiacoli）细胞，或真核细胞，例如动物、植物或真菌细胞。

36. 如权利要求 34 或 35 所述的宿主细胞，其表达如权利要求 32 所述的核酸序列，并借此生产如权利要求 1 至 22 任一项所述的抗体或抗体片段。

37. 一种生产如权利要求 1 至 22 任一项所述的抗体或抗体片段的方法，其包括培养如权利要求 36 所述的宿主细胞，并从宿主细胞回收如权利要求 1 至 22 任一项所述的抗体或抗体片段。

38. 一种制备如权利要求 1 至 22 任一项所述的抗体或抗体片段的变体的方法，该变体保留结合磷酰胆碱和 / 或磷酰胆碱结合物的能力，该方法包括：

（i）提供编码亲代抗体或抗体片段的如权利要求 32 所述的核酸；

（ii）引入一个或多个核苷酸突变至核酸序列的氨基酸编码区，任选地引入至编码 VH 和 / 或 VL 区域的区域内，使得突变的核酸编码与亲代抗体或抗体片段相比具有不同氨基酸序列的变体抗体或抗体片段；

（iii）表达由突变的核酸序列编码的变体抗体或抗体片段；和

（iv）比较变体抗体或抗体片段与亲代抗体或抗体片段结合磷酰胆碱和 / 或磷酰胆碱结合物的能力。

39. 如权利要求 38 所述的方法，其中评估变体抗体或抗体片段结合磷酰胆碱和 / 或磷酰胆碱结合物的能力的步骤进一步包括选择那些与亲代相比具有实质上相等或增强的结

合磷酰胆碱和 / 或磷酰胆碱结合物的能力的变体。

40. 如权利要求 38 或 39 所述的方法，其进一步包括回收包括编码变体抗体或抗体片段的突变的核酸序列的核酸分子；及任选地用包括回收的核酸分子的组合物转化宿主细胞；及进一步任选地从宿主细胞表达变体抗体或抗体片段；并进一步任选地从宿主细胞回收因此表达的变体抗体或抗体片段。

41. 如权利要求 40 所述的方法，其包括从宿主细胞回收因此表达的变体抗体或抗体片段的步骤，并进一步包括将所回收的变体抗体或抗体片段配制成药学上可接受的组合物的步骤。

42. 由权利要求 38 至 40 所述的方法获得的或可获得的变体抗体或抗体片段，或由权利要求 41 所述的方法获得的或可获得的药学上可接受的组合物，其用于医药中和 / 或依照权利要求 25、26 和 / 或 27 的用途中，或用于权利要求 29、30 和 / 或 31 所述的方法中。

抗磷酰胆碱的新抗体

技术领域

[0001] 本发明涉及结合磷酰胆碱(PC)和/或PC结合物且具有惊人体内有效性质的新抗体。

背景技术

[0002] 本说明书中所列或讨论的明显先前公开的文件并不必然被承认是本领域现有技术的一部分或常见的一般知识。

[0003] 尽管存在治疗心血管疾病的选择,急性冠状动脉综合征(ACS)仍为工业化世界死亡的头号原因。ACS的发生是冠状动脉管腔内血栓形成的结果,其与动脉壁内的慢性炎症相关。动脉炎症经由脂质核心的形成以及由导致斑块形成的炎症性细胞的浸润而起始。不稳定的斑块(plaque)含有实质性的坏死核心和凋亡细胞,其破坏内皮且可导致斑块破裂而暴露出底层胶原蛋白、冯威里氏因子(von Willebrand factor,vWF)、组织因子、脂质及平滑肌,而引发血小板黏附、活化和聚集(Libby等人1996.Macrophages and atherosclerotic plaque stability.Curr Opin Lipidol7,330-335)。用抗血小板疗法、降胆固醇药物治疗(如,他汀(statin)类)、抗凝血剂,以及通过经皮冠状动脉介入治疗(percutaneous coronary intervention,PCI)以及支架(stents)植入的外科再通术(recanalization)的组合可治疗ACS。

[0004] 已显示抗血小板疗法例如COX-1抑制剂(如,阿斯匹林)、ADP受体拮抗剂(如,抵克立得(Ticlopedine)及氯吡格雷(clopidogrel))及糖蛋白IIb/IIIa受体拮抗剂在许多不同的临床试验中降低主要不良冠状动脉事件(major adverse caronary events,MACE)的发生率(Dupont等人-2009-Antiplatelet therapies and the role of antiplatelet resistance in acute coronary syndrome.Thromb Res124,6-13)。然而,一部分接受长期抗血小板疗法的患者持续具有心血管事件。另外,慢性预防疗法可能需要多达两年才显示最大有益效果,而且许多患者接下来仍具有疾病复发的高风险。心肌梗塞后有长达6-12个月的期间,在该期间患者易发生进一步的MACE,这经常归因于由于再狭窄而导致的再闭塞(Tabas.2010.Macrophage death and defective inflammation resolution in atherosclerosis.Nat Rev Immunol 10,36-46)。

[0005] 因此,明显需要特异地针对防止进一步斑块进展并促进斑块消退的治疗,从而能够实质性降低在此期间的事件。

[0006] 磷酰胆碱(在某些磷脂上的极性头基团)与心血管疾病有广泛关联。冠状动脉炎症期间产生的活性氧种类造成低密度脂蛋白(LDL)氧化而产生氧化的LDL(oxLDL)。事实上,心血管疾病(CVD),例如动脉粥样硬化、不稳定型心绞痛或急性冠状动脉综合征已显示与血浆的oxLDL水平上升有关联(Itabe和Ueda.2007.Measurement of plasma oxidizedlow-density lipoprotein and its clinical implications.J Atheroscler Thromb14,1-11)。LDL为循环脂蛋白颗粒,其包含带有PC极性头基团的脂类和apoB100蛋白。

[0007] 在LDL氧化期间，产生了含有不存在于未经修饰的LDL上的新抗原表位的PC。巨噬细胞上的清道夫受体(例如CD36)识别oxLDL上新暴露的PC，并且所产生的巨噬细胞吞噬的oxLDL开始进行血管壁中促炎性泡沫细胞(proinflammatory foam cells)的形成。内皮细胞表面的受体也识别氧化的LDL，且已报道会刺激一系列的反应，包含内皮细胞功能失调、细胞凋亡及未折叠蛋白反应(Gora等人2010.Phospholipolyzed LDL induces an inflammatory response in endothelial cells through endoplasmic reticulum stress signaling.FASEB J24 (9):3284-97)。经磷脂酶A2或胺反应性疾病代谢物(例如由糖化蛋白氧化而产生的醛类)修饰后，PC新抗原表位也暴露于LDL上。这些另外修饰的LDL颗粒也是CVD中的促炎性因子。

[0008] 已显示抗磷酰胆碱(PC)的抗体结合氧化的LDL或经其他修饰的LDL，并在体内模型中或体外研究中阻止oxLDL的促炎性活性(Shaw等人2000.Natural antibodies with theT15idiotype may act in atherosclerosis,apoptotic clearance,and protective immunity.J Clin Invest105,1731-1740;Shaw等人2001.Human-derived anti-oxidized LDL autoantibody blocks uptake of oxidized LDL by macrophages and localizes to atherosclerotic lesions in vivo.Arterioscler Thromb Vasc Biol21,1333-1339)。

[0009] 而且，临床数据的检查证实了低水平的天然IgM抗-PC抗体与ACS患者中MACE增高的风险有关(Frostegard,J.2010.Low level natural antibodies against phosphorylcholine:a novel risk marker and potential mechanism in atherosclerosis and cardiovascular disease.Clin Immunol134,47-54)。

[0010] 因此，需要能有效用于治疗的抗-PC抗体分子，特别是适合人类治疗的全人抗-PC抗体。就申请人所知，至今本领域仍未提供治疗上有效的人类抗-PC抗体。鉴定此类抗体受阻于下列事实：用于具有抗-PC结合活性的人类抗体的体外筛选方法对预估体内治疗活性的效果欠佳。

[0011] 鉴于此，本领域需要用于体内系统时提供有效且有利性质的人类抗-PC抗体分子，特别是用于人类治疗时。

发明内容

[0012] 本申请公开了包括能够结合磷酰胆碱和/或磷酰胆碱结合物的新抗原结合区的新抗体及抗体片段的制备与测试。

[0013] 在第一方面中，本发明提供了能够结合磷酰胆碱和/或磷酰胆碱结合物的抗体或抗体片段，其中抗体或抗体片段包括重链可变(VH)区域和/或轻链可变(VL)区域，并且其中：

[0014] (a)VH区域包括包含1、2或优选3个选自下列的互补决定区(CDR)的氨基酸序列：

[0015] CDR1序列，其包括与SEQ ID NO:17的序列具有至少25%、50%、75%或100%序列同一性的氨基酸序列；

[0016] CDR2序列，其包括与SEQ ID NO:18的序列具有至少5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94%或100%序列同一性的氨基酸序列；和

[0017] CDR3序列，其包括与SEQ ID NO:19、20、21或22的序列具有至少4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95%或100%序

列同一性的氨基酸序列；和 / 或

[0018] (b)VL 区域包括包含 1、2 或优选 3 个选自下列的互补决定区(CDR)的氨基酸序列：

[0019] CDR4 序列，其包括与 SEQ ID NO：23 或 24 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列；

[0020] CDR5 序列，其包括与 SEQ ID NO：25 的序列具有至少 14%、28%、42%、57%、71%、85% 或 100% 序列同一性的氨基酸序列；

[0021] CDR6 序列，其包括与 SEQ ID NO：26 的序列具有至少 11%、22%、33%、44%、55%、66%、77%、88% 或 100% 序列同一性的氨基酸序列。

[0022] 在本发明第一方面的一个实施方式中，抗体或抗体片段包括 VH 区域，该 VH 区域包括包含如上所定义的 CDR1 序列、CDR2 及 CDR3 序列的氨基酸序列，和 / 或 VL 区域，该 VL 区域包括包含如上所定义的 CDR4 序列、CDR5 及 CDR6 序列的氨基酸序列。

[0023] 在本发明第一方面的另一个实施方式中，抗体或抗体片段包括：

[0024] VH 区域，其包括包含 CDR1、CDR2 及 CDR3 序列的所有三个的氨基酸序列，而 CDR1、CDR2 及 CDR3 序列存在于选自：SEQ ID NO：1、3、5、7、9、11、13 或 15 的氨基酸序列，或与 SEQ IDNO：1、3、5、7、9、11、13 或 15 的任一个的氨基酸序列具有至少 80％、85％、90％或 95％序列同一性的氨基酸序列；和 / 或

[0025] VL 区域，其包括包含 CDR4、CDR5 及 CDR6 序列的所有三个的氨基酸序列，而 CDR4、CDR5 及 CDR6 序列存在于选自：SEQ ID NO：2、4、6、8、10、12、14 或 16 的氨基酸序列，或与 SEQ ID NO：2、4、6、8、10、12、14 或 16 的任一个的氨基酸序列具有至少 80％、85％、90％或 95％序列同一性的氨基酸序列。

[0026] 在本发明第一方面的另一个实施方式中，抗体或抗体片段包括重链可变 (VH) 区域和 / 或轻链可变 (VL) 区域，其中：

[0027] VH区域包括选自SEQ ID NO：1、3、5、7、9、11、13或15的氨基酸序列，或与SEQID NO：1、3、5、7、9、11、13 或 15 的任一个的氨基酸序列具有至少 50％、60％、70％、80％、85％、90％或 95％序列同一性的氨基酸序列；和

[0028] VL 区域包括选自 SEQ ID NO：2、4、6、8、10、12、14 或 16 的氨基酸序列，或与 SEQ ID NO：2、4、6、8、10、12、14 或 16 的任一个的氨基酸序列具有至少 50％、60％、70％、80％、85％、90％或 95％序列同一性的氨基酸序列。

[0029] SEQ ID NO：1 为如下实施例所示的 X19-A05 抗体重链可变 (VH) 区域并具有序列：

[0030] EVQLLESGGGLVQPGGSLRLSCAASGFTFSGYWMHWVRQAPGKGLEWVS YISPSGGGTHYADSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAVYYCARVRF RSVCSNAVCRPTAYDAFDI WGQGTMVTVSS，

[0031] 并包含互补决定区 (CDR)：

[0032] VH CDR1：GYWM(SEQ ID NO：17)；

[0033] VH CDR2：YISPSGGGTHYADSVKG(SECQ ID NO：18)；

[0034] VH CDR3：VRFRSVCSNAVCRPTAYDAFDI(SEQ ID NO：19)；

[0035] SEQ ID NO：2 为 X19-A05 抗体的轻链可变 (VL) 区域，并具有序列：

[0036] DIVMTQSPDSLAVSLGEIRATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPK LLIYWASTRESGVPDRF SGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT FGQGTKVEIK，

[0037] 并包含互补决定区 (CDR)：

[0038] VL CDR4 :KSSQSVFYQSNKKNYLA(SEQ ID NO :23) ;

[0039] VL CDR5 :WASTRES(SEQ ID NO :25) ;

[0040] VL CDR6 :QQYFNAPRT(SEQ ID NO :26),

[0041] SEQ ID NO :3 为如下实施例所示的 M99-B05 抗体的重链可变 (VH) 区域,并具有序列 :

[0042] EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI SPSGGGTHYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR SVCSNGVCRPTAYDAFDIWGQGTAVTVSS,

[0043] 并包含互补决定区 (CDR) :

[0044] VH CDR1 :GYWM(SEQ ID NO :17) ;

[0045] VH CDR2 :YISPSGGGTHYADSVKG(SEQ ID NO :18) ;

[0046] VH CDR3 :VRFRSVCSNGVCRPTAYDAFDI(SEQ ID NO :20),

[0047] SEQ ID NO :4 为 M99-B05 抗体的轻链可变 (VL) 区域,且具有序列 :

[0048] QDIQMTQSPDSLAVSLGERATI NCKSSQSVFYNSNKKNYLAWYQQKAGQPP KLLIHWASTRESGVPDR FSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPR TFGQGTKVEIK,

[0049] 并包含互补决定区 (CDR) :

[0050] VL CDR4 :KSSQSVFYNSNKKNYLA(SEQ I D NO :24) ;

[0051] VL CDR5 :WASTRES(SEQ ID NO :25) ;

[0052] VL CDR6 :QQYFNAPRT(SEQ ID NO :26),

[0053] SEQ ID NO :5 为如下实施例所示的 X19-A01 抗体的重链可变 (VH) 区域,并具有序列 :

[0054] EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI SPSGGGTHYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR SVCSNGVCRPTAYDAFDIWGQGTAVTVSS,

[0055] 并包含互补决定区 (CDR) :

[0056] VH CDR1 :GYWM(SEQ I D NO :17) ;

[0057] VH CDR2 :YISPSGGGTHYADSVKG(SEQ ID NO :18) ;

[0058] VH CDR3 :VRFRSVCSNGVCRPTAYDAFDI(SEQ ID NO :20),

[0059] SEQ ID NO :6 为 X19-A01 抗体的轻链可变 (VL) 区域,且具有序列 :

[0060] DIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPK LLIHWASTRESGVPDRFS GSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRT FGQGTKVEIK,

[0061] 并包含互补决定区 (CDR) :

[0062] VL CDR4 :KSSQSVFYNSNKKNYLA(SEQ ID NO :24) ;

[0063] VL CDR5 :WASTRES(SEQ I D NO :25) ;

[0064] VL CDR6 :QQYFNAPRT(SEQ ID NO :26),

[0065] SEQ ID NO :7 为如下实施例所示的 X19-A03 抗体的重链可变 (VH) 区域,并具有序列 :

[0066] EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI SPSGGGTHYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR SVCSNAVCRPTAYDAFDIWGQGTMVTVSS,

[0067] 并包含互补决定区 (CDR) :

[0068] VH CDR1 :GYWM(SEQ ID NO :17) ;

[0069] VH CDR2 :YISPSGGGTHYADSVKG(SEQ ID NO :18) ;

[0070] VH CDR3 :VRFRSVCSNAVCRPTAYDAFDI(SEQ ID NO :19),

[0071] SEQ ID NO :8 为 X19-A03 抗体的轻链可变 (VL) 区域,且具有序列 :

[0072] DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPK LLIYWASTRESGVPDRFS GSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT FGQGTKVEIK,

[0073] 并包含互补决定区 (CDR) :

[0074] VL CDR4 :KSSQSVFYQSNKKNYLA(SEQ ID NO :23) ;

[0075] VL CDR5 :WASTRES(SEQ ID NO :25) ;

[0076] VL CDR6 :QQYFNAPRT(SEQ ID NO :26),

[0077] SEQ ID NO :9 为如下实施例所示的 X19-A07 抗体的重链可变 (vH) 区域,并具有序列 :

[0078] EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI SPSGGGTHYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR SVCSNGVCRPTAYDAFDIWGQGTMVTVSS,

[0079] 并包含互补决定区 (CDR) :

[0080] VH CDR1 :GYWM(SEQ ID NO :17) ;

[0081] VH CDR2 :YISPSGGGTHYADSVKG(SEQ ID NO :18) ;

[0082] VH CDR3 :VRFRSVCSNGVCRPTAYDAFDI(SEQ ID NO :20),

[0083] SEQ ID NO :10 为 X19-A07 抗体的轻链可变 (vL) 区域且具有序列 :

[0084] DIVMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKPGQPPK LLIYWASTRESGVPDRFS GSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT FGQGTKVEIK,

[0085] 并包含互补决定区 (CDR) :

[0086] VL CDR4 :KSSQSVFYNSNKKNYLA(SEQ ID NO :24) ;

[0087] VL CDR5 :WASTRES(SEQ I D NO :25) ;

[0088] VL CDR6 :QQYFNAPRT(SEQ ID NO :26),

[0089] SEQ ID NO :11 为如下实施例所示的 X19-A09 抗体的重链可变 (vH) 区域,并具有序列 :

[0090] EVQLLESGGGLVQPGGSLRLSCAASGFTFSGYWMHWVRQAPGKGLEWVS YISPSGGGTHYADSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAVYYCARVRF RSVCSNGVCRPTAYDAFDIWGQGTMVTVSS,

[0091] 并包含互补决定区 (CDR) :

[0092] VH CDR1 :GYWM(SEQ ID NO :17) ;

[0093] VH CDR2 :YISPSGGGTHYADSVKG(SEQ ID NO :18) ;

[0094] VH CDR3 :VRFRSVCSNGVCRPTAYDAFDI(SEQ ID NO :20),

[0095] SEQ ID NO :12 为 X19-A09 抗体的轻链可变 (vL) 区域且具有序列 :

[0096] DIVMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKPGQPPK LLIYWASTRESGVPDRFS GSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT FGQGTKVEIK,

[0097] 并包含互补决定区 (CDR) :

[0098] VL CDR4 :KSSQSVFYNSNKKNYLA(SEQ ID NO :24) ;

[0099] VL CDR5 :WASTRES(SEQ ID NO :25) ;

[0100] VL CDR6 :QQYFNAPRT(SEQ ID NO :26),

[0101] SEQ ID NO :13 为如下实施例所示的 X19-A11 抗体的重链可变 (vH) 区域，并具有序列：

[0102] EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI SPSGGGTHYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR SVSSNGVSRPTAYDAFDIWGQGTAVTVSS，

[0103] 并包含互补决定区 (CDR)：

[0104] VH CDRI :GYWM(SEQ ID NO :17)；

[0105] VH CDR2 :YISPSGGGTHYADSVKG(SEQ ID NO :18)；

[0106] VH CDR3 :VRFRSVSSNGVSRPTAYDAFDI(SEQ ID NO :21)，

[0107] SEQ ID NO :14 为 X19-A11 抗体的轻链可变 (vL) 区域且具有序列：

[0108] DIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPK LLIHVWASTRESGVPDRF SGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRT FGQGTKVEIK，

[0109] 并包含互补决定区 (CDR)：

[0110] VL CDR4 :KSSQSVFYNSNKKNYLA(SEQ ID NO :24)；

[0111] VL CDR5 :WASTRES(SEQ ID NO :25)；

[0112] VL CDR6 :QQYFNAPRT(SEQ ID NO :26)，

[0113] SEQ ID NO :15 为如下实施例所示的 X19-C01 抗体的重链可变 (VH) 区域，并具有序列：

[0114] EVQLLESGGGLVQPGGSLRLSCAASGFTSGYWMHWVRQAPGKGLEWVSYI SPSGGGTHYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARVRFR SVSSNAVSRPTAYDAFDIWGQGTMVTVSS，

[0115] 并包含互补决定区 (CDR)：

[0116] VH CDR1 :GYWM(SEQ ID NO :17)；

[0117] VH CDR2 :YISPSGGGTHYADSVKG(SEQ ID NO :18)；

[0118] VH CDR3 :VRFRSVSSNAVSRPTAYDAFDI(SEQ ID NO :22)，

[0119] SEQ ID NO :16 为 X19-C01 抗体的轻链可变 (VL) 区域且具有序列：

[0120] DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPK LLIYWASTRESGVPDRFS GSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRT FGQGTKVEIK，

[0121] 并包含互补决定区 (CDR)：

[0122] VL CDR4 :KSSQSVFYQSNKKNYLA(SEQ ID NO :23)；

[0123] VL CDR5 :WASTRES(SEQ ID NO :25)；

[0124] VL CDR6 :QQYFNAPRT(SEQ I D NO :26).

[0125] 以上定义的 SEO ID NO 的总结显示如下：

[0126]

	VH	VL	CDR1	CDR2	CDR3	CDR4	CDR5	CDR6
X19-A05	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 19	SEQ ID NO: 23	SEQ ID NO: 25	SEQ ID NO: 26
M99-B05	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 20	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-A01	SEQ ID NO: 5	SEQ ID NO: 6	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 20	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-A03	SEQ ID NO: 7	SEQ ID NO: 8	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 19	SEQ ID NO: 23	SEQ ID NO: 25	SEQ ID NO: 26
X19-A07	SEQ ID NO: 9	SEQ ID NO:10	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 20	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-A09	SEQ ID NO: 11	SEQ ID NO:12	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 20	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-A11	SEQ ID NO: 13	SEQ ID NO:14	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 21	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26
X19-C01	SEQ ID NO: 15	SEQ ID NO:16	SEQ ID NO: 17	SEQ ID NO: 18	SEQ ID NO: 22	SEQ ID NO: 23	SEQ ID NO: 25	SEQ ID NO: 26

[0127] 在本发明第一方面的另一个实施方式中，抗体或抗体片段基于 X19-A05 抗体的 vH 和 / 或 vL 区域，并且因此：

[0128] VH区域 (i) 包括与SEQ ID NO :1 的序列具有至少80％、85％、90％、95％或100％序列同一性的氨基酸序列，和 / 或 (ii) 包括 CDR1 序列、CDR2 序列及 CDR3 序列，该 CDR1 序列包括与 SEQ ID NO :17 的序列具有至少 25％、50％、75％或 100％序列同一性的氨基酸序列，该CDR2 序列包括与 SEQ ID NO :18 的序列具有至少 5％、11％、17％、23％、29％、35％、47％、52％、58％、64％、70％、76％、82％、94％或 100％序列同一性的氨基酸序列，以及该 CDR3 序列包括与 SEQ ID NO :19 的序列具有至少 4％、9％、13％、18％、22％、27％、31％、36％、40％、45％、50％、54％、59％、63％、68％、72％、77％、81％、86％、90％、95％或 100％序列同一性的氨基酸序列 ；和 / 或

[0129] VL区域 (iii) 包括与SEQ ID NO :2 的序列具有至少80％、85％、90％、95％或100％序列同一性的氨基酸序列，和 / 或 (iv) 包括CDR4 序列、CDR5 序列及 CDR6 序列，该CDR4 序列包括与 SEQ ID NO :23 的序列具有至少 5％、11％、17％、23％、29％、35％、47％、52％、58％、64％、70％、76％、82％、94％或 100％序列同一性的氨基酸序列，该 CDR5 序列包括与 SEQ ID NO :25 的序列具有至少 14％、28％、42％、57％、71％、85％或 100％序列同一性的氨基酸序列，及该CDR6 序列包括与SEQ ID NO :26 的序列具有至少 11％、22％、33％、44％、55％、66％、77％、88％或 100％序列同一性的氨基酸序列。优选地，VH 区域包括 SEQ ID NO :1 的序列，以及 VL 区域包括 SEQ ID NO :2 的序列。

[0130] 该实施方式的抗体或抗体片段可进一步包括重链恒定 (CH) 区域或其片段，该片段可包括，例如，至少 CH 区域的 10、20、30、40、50、60、70、80、90、100、120、140、160、180、200、220、240、260、280、300、320 个或更多的氨基酸。CH 区域或其片段可连结于 VH 区域。CH

区域无特别限制，虽然在一个实施方式中其为人类 CH 区域。本领域含有许多人类 CH 区域的实例。用于本文的示例性人类 CH 区域包含：

[0131] ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRWSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCS VMHEALHNHYTQKSLSLSPGK(SEQ ID NO :27) ;和 ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS D IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPG(SEQ ID NO :28).

[0132] SEQ ID NO :27 为 M99-B05 的 CH 区域且具有人类 IgGI(UniProtKB / Swiss-Prot : P01857.1) 的 CH 区域的序列。SEQ ID NO :28 为 X19-A05 的 CH 区域。SEQ ID NO :28 不同于 SEQ ID NO :27 之处在于 SEQ ID NO :28 的 CH 区域中末端 K(Lys) 的移除，其降低或避免可能的肽酶降解。

[0133] 该实施方式的抗体或抗体片段可以另外地，或替代地进一步包括轻链恒定 (CL) 区域或其片段，该片段可包括，例如，CL 区域的至少 10、20、30 和、50、60、70、80、90、100 个或更多个氨基酸。CL 区域或其片段可连结于 VL 区域。(区域无特别限制，虽然在一个实施方式中其为人类 CL 区域。本领域含有许多人类 CL 区域的实例。用于本文的示例性人类 CL 区域包含：

[0134] RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC(SEQ ID NO :29).

[0135] SEQ ID NO :29 为 M99-B05 及 X19-A05 两者的 CL 区域，并具有人类 kappa(UniProtKB / Swiss-Prot :P01834.1) 的 CL 区域的序列。

[0136] 根据此实施方式，优选地，VH 区域包括 SEQ ID NO :1 的序列，其连接于 SEQ ID NO : 28 的 CH 区域，以及 VL 区域包括 SEQ ID NO :2 的序列，其连接于 SEQ ID NO :29 的 CL 区域。

[0137] 本发明第一方面的另一个实施方式中，抗体或抗体片段基于 M99-B05 抗体的 VH 和 / 或 VL 区域，并且因此：

[0138] VH 区域 (i) 包括与 SEQ ID NO :3 的序列具有至少 80％、85％、90％、95％或 100％序列同一性的氨基酸序列，和 / 或 (ii) 包括 CDR1 序列、CDR2 序列及 CDR3 序列，该 CDR1 序列包括与 SEQ ID NO :17 的序列具有至少 25％、50％、75％或 100％序列同一性的氨基酸序列，该 CDR2 序列包括与 SEQ ID NO :18 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列，及该 CDR3 序列包括与 SEQ ID NO :20 的序列具有至少 4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和 / 或

[0139] VL 区域 (iii) 包括与 SEQ ID NO :4 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列，和 / 或 (iv) 包括 CDR4 序列、CDR5 序列及 CDR6 序列，该 CDR4 序列包括与 SEQ ID NO :24 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、

82%、94% 或 100% 序列同一性的氨基酸序列，该 CDR5 序列包括与 SEQ ID NO :25 的序列具有至少 14%、28%、42%、57%、71%、85% 或 100% 序列同一性的氨基酸序列，及该 CDR6 序列包括与 SEQ ID NO :26 的序列具有至少 11%、22%、33%、44%、55%、66%、77%、88% 或 100% 序列同一性的氨基酸序列。优选地，VH 区域包括 SEQ ID NO :3 的序列，以及 VL 区域包括 SEQ ID NO :4 的序列。

[0140] 该实施方式的抗体或抗体片段可进一步包括重链恒定（CH）区域或其片段，该片段可包括，例如，CH 区域的至少 10、20、30、40、50、60、70、80、90、100、120、140、160、180、200、220、240、260、280、300、320 个或更多个氨基酸。该 CH 区域或其片段可连结于 VH 区域。CH 区域无特别限制，虽然在一个实施方式中其为人类 CH 区域。本领域含有许多人类 CH 区域的实例。用于本文的示例性人类 CH 区域包含 SEQ ID NO :27 及 SEQ ID NO :28。

[0141] 该实施方式的抗体或抗体片段可另外地，或替代地进一步包括轻链恒定（CL）区域或其片段，该片段可包括，例如，CL 区域的至少 10、20、30、40、50、60、70、80、90、100 个或更多个氨基酸。该 CL 区域或其片段可连结于 VL 区域。CL 区域无特别限制，虽然在一个实施方式中其为人类 CL 区域。本领域含有许多人类 CL 区域的实例。用于本文的示例性人类 CL 区域包含 SEQ ID NO :29。

[0142] 根据此实施方式，优选地，VH 区域包括 SEQ ID NO :3 的序列，其连接于 SEQ ID NO :27 或 28 的 CH 区域，以及 VL 区域包括 SEQ ID NO :4 的序列，其连接于 SEQ ID NO :29 的 CL 区域。

[0143] 本发明第一方面的另一个实施方式中，抗体或抗体片段基于 X19-A01 抗体的 VH 和 / 或 VL 区域，并且因此 :

[0144] VH 区域（i）包括与 SEQ ID NO :5 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列，和 / 或（ii）包括 CDR1 序列、CDR2 序列及 CDR3 序列，该 CDR1 序列包括与 SEQ ID NO :17 的序列具有至少 25%、50%、75% 或 100% 序列同一性的氨基酸序列，该 CDR2 序列包括与 SEQ ID NO :18 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列，及该 CDR3 序列包括与 SEQ ID NO :20 的序列具有至少 4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和 / 或

[0145] VL 区域（i）包括与 SEQ ID NO :6 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列，和 / 或（iv）包括 CDR4 序列、CDR5 序列及 CDR6 序列，该 CDR4 序列包括与 SEQ ID NO :24 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列，该 CDR5 序列包括与 SEQ ID NO :25 的序列具有至少 14%、28%、42%、57%、71%、85% 或 100% 序列同一性的氨基酸序列，及该 CDR6 序列包括与 SEQ ID NO :26 的序列具有至少 11%、22%、33%、44%、55%、66%、77%、88% 或 100% 序列同一性的氨基酸序列。优选地，VH 区域包括 SEQ ID NO :5 的序列，以及 VL 区域包括 SEQ ID NO :6 的序列。

[0146] 该实施方式的抗体或抗体片段可进一步包括重链恒定（CH）区域或其片段，该片段可包括，例如，CH 区域的至少 10、20、30、40、50、60、70、80、90、100、120、140、160、180、200、220、240、260、280、300、320 个或更多个氨基酸。该 CH 区域或其片段可连结于 VH 区域。CH 区域无特别限制，虽然在一个实施方式中其为人类 CH 区域。本领域含有许多人类 CH 区域

的实例。用于本文的示例性人类 CH 区域包含 SEQ ID NO :27 及 SEQ ID NO :28。

[0147] 该实施方式的抗体或抗体片段可另外地，或替代地进一步包括轻链恒定(CL)区域或其片段，该片段可包括，例如，CL 区域的至少 10、20、30、40、50、60、70、80、90、100 个或更多个氨基酸。该 CL 区域或其片段可连结于 VL 区域。CL 区域无特别限制，虽然在一个实施方式中其为人类 CL 区域。本领域含有许多人类 CL 区域的实例。用于本文的示例性人类 CL 区域包含 SEQ ID NO :29。

[0148] 根据此实施方式，优选地，VH 区域包括 SEQ ID NO :5 的序列，其连接于 SEQ ID NO :27 或 28 的 CH 区域，以及 VL 区域包括 SEQ ID NO :6 的序列，其连接于 SEQ ID NO :29 的 CL 区域。

[0149] 在本发明第一方面的另一个实施方式中，抗体或抗体片段基于 X19-A03 抗体的 VH 和 / 或 VL 区域，并且因此 :

[0150] VH 区域(i)包括与 SEQ ID NO :7 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列，和 / 或(ii) 包括 CDR1 序列、CDR2 序列及 CDR3 序列，该 CDR1 序列包括与 SEQ ID NO :17 的序列具有至少 25%、50%、75% 或 100% 序列同一性的氨基酸序列，该 CDR2 序列包括与 SEQ ID NO :18 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列，及该 CDR3 序列包括与 SEQ ID NO :19 的序列具有至少 4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和 / 或

[0151] VL 区域(iii)包括与 SEQ ID NO :8 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列，和 / 或(iv) 包括 CDR4 序列、CDR5 序列及 CDR6 序列，该 CDR4 序列包括与 SEQ ID NO :23 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列，该 CDR5 序列包括与 SEQ ID NO :25 的序列具有至少 14%、28%、42%、57%、71%、85% 或 100% 序列同一性的氨基酸序列，及该 CDR6 序列包括与 SEQ ID NO :26 的序列具有至少 11%、22%、33%、44%、55%、66%、77%、88% 或 100% 序列同一性的氨基酸序列。优选地，VH 区域包括 SEQ ID NO :7 的序列，以及 VL 区域包括 SEQ ID NO :8 的序列。

[0152] 该实施方式的抗体或抗体片段可进一步包括重链恒定(CH)区域或其片段，该片段可包括，例如，CH 区域的至少 10、20、30、40、50、60、70、80、90、100、120、140、160、180、200、220、240、260、280、300、320 个或更多个氨基酸。该 CH 区域或其片段可连结于 VH 区域。CH 区域无特别限制，虽然在一个实施方式中其为人类 CH 区域。本领域含有许多人类 CH 区域的实例。用于本文的示例性人类 CH 区域包含 SEQ ID NO :27 及 SEQ ID NO :28。

[0153] 该实施方式的抗体或抗体片段可另外地，或替代地进一步包括轻链恒定(CL)区域或其片段，该片段可包括，例如，CL 区域的至少 10、20、30、40、50、60、70、80、90、100 个或更多个氨基酸。该 CL 区域或其片段可连结于 VL 区域。CL 区域无特别限制，虽然在一个实施方式中其为人类 CL 区域。本领域含有许多人类 CL 区域的实例。用于本文的示例性人类 CL 区域包含 SEQ ID NO :29。

[0154] 根据此实施方式，优选地，VH 区域包括 SEQ ID NO :7 的序列，其连接于 SEQ ID NO :27 或 28 的 CH 区域，以及 VL 区域包括 SEQ ID NO :8 的序列，其连接于 SEQ ID NO :29 的 CL 区域。

[0155] 本发明第一方面的另一个实施方式中，抗体或抗体片段基于X19-A07抗体的VH和/或VL区域，并且因此：

[0156] VH区域(i)包括与SEQ ID NO:9的序列具有至少80%、85%、90%、95%或100%序列同一性的氨基酸序列，和/或(ii)包括CDR1序列、CDR2序列及CDR3序列，该CDR1序列包括与SEQ ID NO:17的序列具有至少25%、50%、75%或100%序列同一性的氨基酸序列，该CDR2序列包括与SEQ ID NO:18的序列具有至少5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94%或100%序列同一性的氨基酸序列，及该CDR3序列包括与SEQ ID NO:20的序列具有至少4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95%或100%序列同一性的氨基酸序列；和/或

[0157] VL区域(iii)包括与SEQ ID NO:10的序列具有至少80%、85%、90%、95%或100%序列同一性的氨基酸序列，和/或(iv)包括CDR4序列、CDR5序列及CDR6序列，该CDR4序列包括与SEQ ID NO:24的序列具有至少5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94%或100%序列同一性的氨基酸序列，该CDR5序列包括与SEQ ID NO:25的序列具有至少14%、28%、42%、57%、71%、85%或100%序列同一性的氨基酸序列，及该CDR6序列包括与SEQ ID NO:26的序列具有至少11%、22%、33%、44%、55%、66%、77%、88%或100%序列同一性的氨基酸序列。优选地，VH区域包括SEQ ID NO:9的序列，以及VL区域包括SEQ ID NO:10的序列。

[0158] 该实施方式的抗体或抗体片段可进一步包括重链恒定(CH)区域或其片段，该片段可包括，例如，CH区域的至少10、20、30、40、50、60、70、80、90、100、120、140、160、180、200、220、240、260、280、300、320个或更多个氨基酸。该CH区域或其片段可连结于VH区域。CH区域无特别限制，虽然在一个实施方式中其为人类CH区域。本领域含有许多人类CH区域的实例。用于本文的示例性人类CH区域包含SEQ ID NO:27及SEQ ID NO:28。

[0159] 该实施方式的抗体或抗体片段可另外地，或替代地进一步包括轻链恒定(CL)区域或其片段，该片段可包括，例如，CL区域的至少10、20、30、40、50、60、70、80、90、100或更多个氨基酸。该CL区域或其片段可连结于VL区域。CL区域无特别限制，虽然在一个实施方式中其为人类CL区域。本领域含有许多人类CL区域的实例。用于本文的示例性人类CL区域包含SEQ ID NO:29。

[0160] 根据此实施方式，优选地，VH区域包括SEQ ID NO:9的序列，其连接于SEQ ID NO:27或28的CH区域，以及VL区域包括SEQ ID NO:10的序列，其连接于SEQ ID NO:29的CL区域。

[0161] 本发明第一方面的另一个实施方式中，抗体或抗体片段基于X19-A09抗体的VH和/或VL区域，并且因此：

[0162] VH区域(i)包括与SEQ ID NO:11的序列具有至少80%、85%、90%、95%或100%序列同一性的氨基酸序列，和/或(ii)包括CDR1序列、CDR2序列及CDR3序列，该CDR1序列包括与SEQ ID NO:17的序列具有至少25%、50%、75%或100%序列同一性的氨基酸序列，该CDR2序列包括与SEQ ID NO:18的序列具有至少5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94%或100%序列同一性的氨基酸序列，及该CDR3序列包括与SEQ ID NO:20的序列具有至少4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95%或100%序列同一性的氨基酸序列；和/或

[0163] VL 区域(iii)包括与 SEQ ID NO :12 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列,和 / 或(iv)包括 CDR4 序列、CDR5 序列及 CDR6 序列,该 CDR4 序列包括与 SEQ ID NO :24 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列,该 CDR5 序列包括与 SEQ ID NO :25 的序列具有至少 14%、28%、42%、57%、71%、85% 或 100% 序列同一性的氨基酸序列,及该 CDR6 序列包括与 SEQ ID NO :26 的序列具有至少 11%、22%、33%、44%、55%、66%、77%、88% 或 100% 序列同一性的氨基酸序列。优选地,VH 区域包括 SEQ ID NO :11 的序列,以及 VL 区域包括 SEQ ID NO :12 的序列。

[0164] 该实施方式的抗体或抗体片段可进一步包括重链恒定(CH)区域或其片段,该片段可包括,例如,CH 区域的至少 10、20、30、40、50、60、70、80、90、100、120、140、160、180、200、220、240、260、280、300、320 个或更多个氨基酸。该 CH 区域或其片段可连结于 VH 区域。CH 区域无特别限制,虽然在一个实施方式中其为人类 CH 区域。本领域含有许多人类 CH 区域的实例。用于本文的示例性人类 CH 区域包含 SEQ ID NO :27 及 SEQ ID NO :28。

[0165] 该实施方式的抗体或抗体片段可另外地,或替代地进一步包括轻链恒定(CL)区域或其片段,该片段可包括,例如,CL 区域的至少 10、20、30、40、50、60、70、80、90、100 个或更多个氨基酸。该 CL 区域或其片段可连结于 VL 区域。CL 区域无特别限制,虽然在一个实施方式中其为人类 CL 区域。本领域含有许多人类 CL 区域的实例。用于本文的示例性人类 CL 区域包含 SEQ ID NO :29。

[0166] 根据此实施方式,优选地,VH 区域包括 SEQ ID NO :11 的序列,其连接于 SEQ ID NO :27 或 28 的 CH 区域,以及 VL 区域包括 SEQ ID NO :12 的序列,其连接于 SEQ ID NO :29 的 CL 区域。

[0167] 本发明第一方面的另一个实施方式中,抗体或抗体片段基于 X19-A11 抗体的 VH 和 / 或 VL 区域,并且因此 :

[0168] VH 区域(i)包括与 SEQ ID NO :13 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列,和 / 或(ii)包括 CDR1 序列、CDR2 序列及 CDR3 序列,该 CDR1 序列包括与 SEQ ID NO :17 的序列具有至少 25%、50%、75% 或 100% 序列同一性的氨基酸序列,该 CDR2 序列包括与 SEQ ID NO :18 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列,及该 CDR3 序列包括与 SEQ ID NO :21 的序列具有至少 4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和 / 或

[0169] VL 区域(i)包括与 SEQ ID NO :14 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列,和 / 或(iv)包括 CDR4 序列、CDR5 序列及 CDR6 序列,该 CDR4 序列包括与 SEQ ID NO :24 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列,该 CDR5 序列包括与 SEQ ID NO :25 的序列具有至少 14%、28%、42%、57%、71%、85% 或 100% 序列同一性的氨基酸序列,及该 CDR6 序列包括与 SEQ ID NO :26 的序列具有至少 11%、22%、33%、44%、55%、66%、77%、88% 或 100% 序列同一性的氨基酸序列。优选地,VH 区域包括 SEQ ID NO :13 的序列,以及 VL 区域包括 SEQ ID NO :14 的序列。

[0170] 该实施方式的抗体或抗体片段可进一步包括重链恒定(CH)区域或其片段,该片段

可包括，例如，CH 区域的至少 10、20、30、40、50、60、70、80、90、100、120、140、160、180、200、220、240、260、280、300、320 个或更多个氨基酸。该 CH 区域或其片段可连结于 VH 区域。CH 区域无特别限制，虽然在一个实施方式中其为人类 CH 区域。本领域含有许多人类 CH 区域的实例。用于本文的示例性人类 CH 区域包含 SEQ ID NO :27 及 SEQ ID NO :28。

[0171] 该实施方式的抗体或抗体片段可另外地，或替代地进一步包括轻链恒定(CL)区域或其片段，该片段可包括，例如，CL 区域的至少 10、20、30、40、50、60、70、80、90、100 个或更多个氨基酸。该 CL 区域或其片段可连结于 VL 区域。CL 区域无特别限制，虽然在一个实施方式中其为人类 CL 区域。本领域含有许多人类 CL 区域的实例。用于本文的示例性人类 CL 区域包含 SEQ ID NO :29。

[0172] 根据此实施方式，优选地，VH 区域包括 SEQ ID NO :13 的序列，其连接于 SEQ ID NO :27 或 28 的 CH 区域，以及 VL 区域包括 SEQ ID NO :14 的序列，其连接于 SEQ ID NO :29 的 CL 区域。

[0173] 本发明第一方面的另一个实施方式中，抗体或抗体片段基于 X19-C01 抗体的 VH 和 / 或 VL 区域，并且因此 :

[0174] VH 区域(i)包括与 SEQ ID NO :15 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列，和 / 或(ii)包括 CDR1 序列、CDR2 序列及 CDR3 序列，该 CDR1 序列包括与 SEQ ID NO :17 的序列具有至少 25%、50%、75% 或 100% 序列同一性的氨基酸序列，该 CDR2 序列包括与 SEQ ID NO :18 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列，及该 CDR3 序列包括与 SEQ ID NO :22 的序列具有至少 4%、9%、13%、18%、22%、27%、31%、36%、40%、45%、50%、54%、59%、63%、68%、72%、77%、81%、86%、90%、95% 或 100% 序列同一性的氨基酸序列 ;和 / 或

[0175] VL 区域(iii)包括与 SEQ ID NO :16 的序列具有至少 80%、85%、90%、95% 或 100% 序列同一性的氨基酸序列，和 / 或(iv)包括 CDR4 序列、CDR5 序列及 CDR6 序列，该 CDR4 序列包括与 SEQ ID NO :23 的序列具有至少 5%、11%、17%、23%、29%、35%、47%、52%、58%、64%、70%、76%、82%、94% 或 100% 序列同一性的氨基酸序列，该 CDR5 序列包括与 SEQ ID NO :25 的序列具有至少 14%、28%、42%、57%、71%、85% 或 100% 序列同一性的氨基酸序列，及该 CDR6 序列包括与 SEQ ID NO :26 的序列具有至少 11%、22%、33%、44%、55%、66%、77%、88% 或 100% 序列同一性的氨基酸序列。优选地，VH 区域包括 SEQ ID NO :15 的序列，及 VL 区域包括 SEQ ID NO :16 的序列。

[0176] 该实施方式的抗体或抗体片段可进一步包括重链恒定(CH)区域或其片段，该片段可包括，例如，CH 区域的至少 10、20、30、40、50、60、70、80、90、100、120、140、160、180、200、220、240、260、280、300、320 个或更多个氨基酸。该 CH 区域或其片段可连结于 VH 区域。CH 区域无特别限制，虽然在一个实施方式中其为人类 CH 区域。本领域含有许多人类 CH 区域的实例。用于本文的示例性人类 CH 区域包含 SEQ ID NO :27 及 SEQID NO :28。

[0177] 该实施方式的抗体或抗体片段可另外地，或替代地进一步包括轻链恒定(CL)区域或其片段，该片段可包括，例如，CL 区域的至少 10、20、30、40、50、60、70、80、90、100 或更多个氨基酸。该 CL 区域或其片段可连结于 VL 区域。CL 区域无特别限制，虽然在一个实施方式中其为人类 CL 区域。本领域含有许多人类 CL 区域的实例。用于本文的示例性人类 CL 区域包含 SEQ ID NO :29。

[0178] 根据此实施方式，优选地，VH 区域包括 SEQ ID NO :15 的序列，其连接于 SEQ ID NO :27 或 28 的 CH 区域，及 VL 区域包括 SEQ ID NO :16 的序列，其连接于 SEQ ID NO :29 的 CL 区域。

[0179] 在上述各种实施方式中，CH 区域及其片段的讨论也包含使用任一的变体的选项。该变体包括与所述 CH 区域或其片段具有少于 100% 序列同一性的序列，例如大于 50%、60%、70%、80%、85%、90%、95%、96%、97%、98% 或 99% 序列同一性。因此，与所述 CH 区域或其片段相比，CH 区域或其片段的变体可具有一个或多个(例如 2、3、4、5、6、7、8、9、10、15、20、25、30、40、50、60、70、80、90、100、110、120、130、140、150、160 或更多个)序列变异。与所述 CH 区域及其片段相比，序列中的变异可能归因于一个或多个氨基酸的添加、一个或多个氨基酸的缺失和 / 或一个或多个氨基酸的取代。在多于一个变异的情况下，则该变异可在连续或非连续的位置。

[0180] 同样地，在上述各种实施方式中，CL 区域及其片段的讨论也包含使用任一的变体的选项。该变体包括与所述 CL 区域或其片段具有少于 100% 序列同一性的序列，例如大于 50%、60%、70%、80%、85%、90%、95%、96%、97%、98% 或 99% 序列同一性。因此，与所述 CL 区域或其片段相比，CL 区域或其片段的变体可具有一个或多个(例如 2、3、4、5、6、7、8、9、10、15、20、25、30、40、50、60 或更多个) 序列变异。与所述 CL 区或其片段相比，序列中的变异可能归因于一个或多个氨基酸的添加、一个或多个氨基酸的缺失和 / 或一个或多个氨基酸的取代。在多于一个变异的情况下，则该变异可在连续或非连续的位置。

[0181] 在根据上述实施方式的抗体或抗体片段中，优选地，VH 区域、VL 区域或优选地 VH 及 VL 两区域皆包括与所述 SEQ ID NO，或所述 SEQ ID NO 为对应于单个 CDR 序列的情况下，与一种或多种(例如，2 种或 3 种)各个所述 SEQ ID NO 具有 100% 序列同一性的氨基酸序列。

[0182] 或者，VH 区域、VL 区域或 VH 及 VL 两区域可包括与所述 SEQ ID NO，或在所述 SEQID NO 为对应于单个 CDR 序列的情况下，与一种或多种(例如，2 种或 3 种) 各个所述 SEQID NO 具有少于 100% 序列同一性的氨基酸序列。

[0183] 根据本发明的第一方面，包括与所述 SEQ ID NO 具有少于 100% 序列同一性的氨基酸序列的序列，可以是与所述 SEQ ID NO 相比具有一个或多个(例如 2、3、4、5、6、7、8、9、10 或更多个)序列变异的序列。与所述 SEQ ID NO 相比，序列中的变异可能归因于一个或多个(例如 2、3、4、5、6、7、8、9、10 个或更多) 氨基酸的添加、一个或多个(例如 2、3、4、5、6、7、8、9、10 个或更多) 氨基酸的缺失和 / 或一个或多个(例如 2、3、4、5、6、7、8、9、10 个或更多) 氨基酸的取代。在多于一个变异的情况下，该变异可在连续或非连续的位置。

[0184] 在与选自 SEQ ID NO :1 至 16 的所述 SEQ ID NO 具有少于 100%，但至少 80%、85%、90%、95% 序列同一性的变体抗原结合区的序列中的一种或多种变异可存在于，或只存在于形成一种或多种框架区的氨基酸序列中。框架区包括不形成如本文定义的 CDR 的氨基酸区域。

[0185] 另外地或替代地，在与选自 SEQ ID NO :1 至 16 的所述 SEQ ID NO 具有少于 100%，但至少 80%、85%、90%、95% 序列同一性的抗原结合区的序列中的一种或多种变异可存在于，或只存在于形成一种或多种互补决定区 (CDR) 的氨基酸序列中。SEQ ID NO :1 至 16 中的 CDR 是如上述定义且同时显示于以下的表 2 和表 3 中。

[0186] 在本发明第一方面的所有实施方式中，通常，在框架区比在 CDR 中能承受更高水平的序列修饰而没有实质性改变抗体或抗体片段的结合特性和 / 或体内功效。

[0187] 因此，例如，在另一个实施方式中，本发明第一方面的抗体或抗体片段中的一个(a)、该(the)或各个(each)CDR 与定义于 SEQ ID NO :17 至 26 之一的“亲代”CDR 序列比较，可包括多达 1、2、3、4、5、6、7、8、9 或 10 个氨基酸取代、插入和 / 或缺失，且优选不多于 5、4、3、2 或 1 个氨基酸取代、插入和 / 或缺失 ；优选地，与相应定义的 SEQ ID NO 相比，CDR 序列中的氨基酸取代、插入和 / 或缺失的数目不减少序列同一性至少于 50%、60%、70%、75%、80%、85%、90%、95%。

[0188] 另外地，和 / 或替代地，本发明第一方面的抗体或抗体片段中的一个、该或各个框架区，与存在于 VH 或 VL 定义的任一的 SEQ ID NO :1 至 16 的相应框架序列相比，可包括多达 1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20 或更多个氨基酸取代、插入和 / 或缺失，以及任选地不多于 10、9、8、7、6、5、4、3、2 或 1 个氨基酸取代、插入和 / 或缺失 ；优选地，与相应定义的 SEQ ID NO 相比，在任何框架区的氨基酸取代、插入和 / 或缺失的数目都不减少序列同一性达至少于 10%、20%、30%、40%、50%、60%、70%、75%、80%、85%、90%、95%。

[0189] 取代无论是在一个或多个框架区还是互补决定区，均可为保守或非保守取代。“保守取代”是指例如 Gly、Ala ；Val、Ile、Leu ；Asp、Glu ；Asn、Gln ；Ser、Thr ；Lys、Arg ；及 Phe、Tyr 的组合。

[0190] 例如，可引进序列变异以使抗原结合区序列更接近种系(germline)序列，以改善包括变体抗原结合区的抗体或抗体片段的稳定性，降低包括变体抗原结合区的抗体或抗体片段的免疫原性，和 / 或避免或降低在制造过程中的不利性质。实施例中显示了适合的序列变异的非限制性实例，参考引进 M99-B05 的重链和 / 或轻链序列中的变异以产生 X19-A01、X19-A03、X19-A05、X19-A07、X19-A09、X19-A11 和 / 或 X19-C01。

[0191] 可使用如下所示的蛋白质工程及定点突变的方法或本领域公知的替代方法制备此类变体。

[0192] 在本发明第一方面的抗体或抗体片段的 VH 区域、VL 区域或 VH 及 VL 两区域包括与所述 SEQ ID NO、一种或多种各个所述 SEQ ID NO 具有少于 100% 序列同一性的一种或多种氨基酸序列的情况时，在一个实施方式中，抗体或抗体片段结合磷酰胆碱和 / 或磷酰胆碱结合物的能力可以例如实质上等于(即，至少 80%、85%、90% 或 95%)或大于相应“亲代”抗体或抗体片段的能力，其中相应“亲代”抗体或抗体片段的 VH 区域和 VL 区域各包括与所述 SEQ ID NO 或各个所述 SEQ ID NO 具有 100% 序列同一性的氨基酸序列。

[0193] 因此，例如，在抗体或抗体片段基于 X19-A05 抗体，及 VH 区域包括与 SEQ ID NO :1 具有少于 100% 但至少 80%、85%、90% 或 95% 序列同一性的氨基酸序列 ；和 / 或 VL 区域包括与 SEQ ID NO :2 具有少于 100% 但至少 80%、85%、90% 或 95% 序列同一性的氨基酸序列的情况时，该抗体或抗体片段结合磷酰胆碱和 / 或磷酰胆碱结合物的能力可以例如等于相应“亲代”抗体或抗体片段的结合能力，而该“亲代”抗体或抗体片段具有包括 SEQ ID NO :1 的序列的 VH 区域及包括 SEQ ID NO :2 的序列的 VL 区域。在本文中，“相应“亲代”抗体或抗体片段”是指正在做的“抗体或抗体片段”与“相应“亲代”抗体或抗体片段”间的唯一序列差异在于 VH 和 / 或 VL 区域之一或两者。在一个实施方式中，相应亲代抗体为具有 X19-A05 的 VH、VL、CH 及 CL 区域序列的抗体，即，SEQ ID NO :1 的 VH 区域(其连接于 SEQ ID NO :28 的 CH 区域)及 SEQ ID NO :2 的 VL 区域(其连接于 SEQ ID NO :29 的 CL 区域)。

[0194] 在做必要修正的情况下(muatatis mutandis)，相同内容应用于列于上文的其它

抗体或抗体片段，其中 VH 和 / 或 VL 区域包括与所述 SEQ ID NO 或一种或多种各个所述 SEQ ID NO 具有少于 100% 序列同一性的一种或多种氨基酸序列，而用于测定与磷酰胆碱和 / 或磷酰胆碱结合物的结合等效的“相应“亲代”抗体或抗体片段”只在 VH 和 / 或 VL 区域的序列之一或两者存在不同，并具有包括与该所述 SEQ ID NO 或各个所述 SEQ ID NO 具有 100% 序列同一性的氨基酸序列的该序列或各个序列。

[0195] 因此，在抗体或抗体片段基于 M99-B05 时，那么在一个实施方式中，相应亲代抗体为具有 M99-B05 的 VH、VL、CH 及 CL 区域序列的抗体，即，SEQ ID NO :3 的 VH 区域连接于 SEQ ID NO :27 的 CH 区域，及 SEQ ID NO :4 的 VL 区域连接于 SEQ ID NO :29 的 CL 区域。

[0196] 就这一点而言，抗体或抗体片段结合磷酰胆碱和 / 或磷酰胆碱结合物的能力可利用任何适当的方法，例如表面等离子体共振（SPR）分析来测量抗体或抗体片段与固定（例如经由胺基苯基连接剂）于固体表面例如 Biacore SPR 生物传感器的磷酰胆碱的结合来测定。

[0197] 在另外的实施方式中，本发明第一方面的抗体或抗体片段是与“比较者（comparator）”抗体或抗体片段竞争结合如本文定义的 PC 或 PC 结合物（如，用 ELISA 或 SPR 试验确定的）。在本文中，比较者抗体或抗体片段可包括以下抗体的 VH 及 VL 区域，且视需要也包含 CH 及 CL 区域 :X19-A05（分别由 SEQ ID NO :1、2、28 及 29 所定义）、M99-B05（由 SEQ ID NO :3、4、27 及 29 所定义）、X19-A01（分别由 SEQ ID NO :5、6、27 及 29 所定义）、X19-A03（分别由 SEQ ID NO :7、8、27 及 29 所定义）、X19-A07（分别由 SEQ IDNO :9、10、27 及 29 所定义）、X19-A09（分别由 SEQ ID NO :11、12、27 及 29 所定义）、X19-A11（分别由 SEQ ID NO :13、14、27 及 29 所定义）或 X19-C01（分别由 SEQ ID NO :15、16、27 及 29 所定义），并优选与经测试的抗体或抗体片段只在 VH 和 / 或 VL 区域中的序列变异不同。“竞争”是指，在试验中包含等摩尔量的本发明第一方面的抗体或抗体片段及“比较者”抗体可降低“比较者”抗体结合至 PC 或 PC 结合物的可检测水平为 10%、20%、30%、40%、50%、60%、70%、80%、90%、95% 或更多，例如实质上 100%，其是与比较抗体在没有本发明第一方面的抗体或抗体片段存在下在相同试验中结合 PC 或 PC 结合物的可检测水平相比较。

[0198] 如下述实施例所讨论的，M99-B05 结合胺基苯基磷酰胆碱的表观 Kd 约 150nM。在一个实施方式中，当在提供具有 M99-B05（分别由 SEQ ID NO :3 及 4 所定义）的 VH 及 VL 区域的抗体或抗体片段以表观 Kd 约 150nM 结合在固定的胺基苯基磷酰胆碱的条件下（例如使用在实施例中的 SPR 条件）测验时，本发明的抗体或抗体片段将以表观 Kd 不大于约 500nM、约 400nM、约 300nM、约 250nM、约 200nM、约 190nM、约 180nM、约 170nM、约 160nM、约 155nM、约 150nM 或更少来结合固定的胺基苯基磷酰胆碱。在本文中，术语“约”用于指所述数值的 ±20%、15%、10%、5%、4%、3%、2% 或 1% 以内的数值。

[0199] 也如下述实施例所讨论的，M99-B05 可响应 IC50 在 nM 范围的 oxLDL 的刺激而阻止 MCP-1 从单核细胞释出。另一个实施方式中，当在提供具有 M99-B05（分别由 SEQ ID NO :3 及 4 所定义）的 VH 及 VL 区域的抗体或抗体片段的 IC50 在 0.7 至 2.6nM 范围的条件下（如下列实施例所示）试验时，本发明的抗体或抗体片段以 IC50 少于约 10nM、约 5nM、约 4nM、约 3nM、约 2.8nM、约 2.6nM、约 2.4nM、约 2nM、约 1.8nM、约 1.6nM、约 1.4nM、约 1.3nM、约 1.2nM、约 1.1nM、约 1.0nM、约 0.9nM、约 0.8nM、约 0.7nM 或更少来响应 oxLDL 的刺激而阻止 MCP-1 从单核细胞释出。在本文中，术语“约”用于指所述数值的 ±20%、15%、10%、5%、4%、3%、2% 或 1% 以内的数值。

[0200] 本发明的抗体或抗体片段结合磷酰胆碱结合物的能力可通过用磷酰胆碱结合物置换磷酰胆碱，用与上述方法等价的方法测定。适合的磷酰胆碱结合物包含以上所讨论的，包括连接于载体的磷酰胆碱部分，任选地经由间隔物，例如 PC-BSA 及 PC-KLH 结合物。优选地，在测定抗体或抗体片段结合磷酰胆碱结合物的能力的情况时，其是参照抗体或抗体片段特异性结合磷酰胆碱结合物中的磷酰胆碱部分的能力而测定的。这可由本领域已知的技术而测定，如通过比较抗体或抗体片段结合磷酰胆碱结合物及不含磷酰胆碱部分的对应分子的能力。

[0201] 在一个实施方式中，本发明的抗体或抗体片段可包括线性多肽序列中的 VH 区域及 VL 区域。

[0202] 在另一个实施方式中，本发明的抗体或抗体片段可包括每个均在独立的多肽序列中的 VH 区域及 VL 区域。在此实施方式中，优选地，独立的多肽序列直接或间接结合在一起(例如由独立的多肽序列间的一个或多个二硫键)。

[0203] 在另一个实施方式中，VH 区域可连结至 CH 区域或其片段，该片段可包括，例如，CH 区域如上文所述的 CH 区域的变体或其片段的至少 10、20、30、40、50、60、70、80、90、100、120、140、160、180、200、220、240、260、280、300、320 个或更多个氨基酸。连结可为经肽键的直接融合，以使 VH 区域及 CH 区域以单一多肽存在，或者连结可经由连接子例如肽或其它连接子，或经由肽键以外的直接化学键。CH 区域无特别限制，虽然在一个实施方式中其为人类 CH 区域。所属领域含有许多人类 CH 区域的实例。用于本文的示例性人类 CH 区域包含 SEQ ID NO :27 及 SEQ ID NO :28。当使用任何 CH 区域时，可引入末端氨基酸修饰(包含缺失其它氨基酸或其它化学部分或经由添加而遮蔽其它氨基酸或其它化学部分)来降低或避免可能的肽酶降解。

[0204] 在另一个实施方式中，VL 区域可连结至 CL 区域或其片段，该片段可包括，例如，CL 区域或如上文所述的 CL 区域的变体或其片段的至少 10、20、30、40、50、60、70、80、90、100 个或更多个氨基酸。连结可为经肽键的直接融合，以使 VL 区域及 CL 区域以单一多肽存在，或者连结可经由连接子例如肽或其它连接子，或经由肽键以外的直接化学键。CL 区域无特别限制，虽然在一个实施方式中其为人类 CL 区域。所属领域含有许多人类 CL 区域的实例。使用于本文的示例性人类 CL 区域包含 SEQ ID NO :29。使用的任何 CL 区域可导入末端氨基酸修饰(包含缺失其它氨基酸或其它化学部分或经由添加而遮蔽其它氨基酸或其它化学部分)来降低或避免可能的肽酶降解。

[0205] 在另一个实施方式中，本发明的抗体或抗体片段可包括连结于一个多肽序列中的 CH 区域的 VH 区域，及连结于另一个独立多肽序列中的连结 CL 区域的 VL 区域。在此实施方式中，优选地，独立的多肽序列直接或间接结合在一起(例如通过独立的多肽序列间的一个或多个二硫键)。

[0206] 在另一个实施方式中，本发明的抗体或抗体片段可包括 ：

[0207] 第一重链，其包括连结至第一 CH 区域的第一 VH 区域，

[0208] 第一轻链，其包括连结至第一 CL 区域的第一 VL 区域 ；

[0209] 第二重链，其包括连结至第二 CH 区域的第二 VH 区域，

[0210] 第二轻链，其包括连结至第二 CL 区域的第二 VL 区域 ；并且

[0211] 其中任选地，第一轻链及第一重链直接或间接结合在一起(例如通过独立的多肽

序列间的一个或多个二硫键）且第二轻链及第二重链直接或间接结合在一起（例如通过独立的多肽序列间的一个或多个二硫键），及进一步任选地，其中第一及第二重链直接或间接结合在一起（例如通过独立的多肽序列间的一个或多个二硫键）。

[0212] 在另一个实施方式中，本发明的抗体或抗体片段可为单克隆抗体，更优选为人类单克隆抗体。

[0213] 本发明的抗体或抗体片段可为人源化抗体或嵌合抗体。

[0214] 在一个优选的实施方式中，本发明的抗体或抗体片段为分离的抗体或抗体片段。

[0215] 在另一个实施方式中，本发明的抗体或抗体片段可包括包含通过使用标准蛋白质工程技术嫁接至免疫球蛋白的蛋白支架上的如上所述的 VH、VL、CDR1、CDR2、CDR3、CDR4、CDR5 和 / 或 CDR6 序列的一个或多个氨基酸序列。技术人员理解，各种蛋白支架可用且是本领域所公知的。最后结果为新框架中保存的抗原结合活性。

[0216] 例如，免疫球蛋白支架可衍生自 IgA、IgE、IgG1、IgG2a、IgG2b、IgG3、IgM。支架可衍生自来自任何哺乳动物的免疫球蛋白，例如小鼠、大鼠、兔、山羊、骆驼、骆马、灵长类。优选地，免疫球蛋白支架衍生自人类免疫球蛋白。

[0217] 可利用标准分子生物学技术或通过使用产生此类片段的酶（如，胃蛋白酶或木瓜蛋白酶）裂解经纯化的抗体而产生本发明第一方面的抗体片段。本发明的此类抗体片段的实例为，但不限于，单链抗体、Fv、scFv、Fab、F（ab’）2、Fab’、Fd、dAb、CDR 或 scFv-Fc 片段或纳米抗体及双特异抗体（diabodies），或任何已经经如聚乙二醇化而稳定的片段。

[0218] 本发明的第二方面提供了药物组合物，其包括本发明第一方面所述的抗体或抗体片段及药学上可接受的载体或赋形剂。任选地，组合物中仅有的抗体或抗体片段为本发明第一方面所述的抗体或抗体片段。更优选地，例如，组合物中存在单一类型的抗体或抗体片段，例如其中类型是参照氨基酸序列、分子量和 / 或结合磷酰胆碱的特异性而测定的。关于这点，技术人员理解，例如归因于 N- 端变异和 / 或部分降解而在任何群体中的抗体或抗体片段序列中可能存在低水平的变异；因此，在本文中，组合物可称为含有单一类型的抗体或抗体片段，如果例如该组合物中以重量计至少约 80%、约 90%、约 95%、约 96%、约 97%、约 98%、约 99% 或实质上 100% 的可检测水平的抗体或抗体片段为单一类型，所述检测参照氨基酸序列、分子量和 / 或结合磷酰胆碱的特异性而决定。

[0219] 本发明的第三方面提供本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物，其用于医药中，例如用于在人体或动物体或其离体样品上进行的治疗、外科手术或诊断的方法中。

[0220] 例如，本发明第三方面提供本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物，其用于防止、预防和 / 或治疗哺乳动物（包括人类）的动脉粥样硬化、动脉粥样硬化相关疾病或心血管疾病。

[0221] 换言之，本发明第三方面提供了本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物在制备用于防止、预防和 / 或治疗哺乳动物（包括人类）的动脉粥样硬化、动脉粥样硬化相关疾病或心血管疾病的药物中的用途。

[0222] 也提供的是防止、预防和 / 或治疗哺乳动物（包括人类）的动脉粥样硬化、动脉粥样硬化相关疾病或心血管疾病的方法，该方法包括将本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物施与哺乳动物的步骤。

[0223] 本发明第三方面也提供了本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物，其用于预防、防止和 / 或治疗阿尔茨海默病（Alzheimer）。

[0224] 换言之，本发明第三方面提供了本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物在制备用于预防、防止和 / 或治疗阿尔茨海默病的药物中的用途。

[0225] 也提供的是免疫及预防、防止和 / 或治疗受试者以抗阿尔茨海默病的方法，该方法包括将本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物施与个体的步骤。

[0226] 本发明第三方面也提供了本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物，其用于免疫或预防，或防止或治疗哺乳动物（包括人类）的代谢疾病。

[0227] 换言之，本发明第三方面提供本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物在制备用于预防、防止或治疗哺乳动物（包括人类）的代谢疾病的药物中的用途。

[0228] 也提供的是免疫或预防，或治疗哺乳动物（例如人类）的代谢疾病的方法，该方法包括将本发明第一方面的抗体或抗体片段或本发明第二方面的药物组合物施与哺乳动物的步骤。

[0229] 依据本发明第三方面有待解决和 / 或治疗的代谢疾病可例如为选自下列的症状：代谢综合征、胰岛素抗药性、葡糖耐受不良、高血糖症、I 型糖尿病、II 型糖尿病、高脂血症、高甘油三脂血症、高胆固醇血症、血脂异常及多囊性卵巢综合征（PCOS）。

[0230] 本发明第四方面提供核酸分子，其包括编码本发明第一方面的抗体或抗体片段，或抗体或抗体片段的多肽链形成部分的序列。核酸分子可例如为 DNA 或 RNA。核酸分子可包括位于编码本发明第一方面的抗体或抗体片段或其部分的序列的 5’和 / 或 3’的其他序列。此种 5’和 / 或 3’序列可包含转录和 / 或翻译调节序列，例如本领域公知的启动子和 / 或终止子序列，且可例如被加以选择以在所选择的宿主细胞内具有功能性。因此，核酸分子可包括表达盒，该表达盒在转化至所选择的宿主细胞后可利用宿主细胞的转录和 / 或翻译系统而表达，以产生本发明第一方面所编码的抗体或抗体片段，或抗体或抗体片段的多肽链形成部分。

[0231] 本发明第五方面提供了包括本发明第四方面的一个或多个核酸分子序列的载体或质粒。在抗体或抗体片段包括多于一条多肽链时，载体或质粒可例如包括编码各个多肽链的核酸编码序列，以使经载体或质粒转化的宿主细胞可表达所有存在于抗体或抗体片段内的多肽链。

[0232] 因此，第五方面也提供载体或质粒在转化宿主细胞中的用途。用载体或质粒转化宿主细胞的方法是本领域公知的。为了帮助筛选转化的宿主细胞，载体或质粒可包括筛选标记。

[0233] 本发明第六方面提供包括本发明第五方面的一种或多种载体或质粒的宿主细胞。第六方面也提供包括一种或多种本发明第五方面的载体或质粒的细胞培养物，例如单一培养物，其中全部或实质上全部的细胞包括相同的一种或多种本发明第五方面的载体或质粒。可通过例如以下方式得到此种单一培养物：筛选细胞中一种或多种载体或质粒上一种或多种选择标记的存在，并且任选地，在培养筛选的细胞的生长期间维持筛选压力。

[0234] 在本发明第一方面的抗体或抗体片段包括多于一条多肽链时，可用单一载体或质

粒转化宿主细胞，而该载体或质粒包括编码各个多肽链的核酸编码序列，以使用载体或质粒转化的宿主细胞可表达所有存在于抗体或抗体片段内的多肽链。

[0235] 或者，在本发明第一方面的抗体或抗体片段包括多于一条多肽链时，可用多于一种载体或质粒转化宿主细胞，各个载体或质粒包括编码至少一个多肽链的核酸编码序列，以使用多于一种载体或质粒转化的宿主细胞可表达所有存在于抗体或抗体片段内的多肽链。

[0236] 进一步替代地，在本发明第一方面的抗体或抗体片段包括多于一条多肽链时，可用载体或质粒转化多个宿主细胞的每一个，而各个载体或质粒包括不同核酸编码序列，这些核酸编码序列各自编码形成抗体或抗体片段的不同多肽链的一个或多个成员，并且分开培养的各个不同宿主细胞表达各个多肽链。然后，可组合回收的不同多肽链而制备抗体或抗体片段。

[0237] 任何适当的宿主细胞均可使用在本发明第五方面和 / 或第六方面。例如，宿主细胞可为原核细胞，例如大肠杆菌（Escherichia coli）细胞。宿主细胞可为真核细胞，例如动物细胞、植物细胞及真菌细胞。适当的动物细胞可包含哺乳动物细胞、禽类细胞及昆虫细胞。适当的哺乳动物细胞可包含 CHO 细胞及 COS 细胞。适当的真菌细胞可包含酵母细胞，例如啤酒酵母菌（Saccharomyces cerevisiae）细胞。哺乳动物细胞可包含或不包含人类细胞，并可包含或不包含胚胎细胞。

[0238] 本发明第七方面提供了制备本发明第一方面的抗体或抗体片段、抗原结合序列的方法，其包括培养如上所述的一种或多种转化的宿主细胞，并由此回收本发明第一方面的抗体或抗体片段。

[0239] 本发明第八方面提供了制备本发明第一方面的抗体或抗体片段的变体的方法，该变体保留结合磷酰胆碱和 / 或磷酸胆碱结合物的能力，该方法包括：

[0240] （i）提供本发明第四方面的核酸，其编码亲代抗体或抗体片段、或抗体或抗体片段的多肽链形成部分；

[0241] （ii）引入一个或多个核苷酸突变（任选地，达 50、40、30、20、15、10、9、8、7、6、5、4、3、2 或 1 个核苷酸突变）至核酸序列的氨基酸编码区（任选地在编码 VH 和 / 或 VL 区域的区域内），以使该突变的核酸编码与亲代抗体或抗体片段相比具有不同氨基酸序列的变体抗体或抗体片段；

[0242] （iii）表达由突变的核酸所编码的变体抗体或抗体片段、或变体抗体或抗体片段的多肽链形成部分；和

[0243] （iv）比较变体抗体或抗体片段与亲代抗体或抗体片段结合磷酰胆碱和 / 或磷酰胆碱结合物的能力。

[0244] 根据本发明第八方面，核苷酸突变可随机地或以定点方式引入核酸序列的氨基酸编码区。与突变前的核酸所编码的氨基酸序列比较，此类突变可导致编码区编码下述氨基酸序列：其含有一个或多个氨基酸的添加、一个或多个氨基酸的缺失和 / 或一个或多个氨基酸的取代。

[0245] 此类核苷酸突变可导致或不导致编码下述氨基酸序列的编码区：其含有一个或多个抗原结合区序列中的变异。此类核苷酸突变可例如导致氨基酸序列变异（即，一个或多个氨基酸的添加、一个或多个氨基酸的缺失和 / 或一个或多个氨基酸的取代）存在于，或只存

在于形成一个或多个框架区的氨基酸序列中。另外地或替代地，此类核苷酸突变可例如导致氨基酸序列变异(即，一个或多个氨基酸的添加、一个或多个氨基酸的缺失和/或一个或多个氨基酸的取代)存在于，或只存在于形成一个或多个互补决定区的氨基酸序列中。框架区、CDR和/或VH或VL区域耐受的氨基酸变异/修饰的水平如上述本发明第一方面中所讨论，且可经必要的修正后应用于可根据本发明第八方面的方法引入的变异/修饰的水平。

[0246] 另外地或替代地，此类核苷酸突变可导致或不导致编码区编码下述氨基酸序列：其在抗原结合区以外的抗体或抗体片段的一个或多个部分中含有一个或多个变异，例如在CH1、CH2、CH3、CL区域或其它区域的一个或多个中。

[0247] 在一种或多种核苷酸突变导致编码产物中的一个或多个氨基酸取代时，则该一个或多个取代可分别独立的为保守或非保守取代。“保守取代”是指如Gly、Ala；Val、Ile、Leu；Asp、Glu；Asn、Gln；Ser、Thr；Lys、Arg；及Phe、Tyr的组合。

[0248] 例如，可引进核苷酸突变以使所编码抗体或抗体片段的序列靠近种系序列(germlinesequences)，以改善包括变体抗原结合区的抗体或抗体片段的稳定性，降低包括变体抗原结合区的抗体或抗体片段的免疫原性，和/或避免或降低于制造过程中可能不利的性质。

[0249] 可使用本领域公知的方法制备此类核苷酸突变。

[0250] 根据本发明第八方面，评估变体抗体或抗体片段结合磷酰胆碱和/或磷酰胆碱结合物的能力的步骤可进一步包括选择那些与秦代相比，具有实质上相等或增强的结合磷酰胆碱和/或磷酰胆碱结合物的能力的变体。

[0251] 可用例如上述本发明第一方面中所讨论的方法评估变体和亲代结合磷酰胆碱和/或磷酰胆碱结合物的能力。

[0252] 本发明第八方面的方法可任选地进一步包括回收核酸分子，该核酸分子包括编码变体抗体或抗体片段的突变核酸序列，以及任选地用包括该回收的核酸分子的组合物转化宿主细胞，以及进一步任选地使宿主细胞表达变体抗体或抗体片段，还进一步任选地从宿主细胞回收所表达的变体抗体或抗体片段，还进一步任选地将回收的变体抗体或抗体片段配制成药学上可接受的组合物。

[0253] 本发明第八方面也提供通过本发明第八方面的方法得到或可得到的变体抗体或抗体片段，或通过本发明第八方面的方法得到或可得到的药学上可接受的组合物，其用于医药中。

[0254] 本发明第八方面也提供通过本发明第八方面的方法得到或可得到的变体抗体或抗体片段，或通过本发明第八方面的方法得到或可得到的药学上可接受的组合物，其用于：

[0255] (i)防止、预防和/或治疗哺乳动物(包括人类)的动脉粥样硬化、动脉粥样硬化相关疾病或心血管疾病；

[0256] (ii)预防、防止和/或治疗阿尔茨海默病；和/或

[0257] (iii)免疫或预防，或防止或治疗哺乳动物(包括人类)的代谢疾病。

[0258] 换言之，本发明第八方面也提供利用本发明第八方面的方法得到或可得到的变体抗体或抗体片段，或利用本发明第八方面的方法得到或可得到的药学上可接受的组合物在制备药物中的用途，该药物用于：

[0259] (i)防止、预防和/或治疗哺乳动物(包括人类)的动脉粥样硬化、动脉粥样硬化相关疾病或心血管疾病;

[0260] (ii)预防、防止和/或治疗阿尔茨海默病;和/或

[0261] (iii)免疫或预防针对,或防止或治疗哺乳动物(包括人类)的代谢疾病。

[0262] 因此,本发明第八方面也提供的是方法,其用于:

[0263] (i)防止、预防和/或治疗哺乳动物(包括人类)的动脉粥样硬化、动脉粥样硬化相关疾病或心血管疾病;

[0264] (ii)免疫及预防、防止和/或治疗阿尔茨海默病;和/或

[0265] (iii)免疫或预防针对,或治疗哺乳动物(例如人类)的代谢疾病,

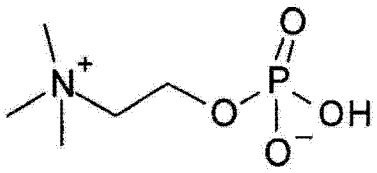
[0266] 该方法包括将利用本发明第八方面的方法得到或可得到的变体抗体或抗体片段或利用本发明第八方面的方法得到或可得到的药学上可接受的组合物施与哺乳动物或受试者的步骤。

[0267] 根据本发明第八方面有待解决和/或有待治疗的代谢疾病可例如为选自下列的症状:代谢综合征、胰岛素抗药性、葡糖耐受不良、高血糖症、I型糖尿病、II型糖尿病、高脂血症、高甘油三脂血症、高胆固醇血症、血脂异常及多囊性卵巢综合征(PCOS)。

[0268] 磷酰胆碱

[0269] 磷酰胆碱(PC)是指根据下述结构式的磷酰胆碱。

[0270]



[0271] 磷酰胆碱结合物是指连接于载体的磷酰胆碱部分,优选经由间隔物连接。磷酰胆碱部分可共价地或非共价地连接于载体。优选地,磷酰胆碱部分经由磷酸基连接于载体。

[0272] 载体可例如为蛋白质、碳水化合物、聚合物、乳胶颗粒或胶体金属。

[0273] 磷酰胆碱结合物可例如为蛋白质-PC结合物,例如人类血清白蛋白(HSA)-PC结合物、运铁蛋白-PC结合物、钥孔血蓝蛋白(keyhole limpet hemocyanin,KLH)-PC结合物或牛血清白蛋白(BSA)-PC结合物。

[0274] 在PC结合物包括经由间隔物连接至载体的PC时,可使用任何合适的间隔物。间隔物的非限制性实例包含耦合剂(典型地,双功能性化合物),例如二羧酸类,如丁二酸及戊二酸,对应的二醛、二胺,例如1,6-二胺己烷,双取代酚类,例如对胺基酚、对重氮酚、对苯二胺、对苯醌等。

[0275] 心血管疾病

[0276] 术语心血管疾病是指包含但不限于动脉粥样硬化、急性冠状动脉综合征、急性心肌梗塞、心肌梗塞(心脏病)、稳定及不稳定型心绞痛、动脉瘤(aneurysms)、冠状动脉疾病(CAD)、缺血性心脏疾病、缺血性心肌、心因性或突发心因性死亡、心肌症、血性心脏衰竭、心脏衰竭、狭窄症、周边动脉疾病(PAD)、间歇性跛行(intermittent claudication)、危急性肢体缺血症状及中风。

[0277] 使用与磷酰胆碱及磷酰胆碱结合物有反应性的抗体治疗或防止心血管疾病已讨

论于，例如，WO2005/100405 及 US2007-0286868，两者的内容均以参考的方式并入本文中。

[0278] 阿尔茨海默病

[0279] 依据本发明，可使用第一方面的抗体或抗体片段治疗或防止有需要的个体的阿尔茨海默病或其风险。

[0280] WO2010/003602 及美国专利申请案第 61/078677 号说明了使用与磷酰胆碱及磷酰胆碱结合物有反应性的抗体治疗或防止阿尔茨海默病，两者的内容均以参考的方式并入本文中，作为可使用第一方面的抗体或抗体片段治疗或防止阿尔茨海默病的方式的进一步公开内容。

[0281] 代谢疾病

[0282] 术语代谢疾病包含但不限于代谢综合征 X、胰岛素抗药性（IRS）、葡糖耐受不良、高血糖症、I 型糖尿病、II 型糖尿病、高脂血症、高甘油三脂血症、高胆固醇血症、血脂异常、多囊性卵巢症（PCOS）及相关疾病。

[0283] WO2012/010291 中进一步讨论了可用与磷酰胆碱及磷酰胆碱结合物有反应性的抗体治疗的代谢疾病，其内容通过引用并入本文，作为可使用第一方面的抗体或抗体片段治疗或防止代谢疾病的方式的进一步公开内容。

[0284] 氨基酸序列同一性

[0285] 两条氨基酸序列间的百分比同一性的测定如下。首先，使用 BLAST2Sequences（Bl2seq）程序将一条氨基酸序列与例如 SEQ ID NO :1 比较，BLAST2Sequences（Bl2seq）程序来自含有 BLASTN2.0.14 版本及 BLASTP2.0.14 版本的 BLASTZ 单机版本。此 BLASTZ 单机版本可从美国政府的国家生物技术信息中心网站（National Centre for Biotechnology Information）ncbi.nlm.nih.gov 获得。可在 BLASTZ 所附的说明文档中找到如何使用 Bl2seq 程序的说明。Bl2seq 使用 BLASTP 算法执行两条氨基酸序列间的比较。为了比较两条氨基酸序列，Bl2seq 的选项设定如下 :-i 设定到含有待比较的第一个氨基酸序列（如，C :\seq1.txt）的文件 ;-j 设定到含有待比较的第二个氨基酸序列（如，C :\seq2.txt）的文件 ;-p 设定到 blastp ;-o 设定到任何所需的文件名（如，C :\output.txt）;以及所有其它选项均保留其默认值。例如，可使用以下指令产生含有两条氨基酸序列间比较的输出文件 :C :\Bl2seq-i c :\seq1.txt-j c :\seq2.txt-p blastp-o c :\output.txt。如果两条比较的序列分享同源性，则该指定的输出文件呈现那些同源性区域为比对（aligned）序列。如果两条比较的序列不分享同源性，则该指定的输出文件不会呈现比对序列。一旦比对，则通过计算两条序列中均存在的相同核苷酸或氨基酸残基的位置数目来测定匹配的数目。

[0286] 同一性百分比通过将相配的数目除以鉴定的序列中所列出的序列长度，随后将所得值乘以 100 而测得。例如，如果一条序列与 SEQ ID NO :A 列出的序列（SEQ ID NO :A 列出的序列长度为 10）比较且匹配的数目为 9，则该序列与 SEQ ID NO :A 列出的序列具有 90%（即，9÷10*100=90）的百分比同一性。

[0287] 抗体

[0288] 本发明说明书中所称的术语“抗体或抗体片段”包含完整的抗体及任何称为“抗原结合区”的抗原结合片段或其单链。

[0289] “抗体”可指包括经二硫键互连的至少两个重（H）链及两个轻（L）链，或其抗原结合部分的蛋白。每条重链由重链可变区域（本文简称为 VH）及重链恒定区域所组成。重链

恒定区域由 3 个区域 CH1、CH2 及 CH3 组成。每条轻链由轻链可变区域(本文简称为 VL)及轻链恒定区域所组成。轻链恒定区域由 1 个区域 CL 所组成。

[0290] VH 及 VL 区域可再细分为称为互补决定区(CDR)的高可变区,在其间散布着更保守的区域(称为框架区(FR))。每个 VH 典型地包括 3 个 CDR 及 4 个 FR,其自氨末端至羧末端按以下列次序排列 :FR1、CDR1、FR2、CDR2、FR3、CDR3、FR4。同样地,每个 VL 一般包括 3 个 CDR 及 4 个 FR,其自氨末端至羧末端按以下列次序排列 :FR5、CDR4、FR6、CDR5、FR7、CDR6、FR8。重链及轻链的可变区域含有与抗原互相作用的结合区域。抗体的恒定区域可介导免疫球蛋白与宿主组织或因子的结合,其包含多种免疫系统细胞(如,效应细胞)及经典补体系统的第一组分(C1q)。

[0291] 本文中使用的术语“抗原结合区”是指抗体的一个或多个保留特异结合抗原的能力的片段。已显示全长抗体的片段可执行抗体的抗原结合功能。术语抗体的“抗原结合区”所涵盖的结合片段的实例包含 :

[0292] (i)Fab 片段,其是由 VL、VH、CL 及 CH1 区域构成的单价片段 ;

[0293] (ii)F (ab')2 片段,其是包括由二硫键于铰链区(hingeregion)连接的两个 Fab 片段的二价片段 ;

[0294] (iii)Fab' 片段,其实质上为带有部分铰链区的 Fab ;

[0295] (iv)由 VH 及 CH1 区域构成的 Fd 片段 ;

[0296] (v)由抗体单臂的 VL 及 VH 区域构成的 Fv 片段 ;

[0297] (vi)由 VH 区域构成的 dAb 片段 ;

[0298] (vii)分离的互补决定区(CDR);及

[0299] (viii)纳米抗体(nanobody),其是含有单一可变区域及 2 个恒定区域的重链可变区。

[0300] 进一步地,虽然 Fv 片段的两个区域 VL 及 VH 是由独立的基因编码,但是它们可使用重组方法并利用合成连接子进行连接,所述连接子可将两者制备为单条蛋白质链,其中 VL 及 VH 区域配对而形成单价分子(称为单链 Fv (scFv))。此类单链抗体也为术语抗体的“抗原结合部分”所涵盖。

[0301] 双特异抗体由两个多肽组成,每条多肽包括以肽连接子连接重链可变(VH)区域与同一多肽链(VH-VL)上的轻链可变(VL)区域。这些抗体片段通过使用本领域技术人员所知的常见技术而得到,且筛选以与完整抗体相同方式起作用的片段。

[0302] 本文中使用的“分离抗体”指实质上无其它具有不同抗原特异性的抗体的抗体(如,特异性结合磷酰胆碱的分离抗体实质上无特异性结合磷酰胆碱以外的抗原的抗体)。此外,分离抗体可实质上无其它细胞物质和 / 或化学物质。

[0303] 本文中使用的术语“单克隆抗体”或“单克隆抗体组合物”是指单一分子组成的抗体分子制备物。单克隆抗体组成显示针对特定表位的单一结合特异性及亲合性。

[0304] 术语“人源化抗体”指其中衍生自另一哺乳动物物种(例如小鼠)的种系的 CDR 序列嫁接至人类框架区序列的抗体。可在人类框架区序列中制备另外的框架区修饰。

[0305] 术语“嵌合抗体”指其中可变区域序列衍生自一个物种而恒定区域序列衍生自另一个物种的抗体,例如其中可变区域序列衍生自小鼠抗体而恒定区域序列衍生自人类抗体的抗体。

[0306] 药物组合物

[0307] 本发明的药物组合物可包括本发明的结合蛋白即药学上可接受的载体和 / 或赋形剂，所述药学上可接受的载体和 / 或赋形剂通常通过考虑预定的给药途径和标准制药实践而加以选择。该组合物可为即释、延迟释放或控释应用的形式。优选地，该配方为含有活性成分每日剂量或单位、每日亚剂量或其适当部分的单位剂量。

[0308] 本发明的药物组合物被或不被配制为适用于胃肠外、静脉内、动脉内、腹膜内、肌内、脑室内或皮下给药的形式，或它们可以经输注技术而施用。其最佳施用形式可以为无菌水溶液形式，无菌水溶液可含有其它物质，例如，足够的盐或葡萄糖以使溶液与血液或脑脊液（CSF）等渗。如有必要，该水溶液可适当地缓冲（优选为 pH3 至 9）。无菌条件下的适当制药配方的制备可以以本领域技术人员公知的标准制药技术容易地完成。

[0309] 此类配方可包含水性及非水性无菌注射溶液，其可含有抗氧化剂、缓冲剂、抑菌剂及可使配方与预定接受者的血液或 CSF 等渗的溶质；及水性及非水性无菌悬浮液，其可包含悬浮剂及增稠剂。可以以单位剂量或多剂量容器提供该配方，例如密封的安瓿和小瓶，且可储存于冷冻干燥（冻干（lyophilised））条件下，而只需在使用前添加无菌液体载体，例如注射用水。临时注射溶液及悬浮液可从先前公开的种类的无菌粉末、颗粒及片剂进行制备。

[0310] 施用于患者（例如人类患者）的本发明的抗体或抗体片段的治疗有效量基于每日剂量水平可为每个成人 0.01 至 1000mg 的抗体或抗体片段（例如，每公斤患者体重约 0.001 至 20mg，例如 0.01 至 10mg/kg，例如大于 0.1mg/kg 及少于 20、10、5、4、3 或 2mg/kg，例如约 1mg/kg），以单次或分次剂量给药。

[0311] 任意事件中的医生均会确定最适合任何个体患者的实际剂量而该剂量会因特定患者的年龄、体重及反应而变化。上述剂量为平均情况的范例。当然，存在较高或较低剂量更为有利的个别实例，这样的实例也包括在本发明范围内。

附图说明

[0312] 图 1 为经由 Biacore 的平衡结合分析评估结合亲合力。

[0313] （◆）M99-B05（批 号 :W21573）（Kd=160±32nM），（○）M99-B05（批 号 :W22595）（Kd=148±8nM）。图比较该抗体的两种不同制剂。

[0314] 图 2 为由 ELISA 测量的结合 PC-BSA 的纯化 IgG。

[0315] （●）M4-G02（EC_{50}=0.14nM），（○）M73-G03（EC_{50}=0.91nM），（△）M99-B05（EC_{50}=0.11nM）。数据以整体 B_{max} 拟合至 4 个参数逻辑方程式而得到 EC_{50} 估值。

[0316] 图 3 为 CD45 阳性白细胞流入股动脉套管小鼠中层（medial）的抑制。

[0317] 以含有 1% 胆固醇及 0.05% 胆酸盐的高胆固醇及高脂质饮食喂食转基因的雄性 ApoE*3Leiden 小鼠，以引发高胆固醇血症。经 3 星期高脂质饮食后，麻醉小鼠并自股动脉周围解剖股动脉且松松地套上非缩窄性聚乙烯套管（Portex，内径 0.40mm，外径 0.80mm 及长度 2.0mm）。于第 0 天时，经 IP 注射来用溶于 PBS 的 10mg/kg 重组抗 -PCIgG 抗体、溶于 PBS 的 10mg/kg 抗链霉亲和素 A2IgG 抗体或只有 PBS 来处理小鼠。手术后 3 天处死小鼠且取下套管的股动脉并以石蜡包埋。自套管的股动脉片段的全长获得连续横断面（5μm），用于组织化学法分析。*p<0.01，n=15。

[0318] 图 4 为股动脉套管小鼠的内膜增厚的抑制。

[0319] 以含有 1% 胆固醇及 0.05% 胆酸盐的高胆固醇及高脂质饮食喂食转基因的雄性 ApoE*3Leiden 小鼠，以引发高胆固醇血症。经 3 星期高脂质饮食后，麻醉小鼠并自股动脉周围解剖股动脉且松松地套上非缩窄性聚乙烯套管（Portex，内径 0.40mm、外径 0.80mm 及长度 2.0mm）。于手术后第 0、3、7 及 10 天时，经 IP 注射来用溶于 PBS 的 10mg/kg 重组抗 -PC IgG 抗体、溶于 PBS 的 10mg/kg 抗链霉亲和素 A2IgG 抗体或只有 PBS 的任一种来处理小鼠。手术后 14 天处死小鼠且取下套管的股动脉并以石蜡包埋。自套管的股动脉片段的全长获得连续横断面（5μm），用于组织化学法分析。

[0320] A. 3 个图中内膜面积（箭头指示处）的比较显示，抗体 M99-B05 降低套管引发的血管损伤 14 天后观察到的内膜增厚。

[0321] B. 内膜增厚（μm）^{2}，n=10，*p<0.05

[0322] 图 5 为使用 ELISA 测量的 M99-B05 突变体的 PC 结合活性。

[0323] （●）M99-B05（EC_{50}=0.28nM），（○）X19-A01（EC_{50}=0.42nM），（▼）X19-A03（EC_{50}=0.54nM），（△）X19-A05（EC_{50}=0.52nM），（■）X19-A07（EC_{50}=0.62nM），（□）X19-A09（EC_{50}=0.58nM），（◆）X19-A11（EC_{50}=0.97nM），（◇）X19-C01（EC_{50}=1.4nM）。

[0324] 图 6 为用抗 - 磷酰胆碱抗体免疫组织化学染色冷冻的人类动脉粥样硬化病变组织。

[0325] 人类动脉粥样硬化病变组织连同正常组织对照购自 Biochain Human 冷冻组织。将该组织与 0.1μg/mL 生物素化 M99-B05 抗 - 磷酰胆碱 IgG 于 4℃孵育过夜。添加链霉亲和素 - 辣根过氧化物酶（HRP）及 HRP 底物后，可显示抗体结合组织。从 HRP 底物产生的颜色显示抗体结合的存在。同型对照并未观察到结合（未显示数据）。

[0326] 图 7 为股动脉套管的小鼠中内膜增厚的抑制。

[0327] 以含有 1% 胆固醇及 0.05% 胆酸盐的高胆固醇及高脂质饮食喂食转基因的雄性 ApoE*3Leiden 小鼠，以引发诱发高胆固醇血症。经 3 星期高脂质饮食后，麻醉小鼠并自股动脉周围解剖股动脉且松松地套上非缩窄性聚乙烯套管（Portex，内径 0.40mm、外径 0.80mm 及长度 2.0mm）。于手术后第 0、3、7 及 10 天时，经 IP 注射来用溶于 PBS 的指定抗体及量来处理小鼠。手术后 14 天处死小鼠且取下套管的股动脉并以石蜡包埋。自套管的股动脉片段的全长获得连续横断面（5μm），用于组织化学法分析并计算内膜增厚（μm）2，n=10，*p<0.05。

实施例

[0328] 本发明包含下列实施例以进一步说明本发明的各个方面。本领域技术人员了解，实施例中所公开的技术遵从了发明人发现的代表性技术和 / 或组合物，其在本发明的实施中运作良好，因此可认为构成实施的优选实施方式。然而，考虑到本公开内容，本领域技术人员了解，在不脱离本发明的精神和范围的情况下，可以在公开的具体实施例中作出许多改变而仍能获得相似或类似的结果。

[0329] 噬菌体展示抗体库的筛选

[0330] 进行噬菌体展示选择及筛选活动以鉴定心血管疾病中逐渐暴露于 oxLDL 上或凋亡的内皮细胞的 PC 并中和 PC 的促炎症活性的人类抗体。

[0331] 通过使用与牛血清白蛋白（BSA）结合的 PC 以及在不同轮次间改为与铁蛋白结合

的 PC 来指导抗 -PC 抗体的选择。

[0332] 利用 ELISA 筛选噬菌体展示选择输出结果来寻找结合 PC-BSA 的个体噬菌体，且击中(hits)进行 DNA 测序以鉴定特定抗体的确切数目；该特定抗体的全部重组转换成 IgG。在进行两个不同噬菌体展示库的选择后，总计鉴定并产生 41 个全人 IgG。这些抗体是在利用 ELISA 筛选总共 10,660 个不同的噬菌体克隆后鉴定的，其中有 1,511 个 ELISA 阳性击中。

[0333] ELISA 击中定义为在固定的目标上(即，PC-BSA)具有至少比背景信号(链霉亲和素涂布的板)大 3 倍的信号。

[0334] 在测序 1,511 个 ELISA 阳性物并将所述抗体自噬菌体上展示的 Fab 片段转换为完全人类 IgG 后，回收 56 个结合 PC 的不同抗体序列，其中 26 个来自第一个噬菌粒(phagemid)库且 30 个来自第二个噬菌体文库。

[0335] IgG 重构(reformatting)、表达及纯化

[0336] 此处公开了从噬菌体上展示的 Fab 重构为全长 IgG 后回收 56 个抗体中的 40 个的结果。

[0337] 制备用于每个 IgG 的 DNA 并转染至人类肾脏 293T 细胞，以在 10 天培养基收获后瞬时产生 IgG。使用蛋白质 A Sepharose (MabSelect)纯化用于体外研究的 IgG 并将缓冲剂替换成 PBS。

[0338] 以蛋白质 A Sepharose 纯化预定用于体内试验的 IgG，随后进行经梯度洗脱(gradientelution)的阳离子交换(Poros HS)。预定用于体内试验的 IgG 抗体的缓冲剂替换为抗体配方缓冲液(0.1M 柠檬酸盐 - 磷酸盐、50mM NaCl、0.01%Tween-80、2% 海藻糖，pH6.0)。以纯化样品在 280nm 的吸光度(1mg/mL=1.40.D.)测定抗体浓度。

[0339] 体外试验

[0340] 40 个 IgG 在一连串的体外试验中进行测试以鉴定具有所需性质的抗体。表 1 总结了筛选全人 IgG 抗 - 磷酰胆碱抗体的结合性质。

[0341] 表 1 第二列(A 列)显示通过只使用添加至固定在 96 孔板表面的 PC-BSA 的 15.6ng/mLIgG 而得到的 ELISA 信号。ELISA 信号 >1 的抗体预期为具有较高亲合力的抗体。

[0342] 表 1 第三列(B 列)显示当将抗体注射于共价固定于生物传感芯片上的胺基苯基磷酰胆碱并使用 Biacore3000 仪器经表面等离子体共振检测结合时得到的信号。Biacore 信号越高，观察到的结合越多。

[0343] 表 1 第四列(C 列)显示通过测验结合共价固定的氨基酚而测定抗体对磷酰胆碱的特异性的测验结果，氨基酚为用于共价偶联磷酰胆碱至 BSA 或生物传感芯片的连接子。一些抗体结合连接子分子与结合氨基苯基磷酰胆碱一样好或更好。这些抗体未必为具有有效治疗性的抗 - 磷酰胆碱抗体。

[0344] 表 1 第五列(D 列)总结了抗体抑制巨噬细胞摄取 oxLDL 的能力的试验结果，巨噬细胞摄取 oxLDL 为心血管炎症的早期病变且导致泡沫细胞的形成。通过使用荧光修饰的 oxLDL、在 80μg/mL 受测抗体存在或不存在下经流式细胞术监测巨噬细胞的摄取。在每个实验中，使用 100μg/mL 亲合纯化的 IgM 抗 -PC 多克隆抗体作为阳性对照。将受测单克隆抗体存在下摄取的 oxLDL 的荧光监测量除以多克隆抗体存在下观察到的荧光量，然后乘以 100。因此，低于 100 的数值表明 80μg/mL 浓度的单克隆抗体在抑制 oxLDL 摄取上比提取自人类血清的浓度为 100μg/mL 的多克隆抗 -PC 更为有效。相似地，高于 100 的值表明抗

体不如多克隆抗 -PC 有效。

[0345] 观察到数个抗体在抑制摄取上与多克隆抗 -PC 对照相似或更好。此外，观察到数个抗体刺激巨噬细胞的 oxLDL 摄取，这是从先导选择（lead selection）排除这些抗体的性质。

[0346] 表 1 最后一列（E 列）显示通过将 IgG 添加至含有 oxLDL 或天然 LDL 之一的 96 孔板的孔中所得到的 ELISA 数据。观察到的每个受测抗体结合 oxLDL 的 ELISA 信号除以用 LDL 观察到的信号的比率列于表 1。明显地，与 LDL 相比，某些抗体是 oxLDL 的更好的结合物。

[0347] 表 1、全人 IgG 抗 -PC 抗体结合性质的总结

[0348] 所有列的标题：

[0349] A）利用 ELISA 检测的 15.6ng/mlAb 与结合于 BSA 的 PC 的结合（OD）

[0350] B）利用 Biacore 检测的与氨基苯基 PC 的结合（RU）

[0351] C）利用 Biacore 检测的与氨基苯基连接子的结合（RU）

[0352] D）在 80μg/ml Ab 存在下巨噬细胞的百分比 oxLDL 摄取（a）

[0353] E）利用 ELISA 检测的与 oxLDL 对与 LDL 的结合（oxLDL 信号 /LDL 信号）（b）

[0354]

样品 ID	A	B	C	D	E
M0004-B02	1.24	366.4	38.6	233.3	6.7
M0004-C02	0.11	44.8	0.2	93	1.2
M0004-G02	1.23	1028.5	15.7	nd	8.4
M0007-H10	0.49	415.8	2.7	105	0.6

[0355]

M0009-A06	0.48	912.1	2.5	80.5	2.8
M0011-F05	1.56	4473.6	155.6	547.5	10.3
M0024-B01	0.26	nd	nd	nd	11.1
M0026-H05	0.03	1.6	17.8	73.7	1.4
M0027-H05	0.03	-3.3	1.4	79.3	1.1
M0028-H05	0.03	1.8	5	86	0.6
M0029-H05	0.08	nd	nd	370	0.9
M0030-H05	0.02	19.1	32.8	nd	nd
M0031-H05	0.03	-4.1	0.2	81	1
M0034-G12	0.84	462.3	14.6	78	nd
M0035-E11	0.14	41.5	2.1	68	0.5
M0039-H05	2.73	-6.4	2.1	80.4	0.7
M0042-G07	nd	-2.9	2.3	93.7	0.8
M0043-D09	1.24	172.7	2.1	1310	16.8
M0050-H09	0.22	279.1	7	71.5	nd
M0073-G03	0.18	46.3	19.9	51.1	1.2
M0077-A11	0.26	836.3	1.3	78.4	0.7
M0086-F02	0.99	1.4	12.6	315	nd
M0086-H01	0.41	51.2	4.9	85	1
M0086-H11	1	-1.1	0.9	74	nd
M0097-B04	0.22	109.5	-0.5	98	1.3
M0097-B05	1.01	699.6	-3.2	80	1.1
M0099-B05	1.03	5219.3	23.3	71	1.5
M0099-D11	0.03	170.7	8.6	560	2.1
M0100-A01	1.53	7532.8	3934.7	nd	1.1
M0102-E11	0.02	1.6	-1.3	83	nd
M0108-H03	nd	532.7	4.5	nd	1.1
M0126-A04	0.03	34.2	-8	nd	2.8
M0126-F10	nd	32.9	-8.3	nd	nd
M0126-H08	0.03	114.3	566.1	98	nd
M0127-A09	0.03	18.2	-8.7	160	1.6
M0127-B07	0.05	16.3	-7	67	nd
M0127-E06	nd	21.9	-4.2	nd	nd
M0127-E07	nd	15.4	-6.2	nd	1.8
M0127-F01	0.02	9.6	3.6	77	nd
X0009-A01	0.23	198.1	2	95	1.5

[0356] a）巨噬细胞的 OxLDL 摄取。研究了衍生自人类 THP-1 单核细胞（ATCC，美国）的巨噬细胞中 Dil- 标记（1,1’- 双十八烷基 -3,3,3’,3’- 四甲基吲哚羰花青高氯酸盐）且

Cu- 氧化的 LDL 的摄取(oxLDL，IntracelCorp，美国)。通过用 RPMI 及 10%FCS 中的 100nM PMA(Sigma-Aldrich)孵育培养 24h 而引发分化，之后置换培养基并让细胞再留置 48 小时。然后将细胞与所述抗体于 37℃培养 50 至 60 分钟。之后，添加 20μg/ml oxLDL 并继续孵育 5 小时。孵育终末时，用冰冷的 PBS/0.2%BSA 洗涤细胞两次并用 PBS 洗涤一次。在含 2%PFA 的 PBS 中收获细胞。使用具备 Cell Quest 软件的 FACS Calibur 取得数据并分析。每个样品最少分析 10,000 个细胞。

[0357] b)OxLDL ELISA。 将 hLDL (Kalen Biomedical#770200-4)、oxLDL (Kalen Biomedical#770252-7)(未显示这些数据)以 10μg/ml 的浓度及 100μl/ 孔的体积于 4℃过夜涂布于 ELISA 板上(Immulon2HB)。室温下以 1%BSA 溶液(300μl/ 孔)封闭板两个小时。洗涤后，将板与所述抗体(100μl/ 孔 ;25 至 100nM)于室温孵育 1 小时。将偶联 AP 的山羊抗人二抗(ThermoScientific#31316)以 1 :5000 稀释后、以 100μl/ 孔添加至洗涤后的孔板中并于室温孵育 1 小时。加入检测剂(ThermoScientific#37621)(100μl/ 孔)并立即于温度 30℃及 405nm 以动态模式(kinetic mode)读板。以 ODoxLDL/ODLDL 显示结果。

[0358] 利用 SPR 分析抗 -PC IgG 与 PC 的亲合力。

[0359] 通过使用 Biacore 表面等离子体共振(SPR)生物传感器筛选结合 PC 的 IgG。氨基苯基磷酰胆碱(Biosearch Technologies)通过游离氨基偶联至 CM5 芯片的一个流动池(flowcell)，使密度为 120RU。将氨基酚连接子偶联至相同 CM5 芯片的另一个流动池，使密度为约 120RU。也将 PC-KLH 及 PC-BSA 偶联至 CM5 芯片的不同流动池。

[0360] 在不同情况下使用具有固定的 PC 的这些表面，以 100nM 及 50μL/min 注射抗体而得到结合传感图(sensorgrams)。通过以 50μL/min 使不同浓度的抗体流过表面而探讨了 M99-B05 的亲合力。抗体对该固定的抗原表现出快速连接速率(fast on rate)及快速解离速率(fast offrate)，其妨碍我们从动态传感图获得可信赖的 k_{on} 及 k_{off} 估值。

[0361] 接近注射结束时所观察到的每个抗体浓度的信号对抗体浓度绘图并将该数据拟合至标准双曲线平衡结合方程式(图 1)。如图 1 所示，M99-B05 以约 150nM 的表观 Kd 结合氨基苯基 PC。两个测试的制备物具有等价的 Kd 值但 Rmax (最大反应)不同。于此表面观察到的每个抗体的表观 Kd 值可以或不可以代表在更生理性底物上观察到的亲合力。

[0362] 纯化的抗 -PCIgG 的 ELISA 筛选

[0363] 也通过使用 PC-BSA 的 ELISA 筛选了结合 PC 的纯化的 IgG。拟合此数据以提供估算的 EC50 值(图 2)。

[0364] 抑制 oxLDL 引发的 MCP-1 自单核细胞的释放

[0365] 测试数种抗体阻断响应 oxLDL 刺激而自单核细胞释放趋化因子 MCP-1 的能力。如表 2 所示，M99-B05 对阻断 oxLDL 引发的 MCP-1 释放非常有效。此抗体以 nM 范围内的 IC_{50} 强力抑制 MCP-1 的释放。

[0366] MCP-1 是强力的促炎性趋化因子，其促进动脉粥样硬化病变部位的白细胞流入(Reape 和 Groot.1999.Chemokines and atherosclerosis.Atherosclerosis147,213-225)。作为阴性对照的对照 IgG 抗链霉亲和素 A2 未显示抑制 oxLDL 引发的 MCP-1 自单核细胞的释放(未显示数据)。

[0367] 表 2、抗 -PC 抑制 oxLDL 引发的 MCP-1 自人类单核细胞的分泌

[0368]

	M99-B05 的 IC_{50}
供体 1	1.8±0.74nM
供体 2	1.3±0.7nM

[0369] 自人类血液分离单核细胞并在存在或缺少 10pM 至 40nM 抗 -PCIgG 的情况下以 2μg/mL 铜氧化的 oxLDL 刺激单核细胞。通过使用市售 MCP-1 特异性 ELISA 试剂盒定量细胞培养基中的 MCP-1 水平。

[0370] 也显示抗体(M99-B05)结合人类动脉粥样硬化病变组织(图 6)。

[0371] 体内试验

[0372] 基于有利的体外结合性质及体外试验的功能性的组合,进一步以冠状动脉炎症体内模型测验了抗体 M4-G2、M73-G03 及 M99-B05。

[0373] 此小鼠模型测量进入内皮下组织(即,中膜(media))的炎症细胞流入,该炎症细胞流入是对通过置放限制性套管于暴露的股动脉周围而引发的血管损伤的反应(图 3)。从图 3 明显看出 M99-B05 减少白细胞流入内皮下层,观察到的降低是统计学上显著的。相反地,尽管其在体外结合性质及体外试验的功能性中是有利的,M4-G2 或 M73-G03 均未显示比对照抗体(称为“HuIgG1a-A2”的抗链霉亲和素 A2IgG)有任何统计学上显著的降低。

[0374] 发明人并未预期到且感到惊讶的是在此分析中与 M4-G2 及 M73-G03 比较 M99-B05 的非常独特的效用。这证明体外阳性数据并不能预期抗 -PC 抗体的体内效用。

[0375] 因此,以小鼠血管再狭窄模型测验 M99-B05,其中通过在股动脉周围放置套管而再次引发伤害,但使试验进行 14 天而不是 3 天。然后利用组织化学分析狭窄量,该狭窄量为观察到的受影响动脉的血管中层的增厚(图 4)。从图 4 明显看出 M99-B05 显著抑制套管引发的血管损伤后的血管壁增厚。这进一步证明 M99-B05 在体内是高效的。

[0376] 种系及稳定性突变体的构建

[0377] M99-B05 抗体的重链及轻链可变区域的氨基酸序列分析鉴定了氨基酸取代,以降低可能的免疫原性并避免于抗体表达及纯化期间可能发生的敏感的氨基酸修饰。

[0378] 下列表格显示了通过使用 Kabat 数据库比对的可变区域的氨基酸序列和其最紧密相关的种系抗体序列。表中也强调的是抗体中所制备的使其更接近种系的氨基酸取代,除了移除可能的脱酰胺化位点(HCDR3 二硫键)的突变体外,所有这些均可能引起可制造性的关注(所谓”稳定性突变体”)。

[0379] M99-B05 的突变体

[0380] X19-A01 突变体具有与野生型 M99-B05 同样的重链及轻链氨基酸序列,只是 M99-B05 轻链中的第一个氨基酸(谷氨酰胺)在 X19-A01 中缺失,以更好地匹配种系序列。

[0381] X19-A03 突变体的序列编码相对于 VH3-23、JH3 重链及 VK4-B3、JK1 轻链种系序列而言完全种系化的抗体,而在重链可变区域的框架区 1 (HV-FR1)中没有插入的苯丙氨酸(F)以及可能在表达及纯化期间降低蛋白质氨基酸修饰的氨基酸取代(稳定性突变)。发现 M99-B05 抗体在相对于种系序列的 HV-FR1 的尾部具有删除的 F 氨基酸。将 F 插入此位置使抗体更接近种系序列并且可能较少为免疫原性的。在该插入影响 PC 结合的情况下,构建 X19-A03 突变体使其含有除 F 插入以外的所有其它种系取代。此类稳定性突变体在 HV-CDR3

中含有 G 至 A 的突变，进行该突变以破坏潜在的脱酰胺化位点（NG）及 LV-CDR1 中的 N 至 Q 取代以移除另一个潜在的脱酰胺化位点。

[0382] X19-A05 突变体的序列含有全部种系取代，其包含 HV-FR1 中插入的 F 及稳定性突变。X19-A05 抗体为本实施例所产生的含全部种系取代及稳定性突变的唯一突变体抗体。

[0383] X19-A11 突变体具有与 X19-A01 相同的序列，但是在 HV-CDR3 中具有两个 C 至 S 的取代以移除预期可形成于此区的二硫键。

[0384] X19-C01 为种系化的、没有 F 插入且有 C 至 S 取代的稳定性突变体，以移除二硫键。可比较的抗体（前二硫键移除）为 X19-A03。

[0385] 表 3、M99-B05 重链序列的优化

[0386]

```
M99-B05      EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
X19-A01      EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
X19-A03      EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
X19-A05      EVQLLESGGGLVQPGGSLRLSCAASGFTFSGYWMHWVRQAPGKGLEWVS
X19-A07      EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
X19-A09      EVQLLESGGGLVQPGGSLRLSCAASGFTFSGYWMHWVRQAPGKGLEWVS
X19-A11      EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
X19-C01      EVQLLESGGGLVQPGGSLRLSCAASGFT-SGYWMHWVRQAPGKGLEWVS
             **************************** ********************

M99-B05      YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A01      YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A03      YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A05      YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A07      YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A09      YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-A11      YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
X19-C01      YISPSGGGTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
             ************************************************
```

[0387]

M99-B05	VRFRSVCSNGVCRPTAYDAFDIWGQGTAVTVSS	SEQ ID NO: 3
X19-A01	VRFRSVCSNGVCRPTAYDAFDIWGQGTAVTVSS	SEQ ID NO: 5
X19-A03	VRFRSVCSNAVCRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 7
X19-A05	VRFRSVCSNAVCRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 1
X19-A07	VRFRSVCSNGVCRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 9
X19-A09	VRFRSVCSNGVCRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 11
X19-A11	VRFRSVSSNGVSRPTAYDAFDIWGQGTAVTVSS	SEQ ID NO: 13
X19-C01	VRFRSVSSNAVSRPTAYDAFDIWGQGT**M**VTVSS	SEQ ID NO: 15
	******.**.*.*************** *****	

[0388] 种系化的序列突变以粗体字显示。可减少可能的制造问题的残基突变于其下方划线。CDR 区则加框。

[0389] 表 4、M99-B05 轻链序列的优化

[0390]

```
M99-B05     QDIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPKL
X19-A01     -DIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPKL
X19-A03     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPKL
X19-A05     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPKL
X19-A07     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKPGQPPKL
X19-A09     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKPGQPPKL
X19-A11     -DIQMTQSPDSLAVSLGERATINCKSSQSVFYNSNKKNYLAWYQQKAGQPPKL
X19-C01     -DIVMTQSPDSLAVSLGERATINCKSSQSVFYQSNKKNYLAWYQQKPGQPPKL
             ** ****************************:*************.******

M99-B05      LIHWASTRESGVPDRFSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRTF
X19-A01      LIHWASTRESGVPDRFSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRTF
X19-A03      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
X19-A05      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
X19-A07      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
X19-A09      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
X19-A11      LIHWASTRESGVPDRFSGSGSGTDFTLTISNLQAEDVALYYCQQYFNAPRTF
X19-C01      LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFNAPRTF
             **:***************************.*******:*************

M99-B05      GQGTKVEIK          SEQ ID NO:  4
X19-A01      GQGTKVEIK          SEQ ID NO:  6
X19-A03      GQGTKVEIK          SEQ ID NO:  8
X19-A05      GQGTKVEIK          SEQ ID NO:  2
X19-A07      GQGTKVEIK          SEQ ID NO: 10
X19-A09      GQGTKVEIK          SEQ ID NO: 12
X19-A11      GQGTKVEIK          SEQ ID NO: 14
X19-C01      GQGTKVEIK          SEQ ID NO: 16
             *********
```

[0391] 种系化的序列突变以粗体字显示。可减少可能的制造问题的残基突变于其下方划线。CDR 区则加框。

[0392] 为避免疑问，在本申请中提出的序列间如有任何未注意的不一致的情况时，表 3 及 4 中提供的 VH 及 VL 区域的序列及各种 CDR 序列是最终的序列。

[0393] M99-B05 突变体的 PC 结合

[0394] 利用 ELISA 评估了 M99-B05 突变体的 PC 结合(图 5)。从 ELISA 数据(图 5)明显看出，M99-B05 中的许多突变并未显著影响结合 PC。然而，以丝氨酸替换 Hv-CDR3 中的半胱

氨酸残基(X19-A11 及 X19-C01)确实降低了亲合力。这些半胱氨酸残基预期会形成二硫键并存在于 VK4-B3 所编码的种系抗体序列中。所观察到的结合信号的差异可能归因于每个制备物中活性抗体量的差异和 / 或浓度测量中的轻微误差。含有最大数目的可允许序列优化的取代的 M99-B05 的抗体突变体为 X19-A05。此抗体含有所有稳定性及种系取代但保留 Hv-CDR3 二硫键。

[0395] M99-B05 及 X19-A05 的体内功效比较

[0396] 以小鼠血管再狭窄模型测验 M99-B05 及 X19-A05,其中通过在股动脉周围放置套管而再次引发伤害,但使试验进行 14 天而不是 3 天。然后利用组织化学分析狭窄量,该狭窄量为观察到的受影响动脉的血管中层的增厚,并计算内膜增厚(图 7)。从图 7 明显看出 X19-A05 显著抑制套管引发的血管损伤后的血管壁增厚,达到与 M99-B05 相似的程度。X19-A05 的作用也显示清楚的剂量 - 反应关系。

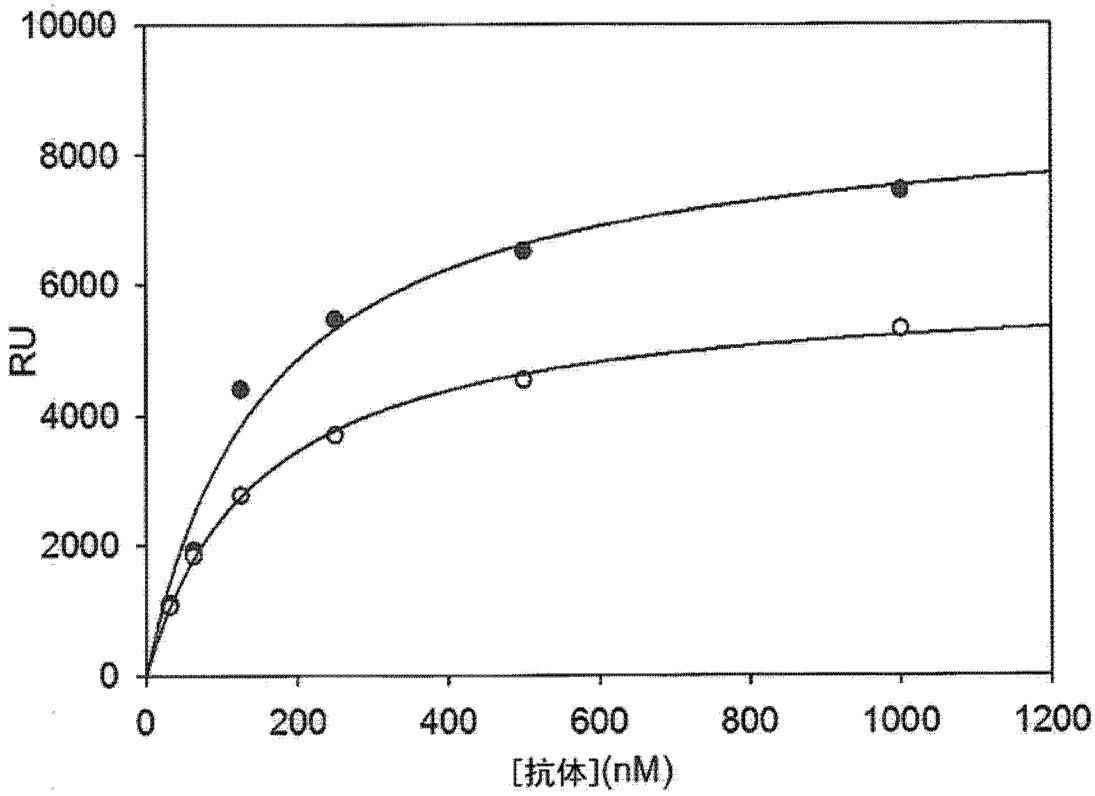


图 1

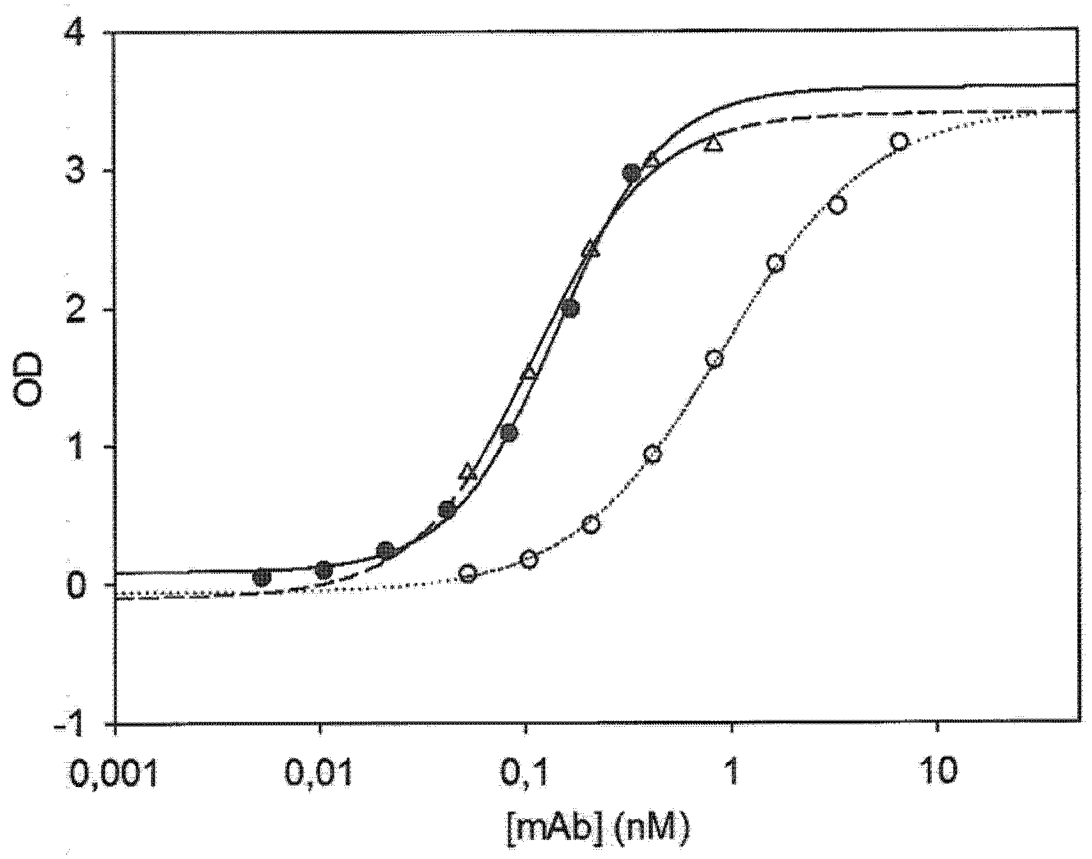

4
3
2
1
0
-1
OD
0,001
0,01
0,1
1
10
[mAb] (nM)

图 2

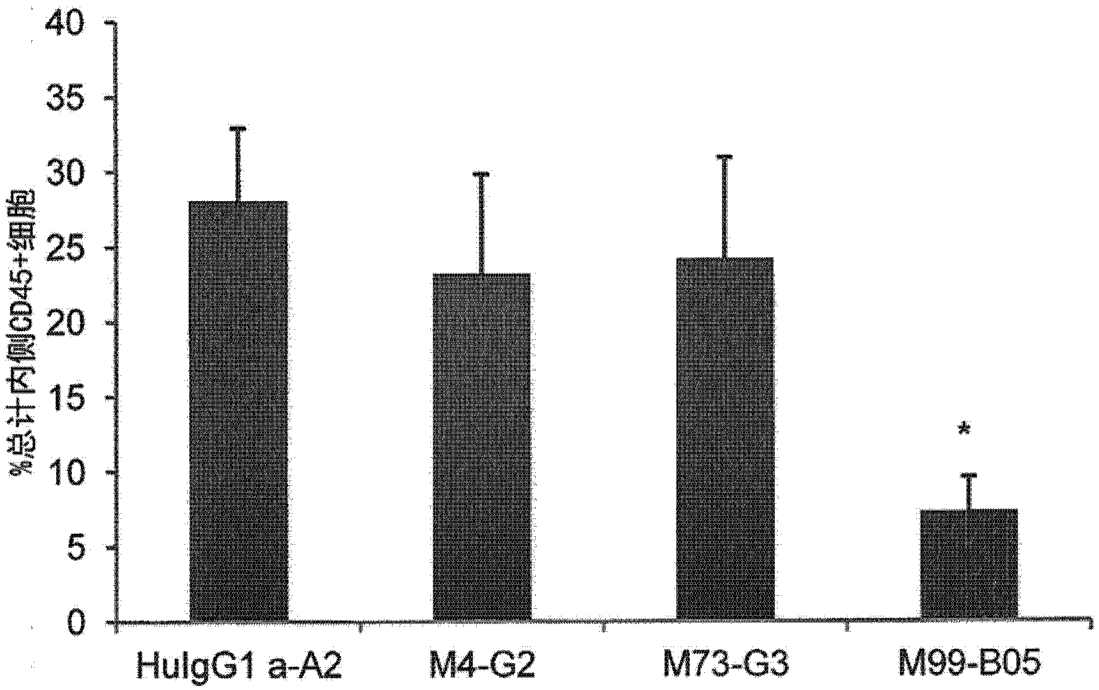

40
35
30
25
20
15
10
5
0
%总计内侧CD45+细胞
*
HulgG1 a-A2
M4-G2
M73-G3
M99-B05

图 3

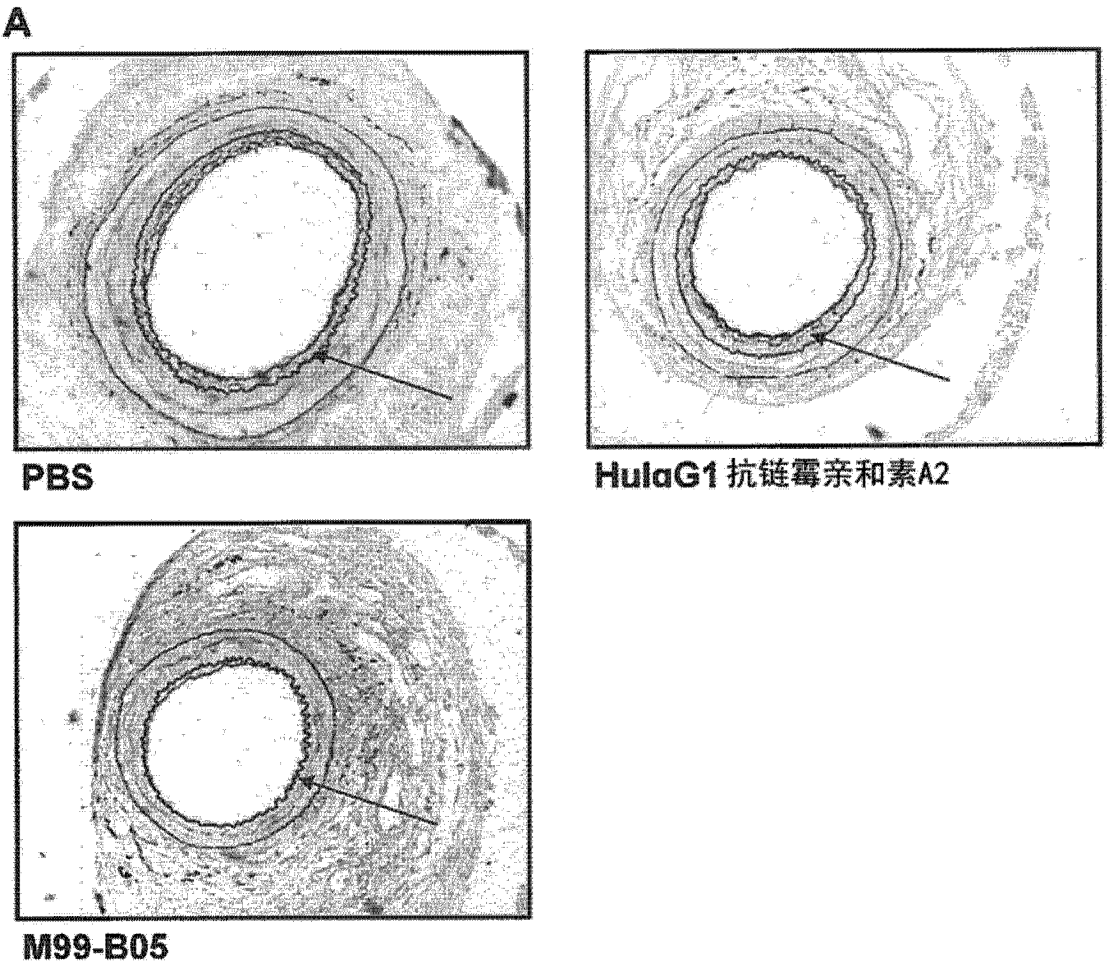

A
PBS
HulaG1 抗链霉亲和素A2
M99-B05
B
新生内膜增厚(μm)²
8000
6000
4000
2000
0
*
PBS
抗链霉亲和素A2
M99-B05

图 4

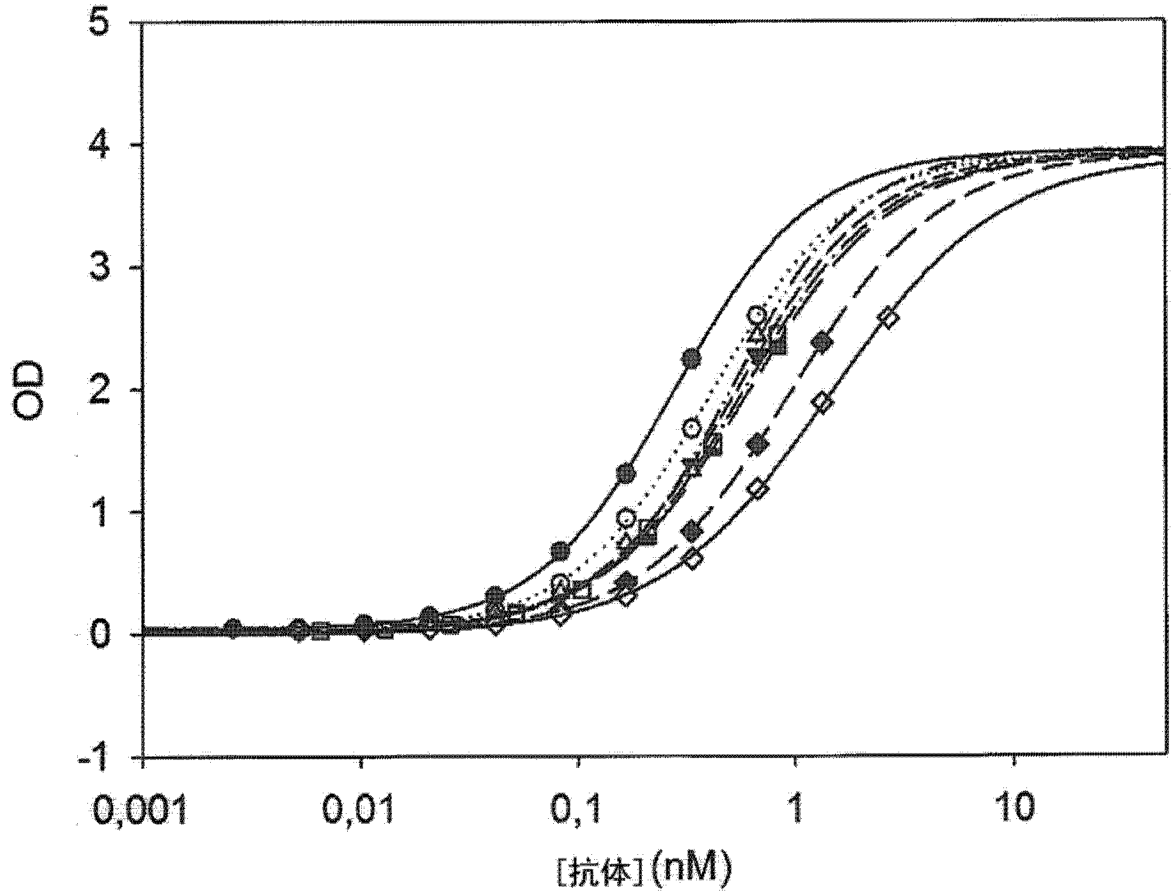


图 5

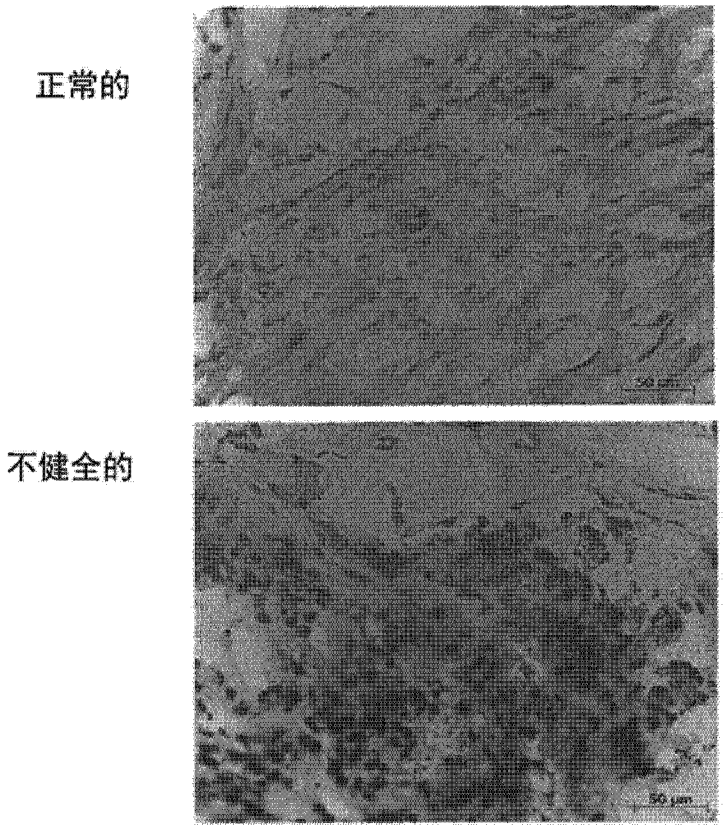


图 6

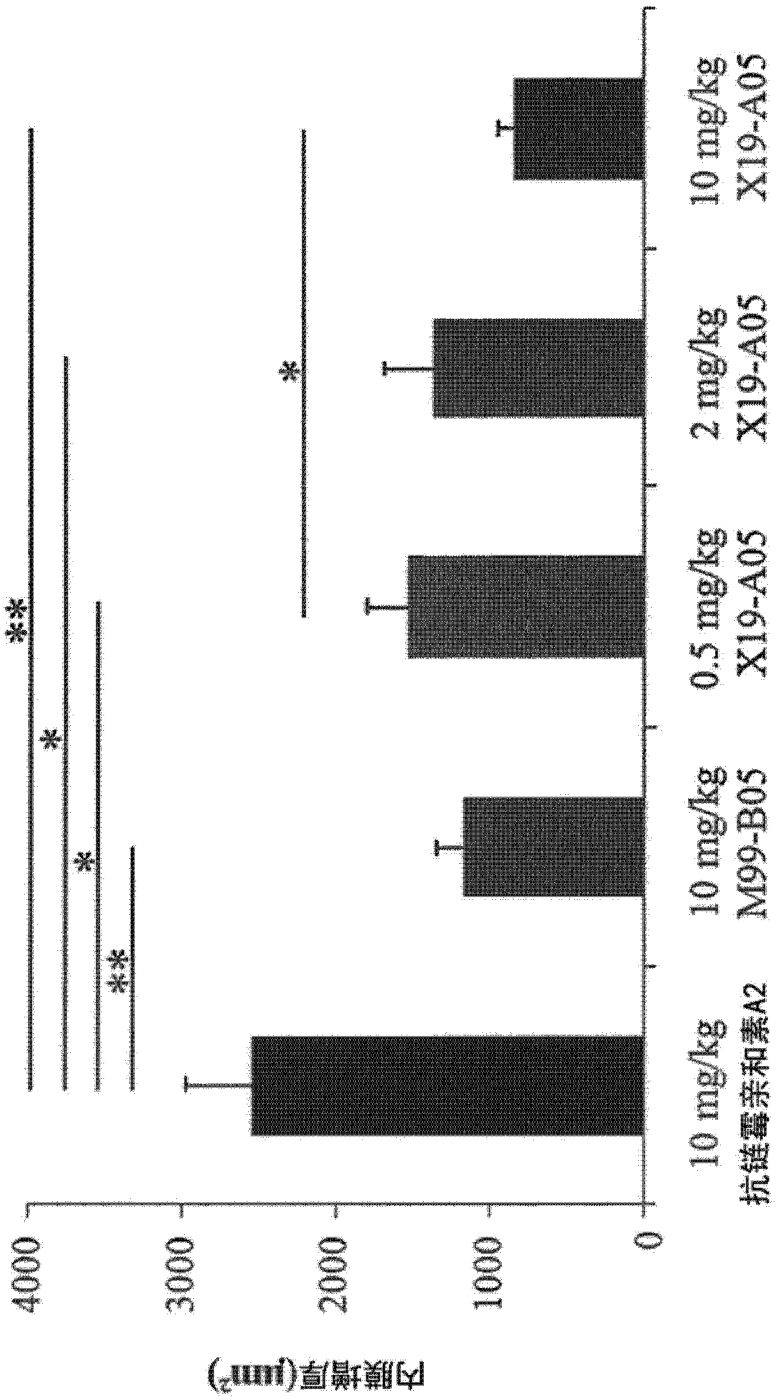

内膜增厚(μm²)
4000
3000
2000
1000
0
**
*
*
**
*
10 mg/kg
抗链霉亲和素A2
10 mg/kg
M99-B05
0.5 mg/kg
X19-A05
2 mg/kg
X19-A05
10 mg/kg
X19-A05

图 7