TITLE OF THE INVENTION

Human binding molecules capable of neutralizing influenza virus H3N2 and uses thereof

FIELD OF THE INVENTION

The invention relates to medicine. The invention in particular relates to human binding molecules capable of neutralizing various influenza A subtypes, including neutralizing binding molecules against influenza viruses comprising HA of the H3 subtype, such as influenza virus H3N2. In particular, the invention relates to the diagnosis, prophylaxis and/or treatment of an infection by an influenza virus comprising HA of the H3 subtype, in particular influenza virus H3N2.

BACKGROUND OF THE INVENTION

Influenza viruses are RNA orthomyxoviruses and consist of three types, A, B and C. Whereas influenza viruses of types B and C are predominantly human pathogens, influenza A viruses infect a wide variety of birds and mammals, including humans, horses, marine mammals, pigs, ferrets, and chickens. In animals most influenza A viruses cause mild localized infections of the respiratory and intestinal tract. However, also highly pathogenic influenza A subtypes, such as H5N1, exist, that cause systemic infections in poultry in which mortality may reach 100%. Several subtypes of influenza A viruses also may cause severe illness in man.

Influenza A viruses can be classified into subtypes based on allelic variations in antigenic regions of two genes that encode surface glycoproteins, namely, hemagglutinin (HA) and neuraminidase (NA) which are required for viral attachment and cellular release. Other major viral proteins include the nucleoprotein, the nucleocapsid structural protein, membrane proteins (M1 and M2), polymerases (PA, PB and PB2) and non-structural proteins (NS1 and NS2). Currently, sixteen subtypes of HA (H1-H16) and nine NA (N1-N9) antigenic variants are known in influenza A virus. Influenza virus subtypes can further be classified by reference to their phylogenetic group. Phylogenetic analysis (Fouchier et al., 2005) has demonstrated a subdivision of HA's that falls into two main groups (Air, 1981): *inter alia* the H1, H2, H5 and H9 subtypes in phylogenetic group 1 and *inter alia* the H3, H4 and H7 subtypes in phylogenetic group 2 (Figure 1).

Only some of the influenza A subtypes (i.e. H1N1, H1N2 and H3N2) circulate among people, but all combinations of the 16 HA and 9 NA subtypes have been identified in avian species. Animals infected with influenza A often act as a reservoir for the influenza viruses and certain subtypes have been shown to cross the species barrier to humans, such as the highly pathogenic influenza A strain H5N1.

Influenza infection is one of the most common diseases known to man causing between three and five million cases of severe illness and between 250,000 and 500,000 deaths every year around the world. Influenza rapidly spreads in seasonal epidemics affecting 5-15% of the population and the burden on health care costs and lost productivity are extensive (World Healthcare Organization (WHO)). Hospitalization and deaths mainly occur in high-risk groups (elderly, chronically ill).

Annual epidemics of influenza occur when the antigenic properties of the viral surface protein HA and NA are altered. The mechanism of altered antigenicity is twofold: antigenic shift, caused by genetic rearrangement between human and animal viruses after double infection of host cells, which can cause a pandemic; and antigenic drift, caused by small changes in the HA and NA proteins on the virus surface, which can cause influenza epidemics. The emergence of variant virus strains by these two mechanisms is the cause of influenza epidemics. Three times in the last century, the influenza A viruses have undergone major genetic changes mainly in their HA-component, resulting in global pandemics and large tolls in terms of both disease and deaths. The most infamous pandemic was “Spanish Flu”, caused by influenza virus H1N1, which affected large parts of the world population and is thought to have killed at least 40 million people in 1918-1919. More recently, two other influenza A pandemics occurred, in 1957 (“Asian influenza”, caused by influenza virus H2N2) and 1968 (“Hong Kong influenza”, caused by influenza virus H3N2), and caused significant morbidity and mortality globally. In contrast to current seasonal influenza epidemics, these pandemics were associated with severe outcomes also among healthy younger persons.

Current approaches to dealing with annual influenza epidemics include annual vaccination, preferably generating heterotypic cross-protection. However, as indicated above, circulating influenza viruses in humans are subject to permanent antigenic changes which require annual adaptation of the influenza vaccine formulation to ensure the closest possible match between the influenza vaccine strains and the circulating influenza strains.

Although yearly vaccination with the flu vaccine is the best way to prevent the flu, antiviral drugs, such as oseltamivir (Tamiflu®) can be effective for prevention and treatment of the flu. However, the number of influenza virus strains showing resistance against such oseltamivir is increasing.

An alternative approach is the development of antibody-based prophylactic or therapeutic means to neutralize various seasonal influenza viruses. The primary target of neutralizing antibodies that protect against influenza virus infection is the globular head (HA1 part) of the viral HA protein which contains the receptor binding site, but which is subject to continuing genetic evolution with amino acid substitutions in antibody-binding sites (antigenic drift). Cross-neutralizing antibodies recognizing the more conserved stem-region of HA of influenza A viruses of phylogenetic group 1 (e.g. H1 and H5) have recently been identified (e.g. WO2008/028946). There has, however, been limited success in identifying antibodies that neutralize one or more influenza A virus subtypes of phylogenetic group 2, such as H3 viruses, and their breadth of neutralization is narrow and their potency low.

Antibodies specifically recognizing H3N2 influenza virus strains have been described. Thus, a human monoclonal antibody, C28, capable of binding to and neutralizing several H3N2 influenza virus strains from the years between 1968 and 1980 has been described by Östberg and Pursch (1983). Wang et al. (2010), have described an anti-HA2 murine antibody neutralizing H3 viruses spanning several decades, but which was shown not to neutralize any non-H3 subtype viruses.

Cross-reactive anti-HA2 murine antibodies recognizing HA of the H3 subtype, as well as of the H4 and H7 subtype, and capable of in vitro reducing influenza virus replication of H3 and H4 influenza viruses have been described by Stropkovská et al., (2009). It was demonstrated that the accessibility of the HA2 epitopes to these antibodies in the native virus was low, and that the antibodies have a higher reactivity with HA after its trypsin cleavage and pH 5 treatment (Varečková et al., 2003a), which may explain the observation that the in vitro inhibition of virus replication (Varečková et al., 2003b), as well as in vivo potency of these antibodies was relatively low (Gocník et al., 2007).

In WO2009/115972, a human monoclonal antibody, Fab28, has been disclosed, which recognizes an epitope on the stem region of HA and displays a neutralizing activity against H1N1 but less neutralizing acitivity against H3N2.

In view of the severity of the respiratory illness caused by certain influenza A viruses, and the always existing threat of a potential pandemic, as well has the high economic impact of the seasonal epidemics, there is an ongoing need for effective means for the prevention and treatment of the various influenza A subtypes. There is thus a need for binding molecules, preferably broadly neutralizing human binding molecules, capable of neutralizing seasonal influenza virus subtypes, including influenza viruses comprising HA of the H3 subtype, preferably H3N2, and that have no or less of the drawbacks of the antibodies known in the prior art.

The present invention provides these binding molecules which can be used in medicine, in particular for diagnosis, prevention and/or treatment of infection with influenza virus comprising HA of the H3 subtype, preferably H3N2 infections. Some of the binding molecules of the invention are unique in their breadth of neutralizing activity within the H3 subtype. Thus, some of the binding molecules identified herein are capable of neutralizing several, including at least one or more recent, strains within the H3N2 subtype and may be used as a universal prophylactic and/or treatment agent for seasonal influenza, irrespective of the causative influenza H3N2 strain. At least some of the binding molecules are capable of preventing *in vitro* the cleavage of the HA precursor molecule HA0 by trypsin. Furthermore, at least some of the binding molecules of the present invention are capable of preventing the conformational change of the HA protein, thought to be involved in membrane fusion of the influenza viral membrane and the endosomal membrane of an infected cell. Furthermore, at least some binding molecules of the invention are unique in that they are also capable of cross-neutralizing influenza viruses of at least one other subtype, including influenza viruses comprising HA of the H7 and/or H10 subtypes, and thus can be used as a universal prophylactic, diagnostic and/or treatment agent for influenza viruses, even irrespective of the causative influenza subtype.

DESCRIPTION OF THE FIGURES

FIG. 1 shows a phylogenetic tree of amino acid sequences at the subtype level. Division of subtypes by group is indicated. The H1 clade, comprising inter alia the H1 subtypes, and the H9 clade, comprising the H9 subtypes, form phylogenetic group 1, and the H7, comprising inter alia the H7 subtypes and the H3 clade, comprising inter alia the H3 subtypes, form phylogenetic group 2.

FIG. 2 is a bar diagram showing binding of IgG1 to surface expressed H3 rHA, measured by FACS analysis, after sequential treatment with trypsin (striped bars), pH 4.9 buffered medium (solid white bars) and DTT (crossed bars) and expressed as percentage binding to untreated rHA (solid black bars).

FIG. 3 shows the results of an *in vitro* protease susceptibility assay. Samples were run on a 4-12% BisTris gel in 1x MOPS buffer. Protein bands were visualized by colloidal blue staining.

FIG. 4 is a schematic representation of the different conformations of the HA protein during the infection process.

FIG. 5 is a bar diagram showing binding of the H3 mAbs to HA expressing cells after different treatments measured by FACS analysis, after sequential treatment with trypsin (striped bars), pH 4.9 buffered medium (solid white bars) and DTT (crossed bars) and expressed as percentage binding to untreated rHA (solid black bars).

FIG. 6 shows the result of the time course experiment described in Example 11. to determine the incubation time of HA with trypsin to achieve cleavage of H3 HA.

FIG. 7 shows the results of trypsin digestion of H3 HA samples pre-incubated with mAbs, as described in Example 11.

FIG. 8 is a bar diagram demonstrating that CR8043 inhibits pH-induced conformational change in H3 HA.

FIG. 9 shows that CR8020 and CR8041 are also capable of blocking the pH-induced conformational change of HA: A. mAbs added before Trypsin cleavage; B. mAbs added after trypsin cleavage; C. mAbs added after all treatments.

FIG. 10 shows the Kaplan-Meier survival probability curves. Antibody was administered intravenously at day -1 before challenge using a dose range from 30 down to 1 mg/kg. Control Ab was administered at 30 mg/kg (grey), followed by a lethal challenge at day 0 with 25 LD50 A/HK/1/68-MA20 (H3N2). CR8020 (A) was tested in a separate study from CR8041 (B) and CR8043 (C), which were evaluated in 1 experiment. Therefore the same control antibody group is used for B and C.

FIG. 11 shows the mean body weight change (%) relative to day 0. Antibody was administered intravenously at day -1 before challenge using a dose range from 30 down to 1 mg/kg. Control Ab was administered at 30 mg/kg (grey), followed by a lethal challenge at day 0 with 25 LD50 A/HK/1/68-MA20 (H3N2). Bars represent the 95% CI of the mean. If a mouse died / was euthanized during follow-up of the study, the last observed body weight was carried forward. CR8020 (A) was tested in a

separate study from CR8041 (B) and CR8043 (C), which were evaluated in 1 experiment. Therefore, the same control antibody group is used for B and C.

FIG. 12 shows the median clinical score. Antibody was administered intravenously at day -1 before challenge using a dose range from 30 down to 1 mg/kg. Control Ab was administered at 30 mg/kg (grey), followed by a lethal challenge at day 0 with 25 LD50 A/HK1/68-MA20 (H3N2). Bars represent interquartile ranges. CR8020 (A) was tested in a separate study from CR8041 (B) and CR8043 (C), which were evaluated in 1 experiment. Therefore the same control antibody group is used for B and C. Clinical score explanation: 0 = no clinical signs; 1 = rough coat; 2 = rough coat, less reactive, passive during handling; 3 = rough coat, rolled up, laboured breathing, passive during handling; 4 = rough coat, rolled up, laboured breathing, does not roll back on stomach when laid down on its back. Mice observed with clinical score 4 were euthanized on the same day.

FIG. 13 demonstrates the therapeutic efficacy of mAb CR8020 in the mouse lethal challenge model with influenza A/HK/1/68-MA20 (H3N2). A single dose of mAb CR8020 (15 mg/kg) was administered intravenously either at day -1 pre-challenge or at day 1, 2, 3, 4, 5, or 6 post challenge in 129X1/SvJ mice (n=10 /group). Control mAb (15 mg/kg) was administered at day 1 post challenge. Mice were challenged at day 0 with 25 LD50 A/HK/1/68-MA20 (H3N2) and monitored for 21 days. Panel A: Kaplan-Meier survival probability curves. Panel B: Mean body weight change (%) relative to day 0. Bars represent the 95% CI of the mean. If a mouse died / was euthanized during follow-up of the study, the last observed body weight was carried forward. Panel C: Median clinical score. Bars represent interquartile ranges. 0 = no clinical signs; 1 = rough coat; 2 = rough coat, less reactive, passive during handling; 3 = rough coat, rolled up, laboured breathing, passive during handling; 4 = rough coat, rolled up, laboured breathing, does not roll back on stomach when laid down on its back. Mice observed with clinical score 4 were euthanized on the same day.

FIG. 14 shows the prophylactic efficacy of mAb CR8020 in the mouse lethal challenge model with mouse adapted influenza A/CH/NL/621557/03 (H7N7). A: Kaplan-Meier survival probability curves. Panel B: Mean body weight change (%) relative to day 0. Bars represent the 95% CI of the mean. If a mouse died / was euthanized during follow-up of the study, the last observed body weight was carried forward. Panel C: Median clinical score. Bars represent interquartile ranges. 0 = no clinical signs; 1 = rough coat; 2 = rough coat, less reactive, passive during handling; 3

= rough coat, rolled up, laboured breathing, passive during handling; 4 = rough coat, rolled up, laboured breathing, does not roll back on stomach when laid down on its back. Mice observed with clinical score 4 were euthanized on the same day.

FIG. 15 shows the prophylactic efficacy of mAbs CR8020, CR8041 and CR8043 in the mouse lethal challenge model with mouse adapted influenza A/CH/NL/621557/03 (H7N7) MAbs were administered intravenously at day -1 before challenge in female Balb/c mice (n=8/group) using a dose range from 10 down to 1 mg/kg (CR8020) or 30 down to 1 mg/kg (CR8041 and CR8043). Control mAb was administered at day -1 at 30 mg/kg (grey). At day 0 a lethal challenge was given by intranasal inoculation with 25 LD_{50} mouse adapted A/CH/NL/621557/03 (H7N7) and the mice were subsequently monitored for 21 days. Panel A: Kaplan-Meier survival probability curves. Panel B: Mean body weight change (%) relative to day 0. Bars represent the 95% CI of the mean. If a mouse died / was euthanized during follow-up of the study, the last observed body weight was carried forward. Panel C: Median Clinical score. Bars represent interquartile ranges. 0 = no clinical signs; 1 = rough coat; 2 = rough coat, less reactive, passive during handling; 3 = rough coat, rolled up, laboured breathing, passive during handling; 4 = rough coat, rolled up, laboured breathing, does not roll back on stomach when laid down on its back. Mice observed with clinical score 4 were euthanized on the same day.

FIG. 16 shows the therapeutic efficacy of mAb CR8020 in the mouse lethal challenge model with mouse adapted influenza A/CH/NL/621557/03 (H7N7). A single dose of mAb CR8020 (15 mg/kg) was administered intravenously either at day -1 pre-challenge or at day 1, 2, 3, 4, 5, or 6 post challenge in female Balb/c mice (n=8/group). Control mAb (15 mg/kg) was administered at day 1 post challenge. Mice were challenged at day 0 with 25 LD_{50} mouse adapted A/CH/NL/621557/03 (H7N7) and monitored for 21 days. Panel A: Kaplan-Meier survival probability curves. Panel B: Mean body weight change (%) relative to day 0. Bars represent the 95% CI of the mean. If a mouse died / was euthanized during follow-up of the study, the last observed body weight was carried forward. Panel C: Median clinical score. Bars represent interquartile ranges. 0 = no clinical signs; 1 = rough coat; 2 = rough coat, less reactive, passive during handling; 3 = rough coat, rolled up, laboured breathing, passive during handling; 4 = rough coat, rolled up, laboured breathing, does not roll back on stomach when laid down on its back. Mice observed with clinical score 4 were euthanized on the same day.

DESCRIPTION OF THE INVENTION

Definitions of terms as used in the invention are given below.

The term "included" or "including" as used herein is deemed to be followed by the words "without limitation".

As used herein the term "binding molecule" refers to an intact immunoglobulin including monoclonal antibodies, such as chimeric, humanized or human monoclonal antibodies, or to an antigen-binding and/or variable domain comprising fragment of an immunoglobulin that competes with the intact immunoglobulin for specific binding to the binding partner of the immunoglobulin, *e.g.* H3. Regardless of structure, the antigen-binding fragment binds with the same antigen that is recognized by the intact immunoglobulin. An antigen-binding fragment can comprise a peptide or polypeptide comprising an amino acid sequence of at least 2, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, or 250 contiguous amino acid residues of the amino acid sequence of the binding molecule.

The term "binding molecule", as used herein includes all immunoglobulin classes and subclasses known in the art. Depending on the amino acid sequence of the constant domain of their heavy chains, binding molecules can be divided into the five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4.

Antigen-binding fragments include, *inter alia*, Fab, F(ab'), F(ab')2, Fv, dAb, Fd, complementarity determining region (CDR) fragments, single-chain antibodies (scFv), bivalent single-chain antibodies, single-chain phage antibodies, diabodies, triabodies, tetrabodies, (poly)peptides that contain at least a fragment of an immunoglobulin that is sufficient to confer specific antigen binding to the (poly)peptide, etc. The above fragments may be produced synthetically or by enzymatic or chemical cleavage of intact immunoglobulins or they may be genetically engineered by recombinant DNA techniques. The methods of production are well known in the art and are described, for example, in Antibodies: A Laboratory Manual, Edited by: E. Harlow and D, Lane (1988), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, which is incorporated herein by reference. A binding molecule or antigen-binding fragment thereof may have one or more binding sites. If

there is more than one binding site, the binding sites may be identical to one another or they may be different.

The binding molecule can be a naked or unconjugated binding molecule but can also be part of an immunoconjugate. A naked or unconjugated binding molecule is intended to refer to a binding molecule that is not conjugated, operatively linked or otherwise physically or functionally associated with an effector moiety or tag, such as *inter alia* a toxic substance, a radioactive substance, a liposome, an enzyme. It will be understood that naked or unconjugated binding molecules do not exclude binding molecules that have been stabilized, multimerized, humanized or in any other way manipulated, other than by the attachment of an effector moiety or tag. Accordingly, all post-translationally modified naked and unconjugated binding molecules are included herewith, including where the modifications are made in the natural binding molecule-producing cell environment, by a recombinant binding molecule-producing cell, and are introduced by the hand of man after initial binding molecule preparation. Of course, the term naked or unconjugated binding molecule does not exclude the ability of the binding molecule to form functional associations with effector cells and/or molecules after administration to the body, as some of such interactions are necessary in order to exert a biological effect. The lack of associated effector group or tag is therefore applied in definition to the naked or unconjugated binding molecule *in vitro*, not *in vivo*.

As used herein, the term "biological sample" encompasses a variety of sample types, including blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures, or cells derived there from and the progeny thereof. The term also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides. The term encompasses various kinds of clinical samples obtained from any species, and also includes cells in culture, cell supernatants and cell lysates.

The term "complementarity determining regions" (CDR) as used herein means sequences within the variable regions of binding molecules, such as immunoglobulins, that usually contribute to a large extent to the antigen binding site which is complementary in shape and charge distribution to the epitope recognized on

the antigen. The CDR regions can be specific for linear epitopes, discontinuous epitopes, or conformational epitopes of proteins or protein fragments, either as present on the protein in its native conformation or, in some cases, as present on the proteins as denatured, *e.g.*, by solubilization in SDS. Epitopes may also consist of posttranslational modifications of proteins.

The term "deletion", as used herein, denotes a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent as compared to the reference, often the naturally occurring, molecule.

The term "expression-regulating nucleic acid sequence" as used herein refers to polynucleotide sequences necessary for and/or affecting the expression of an operably linked coding sequence in a particular host organism. The expression-regulating nucleic acid sequences, such as *inter alia* appropriate transcription initiation, termination, promoter, enhancer sequences; repressor or activator sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*e.g.*, ribosome binding sites); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion, can be any nucleic acid sequence showing activity in the host organism of choice and can be derived from genes encoding proteins, which are either homologous or heterologous to the host organism. The identification and employment of expression-regulating sequences is routine to the person skilled in the art.

The term "functional variant", as used herein, refers to a binding molecule that comprises a nucleotide and/or amino acid sequence that is altered by one or more nucleotides and/or amino acids compared to the nucleotide and/or amino acid sequences of the reference binding molecule and that is still capable of competing for binding to the binding partner, *e.g.* H3N2, with the reference binding molecule. In other words, the modifications in the amino acid and/or nucleotide sequence of the reference binding molecule do not significantly affect or alter the binding characteristics of the binding molecule encoded by the nucleotide sequence or containing the amino acid sequence, *i.e.* the binding molecule is still able to recognize

and bind its target. The functional variant may have conservative sequence modifications including nucleotide and amino acid substitutions, additions and deletions. These modifications can be introduced by standard techniques known in the art, such as site-directed mutagenesis and random PCR-mediated mutagenesis, and may comprise natural as well as non-natural nucleotides and amino acids.

Conservative amino acid substitutions include the ones in which the amino acid residue is replaced with an amino acid residue having similar structural or chemical properties. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.,* aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), non-polar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan). It will be clear to the skilled artisan that other classifications of amino acid residue families than the one used above can also be employed. Furthermore, a variant may have non-conservative amino acid substitutions, *e.g.*, replacement of an amino acid with an amino acid residue having different structural or chemical properties. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing immunological activity may be found using computer programs well known in the art.

A mutation in a nucleotide sequence can be a single alteration made at a locus (a point mutation), such as transition or transversion mutations, or alternatively, multiple nucleotides may be inserted, deleted or changed at a single locus. In addition, one or more alterations may be made at any number of loci within a nucleotide sequence. The mutations may be performed by any suitable method known in the art. The term “influenza virus subtype” as used herein refers to influenza A virus variants that are characterized by various combinations of the hemagglutinin (H) and neuramidase (N) viral surface proteins. According to the present invention influenza virus subtypes may be referred to by their H number, such as for example “influenza virus comprising HA of the H3 subtype”, or “H3 influenza”, or by a combination of a H number and an N number, such as for example “influenza virus subtype H3N2” or “H3N2”. The term “subtype” specifically includes all individual “strains” within each

subtype, which usually result from mutations and show different pathogenic profiles. Such strains may also be refered to as various "isolates" of a viral subtype. Accordingly, as used herein, the terms "strains" and "isolates" may be used interchangeably. The current nomenclature for human influenza virus strains or isolates includes the geographical location of the first isolation, strain number and year of isolation, usually with the antigenic description of HA and NA given in brackets, e.g. A/Moscow/10/00 (H3N2). Non-human strains also include the host of origin in the nomenclature.

The influenza virus subtypes can further be classified by reference to their phylogenetic group. Phylogenetic analysis (Fouchier et al., 2005) has demonstrated a subdivision of HA's that falls into two main groups (Air, 1981): *inter alia* the H1, H2, H5 and H9 subtypes in phylogenetic group 1 and *inter alia* the H3, H4 and H7 subtypes in phylogenetic group 2 (Figure 1).

The term "neutralizing" as used herein in relation to the binding molecules of the invention refers to binding molecules that inhibit an influenza virus from replicatively infecting a target cell, regardless of the mechanism by which neutralization is achieved. Thus, neutralization can e.g. be achieved by inhibiting the attachment or adhesion of the virus to the cell surface, or by inhibition of the fusion of viral and cellular membranes following attachement of the virus to the target cell, and the like.

The term "cross-neutralizing" or "cross-neutralization" as used herein in relation to the binding molecules of the invention refers to the ability of the binding molecules of the invention to neutralize influenza A viruses of different subtypes, such as for example influenza viruses comprising HA of the H3, H7 and/or H10 subtype.

The term "host", as used herein, is intended to refer to an organism or a cell into which a vector such as a cloning vector or an expression vector has been introduced. The organism or cell can be prokaryotic or eukaryotic. Preferably, the hosts isolated host cells, e.g. host cells in culture. The term "host cells" merely signifies that the cells are modified for the (over)-expression of the binding molecules of the invention and include B-cells that originally express these binding molecules and which cells have been modified to over-express the binding molecule by immortalization, amplification, enhancement of expression etc. It should be understood that the term

host is intended to refer not only to the particular subject organism or cell but to the progeny of such an organism or cell as well. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent organism or cell, but are still included within the scope of the term "host" as used herein.

The term "human", when applied to binding molecules as defined herein, refers to molecules that are either directly derived from a human or based upon a human sequence. When a binding molecule is derived from or based on a human sequence and subsequently modified, it is still to be considered human as used throughout the specification. In other words, the term human, when applied to binding molecules is intended to include binding molecules having variable and constant regions derived from human germline immunoglobulin sequences or based on variable or constant regions occurring in a human or human lymphocyte and modified in some form. Thus, the human binding molecules may include amino acid residues not encoded by human germline immunoglobulin sequences, comprise substitutions and/or deletions (*e.g.*, mutations introduced by for instance random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). "Based on" as used herein refers to the situation that a nucleic acid sequence may be exactly copied from a template, or with minor mutations, such as by error-prone PCR methods, or synthetically made matching the template exactly or with minor modifications. Semi-synthetic molecules based on human sequences are also considered to be human as used herein.

The term "insertion", also known as the term "addition", denotes a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the parent sequence.

The term "isolated", when applied to binding molecules as defined herein, refers to binding molecules that are substantially free of other proteins or polypeptides, particularly free of other binding molecules having different antigenic specificities, and are also substantially free of other cellular material and/or chemicals. For example, when the binding molecules are recombinantly produced, they are preferably substantially free of culture medium components, and when the binding molecules are produced by chemical synthesis, they are preferably substantially free

of chemical precursors or other chemicals, *i.e.*, they are separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. The term "isolated" when applied to nucleic acid molecules encoding binding molecules as defined herein, is intended to refer to nucleic acid molecules in which the nucleotide sequences encoding the binding molecules are free of other nucleotide sequences, particularly nucleotide sequences encoding binding molecules that bind binding partners other than H5N1. Furthermore, the term "isolated" refers to nucleic acid molecules that are substantially separated from other cellular components that naturally accompany the native nucleic acid molecule in its natural host, *e.g.*, ribosomes, polymerases, or genomic sequences with which it is naturally associated. Moreover, "isolated" nucleic acid molecules, such as cDNA molecules, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

The term "monoclonal antibody" as used herein refers to a preparation of antibody molecules of single specificity. A monoclonal antibody displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to an antibody displaying a single binding specificity which has variable and constant regions derived from or based on human germline immunoglobulin sequences or derived from completely synthetic sequences. The method of preparing the monoclonal antibody is not relevant for the binding specificity.

The term "naturally occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

The term "nucleic acid molecule" as used in the present invention refers to a polymeric form of nucleotides and includes both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide, deoxynucleotide or a modified form of either

type of nucleotide. The term also includes single- and double-stranded forms of DNA. In addition, a polynucleotide may include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages. The nucleic acid molecules may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, etc.), pendent moieties (*e.g.*, polypeptides), intercalators (*e.g.*, acridine, psoralen, etc.), chelators, alkylators, and modified linkages (*e.g.*, alpha anomeric nucleic acids, etc.). The above term is also intended to include any topological conformation, including single-stranded, double-stranded, partially duplexed, triplex, hairpinned, circular and padlocked conformations. Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule. A reference to a nucleic acid sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid molecule having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence. The complementary strand is also useful, *e.g.*, for anti-sense therapy, hybridisation probes and PCR primers.

The term "operably linked" refers to two or more nucleic acid sequence elements that are usually physically linked and are in a functional relationship with each other. For instance, a promoter is operably linked to a coding sequence, if the promoter is able to initiate or regulate the transcription or expression of a coding sequence, in which case the coding sequence should be understood as being "under the control of" the promoter.

By "pharmaceutically acceptable excipient" is meant any inert substance that is combined with an active molecule such as a drug, agent, or binding molecule for preparing an agreeable or convenient dosage form. The "pharmaceutically acceptable

excipient" is an excipient that is non-toxic to recipients at the dosages and concentrations employed, and is compatible with other ingredients of the formulation comprising the drug, agent or binding molecule. Pharmaceutically acceptable excipients are widely applied in the art.

The term "specifically binding", as used herein, in reference to the interaction of a binding molecule, *e.g.* an antibody, and its binding partner, *e.g.* an antigen, means that the interaction is dependent upon the presence of a particular structure, *e.g.* an antigenic determinant or epitope, on the binding partner. In other words, the antibody preferentially binds or recognizes the binding partner even when the binding partner is present in a mixture of other molecules or organisms. The binding may be mediated by covalent or non-covalent interactions or a combination of both. In yet other words, the term "specifically binding" means immunospecifically binding to an antigenic determinant or epitope and not immunospecifically binding to other antigenic determinants or epitopes. A binding molecule that immunospecifically binds to an antigen may bind to other peptides or polypeptides with lower affinity as determined by, *e.g.*, radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), BIACORE, or other assays known in the art. Binding molecules or fragments thereof that immunospecifically bind to an antigen may be cross-reactive with related antigens, carrying the same epitope. Preferably, binding molecules or fragments thereof that immunospecifically bind to an antigen do not cross-react with other antigens.

A "substitution", as used herein, denotes the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

The term "therapeutically effective amount" refers to an amount of the binding molecule as defined herein that is effective for preventing, ameliorating and/or treating a condition resulting from infection with influenza of the H3 subtype. Ameloriation as used in herein may refer to the reduction of visible or perceptible disease symptoms, viremaia, or any other measurable manifestation of influenza infection.

The term "treatment" refers to therapeutic treatment as well as prophylactic or preventative measures to cure or halt or at least retard disease progress. Those in need of treatment include those already inflicted with a condition resulting from infection with influenza virus comprising HA of the H3 subtype as well as those in which infection with influenza virus comprising HA of the H3 subtype is to be prevented. Subjects partially or totally recovered from infection with H3 influenza might also be in need of treatment. Prevention encompasses inhibiting or reducing the spread of influenza virus comprising HA of the H3 subtype or inhibiting or reducing the onset, development or progression of one or more of the symptoms associated with infection with H3 influenza.

The term "vector" denotes a nucleic acid molecule into which a second nucleic acid molecule can be inserted for introduction into a host where it will be replicated, and in some cases expressed. In other words, a vector is capable of transporting a nucleic acid molecule to which it has been linked. Cloning as well as expression vectors are contemplated by the term "vector", as used herein. Vectors include, but are not limited to, plasmids, cosmids, bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC) and vectors derived from bacteriophages or plant or animal (including human) viruses. Vectors comprise an origin of replication recognized by the proposed host and in case of expression vectors, promoter and other regulatory regions recognized by the host. A vector containing a second nucleic acid molecule is introduced into a cell by transformation, transfection, or by making use of viral entry mechanisms. Certain vectors are capable of autonomous replication in a host into which they are introduced (*e.g.*, vectors having a bacterial origin of replication can replicate in bacteria). Other vectors can be integrated into the genome of a host upon introduction into the host, and thereby are replicated along with the host genome.

SUMMARY OF THE INVENTION

The invention provides human binding molecules capable of specifically binding to influenza virus strains comprising HA of the H3 subtype, including H3N2, and exhibiting neutralizing activity against such influenza virus. In an embodiment, the binding molecules of the invention are unique in that they are capable of

neutralizing several, including at least one or more recent, strains, preferably all known strains, of influenza virus subtype H3, the most common epidemic subtype in humans, with high potency. In an embodiment the binding molecules bind to a conserved epitope in the stem region of the H3 HA protein. In an embodiment, the binding molecules have hemagglutination inhibiting activity. In an embodiment, the binding molecules are capable of preventing *in vitro* cleavage of the HA precursor molecule HA0. In an embodiment the binding molecules of the present invention are capable of preventing the conformational change of the HA protein required for fusion of the influenza viral membrane with the endosomal membrane of an infected cell.

The invention also provides binding molecules that bind to an epitope in the haemagglutinin protein that is shared between influenza subtypes within the phylogenetic group 2 to which H3 subtypes belong, and therefore relates to binding molecules that cross-react between H3-, H7-, and/or H10 influenza based subtypes, and other influenza subtypes that contain the HA protein with these particular epitopes, preferably all subtypes of phylogenetic group 2. Several binding molecules of the present invention are thus unique in that they posses cross-neutralizing activity against one or more other influenza virus A subtypes, such as influenza viruses comprising HA of the H7 and/or the H10 subtype. Preferably, the binding molecules of the present invention are capable of cross-neutralizing all influenza virus subtypes of phylogenetic group 2, encompassing the H3, H7 and H10 subtypes, and thus can be used as a universal prophylactic, diagnostic and/or treatment agent for influenza viruses belonging to phylogenetic group 2, even irrespective of the causative influenza subtype within that phylogenetic group.

It is surmised that the binding molecules according to the present invention bind to hitherto unkown conserved epitopes that are not or much less prone to antigenic drift or shift. Hence it is also encompassed to use the binding molecules of the invention to identify and/or characterize these epitopes. The invention also pertains to nucleic acid molecules encoding at least the binding region of the human binding molecules. The invention further provides for the use of the human binding molecules of the invention in the prophylaxis and/or treatment of a subject having, or at risk of developing, an H3 influenza infection, such as a H3N2 influenza infection. Furthermore, the invention pertains to the use of the human binding molecules of the invention in the diagnosis/detection of such influenza infection.

DETAILED DESCRIPTION

In a first aspect the present invention encompasses binding molecules capable of specifically binding to and having neutralizing activity against influenza virus A, particularly influenza virus A comprising HA of the H3 subtype, in particular H3N2. Preferably, the binding molecules are human binding molecules. In an embodiment, the binding molecules of the invention are capable of specifically binding to and/or have neutralizing activity against several influenza virus H3N2 strains, preferably two or more different H3N2 strains, more preferably three or more, more preferably four or more, more preferably five or more different H3N2 strains. The strains may be obtained from both humans or from non-human animals, e.g birds. In an embodiment, the binding molecules bind to and neutralize at least one or more of the recent H3N2 strains selected from the group consisting of A/Wisconsin/67/2005, A/Hiroshima/52/2005, A/Panama/2007/99, and A/Johannesburg/33/94. In another embodiment, the binding molecules also bind to and neutralize the H3N2 strain A/Hong Kong/1/68. Most preferably, the binding molecules bind to and have neutralizing activity against all influenza H3N2 strains from the years between 1968 and 2005. Preferably, the binding molecules have neutralizing activity against at least all naturally occurring isolates of influenza virus H3N2 known before January 20, 2010.

The binding molecules of the invention may be capable of specifically binding to the HA0, HA1 and/or HA2 subunit of the HA protein. They may be capable of specifically binding to linear or structural and/or conformational epitopes on the HA0, HA1 and/or HA2 subunit of the HA protein. The HA molecule may be purified from viruses or recombinantly produced and optionally isolated before use. Alternatively, HA may be expressed on the surface of cells. Preferably, the binding molecules of the invention bind to an epitope comprising one or more of the the amino acids at position 19, 25, 27, 33 and 34 of the HA2 polypeptide of the H3 HA protein. In an embodiment, the binding molecules bind to said epitope on HA2, when the amino acid on position 19 is aspartic acid (D), the amino acid on position 25 is glutamine (Q), the amino acid on position 27 is glycine (G), the amino acid at position 33 is glycine (G) and/or the amino acid at position 34 is glutamine (numbering of HA2 starting at position 1 just following the Arginine residue that constitutes the cleavage

site between HA1 and HA2). In an embodiment, the binding molecules do not bind to said epitope on HA2 when one or more of said amino acids have changed.

In another aspect, the present invention encompasses binding molecules that are capable of, at least *in vitro,* preventing the trypsin cleavage of the H3 HA precursor molecule HA0 in HA1 and HA2.

In another aspect, the present invention encompasses binding molecules that are capable of preventing the conformational change of the H3 HA protein, required for membrane fusion of the influenza viral membrane and the endosomal membrane of an infected cell, at least *in vitro*.

In another aspect, the binding molecules have some or all of the properties listed above, i.e. cross-neutralizing activity, binding to a conserved epitope on the stem region of HA2, inhibiting *in vitro* trypsin cleavage of HA0, and/or inhibiting conformational change.

In an embodiment, the binding molecules of the invention have all or some of the properties above and, in addition, are not capable of binding to and/or neutralizing influenza virus A comprising HA of the H1 subtype, such as H1N1.

The binding molecules of the invention may be capable of specifically binding to e.g. influenza virus H3N2 that are viable, living and/or infective or that are in inactivated/attenuated form. Methods for inactivating/attenuating virus, e.g. influenza virus H3N2, are well known in the art and include, but are not limited to, treatment with formalin, β-propiolactone (BPL), merthiolate, and/or ultraviolet light.

The binding molecules of the invention may also be capable of specifically binding to one or more fragments of the influenza viruses, such as *inter alia* a preparation of one or more proteins and/or (poly)peptides derived from subtype H3N2 or one or more recombinantly produced proteins and/or polypeptides of H3N2. For methods of treatment and/or prevention of H3N2 infections the binding molecules are preferably capable of specifically binding to surface accessible proteins of H3N2 such as the surface glycoproteins, hemagglutinin (HA), which is required for viral attachment and cellular release.

The nucleotide and/or amino acid sequence of proteins of various H3N2 strains can be found in the GenBank-database, NCBI Influenza Virus Sequence Database, Influenza Sequence Database (ISD), EMBL-database and/or other databases. It is well within the reach of the skilled person to find such sequences in the respective databases.

In another embodiment the binding molecules of the invention are capable of specifically binding to a fragment of the above-mentioned proteins and/or polypeptides, wherein the fragment at least comprises an epitope recognized by the binding molecules of the invention. An "epitope" as used herein is a moiety that is capable of binding to a binding molecule of the invention with sufficiently high affinity to form a detectable antigen-binding molecule complex.

The binding molecules of the invention may or may not be capable of specifically binding to the extracellular part of HA (also called herein soluble HA (sHA)).

The binding molecules of the invention can be intact immunoglobulin molecules such as polyclonal or monoclonal antibodies or the binding molecules can be antigen-binding fragments including, but not limited to, Fab, F(ab'), $F(ab')_2$, Fv, dAb, Fd, complementarity determining region (CDR) fragments, single-chain antibodies (scFv), bivalent single-chain antibodies, single-chain phage antibodies, diabodies, triabodies, tetrabodies, and (poly)peptides that contain at least a fragment of an immunoglobulin that is sufficient to confer specific antigen binding to influenza virus H3N2 strains or a fragment thereof. In a preferred embodiment the binding molecules of the invention are human monoclonal antibodies.

The binding molecules of the invention can be used in non-isolated or isolated form. Furthermore, the binding molecules of the invention can be used alone or in a mixture comprising at least one binding molecule (or variant or fragment thereof) of the invention. In other words, the binding molecules can be used in combination, *e.g.*, as a pharmaceutical composition comprising two or more binding molecules of the invention, variants or fragments thereof. For example, binding molecules having different, but complementary activities can be combined in a single therapy to achieve a desired prophylactic, therapeutic or diagnostic effect, but alternatively, binding molecules having identical activities can also be combined in a single therapy to achieve a desired prophylactic, therapeutic or diagnostic effect. Optionally, the mixture further comprises at least one other therapeutic agent. Preferably, the therapeutic agent such as, *e.g.*, M2 inhibitors (*e.g.*, amantidine, rimantadine) and/or neuraminidase inhibitors (*e.g.*, zanamivir, oseltamivir) is useful in the prophylaxis and/or treatment of an influenza virus H3N2 infection

Typically, binding molecules according to the invention can bind to their binding partners, *i.e.* influenza virus H3N2 or fragments thereof, with an affinity

constant (K_d-value) that is lower than $0.2x10^{-4}$ M, $1.0x10^{-5}$ M, $1.0x10^{-6}$ M, $1.0x10^{-7}$ M, preferably lower than $1.0x10^{-8}$ M, more preferably lower than $1.0x10^{-9}$ M, more preferably lower than $1.0x10^{-10}$ M, even more preferably lower than $1.0x10^{-11}$ M, and in particular lower than $1.0x10^{-12}$ M. The affinity constants can vary for antibody isotypes. For example, affinity binding for an IgM isotype refers to a binding affinity of at least about $1.0x10^{-7}$ M. Affinity constants can for instance be measured using surface plasmon resonance, for example using the BIACORE system (Pharmacia Biosensor AB, Uppsala, Sweden).

Typically, the binding molecules according to the invention have a neutralizing activity of 10 μg/ml or less, preferably 5 μg/ml or less, more preferably 2 μg/ml or less, even more preferably 1 μg/ml or less, as determined in an *in vitro* virus neutralization assay (VNA) as described in Example 6.

The binding molecules according to the invention may bind to influenza virus H3N2 or a fragment thereof in soluble form such as for instance in a sample or in suspension or may bind to influenza virus H3N2 or a fragment thereof bound or attached to a carrier or substrate, *e.g.*, microtiter plates, membranes and beads, etc. Carriers or substrates may be made of glass, plastic (*e.g.*, polystyrene), polysaccharides, nylon, nitrocellulose, or Teflon, etc. The surface of such supports may be solid or porous and of any convenient shape. Furthermore, the binding molecules may bind to influenza virus H3N2 in purified/isolated or non-purified/non-isolated form.

The binding molecules of the invention exhibit neutralizing activity. Neutralizing activity can for instance be measured as described herein. Alternative assays measuring neutralizing activity are described in for instance WHO Manual on Animal Influenza Diagnosis and Surveillance, Geneva: World Health Organisation, 2005, version 2002.5.

The present invention relates to an isolated human binding molecule that is able to recognize and bind to an epitope in the influenza haemagglutinin protein (HA), characterized in that said binding molecule has neutralizing activity against an influenza virus A, comprising HA of the H3 subtype. An example of an influenza subtype that contains HA of the H3 subtype is H3N2. Particularly preferred are binding molecules that neutralize the H3N2 influenza subtype. In an embodiment the binding molecules neutralizes at least one or more of the recent H3N2 strains. In an

embodiment, the binding molecules thus at least bind to and neutralize one or more H3N2 strains selected from the group consisting of A/Wisconsin/67/2005, A/Hiroshima/52/2005, A/Panama/2007/99, and A/Johannesburg/33/94. In another embodiment, the binding molecules also bind to and neutralize the H3N2 strain A/Hong Kong/1/68. Most preferably, the binding molecules bind to and have neutralizing activity against all influenza H3N2 strains from the years between 1968 and 2005, preferably all known strains of said influenza virus subtype.

In another embodiment, the binding molecules according to the invention also have neutralizing activity against influenza viruses of other influenza virus A subtypes, preferably at least influenza viruses comprising HA of the H7 subtype, such as the strain A/Mallard/Netherlands/12/2000, and/or H10 subtype, such as the strain A/chick/Germany/N/49. It thus has been shown that some of the binding molecules of the present invention cross-neutralize these influenza subtypes. The invention thus also provides binding molecules that bind to an epitope in the haemagglutinin protein that is shared and conserved between influenza subtypes and therefore relates to binding molecules that cross-react between H3-, H7-, and/or H10 influenza based subtypes, and other influenza subtypes that contain the HA protein with these particular epitopes, preferably all influenza viruses of phylogenetic group 2.. The cross-neutralizing binding molecules preferably bind to and neutralize several strains of the H3-, H7, and/or H10-subtypes. In an embodiment, these cross-neutralizing binding molecules bind to and neutralize at least one or more of the recent H3N2 strains selected from the group consisting of A/Wisconsin/67/2005, A/Hiroshima/52/2005, A/Johannesburg/33/94, and A/Panama/2007/99. In another embodiment, the binding molecules also bind to and neutralize the H3N2 strain A/Hong Kong/1/68. Most preferably, the binding molecules bind to and have neutralizing activity against all influenza H3N2 strains from the years between 1968 and 2005, preferably all known, and even more preferably also future H3N2 strains. In a further embodiment the binding molecules neutralize substantially all isolates of said other influenza virus subtypes.

In an embodiment, the binding molecules bind to and neutralize all influenza virus subtypes of phylogenetic group 2.

The skilled person, based on what has been disclosed herein, can determine whether an antibody indeed cross-reacts with HA proteins from different subtypes and

also determine whether they are able to neutralize influenza viruses of different subtypes *in vivo*.

Influenza viruses infect cells by binding to sialic acid residues on the cell surface of target cells, and following transfer into endosomes, by fusing their membranes with the endosomal membranes and releasing the genome-transcriptase complex into the cell. Both receptor binding and membrane fusion process are mediated by the HA glycoprotein. The HA of influenza virus A comprises two structurally distinct regions, i.e. a globular head region, which contains a receptor binding site which is responsible for virus attachment to the target cell, and is involved in the haemagglutination activity of HA, and a stem region, containing a fusion peptide which is necessary for membrane fusion between the viral envelope and the endosomal membrane of the cell. The HA protein is a trimer in which each monomer consists of two disulphide - linked glycopolypeptides, HA1 and HA2, that are produced during infection by proteolytic cleavage of a precursor (HA0). Cleavage is necessary for virus infectivity since it is required to prime the HA for membrane fusion, to allow conformational change. Activation of the primed molecule occurs at low pH in endosomes, between pH5 and pH6, and requires extensive changes in HA structure. The 3-dimensional structure of the pre-fusion uncleaved (**I**), pre-fusion cleaved (**II**) and post-fusion HA (**III**) conformations are schematically shown in Figure 4. Each of the stages in the priming and activation of HA for its participation in the membrane fusion process presents a different target for inhibition, e.g. by monoclonal antibodies.

In an embodiment of the present invention, the binding molecules are at least capable of preventing the cleavage of the HA precursor molecule HA0 in an *in vitro* assay., e.g. an assay as described below in the Examples. As explained above, cleavage of the HA precursor molecule HA0 into HA1 and HA2 by host proteases is required to activate virus infectivity. The prevention of cleavage of the HA precursor molecule HA0 by the binding molecules of the invention thus may prevent infection by the influenza virus.

In an embodiment, the binding molecules bind to an epitope comprising the amino acid at position 19, 25, 27, 33 and/or 34 of the HA2 polypeptide of the H3 HA protein. In an embodiment, the binding molecules bind to said epitope on HA2, when the amino acid on position 19 is aspartic acid (D), the amino acid on position 25 is

glutamine (Q), the amino acid on position 27 is glycine (G), the amino acid at position 33 is glycine (G) and/or the amino acid at position 34 is glutamine. Preferably, the binding molecules do not bind to said epitope on HA2 when one or more of said amino acids have changed. The numbering of the amino acids is based on the hemagglutin sequence from Uniprot database number Q91MA7 (SEQ ID NO: 193). Q91MA7 gives the full length sequence of immature HA from A/Hong Kong/1/1968. The HA2 sequence starts at G346 of the uncleaved HA immature protein. In the numbering above the G346 is G1 in HA2 sequence.

Preferred is a binding molecule according to the present invention that is selected from the group consisting of:

a) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:81, a heavy chain CDR2 region of SEQ ID NO:82, and a heavy chain CDR3 region of SEQ ID NO:83,

b) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:87, a heavy chain CDR2 region of SEQ ID NO:88, and a heavy chain CDR3 region of SEQ ID NO:89,

c) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:103, a heavy chain CDR2 region of SEQ ID NO:104, and a heavy chain CDR3 region of SEQ ID NO:105,

d) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:109, a heavy chain CDR2 region of SEQ ID NO:110, and a heavy chain CDR3 region of SEQ ID NO:111,

e) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:115, a heavy chain CDR2 region of SEQ ID NO:116, and a heavy chain CDR3 region of SEQ ID NO:117,

f) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:121, a heavy chain CDR2 region of SEQ ID NO:122, and a heavy chain CDR3 region of SEQ ID NO:123,

g) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:126, a heavy chain CDR2 region of SEQ ID NO:127, and a heavy chain CDR3 region of SEQ ID NO:128,

h) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:132, a heavy chain CDR2 region of SEQ ID NO:133, and a heavy chain CDR3 region of SEQ ID NO:134,

i) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:138, a heavy chain CDR2 region of SEQ ID NO:139, and a heavy chain CDR3 region of SEQ ID NO:140,

j) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:144, a heavy chain CDR2 region of SEQ ID NO:145, and a heavy chain CDR3 region of SEQ ID NO:146,

k) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:150, a heavy chain CDR2 region of SEQ ID NO:151, and a heavy chain CDR3 region of SEQ ID NO:152,

l) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:156, a heavy chain CDR2 region of SEQ ID NO:157, and a heavy chain CDR3 region of SEQ ID NO:158,

m) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:162, a heavy chain CDR2 region of SEQ ID NO:163, and a heavy chain CDR3 region of SEQ ID NO:164,

n) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:168, a heavy chain CDR2 region of SEQ ID NO:169, and a heavy chain CDR3 region of SEQ ID NO:170,

o) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:173, a heavy chain CDR2 region of SEQ ID NO:174, and a heavy chain CDR3 region of SEQ ID NO:175, and

p) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:179, a heavy chain CDR2 region of SEQ ID NO:180, and a heavy chain CDR3 region of SEQ ID NO:181.

In a preferred embodiment, the binding molecule according to the invention is for a use as a medicament and preferably for the diagnostic, therapeutic and/or prophylactic treatment of influenza infection. Preferably, the influenza virus that causes the influenza infection and that can be treated by the binding molecules of the present invention, is influenza virus subtype H3N2. The present invention also relates to a

pharmaceutical composition comprising a binding molecule according to the invention, and a pharmaceutically acceptable excipient.

In yet another embodiment the invention relates to a use of a binding molecule according to the invention in the preparation of a medicament for the diagnosis, prophylaxis, and/or treatment of an influenza virus infection. Such infections can occur in small populations, but can also spread around the world in seasonal epidemics or, worse, in global pandemics where millions of individuals are at risk. The invention provides binding molecules that can neutralize the infection of influenza strains that cause such seasonal epidemics, as well as potential pandemics. Importantly, protection and treatment can be envisioned now with the binding molecules of the present invention in relation to various influenza subtypes as it has been disclosed that the binding molecules of the present invention are capable of cross-neutralizing various influenza subtypes of phylogenetic group 2, encompassing subtypes H3, H7 and H10.

In a preferred embodiment, the human binding molecules according to the invention are characterized in that the human binding molecules are selected from the group consisting of:

a) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:81, a heavy chain CDR2 region of SEQ ID NO:82, and a heavy chain CDR3 region of SEQ ID NO:83, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:84, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:85, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:86,

b) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:87, a heavy chain CDR2 region of SEQ ID NO:88, and a heavy chain CDR3 region of SEQ ID NO:89, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:90, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:91, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:92,

c) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:87, a heavy chain CDR2 region of SEQ ID NO:88, and a heavy chain CDR3 region of SEQ ID NO:89, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:93, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:94, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:95,

d) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:87, a heavy chain CDR2 region of SEQ ID NO:88, and a heavy chain CDR3 region of SEQ ID NO:89, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:96, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:97, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:98,

e) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:87, a heavy chain CDR2 region of SEQ ID NO:88, and a heavy chain CDR3 region of SEQ ID NO:89, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:99, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:100, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:101,

f) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:87, a heavy chain CDR2 region of SEQ ID NO:88, and a heavy chain CDR3 region of SEQ ID NO:89, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:102, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:85, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:86,

g) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:103, a heavy chain CDR2 region of SEQ ID NO:104, and a heavy chain CDR3 region of SEQ ID NO:105, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:106, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:107, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:108,

h) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:109, a heavy chain CDR2 region of SEQ ID NO:110, and a heavy chain CDR3 region of SEQ ID NO:111, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:112, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:113, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:114,

i) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:115, a heavy chain CDR2 region of SEQ ID NO:116, and a heavy chain CDR3 region of SEQ ID NO:117, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:118, a light chain CDR2 region having the amino acid sequence of SEQ ID

NO:119, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:120,

j) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:121, a heavy chain CDR2 region of SEQ ID NO:122, and a heavy chain CDR3 region of SEQ ID NO:123, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:124, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:119, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:125,

k) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:126, a heavy chain CDR2 region of SEQ ID NO:127, and a heavy chain CDR3 region of SEQ ID NO:128, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:129, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:130, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:131,

l) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:132, a heavy chain CDR2 region of SEQ ID NO:133, and a heavy chain CDR3 region of SEQ ID NO:134, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:135, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:136, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:137,

m) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:138, a heavy chain CDR2 region of SEQ ID NO:139, and a heavy chain CDR3 region of SEQ ID NO:140, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:141, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:142, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:143,

n) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:144, a heavy chain CDR2 region of SEQ ID NO:145, and a heavy chain CDR3 region of SEQ ID NO:146, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:147, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:148, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:149,

o) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:150, a heavy chain CDR2 region of SEQ ID NO:151, and a heavy chain CDR3 region of

SEQ ID NO:152, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:153, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:154, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:155,

p) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:156, a heavy chain CDR2 region of SEQ ID NO:157, and a heavy chain CDR3 region of SEQ ID NO:158, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:159, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:160, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:161,

q) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:162, a heavy chain CDR2 region of SEQ ID NO:163, and a heavy chain CDR3 region of SEQ ID NO:164, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:165, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:166, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:167,

r) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:168, a heavy chain CDR2 region of SEQ ID NO:169, and a heavy chain CDR3 region of SEQ ID NO:170, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:171, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:172, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:137,

s) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:173, a heavy chain CDR2 region of SEQ ID NO:174, and a heavy chain CDR3 region of SEQ ID NO:175, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:176, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:177, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:178,

and

t) a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:179, a heavy chain CDR2 region of SEQ ID NO:180, and a heavy chain CDR3 region of SEQ ID NO:181, a light chain CDR1 region having the amino acid sequence of SEQ ID NO:182, a light chain CDR2 region having the amino acid sequence of SEQ ID

NO:183, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:184.

In a specific embodiment the binding molecules of the invention is selected from the group consisting of a binding molecule comprising a heavy chain CDR1 region having the amino acid sequence of SEQ ID NO: 81, a heavy chain CDR2 region having the amino acid sequence of SEQ ID NO: 82 and a heavy chain CDR3 region having the amino acid sequence of SEQ ID NO: 83; a binding molecule comprising a heavy chain CDR1 region having the amino acid sequence of SEQ ID NO: 109, a heavy chain CDR2 region having the amino acid sequence of SEQ ID NO: 110 and a heavy chain CDR3 region having the amino acid sequence of SEQ ID NO: 111; a binding molecule comprising a heavy chain CDR1 region having the amino acid sequence of SEQ ID NO: 138, a heavy chain CDR2 region having the amino acid sequence of SEQ ID NO: 139 and a heavy chain CDR3 region having the amino acid sequence of SEQ ID NO: 140; a binding molecule comprising a heavy chain CDR1 region having the amino acid sequence of SEQ ID NO: 144, a heavy chain CDR2 region having the amino acid sequence of SEQ ID NO: 145 and a heavy chain CDR3 region having the amino acid sequence of SEQ ID NO: 146; and a binding molecule comprising a heavy chain CDR1 region having the amino acid sequence of SEQ ID NO: 173, a heavy chain CDR2 region having the amino acid sequence of SEQ ID NO: 174 and a heavy chain CDR3 region having the amino acid sequence of SEQ ID NO: 175.

The CDR regions of the binding molecules of the invention are shown in Table 1. CDR regions are according to Kabat *et al.* (1991) as described in Sequences of Proteins of Immunological Interest. In an embodiment of the present invention binding molecules may comprise one, two, three, four, five or all six CDR regions as disclosed herein. Preferably, a binding molecule according to the present invention comprises at least two of the CDRs disclosed herein.

In yet another embodiment, the binding molecules according to the invention comprise a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:22, SEQ ID NO:26, SEQ ID NO:30, SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:42, SEQ ID NO:46, SEQ ID NO:50, SEQ ID

NO:54, SEQ ID NO:58, SEQ ID NO:62, SEQ ID NO:66, SEQ ID NO:70, SEQ ID NO:74, and SEQ ID NO:78. In a further embodiment, the binding molecules according to the invention comprise a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:32, SEQ ID NO:36, SEQ ID NO:40, SEQ ID NO:44, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:56, SEQ ID NO:60, SEQ ID NO:64, SEQ ID NO:68, SEQ ID NO:72, SEQ ID NO:76, and SEQ ID NO:80.

Another aspect of the invention includes functional variants of the binding molecules as defined herein. Molecules are considered to be functional variants of a binding molecule according to the invention, if the variants are capable of competing for specifically binding to influenza virus H3N2 or a fragment thereof with the "parental" or "reference" binding molecules. In other words, when the functional variants are still capable of binding to the same or overlapping epitope of the influenza virus H3N2 or a fragment thereof. For the sake of this application "parental" and "reference" will be used as synonyms meaning that the information of the reference or parental molecule, or the physical molecule itself may form the basis for the variation. Preferably, the functional variants are capable of competing for specifically binding to at least two (or more) different influenza virus H3N2 strains or fragments thereof that are specifically bound by the reference binding molecules. Furthermore, molecules are considered to be functional variants of a binding molecule according to the invention, if they have neutralizing activity against influenza virus H3N2, preferably against the at least two (or more) influenza virus H3N2 strains against which the parental binding molecule exhibits neutralizing activity. Functional variants include, but are not limited to, derivatives that are substantially similar in primary structural sequence, including those that have modifications in the Fc receptor or other regions involved with effector functions, and/or which contain *e.g. in vitro* or *in vivo* modifications, chemical and/or biochemical, that are not found in the parental binding molecule. Such modifications include *inter alia* acetylation, acylation, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, cross-linking, disulfide bond formation, glycosylation, hydroxylation, methylation, oxidation, pegylation, proteolytic processing, phosphorylation, and the like.

Alternatively, functional variants can be binding molecules as defined in the present invention comprising an amino acid sequence containing substitutions, insertions, deletions or combinations thereof of one or more amino acids compared to the amino acid sequences of the parental binding molecules. Furthermore, functional variants can comprise truncations of the amino acid sequence at either or both the amino or carboxyl termini. Functional variants according to the invention may have the same or different, either higher or lower, binding affinities compared to the parental binding molecule but are still capable of binding to influenza virus H3N2 or a fragment thereof. For instance, functional variants according to the invention may have increased or decreased binding affinities for influenza virus H3N2 or a fragment thereof compared to the parental binding molecules. Preferably, the amino acid sequences of the variable regions, including, but not limited to, framework regions, hypervariable regions, in particular the CDR3 regions, are modified. Generally, the light chain and the heavy chain variable regions comprise three hypervariable regions, comprising three CDRs, and more conserved regions, the so-called framework regions (FRs). The hypervariable regions comprise amino acid residues from CDRs and amino acid residues from hypervariable loops. Functional variants intended to fall within the scope of the present invention have at least about 50% to about 99%, preferably at least about 60% to about 99%, more preferably at least about 70% to about 99%, even more preferably at least about 80% to about 99%, most preferably at least about 90% to about 99%, in particular at least about 95% to about 99%, and in particular at least about 97% to about 99% amino acid sequence homology with the parental binding molecules as defined herein. Computer algorithms such as *inter alia* Gap or Bestfit known to a person skilled in the art can be used to optimally align amino acid sequences to be compared and to define similar or identical amino acid residues. Functional variants can be obtained by altering the parental binding molecules or parts thereof by general molecular biology methods known in the art including, but not limited to, error-prone PCR, oligonucleotide-directed mutagenesis, site-directed mutagenesis and heavy and/or light chain shuffling. In an embodiment the functional variants of the invention have neutralizing activity against influenza virus H3N2. The neutralizing activity may either be identical, or be higher or lower compared to the parental binding molecules. Henceforth, when the term (human) binding molecule is used, this also encompasses functional variants of the (human) binding molecule.

In yet a further aspect, the invention includes immunoconjugates, *i.e.* molecules comprising at least one binding molecule as defined herein and further comprising at least one tag, such as *inter alia* a detectable moiety/agent. Also contemplated in the present invention are mixtures of immunoconjugates according to the invention or mixtures of at least one immunoconjugates according to the invention and another molecule, such as a therapeutic agent or another binding molecule or immunoconjugate. In a further embodiment, the immunoconjugates of the invention may comprise more than one tag. These tags can be the same or distinct from each other and can be joined/conjugated non-covalently to the binding molecules. The tag(s) can also be joined/conjugated directly to the human binding molecules through covalent bonding. Alternatively, the tag(s) can be joined/conjugated to the binding molecules by means of one or more linking compounds. Techniques for conjugating tags to binding molecules are well known to the skilled artisan.

The tags of the immunoconjugates of the present invention may be therapeutic agents, but they can also be detectable moieties/agents. Tags suitable in therapy and/or prevention may be toxins or functional parts thereof, antibiotics, enzymes, other binding molecules that enhance phagocytosis or immune stimulation. Immunoconjugates comprising a detectable agent can be used diagnostically to, for example, assess if a subject has been infected with an influenza virus H3N2 strain or monitor the development or progression of an influenza virus H3N2 infection as part of a clinical testing procedure to, *e.g.*, determine the efficacy of a given treatment regimen. However, they may also be used for other detection and/or analytical and/or diagnostic purposes. Detectable moieties/agents include, but are not limited to, enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals, and non-radioactive paramagnetic metal ions. The tags used to label the binding molecules for detection and/or analytical and/or diagnostic purposes depend on the specific detection/analysis/diagnosis techniques and/or methods used such as *inter alia* immunohistochemical staining of (tissue) samples, flow cytometric detection, scanning laser cytometric detection, fluorescent immunoassays, enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), bioassays (*e.g.*, phagocytosis assays), Western blotting applications, etc. Suitable labels for the

detection/analysis/diagnosis techniques and/or methods known in the art are well within the reach of the skilled artisan.

Furthermore, the human binding molecules or immunoconjugates of the invention can also be attached to solid supports, which are particularly useful for *in vitro* immunoassays or purification of influenza virus H3N2 or a fragment thereof. Such solid supports might be porous or nonporous, planar or non-planar. The binding molecules of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. Examples include, but are not limited to, the hexa-histidine tag, the hemagglutinin (HA) tag, the myc tag or the flag tag. Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate. In another aspect the binding molecules of the invention may be conjugated/attached to one or more antigens. Preferably, these antigens are antigens which are recognized by the immune system of a subject to which the binding molecule-antigen conjugate is administered. The antigens may be identical, but may also differ from each other. Conjugation methods for attaching the antigens and binding molecules are well known in the art and include, but are not limited to, the use of cross-linking agents. The binding molecules of the invention will bind to influenza virus H3N2 and the antigens attached to the binding molecules will initiate a powerful T-cell attack on the conjugate, which will eventually lead to the destruction of the influenza virus H3N2.

Next to producing immunoconjugates chemically by conjugating, directly or indirectly, via for instance a linker, the immunoconjugates can be produced as fusion proteins comprising the binding molecules of the invention and a suitable tag. Fusion proteins can be produced by methods known in the art such as, *e.g.*, recombinantly by constructing nucleic acid molecules comprising nucleotide sequences encoding the binding molecules in frame with nucleotide sequences encoding the suitable tag(s) and then expressing the nucleic acid molecules.

It is another aspect of the present invention to provide a nucleic acid molecule encoding at least a binding molecule, functional variant or immunoconjugate according to the invention. Such nucleic acid molecules can be used as intermediates for cloning purposes, *e.g.* in the process of affinity maturation as described above. In a preferred embodiment, the nucleic acid molecules are isolated or purified.

The skilled man will appreciate that functional variants of these nucleic acid molecules are also intended to be a part of the present invention. Functional variants are nucleic acid sequences that can be directly translated, using the standard genetic code, to provide an amino acid sequence identical to that translated from the parental nucleic acid molecules.

Preferably, the nucleic acid molecules encode binding molecules comprising the CDR regions as described above. In a further embodiment the nucleic acid molecules encode binding molecules comprising two, three, four, five or even all six CDR regions of the binding molecules of the invention.

In another embodiment, the nucleic acid molecules encode binding molecules comprising a heavy chain comprising the variable heavy chain sequences as described above. In another embodiment the nucleic acid molecules encode binding molecules comprising a light chain comprising the variable light chain sequences as described above. The nucleotide sequences and the amino acid sequences of the heavy and light chain variable regions of the binding molecules of the invention are given below.

It is another aspect of the invention to provide vectors, *i.e.* nucleic acid constructs, comprising one or more nucleic acid molecules according to the present invention. Vectors can be derived from plasmids such as *inter alia* F, R1, RP1, Col, pBR322, TOL, Ti, etc; cosmids; phages such as lambda, lambdoid, M13, Mu, P1, P22, Qβ, T-even, T-odd, T2, T4, T7, etc; plant viruses. Vectors can be used for cloning and/or for expression of the binding molecules of the invention and might even be used for gene therapy purposes. Vectors comprising one or more nucleic acid molecules according to the invention operably linked to one or more expression-regulating nucleic acid molecules are also covered by the present invention. The choice of the vector is dependent on the recombinant procedures followed and the host used. Introduction of vectors in host cells can be effected by *inter alia* calcium phosphate transfection, virus infection, DEAE-dextran mediated transfection, lipofectamin transfection or electroporation. Vectors may be autonomously replicating or may replicate together with the chromosome into which they have been integrated. Preferably, the vectors contain one or more selection markers. The choice of the markers may depend on the host cells of choice, although this is not critical to the invention as is well known to persons skilled in the art. They include, but are not limited to, kanamycin, neomycin, puromycin, hygromycin, zeocin, thymidine kinase gene from Herpes simplex virus (HSV-TK), dihydrofolate reductase gene from mouse

(dhfr). Vectors comprising one or more nucleic acid molecules encoding the human binding molecules as described above operably linked to one or more nucleic acid molecules encoding proteins or peptides that can be used to isolate the human binding molecules are also covered by the invention. These proteins or peptides include, but are not limited to, glutathione-S-transferase, maltose binding protein, metal-binding polyhistidine, green fluorescent protein, luciferase and beta-galactosidase.

Hosts containing one or more copies of the vectors mentioned above are an additional subject of the present invention. Preferably, the hosts are host cells. Host cells include, but are not limited to, cells of mammalian, plant, insect, fungal or bacterial origin. Bacterial cells include, but are not limited to, cells from Gram-positive bacteria or Gram-negative bacteria such as several species of the genera *Escherichia*, such as *E. coli*, and *Pseudomonas*. In the group of fungal cells preferably yeast cells are used. Expression in yeast can be achieved by using yeast strains such as *inter alia Pichia pastoris*, *Saccharomyces cerevisiae* and *Hansenula polymorpha*. Furthermore, insect cells such as cells from Drosophila and Sf9 can be used as host cells. Besides that, the host cells can be plant cells such as *inter alia* cells from crop plants such as forestry plants, or cells from plants providing food and raw materials such as cereal plants, or medicinal plants, or cells from ornamentals, or cells from flower bulb crops. Transformed (transgenic) plants or plant cells are produced by known methods, for example, Agrobacterium-mediated gene transfer, transformation of leaf discs, protoplast transformation by polyethylene glycol-induced DNA transfer, electroporation, sonication, microinjection or bolistic gene transfer. Additionally, a suitable expression system can be a baculovirus system. Expression systems using mammalian cells such as Chinese Hamster Ovary (CHO) cells, COS cells, BHK cells, NSO cells or Bowes melanoma cells are preferred in the present invention. Mammalian cells provide expressed proteins with posttranslational modifications that are most similar to natural molecules of mammalian origin. Since the present invention deals with molecules that may have to be administered to humans, a completely human expression system would be particularly preferred. Therefore, even more preferably, the host cells are human cells. Examples of human cells are *inter alia* HeLa, 911, AT1080, A549, 293 and HEK293T cells. In preferred embodiments, the human producer cells comprise at least a functional part of a nucleic acid sequence encoding an adenovirus E1 region in expressible format. In even more preferred embodiments, said host cells are derived from a human retina and immortalized with

nucleic acids comprising adenoviral E1 sequences, such as 911 cells or the cell line deposited at the European Collection of Cell Cultures (ECACC), CAMR, Salisbury, Wiltshire SP4 OJG, Great Britain on 29 February 1996 under number 96022940 and marketed under the trademark PER.C6® (PER.C6 is a registered trademark of Crucell Holland B.V.). For the purposes of this application "PER.C6 cells" refers to cells deposited under number 96022940 or ancestors, passages up-stream or downstream as well as descendants from ancestors of deposited cells, as well as derivatives of any of the foregoing. Production of recombinant proteins in host cells can be performed according to methods well known in the art. The use of the cells marketed under the trademark PER.C6® as a production platform for proteins of interest has been described in WO 00/63403 the disclosure of which is incorporated herein by reference in its entirety.

Binding molecules can be prepared by various means. A method of producing a binding molecule according to the invention is an additional part of the invention. The method comprises the steps of a) culturing a host according to the invention under conditions conducive to the expression of the binding molecule, and b) optionally, recovering the expressed binding molecule. The expressed binding molecules can be recovered from the cell free extract, but preferably they are recovered from the culture medium. The above method of producing can also be used to make functional variants of the binding molecules and/or immunoconjugates of the present invention. Methods to recover proteins, such as binding molecules, from cell free extracts or culture medium are well known to the man skilled in the art. Binding molecules, functional variants and/or immunoconjugates as obtainable by the above-described method are also a part of the present invention.

Alternatively, next to the expression in hosts, such as host cells, the binding molecules and immunoconjugates of the invention can be produced synthetically by conventional peptide synthesizers or in cell-free translation systems using RNA nucleic acid derived from DNA molecules according to the invention. Binding molecules and immunoconjugates as obtainable by the above described synthetic production methods or cell-free translation systems are also a part of the present invention.

In yet another embodiment, binding molecules of the present invention can also be produced in transgenic, non-human, mammals such as *inter alia* rabbits, goats or cows, and secreted into for instance the milk thereof.

In yet another alternative embodiment, binding molecules according to the present invention, preferably human binding molecules specifically binding to influenza virus H3N2 or a fragment thereof, may be generated by transgenic non-human mammals, such as for instance transgenic mice or rabbits, that express human immunoglobulin genes. Preferably, the transgenic non-human mammals have a genome comprising a human heavy chain transgene and a human light chain transgene encoding all or a portion of the human binding molecules as described above. The transgenic non-human mammals can be immunized with a purified or enriched preparation of influenza virus H3N2 or a fragment thereof. Protocols for immunizing non-human mammals are well established in the art. See Using Antibodies: A Laboratory Manual, Edited by: E. Harlow, D. Lane (1998), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York and Current Protocols in Immunology, Edited by: J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober (2001), John Wiley & Sons Inc., New York, the disclosures of which are incorporated herein by reference. Immunization protocols often include multiple immunizations, either with or without adjuvants such as Freund's complete adjuvant and Freund's incomplete adjuvant, but may also include naked DNA immunizations. In another embodiment, the human binding molecules are produced by B-cells, plasma and/or memory cells derived from the transgenic animals. In yet another embodiment, the human binding molecules are produced by hybridomas, which are prepared by fusion of B-cells obtained from the above-described transgenic non-human mammals to immortalized cells. B-cells, plasma cells and hybridomas as obtainable from the above-described transgenic non-human mammals and human binding molecules as obtainable from the above-described transgenic non-human mammals, B-cells, plasma and/or memory cells and hybridomas are also a part of the present invention.

In a further aspect, the invention provides a method of identifying a binding molecule, such as a human binding molecule, *e.g.* a human monoclonal antibody or fragment thereof, specifically binding to influenza virus H3N2 or nucleic acid molecules encoding such binding molecules and comprises the steps of: (a) contacting a collection of binding molecules on the surface of replicable genetic packages with

influenza virus H3N2 or a fragment thereof under conditions conducive to binding, (b) selecting at least once for a replicable genetic package binding to influenza virus H3N2 or a fragment thereof, (c) separating and recovering the replicable genetic package binding to influenza virus H3N2 or a fragment thereof from replicable genetic packages that do not bind to influenza virus H3N2 or a fragment thereof. A replicable genetic package as used herein can be prokaryotic or eukaryotic and includes cells, spores, yeasts, bacteria, viruses, (bacterio)phage, ribosomes and polysomes. A preferred replicable genetic package is a phage. The binding molecules, such as for instance single chain Fvs, are displayed on the replicable genetic package, *i.e.* they are attached to a group or molecule located at an exterior surface of the replicable genetic package. The replicable genetic package is a screenable unit comprising a binding molecule to be screened linked to a nucleic acid molecule encoding the binding molecule. The nucleic acid molecule should be replicable either *in vivo* (*e.g.*, as a vector) or *in vitro* (*e.g.*, by PCR, transcription and translation). *In vivo* replication can be autonomous (as for a cell), with the assistance of host factors (as for a virus) or with the assistance of both host and helper virus (as for a phagemid). Replicable genetic packages displaying a collection of binding molecules is formed by introducing nucleic acid molecules encoding exogenous binding molecules to be displayed into the genomes of the replicable genetic packages to form fusion proteins with endogenous proteins that are normally expressed from the outer surface of the replicable genetic packages. Expression of the fusion proteins, transport to the outer surface and assembly results in display of exogenous binding molecules from the outer surface of the replicable genetic packages.

The selection step(s) in the methods according to the present invention can be performed with influenza H3N2 viruses that are live and still infective or inactivated. Inactivation of influenza virus H3N2 may be performed by viral inactivation methods well known to the skilled artisan such as *inter alia* treatment with formalin, β-propiolactone (BPL), merthiolate, and/or ultraviolet light. Methods to test, if influenza virus H3N2 is still alive, infective and/or viable or partly or completely inactivated are well known to the person skilled in the art. The influenza virus H3N2 used in the above method does not need to be in purified form, *e.g.* may be present in serum and/or blood of an infected individual. The influenza virus H3N2 used may also be isolated from cell culture in a suitable medium.

In an embodiment the influenza virus H3N2 is in suspension when contacted with the replicable genetic packages. Alternatively, they may also be coupled to a carrier when contact takes place. In an embodiment a first and further selection may take place against one influenza virus H3N2 strain. Alternatively, first and further selection rounds may be performed against different influenza virus H3N2 strains. Alternatively, the selection step(s) may be performed in the presence of a fragment of influenza virus H3N2 such as *e.g.* cell membrane preparations, recombinant H3N2 proteins or polypeptides, fusion proteins comprising H3N2 proteins or polypeptides, cells expressing recombinant H3N2 proteins or polypeptides, and the like. Extracellularly exposed parts of these proteins or polypeptides can also be used as selection material. The fragments of influenza virus H3N2 may be immobilized to a suitable material before use or may be used in suspension. In an embodiment the selection can be performed on different fragments of influenza virus H3N2 or fragments of different influenza virus H3N2 strains. Finding suitable selection combinations are well within the reach of the skilled artisan. Selections may be performed by ELISA or FACS.

In yet a further aspect, the invention provides a method of obtaining a binding molecule specifically binding to an influenza virus H3N2 strain or fragment thereof or a nucleic acid molecule encoding such a binding molecule, wherein the method comprises the steps of a) performing the above described method of identifying binding molecules, and b) isolating from the recovered replicable genetic package the binding molecule and/or the nucleic acid molecule encoding the binding molecule. The collection of binding molecules on the surface of replicable genetic packages can be a collection of scFvs or Fabs. Once a new scFv or Fab has been established or identified with the above-mentioned method of identifying binding molecules or nucleic acid molecules encoding the binding molecules, the DNA encoding the scFv or Fab can be isolated from the bacteria or phages and combined with standard molecular biological techniques to make constructs encoding scFvs, bivalent scFvs, Fabs or complete human immunoglobulins of a desired specificity (*e.g.* IgG, IgA or IgM). These constructs can be transfected into suitable cell lines and complete human monoclonal antibodies can eventually be produced (see Huls *et al.,* 1999; Boel *et al.*, 2000).

As mentioned before the preferred replicable genetic package is a phage. Phage display methods for identifying and obtaining (human) binding molecules, *e.g.*

(human) monoclonal antibodies, are by now well-established methods known by the person skilled in the art. They are, *e.g.*, described in US Patent Number 5,696,108; Burton and Barbas, 1994; de Kruif *et al.*, 1995b; and Phage Display: A Laboratory Manual. Edited by: CF Barbas, DR Burton, JK Scott and GJ Silverman (2001), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. All these references are herewith incorporated herein in their entirety. For the construction of phage display libraries, collections of human monoclonal antibody heavy and light chain variable region genes are expressed on the surface of bacteriophage, preferably filamentous bacteriophage, particles, in for example single-chain Fv (scFv) or in Fab format (see de Kruif *et al.*, 1995b). Large libraries of antibody fragment-expressing phages typically contain more than $1.0x10^9$ antibody specificities and may be assembled from the immunoglobulin V-regions expressed in the B-lymphocytes of immunized- or non-immunized individuals. In a specific embodiment of the invention the phage library of binding molecules, preferably scFv phage library, is prepared from RNA isolated from cells obtained from a subject that has been vaccinated against influenza virus, recently vaccinated against an unrelated pathogen, recently suffered from a influenza virus H3N2 infection or from a healthy individual. RNA can be isolated from *inter alia* bone marrow or peripheral blood, preferably peripheral blood lymphocytes or isolated B-cells or even subpopulations of B-cells such as memory B-cells, identified as CD24+/CD27+ B-cells. The subject can be an animal, preferably a human. In a preferred embodiment the libraries may be assembled from the immunoglobulin V-regions expressed by IgM memory B-cells, identified as IgM+/CD24+/CD27+ cells.

Alternatively, phage display libraries may be constructed from immunoglobulin variable regions that have been partially assembled *in vitro* to introduce additional antibody diversity in the library (semi-synthetic libraries). For example, *in vitro* assembled variable regions contain stretches of synthetically produced, randomized or partially randomized DNA in those regions of the molecules that are important for antibody specificity, *e.g.* CDR regions. Phage antibodies specific for influenza virus H3N2 can be selected from the library by exposing the virus or fragment thereof to a phage library to allow binding of phages expressing antibody fragments specific for the virus or fragment thereof. Non-bound phages are removed by washing and bound phages eluted for infection of *E.coli* bacteria and subsequent propagation. Multiple rounds of selection and propagation are usually

required to sufficiently enrich for phages binding specifically to the virus or fragment thereof. If desired, before exposing the phage library to the virus or fragment thereof the phage library can first be subtracted by exposing the phage library to non-target material such as viruses or fragments thereof of a different strain, *i.e.* non-H3N2 influenza viruses. These subtractor viruses or fragments thereof can be bound to a solid phase or can be in suspension. Phages may also be selected for binding to complex antigens such as complex mixtures of H3N2 proteins or (poly)peptides optionally supplemented with other material. Host cells expressing one or more proteins or (poly)peptides of influenza virus H3N2 may also be used for selection purposes. A phage display method using these host cells can be extended and improved by subtracting non-relevant binders during screening by addition of an excess of host cells comprising no target molecules or non-target molecules that are similar, but not identical, to the target, and thereby strongly enhance the chance of finding relevant binding molecules. Of course, the subtraction may be performed before, during or after the screening with virus or fragments thereof. The process is referred to as the MABSTRACT® process (MABSTRACT® is a registered trademark of Crucell Holland B.V., see also US Patent Number 6,265,150 which is incorporated herein by reference).

In yet another aspect the invention provides a method of obtaining a binding molecule potentially having neutralizing activity against influenza virus H3N2, wherein the method comprises the steps of (a) performing the method of obtaining a binding molecule specifically binding to influenza virus H3N2 or a fragment thereof or a nucleic acid molecule encoding such a binding molecule as described above, and (b) verifying if the binding molecule isolated has neutralizing activity against the virus, preferably against at least one or more influenza virus H3N2 strains selected from the group consisting of A/Hong Kong/1/68, A/Johannesburg/33/94, A/Panama/2007/99, A/Wisconsin/67/2005 and A/Hiroshima/52/2005, preferably all strains of H3N2, in particular all known and future H3N2 strains. Assays for verifying if a binding molecule has neutralizing activity are well known in the art (see WHO Manual on Animal Influenza Diagnosis and Surveillance, Geneva: World Health Organisation, 2005 version 2002.5).

In a further aspect the invention pertains to a human binding molecule having neutralizing activity against at least influenza virus A comprising HA of the H3 subtype, obtainable by one of the methods as described above.

In yet a further aspect, the invention provides compositions comprising at least a binding molecule preferably a human monoclonal antibody according to the invention, at least a functional variant thereof, at least an immunoconjugate according to the invention and/or a combination thereof. In addition to that, the compositions may comprise *inter alia* stabilizing molecules, such as albumin or polyethylene glycol, or salts. Preferably, the salts used are salts that retain the desired biological activity of the binding molecules and do not impart any undesired toxicological effects. If necessary, the human binding molecules of the invention may be coated in or on a material to protect them from the action of acids or other natural or non-natural conditions that may inactivate the binding molecules.

In yet a further aspect, the invention provides compositions comprising at least a nucleic acid molecule as defined in the present invention. The compositions may comprise aqueous solutions such as aqueous solutions containing salts (*e.g.*, NaCl or salts as described above), detergents (*e.g.*, SDS) and/or other suitable components.

Furthermore, the present invention pertains to pharmaceutical compositions comprising at least a binding molecule such as a human monoclonal antibody of the invention (or functional fragment or variant thereof), at least an immunoconjugate according to the invention, at least a composition according to the invention, or combinations thereof. The pharmaceutical composition of the invention further comprises at least one pharmaceutically acceptable excipient. Pharmaceutically acceptable excipients are well known to the skilled person. The pharmaceutical composition according to the invention may further comprise at least one other therapeutic agent. Suitable agents are also well known to the skilled artisan.

In a preferred embodiment the pharmaceutical composition according to the invention comprises at least one additional binding molecule, *i.e.* the pharmaceutical composition can be a cocktail or mixture of binding molecules. The pharmaceutical composition may comprise at least two binding molecules according to the invention, or at least one binding molecule according to the invention and at least one further influenza virus binding and/or neutralizing molecule. In another embodiment the additional binding molecule may be formulated for simultaneous separate or sequential administration.

In an embodiment the pharmaceutical compositions may comprise two or more binding molecules that have neutralizing activity against influenza virus A comprising HA of the H3 subtype, such as H3N2. In an embodiment, the binding molecules exhibit synergistic neutralizing activity, when used in combination. In other words, the compositions may comprise at least two binding molecules having neutralizing activity, characterized in that the binding molecules act synergistically in neutralizing influenza virus H3N2. As used herein, the term "synergistic" means that the combined effect of the binding molecules when used in combination is greater than their additive effects when used individually. The synergistically acting binding molecules may bind to different structures on the same or distinct fragments of influenza virus H3N2. A way of calculating synergy is by means of the combination index. The concept of the combination index (CI) has been described by Chou and Talalay (1984). The compositions may e.g. comprise one binding molecule having neutralizing activity and one non-neutralizing H3N2-specific binding molecule. The non-neutralizing and neutralizing H3N2-specific binding molecules may also act synergistically in neutralizing influenza virus H3N2.

In an embodiment, the pharmaceutical composition may comprise at least two influenza virus neutralizing binding molecules, wherein at least one binding molecules is capable of neutralizing one or more influenza virus subtypes of phylogenetic group 1 and wherein at least one binding molecules is capable of neutralizing one or more influenza virus subtypes of phylogenetic group 2.

In an embodiment, the pharmaceutical composition may comprise at least one binding molecule according to the invention and at least one further influenza virus neutralizing binding molecule.

In another embodiment, the further influenza virus neutralizing binding molecule preferably is capable of binding to and neutralizing an influenza virus of a different subtype, preferably an influenza virus comprising HA of the H1, such as H1N1, and/or HA of the H5 sybtype, such as H5N1, such as the binding molecules as disclosed in WO 2008/028946. Even more preferably said further binding molecule is a cross-neutralizing binding molecule against (all) influenza virus subtypes of phylogenetic group 1, including H1, H2, H5, H9. In a preferred embodiment, the further binding molecule is the binding molecule identified as CR6261 in WO 2008/028946, comprising a heavy chain variable region comprising amino acids 1-121 of amino acid sequence of SEQ ID NO: 186, or a functional variant thereof,

and/or a light chain variable region comprising amino acids 1-112 of SEQ ID NO:188. In yet another embodiment the binding molecule comprises a heavy and light chain comprising the amino acid sequences of SEQ ID NO: 186 and SEQ ID NO: 188, respectively. The binding molecules in the pharmaceutical composition thus preferably are capable of reacting with influenza viruses of different subtypes. The binding molecules should be of high affinity and should have a broad specificity. Preferably, both binding molecules are cross-neutralizing molecules in that they each neutralize influenza viruses of different subtypes. In addition, preferably they neutralize as many strains of each of the different influenza virus subtypes as possible.

A pharmaceutical composition according to the invention can further comprise at least one other therapeutic, prophylactic and/or diagnostic agent. Preferably, the pharmaceutical composition comprises at least one other prophylactic and/or therapeutic agent. Preferably, said further therapeutic and/or prophylactic agents are agents capable of preventing and/or treating an influenza virus H3N2 infection and/or a condition resulting from such an infection. Therapeutic and/or prophylactic agents include, but are not limited to, anti-viral agents. Such agents can be binding molecules, small molecules, organic or inorganic compounds, enzymes, polynucleotide sequences, anti-viral peptides, etc. Other agents that are currently used to treat patients infected with influenza virus H3N2 are M2 inhibitors (*e.g.*, amantidine, rimantadine) and/or neuraminidase inhibitors (*e.g.*, zanamivir, oseltamivir). These can be used in combination with the binding molecules of the invention. "In combination" herein means simultaneously, as separate formulations, or as one single combined formulation, or according to a sequential administration regimen as separate formulations, in any order. Agents capable of preventing and/or treating an infection with influenza virus H3N2 and/or a condition resulting from such an infection that are in the experimental phase might also be used as other therapeutic and/or prophylactic agents useful in the present invention.

The binding molecules or pharmaceutical compositions of the invention can be tested in suitable animal model systems prior to use in humans. Such animal model systems include, but are not limited to, mouse, ferret and monkey.

Typically, pharmaceutical compositions must be sterile and stable under the conditions of manufacture and storage. The binding molecules, immunoconjugates, nucleic acid molecules or compositions of the present invention can be in powder form for reconstitution in the appropriate pharmaceutically acceptable excipient

before or at the time of delivery. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Alternatively, the binding molecules, immunoconjugates, nucleic acid molecules or compositions of the present invention can be in solution and the appropriate pharmaceutically acceptable excipient can be added and/or mixed before or at the time of delivery to provide a unit dosage injectable form. Preferably, the pharmaceutically acceptable excipient used in the present invention is suitable to high drug concentration, can maintain proper fluidity and, if necessary, can delay absorption.

The choice of the optimal route of administration of the pharmaceutical compositions will be influenced by several factors including the physico-chemical properties of the active molecules within the compositions, the urgency of the clinical situation and the relationship of the plasma concentrations of the active molecules to the desired therapeutic effect. For instance, if necessary, the binding molecules of the invention can be prepared with carriers that will protect them against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can *inter alia* be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Furthermore, it may be necessary to coat the binding molecules with, or co-administer the binding molecules with, a material or compound that prevents the inactivation of the human binding molecules. For example, the binding molecules may be administered to a subject in an appropriate carrier, for example, liposomes or a diluent.

The routes of administration can be divided into two main categories, oral and parenteral administration. The preferred administration route is intravenous or by inhalation.

Oral dosage forms can be formulated *inter alia* as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard capsules, soft gelatin capsules, syrups or elixirs, pills, dragees, liquids, gels, or slurries. These formulations can contain pharmaceutically excipients including, but not limited to, inert diluents, granulating and disintegrating agents, binding agents,

lubricating agents, preservatives, colouring, flavouring or sweetening agents, vegetable or mineral oils, wetting agents, and thickening agents.

The pharmaceutical compositions of the present invention can also be formulated for parenteral administration. Formulations for parenteral administration can be *inter alia* in the form of aqueous or non-aqueous isotonic sterile non-toxic injection or infusion solutions or suspensions. The solutions or suspensions may comprise agents that are non-toxic to recipients at the dosages and concentrations employed such as 1,3-butanediol, Ringer's solution, Hank's solution, isotonic sodium chloride solution, oils, fatty acids, local anaesthetic agents, preservatives, buffers, viscosity or solubility increasing agents, water-soluble antioxidants, oil-soluble antioxidants and metal chelating agents.

In a further aspect, the binding molecules such as human monoclonal antibodies (functional fragments and variants thereof), immunoconjugates, compositions, or pharmaceutical compositions of the invention can be used as a medicament. So, a method of diagnosis, treatment and/or prevention of an influenza virus H3N2 infection using the binding molecules, immunoconjugates, compositions, or pharmaceutical compositions of the invention is another part of the present invention. The above-mentioned molecules can *inter alia* be used in the diagnosis, prophylaxis, treatment, or combination thereof, of an influenza virus H3N2 infection. They are suitable for treatment of yet untreated patients suffering from an influenza virus H3N2 infection and patients who have been or are treated for an influenza virus H3N2 infection.

The above-mentioned molecules or compositions may be employed in conjunction with other molecules useful in diagnosis, prophylaxis and/or treatment. They can be used *in vitro, ex vivo* or *in vivo*. For instance, the binding molecules such as human monoclonal antibodies (or functional variants thereof), immunoconjugates, compositions or pharmaceutical compositions of the invention can be co-administered with a vaccine against influenza virus H3N2 (if available). Alternatively, the vaccine may also be administered before or after administration of the molecules of the invention. Instead of a vaccine, anti-viral agents can also be employed in conjunction with the binding molecules of the present invention. Suitable anti-viral agents are mentioned above.

The molecules are typically formulated in the compositions and pharmaceutical compositions of the invention in a therapeutically or diagnostically

effective amount. Alternatively, they may be formulated and administered separately. For instance the other molecules such as the anti-viral agents may be applied systemically, while the binding molecules of the invention may be applied intravenously.

Treatment may be targeted at patient groups that are susceptible to H3N2 infection. Such patient groups include, but are not limited to e.g., the elderly (e.g. ≥ 50 years old, ≥ 60 years old, and preferably ≥ 65 years old), the young (e.g. ≤ 5 years old, ≤ 1 year old), hospitalized patients and patients who have been treated with an antiviral compound but have shown an inadequate antiviral response.

Dosage regimens can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). A suitable dosage range may for instance be 0.1-100 mg/kg body weight, preferably 1-50 mg/kg body weight, preferably 0.5-15 mg/kg body weight. Furthermore, for example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. The molecules and compositions according to the present invention are preferably sterile. Methods to render these molecules and compositions sterile are well known in the art. The other molecules useful in diagnosis, prophylaxis and/or treatment can be administered in a similar dosage regimen as proposed for the binding molecules of the invention. If the other molecules are administered separately, they may be administered to a patient prior to (*e.g.*, 2 min, 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 10 hrs, 12 hrs, 14 hrs, 16 hrs, 18 hrs, 20 hrs, 22 hrs, 24 hrs, 2 days, 3 days, 4 days, 5 days, 7 days, 2 weeks, 4 weeks or 6 weeks before), concomitantly with, or subsequent to (*e.g.*, 2 min, 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 10 hrs, 12 hrs, 14 hrs, 16 hrs, 18 hrs, 20 hrs, 22 hrs, 24 hrs, 2 days, 3 days, 4 days, 5 days, 7 days, 2 weeks, 4 weeks or 6 weeks after) the administration of one or more of the human binding molecules or pharmaceutical compositions of the invention. The exact dosing regimen is usually sorted out during clinical trials in human patients.

Human binding molecules and pharmaceutical compositions comprising the human binding molecules are particularly useful, and often preferred, when to be administered to human beings as *in vivo* therapeutic agents, since recipient immune response to the administered antibody will often be substantially less than that

occasioned by administration of a monoclonal murine, chimeric or humanized binding molecule.

In another aspect, the invention concerns the use of the binding molecules such as neutralizing human monoclonal antibodies (functional fragments and variants thereof), immunoconjugates, nucleic acid molecules, compositions or pharmaceutical compositions according to the invention in the preparation of a medicament for the diagnosis, prophylaxis, treatment, or combination thereof, of an influenza virus H3N2 infection.

Next to that, kits comprising at least a binding molecule such as a neutralizing human monoclonal antibody (functional fragments and variants thereof), at least an immunoconjugate, at least a nucleic acid molecule, at least a composition, at least a pharmaceutical composition, at least a vector, at least a host according to the invention or a combination thereof are also a part of the present invention. Optionally, the above-described components of the kits of the invention are packed in suitable containers and labelled for diagnosis, prophylaxis and/or treatment of the indicated conditions. The above-mentioned components may be stored in unit or multi-dose containers as an aqueous, preferably sterile, solution or as a lyophilised, preferably sterile, formulation for reconstitution. The containers may be formed from a variety of materials such as glass or plastic and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The kit may further comprise more containers comprising a pharmaceutically acceptable buffer. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, culture medium for one or more of the suitable hosts and, possibly, even at least one other therapeutic, prophylactic or diagnostic agent. Associated with the kits can be instructions customarily included in commercial packages of therapeutic, prophylactic or diagnostic products, that contain information about for example the indications, usage, dosage, manufacture, administration, contra-indications and/or warnings concerning the use of such therapeutic, prophylactic or diagnostic products.

The binding molecules according to the present invention can also be advantageously used as a diagnostic agent in an *in vitro* method for the detection of phylogenetic group 2 subtype influenza virus. The invention thus further pertains to a method of detecting influenza virus phylogenetic group 2 subtype influenza virus in a

sample, wherein the method comprises the steps of (a) contacting a sample with a diagnostically effective amount of a binding molecule (functional fragments and variants thereof) or an immunoconjugate according to the invention, and (b) determining whether the binding molecule or immunoconjugate specifically binds to a molecule of the sample. The sample may be a biological sample including, but not limited to blood, serum, stool, sputum, nasophargyal aspirates, bronchial lavages, urine, tissue or other biological material from (potentially) infected subjects, or a non-biological sample such as water, drink, etc. The (potentially) infected subjects may be human subjects, but also animals that are suspected as carriers of influenza virus phylogenetic group 2 subtype influenza virus might be tested for the presence of the virus using the human binding molecules or immunoconjugates of the invention. The sample may first be manipulated to make it more suitable for the method of detection. Manipulation means *inter alia* treating the sample suspected to contain and/or containing the virus in such a way that the virus will disintegrate into antigenic components such as proteins, (poly)peptides or other antigenic fragments. Preferably, the human binding molecules or immunoconjugates of the invention are contacted with the sample under conditions which allow the formation of an immunological complex between the human binding molecules and the virus or antigenic components thereof that may be present in the sample. The formation of an immunological complex, if any, indicating the presence of the virus in the sample, is then detected and measured by suitable means. Such methods include, *inter alia*, homogeneous and heterogeneous binding immunoassays, such as radio-immunoassays (RIA), ELISA, immunofluorescence, immunohistochemistry, FACS, BIACORE and Western blot analyses.

Preferred assay techniques, especially for large-scale clinical screening of patient sera and blood and blood-derived products are ELISA and Western blot techniques. ELISA tests are particularly preferred. For use as reagents in these assays, the binding molecules or immunoconjugates of the invention are conveniently bonded to the inside surface of microtiter wells. The binding molecules or immunoconjugates of the invention may be directly bonded to the microtiter well. However, maximum binding of the binding molecules or immunoconjugates of the invention to the wells might be accomplished by pre-treating the wells with polylysine prior to the addition of the binding molecules or immunoconjugates of the invention. Furthermore, the binding molecules or immunoconjugates of the invention may be covalently attached

by known means to the wells. Generally, the binding molecules or immunoconjugates are used between 0.01 to 100 μg/ml for coating, although higher as well as lower amounts may also be used. Samples are then added to the wells coated with the binding molecules or immunoconjugates of the invention.

Furthermore, binding molecules of the invention can be used to identify specific binding structures of influenza virus H3N2. The binding structures can be epitopes on proteins and/or polypeptides. They can be linear, but also structural and/or conformational. In one embodiment, the binding structures can be analysed by means of PEPSCAN analysis (see *inter alia* WO 84/03564, WO 93/09872, Slootstra *et al.*, 1996). Alternatively, a random peptide library comprising peptides from a protein of influenza virus H3N2 can be screened for peptides capable of binding to the binding molecules of the invention. The binding structures/peptides/epitopes found can be used as vaccines and for the diagnosis of influenza virus H3N2 infections. In case fragments other than proteins and/or polypeptides are bound by the binding molecules, binding structures can be identified by mass spectrometry, high performance liquid chromatography and nuclear magnetic resonance.

In a further aspect, the invention provides a method of screening a binding molecule (or a functional fragment or variant thereof) for specific binding to the same epitope of influenza virus H3N2, as the epitope bound by a human binding molecule of the invention, wherein the method comprises the steps of (a) contacting a binding molecule to be screened, a binding molecule of the invention and influenza virus H3N2 or a fragment thereof, (b) measure if the binding molecule to be screened is capable of competing for specifically binding to influenza virus H3N2 or a fragment thereof with the binding molecule of the invention. In a further step it may be determined, if the screened binding molecules that are capable of competing for specifically binding to influenza virus H3N2 or a fragment thereof have neutralizing activity. A binding molecule that is capable of competing for specifically binding to influenza virus H3N2 or a fragment thereof with the binding molecule of the invention is another part of the present invention. In the above-described screening method, "specifically binding to the same epitope" also contemplates specific binding to substantially or essentially the same epitope as the epitope bound by the a binding molecule of the invention. The capacity to block, or compete with, the binding of the binding molecules of the invention to influenza virus H3N2 typically indicates that a binding molecule to be screened binds to an epitope or binding site on influenza virus

H3N2 that structurally overlaps with the binding site on influenza virus H3N2 that is immunospecifically recognized by the binding molecules of the invention. Alternatively, this can indicate that a binding molecule to be screened binds to an epitope or binding site which is sufficiently proximal to the binding site immunospecifically recognized by the binding molecules of the invention to sterically or otherwise inhibit binding of the binding molecules of the invention to influenza virus H3N2.

In general, competitive inhibition is measured by means of an assay, wherein an antigen composition, *i.e.* a composition comprising influenza virus H3N2 or fragments thereof, is admixed with reference binding molecules, *i.e.* the binding molecules of the invention, and binding molecules to be screened. Usually, the binding molecules to be screened are present in excess. Protocols based upon ELISAs and Western blotting are suitable for use in such simple competition studies. By using species or isotype secondary antibodies one will be able to detect only the bound reference binding molecules, the binding of which will be reduced by the presence of a binding molecule to be screened that recognizes substantially the same epitope. In conducting a binding molecule competition study between a reference binding molecule and any binding molecule to be screened (irrespective of species or isotype), one may first label the reference binding molecule with a detectable label, such as, *e.g.*, biotin, an enzymatic, a radioactive or other label to enable subsequent identification. Binding molecules identified by these competition assays ("competitive binding molecules" or "cross-reactive binding molecules") include, but are not limited to, antibodies, antibody fragments and other binding agents that bind to an epitope or binding site bound by the reference binding molecule, *i.e.* a binding molecule of the invention, as well as antibodies, antibody fragments and other binding agents that bind to an epitope or binding site sufficiently proximal to an epitope bound by the reference binding molecule for competitive binding between the binding molecules to be screened and the reference binding molecule to occur. Preferably, competitive binding molecules of the invention will, when present in excess, inhibit specific binding of a reference binding molecule to a selected target species by at least 10%, preferably by at least 25%, more preferably by at least 50%, and most preferably by at least 75%-90% or even greater. The identification of one or more competitive binding molecules that bind to about, substantially, essentially or at the same epitope as the binding molecules of the invention is a straightforward technical matter. As the

identification of competitive binding molecules is determined in comparison to a reference binding molecule, *i.e.* a binding molecule of the invention, it will be understood that actually determining the epitope to which the reference binding molecule and the competitive binding molecule bind is not in any way required in order to identify a competitive binding molecule that binds to the same or substantially the same epitope as the reference binding molecule. The invention is further illustrated in the following examples and figures. The examples are not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1 *Construction of scFv phage display libraries using RNA extracted from memory B cells*

Peripheral blood was collected from normal healthy donors by venapuncture in EDTA anti-coagulation sample tubes. scFv phage display libraries were obtained as described in WO 2008/028946, which is incorporated by reference herein. Memory B cells (CD24+/CD27+) were separated from naive B cells (CD24+/CD27-) and memory T cells (CD24-/CD27+) and in a next step, IgM memory B cells (IgM+) were separated from switch memory B cells (IgM-) using IgM expression. RNA was isolated from the IgM memory B cells and cDNA prepared.

A two round PCR amplification approach was applied using the primer sets shown in Tables 1 and 2 to isolate the immunoglobulin VH and VL regions from the respective donor repertoire.

First round amplification on the respective cDNA using the primer sets mentioned in Table 1 yielded 7, 6 and 9 products of about 650 base pairs for respectively VH, Vkappa and Vlambda regions. For IgM memory B cell VH region amplification the OCM constant primer was used in combination with OH1 to OH7. The thermal cycling program for first round amplifications was: 2 min 96°C (denaturation step), 30 cycles of 30 sec 96°C/ 30 sec 55°C/ 60 sec 72°C, 10 min 72°C final elongation and 4°C refrigeration. The products were loaded on and isolated from a 1% agarose gel using gel-extraction columns (Qiagen) and eluted in 50 µl 1 mM Tris-HCl pH 8.0. Ten percent of first round products (5 µl) was subjected to second round amplification using the primers mentioned in Table 2. These primers were extended with restriction sites enabling the directional cloning of the respective VL

and VH regions into phage display vector PDV-C06. The PCR program for second round amplifications was as follows: 2 min 96°C (denaturation step), 30 cycles of 30 sec 96°C/ 30 sec 60°C/ 60 sec 72°C, 10 min 72°C final elongation and 4°C refrigeration. The second round products (~350 base pairs) were first pooled according to natural occurrence of J segments found in immunoglobulin gene products, resulting in 7, 6 and 9 pools for respectively the VH, Vkappa and Vlambda variable regions (see Tables 3 and 4). To obtain a normalized distribution of immunoglobulin sequences in the immune library the 6 Vkappa and 9 Vlambda light chain pools were mixed according to the percentages mentioned in Table 3. This single final VL pool (3 μg) was digested overnight with SalI and NotI restriction enzymes, loaded on and isolated from a 1.5% agarose gel (~350 base pairs) using Qiagen gel-extraction columns and ligated in SalI-NotI cut PDV-C06 vector (~5000 base pairs) as follows: 10 μl PDV-C06 vector (50 ng/μl), 7 μl VL insert (10 ng/μl), 5 μl 10X ligation buffer (NEB), 2.5 T4 DNA Ligase (400 U/μl) (NEB), 25.5 μl ultrapure water (vector to insert ratio was 1:2). Ligation was performed overnight in a water bath of 16°C. Next, the volume was doubled with water, extracted with an equal volume of phenol-chloroform-isoamylalcohol (75:24:1) (Invitrogen) followed by chloroform (Merck) extraction and precipitated with 1 μl Pellet Paint (Novogen), 10 μl sodium acetate (3 M pH 5.0) and 100 μl isopropanol for 2 hrs at -20°C. The obtained sample was subsequently centrifuged at 20.000xg for 30 min at 4°C. The obtained precipitate was washed with 70% ethanol and centrifuged for 10 min at 20.000xg at room temperature. Ethanol was removed by vacuum aspiration and the pellet was air dried for several min and then dissolved in 50 μl buffer containing 10 mM Tris-HCl, pH 8.0. 1 μl ligation mixture was used for the transformation of 40 μl TG-1 electro-competent cells (Stratagene) in a chilled 0.1 cm electroporation cuvette (Biorad) using a Genepulser II apparatus (Biorad) set at 1.7 kV, 200 Ohm, 25 μF (time constant ~4,5 msec). Directly after pulse, the bacteria were flushed from the cuvette with 1000 μl SOC medium (Invitrogen) containing 5% (w/v) glucose (Sigma) at 37°C and transferred to a 15 ml round bottom culture tube. Another 500 μl SOC/glucose was used to flush residual bacteria from the cuvette and was added to the culture tube. Bacteria were recovered by culturing for exactly one hr at 37°C in a shaker incubator at 220 rpm. The transformed bacteria were plated over large 240 mm square petridishes (NUNC) containing 200 ml 2TY agar (16 g/l bacto-tryptone, 10 g/l bacto-yeast extract, 5 g/l NaCl, 15 g/l agar, pH 7.0) supplemented with 50 μg/ml

ampicillin and 5% (w/v) glucose (Sigma). A 1 to 1000 dilution was plated for counting purposes on 15 cm petridishes containing the same medium. This transformation procedure was repeated sequentially twenty times and the complete library was plated over a total of thirty large square petridishes and grown overnight in a 37°C culture stove. Typically, around $1x10^7$ cfu were obtained using the above protocol. The intermediate VL light chain library was harvested from the plates by mildly scraping the bacteria into 10 ml 2TY medium per plate. The cell mass was determined by OD600 measurement and two times 500 OD of bacteria was used for maxi plasmid DNA preparation using two P500 maxiprep columns (Qiagen) according to manufacturer's instructions.

Analogous to the VL variable regions, the second round VH-JH products were first mixed together to obtain the normal J segment usage distribution (see Table 4), resulting in 7 VH subpools called PH1 to PH7. The pools were mixed to acquire a normalized sequence distribution using the percentages depicted in Table 4, obtaining one VH fraction that was digested with SfiI and XhoI restriction enzymes and ligated in SfiI-XhoI cut PDV-VL intermediate library obtained as described above. The ligation set-up, purification method, subsequent transformation of TG1 and harvest of bacteria was exactly as described for the VL intermediate library (see above). The final library (approximately $5x10^6$ cfu) was checked for insert frequency with a colony PCR using a primer set flanking the inserted VH-VL regions. More than 95% of the colonies showed a correct length insert (see Table 5). The colony PCR products were used for subsequent DNA sequence analysis to check sequence variation and to assess the percentage of colonies showing a complete ORF. This was typically above 70% (see Table 5). The frequency of mutations in the V genes was also analysed. Out of 50 sequences, 47 (94%) were not in germline configuration indicative of a maturation process and consistent with the memory phenotype of the B cells used as an RNA source for the library. Finally, the library was rescued and amplified by using CT helper phages (see WO 02/103012) and was used for phage antibody selection by panning methods as described below.

Example 2

Selection of phages carrying single chain Fv fragments against Influenza A subtypes H3 and H7 and Influenza B.

Antibody fragments were selected using antibody phage display libraries constructed essentially as described above and general phage display technology and MABSTRACT® technology essentially as described in US Patent Number 6,265,150 and in WO 98/15833 (both of which are incorporated by reference herein). Furthermore, the methods and helper phages as described in WO 02/103012 (which is incorporated by reference herein) were used in the present invention.

Selection was performed against recombinant hemagglutinin (HA) of influenza A subtype H3 (A/Wisconsin/67/2005) and H7 (A/Netherlands/219/2003) or influenza B (B/Ohio/01/2005). HA antigens were diluted in PBS (5.0 μg/ml), added to MaxiSorp™ Nunc-Immuno Tubes (Nunc) and incubated overnight at 4°C on a rotating wheel. The immunotubes were emptied and washed three times in block buffer (2% non-fat dry milk (ELK) in PBS). Subsequently, the immunotubes were filled completely with block buffer and incubated for 1-2 hrs at room temperature. Aliquots of phage display library (500-1000 μl, $0.5x10^{13}$ – $1x10^{13}$ cfu, amplified using CT helper phage (see WO 02/103012)) were blocked in blocking buffer supplemented with 10% non-heat inactivated fetal bovine serum and 2% mouse serum for 1-2 hrs at room temperature. The blocked phage library was added to the immunotubes, incubated for 2 hrs at room temperature, and washed with wash buffer (0.05% (v/v) Tween-20 in PBS) to remove unbound phages. Bound phages were eluted from the respective antigen by incubation with 1 ml of 100 mM triethylamine (TEA) for 10 min at room temperature. Subsequently, the eluted phages were mixed with 0.5 ml of 1 M Tris-HCl pH 7.5 to neutralize the pH. This mixture was used to infect 5 ml of an XL1-Blue *E.coli* culture that had been grown at 37°C to an OD 600 nm of approximately 0.3. The phages were allowed to infect the XL1-Blue bacteria for 30 min at 37°C. Then, the mixture was centrifuged for 10 min at 3000xg at room temperature and the bacterial pellet was resuspended in 0.5 ml 2-trypton yeast extract (2TY) medium. The obtained bacterial suspension was divided over two 2TY agar plates supplemented with tetracycline, ampicillin and glucose. After incubation overnight of the plates at 37°C, the colonies were scraped from the plates and used to prepare an enriched phage library, essentially as described by De Kruif *et al.* (1995a) and WO 02/103012. Briefly, scraped bacteria were used to inoculate 2TY medium containing ampicillin, tetracycline and glucose and grown at a temperature of 37°C to an OD 600 nm of ~0.3. CT helper phages were added and allowed to infect the

bacteria after which the medium was changed to 2TY containing ampicillin, tetracycline and kanamycin. Incubation was continued overnight at 30°C. The next day, the bacteria were removed from the 2TY medium by centrifugation after which the phages in the medium were precipitated using polyethylene glycol (PEG) 6000/NaCl. Finally, the phages were dissolved in 2 ml of PBS with 1% bovine serum albumin (BSA), filter-sterilized and used for the next round of selection. The second round of selection is performed either on the same HA subtype or on HA of a different subtype.

Two consecutive rounds of selections were performed before isolation of individual single-chain phage antibodies. After the second round of selection, individual *E.coli* colonies were used to prepare monoclonal phage antibodies. Essentially, individual colonies were grown to log-phase in 96 well plate format and infected with VCS-M13 helper phages after which phage antibody production was allowed to proceed overnight. The supernatants containing phage antibodies were used directly in ELISA for binding to HA antigens. Alternatively, phage antibodies were PEG/NaCl-precipitated and filter-sterilized for both elisa and flow cytometry analysis.

Example 3

Validation of the HA specific single-chain phage antibodies

Selected supernatants containing single-chain phage antibodies that were obtained in the screenings described above were validated in ELISA for specificity, *i.e.* binding to different HA antigens. For this purpose, baculovirus expressed recombinant H3 (A/Wisconsin/67/2005), H7 (A/Netherlands/219/2003) and B (B/Ohio/01/2005) HA's (Protein Sciences, CT, USA) were coated to Maxisorp™ ELISA plates. After coating, the plates were washed three times with PBS containing 0.1% v/v Tween-20 and blocked in PBS containing 3% BSA or 2% ELK for 1 hr at room temperature. The selected single-chain phage antibodies were incubated for 1 hr in an equal volume of PBS containing 4% ELK to obtain blocked phage antibodies. The plates were emptied, washed three times with PBS/0.1% Tween-20 and the blocked single-chain phage antibodies were added to the wells. Incubation was allowed to proceed for one hr, the plates were washed with PBS/0.1% Tween-20 and bound phage antibodies were detected (using OD 492nm measurement) using an anti-M13 antibody

conjugated to peroxidase. As a control, the procedure was performed simultaneously without single-chain phage antibody and with an unrelated negative control single-chain phage antibody. From the selections on the different HA antigens with the IgM memory B cell libraries, 6 unique single-chain phage antibodies specific for both recombinant H3 HA and H7 HA were obtained (SC08-001, SC08-003, SC08-006, SC08-014, SC08-017 and SC08-018). In addition, 2 unique single-chain phage antibodies specific for recombinant H3 HA (SC08-015 and SC08-016) and 5 for recombinant H7 HA (SC08-007, SC08-009, SC08-010, SC08-011 and SC08-013) were isolated. See table 6.

Alternatively, PEG/NaCl-precipitated and filter-sterilized phage antibodies were used to validate elisa binding and specificity. For this purpose, baculovirus expressed recombinant influenza A H1 (A/New Caledonia/20/1999), H3 (A/Wisconsin/67/2005), H5 (A/Vietnam/1203/2004), H7 (A/Netherlands/219/2003) and influenza B (B/Ohio/01/2005, B/Malaysia/2506/2004, B/Jilin/219/2003) HA's (Protein Sciences, CT, USA) were coated to Maxisorp™ ELISA plates. After coating, the plates were washed three times with PBS containing 0.1% v/v Tween-20 and blocked in PBS containing 3% BSA or 2% ELK for 1 hr at room temperature. The selected single-chain phage antibodies were incubated for 1 hr in an equal volume of PBS containing 4% ELK to obtain blocked phage antibodies. The plates were emptied, washed three times with PBS/0.1% Tween-20 and the blocked single-chain phage antibodies were added to the wells. Incubation was allowed to proceed for one hr, the plates were washed with PBS/0.1% Tween-20 and bound phage antibodies were detected (using OD 492nm measurement) using an anti-M13 antibody conjugated to peroxidase. As a control, the procedure was performed simultaneously without single-chain phage antibody and with a negative control single-chain phage antibody. From the selections on the different HA antigens with the IgM memory B cell libraries, 2 unique single-chain phage antibody specific for recombinant H1, H3 and H7 HA were obtained (SC08-001 and SC08-014). In addition, 6 unique single-chain phage antibodies specific for recombinant H3 HA (SC08-003, SC08-006, SC08-015, SC08-016, SC08-017 and SC08-018), and 5 for recombinant H7 HA (SC08-007, SC08-009, SC08-010, SC08-011 and SC08-013) were isolated. See table 7.

Alternatively, PEG/NaCl-precipitated and filter-sterilized phage antibodies were used to validate binding and specificity by FACS analysis. For this purpose, full-length recombinant influenza A subtypes H1 (A/New Caledonia/20/1999), H3

(A/Wisonsin/67/2005), H5(TV), H7 (A/Netherlands/219/2003) and influenza B (B/Ohio/o1/2005) HA's were expressed on the surface of PER.C6 cells. The cells were incubated with single-chain phage antibodies for 1 hr followed by three wash steps with PBS+0.1%BSA. Bound phages were detected using FITC conjugated M13-antibody. From the selections on the different HA antigens with the IgM memory B cell libraries, one single-chain phage antibody specific for influenza A subtypes H1, H3 and H7 HA was isolated (SC08-001). In addition, 6 unique single-chain phage antibodies specific for H3 HA (SC08-003, SC08-006, SC08-015, SC08-016, SC08-017 and SC08-018), 4 unique single-chain phage antibodies specific for H7 HA (SC08-007, SC08-010, SC08-011 and SC08-013) were isolated. See table 8. Of these, six phage antibodies (SC08-001, SC08-003, SC08-015, SC08-016, SC08-017, SC08-018) were used for construction of fully human immunoglobulins for further characterization (see Example 5).

Example 4

Selection and validation of Influenza A (H3N2) HA specific immortalized B-cell clones

In addition to phage display, the binding molecules of the present invention can also be isolated by other methods, for example using immortalized B cells, as described in e.g. WO 2007067046**.** Immortalized IgM memory cells (CD19+/CD27+, IgD+), derived from vaccinated donors, were stained with APC labeled H3 HA and single cell sorted into limiting dilution culture. After recovery and cell expansion the supernatants of the H3 HA sorted cells were measured by solid phase ELISA for H1, H3 and H7 immunoreactivity.

Subsequently, the target specific B cells were characterized for binding activity and neutralization. The B cells were cloned by limiting dilution to yield single clones. The clones were seeded into culture plates and the cells cultured for 14 days. Supernatants of the clones were screened for production of anti-HA monoclonal antibodies that bind to HA-transfected 293 cells expressing H1, H3, H5 and H7 derived HA. As a control for aspecific or background staining, untransfected 293 cells were used.

In order to determine whether the selected B-cell clone supernatants containing either IgM or IgG antibodies that were obtained in the screenings described above were capable of blocking influenza A (H3N2) infection, an *in vitro*

virus neutralization assay (VNA) was performed. The VNA was performed on MDCK cells (ATCC CCL-34). MDCK cells were cultured in MDCK cell culture medium (MEM medium supplemented with antibiotics, 20 mM Hepes and 0.15% (w/v) sodium bicarbonate (complete MEM medium), supplemented with 10% (v/v) fetal bovine serum). The H3N2 (A/Wisconsin/67/2005), strain which was used in the assay was diluted to a titer of 5,7 $x10^3$ TCID50/ml (50% tissue culture infective dose per ml), with the titer calculated according to the method of Spearman and Karber. The IgG or IgM preparations were serially 2-fold diluted (1:2 - 1:64) in complete MEM medium in quadruplicate wells. 25 µl of the respective IgG dilution was mixed with 25 µl of virus suspension (100 TCID50/25 µl) and incubated for one hr at 37°C. The suspension was then transferred in quadruplicate onto 96-well plates containing confluent MDCK cultures in 50 µl complete MEM medium. Prior to use, MDCK cells were seeded at $3x10^4$ cells per well in MDCK cell culture medium, grown until cells had reached confluence, washed with 300-350 µl PBS, pH 7.4 and finally 50 µl complete MEM medium was added to each well. The inoculated cells were cultured for 3-4 days at 37°C and observed daily for the development of cytopathogenic effect (CPE). CPE was compared to the positive control.

Of the 187 IgG supernatants tested, 43 were found to neutralize the H3N2 (A/Wisconsin/67/2005) strain used in this assay. Of these, 14 were used for construction of human IgG immunoglobulins as described in example 5.

Example 5

Construction of fully human immunoglobulin molecules (human monoclonal antibodies) from the selected single chain Fvs and B-cell clones

From the selected specific single-chain phage antibodies (scFv) clones plasmid DNA was obtained and nucleotide and amino acid sequences were determined according to standard techniques. Heavy and light chain variable regions of the scFvs were cloned directly by restriction digest for expression in the IgG expression vectors pIg-C911-HCgamma1 (see SEQ ID No: 189), pIG-C909-Ckappa (see SEQ ID NO: 190), or pIg-C910-Clambda (see SEQ ID No: 191). Heavy and light chain variable regions of the B-cell clones were PCR-amplified and cloned directly by restriction digest for expression in the IgG expression vectors pIg-C911-HCgamma1 (see SEQ ID No: 190), pIG-C909-Ckappa (see SEQ ID NO: 191), or pIg-C910-Clambda (see SEQ ID No: 192). The VH and VL gene identity (see Tomlinson IM et al. V-BASE Sequence

Directory. Cambridge United Kingdom: MRC Centre for Protein Engineering (1997)) of the scFvs were determined (see Table 9).

Nucleotide sequences for all constructs were verified according to standard techniques known to the skilled artisan. The resulting expression constructs encoding the human IgG1 heavy and light chains were transiently expressed in combination in 293T cells and supernatants containing human IgG1 antibodies were obtained and produced using standard purification procedures. The human IgG1 antibodies were titrated in a concentration range of between 10 and 0.003 μg/ml against H3, H7 or B antigen (data not shown). An unrelated antibody was included as a control antibody.

The amino acid sequence of the CDRs of the heavy and light chains of the selected immunoglobulin molecules is given in Table 9. The nucleotide sequence and amino acid sequence of the heavy and light chain variable regions are given below. The immunoglobulins comprise the heavy and light chain constant region of CR6261, as given below.

Example 6

In vitro neutralization of influenza virus by H3N2 binding IgGs (virus neutralization assay)

In order to determine whether the selected IgGs were capable of blocking influenza A (H3N2) infection, an *in vitro* virus neutralization assay (VNA) was performed. The VNA was performed on MDCK cells (ATCC CCL-34). MDCK cells were cultured in MDCK cell culture medium (MEM medium supplemented with antibiotics, 20 mM Hepes and 0.15% (w/v) sodium bicarbonate (complete MEM medium), supplemented with 10% (v/v) fetal bovine serum). The H3N2 (A/Wisconsin/67/2005) strain which was used in the assay was diluted to a titer of 5,7 $x10^3$ TCID50/ml (50% tissue culture infective dose per ml), with the titer calculated according to the method of Spearman and Karber. The IgG preparations (200 μg/ml) were serially 2-fold diluted (1:2 - 1:512) in complete MEM medium in quadruplicate wells. 25 μl of the respective IgG dilution was mixed with 25 μl of virus suspension (100 TCID50/25 μl) and incubated for one hr at 37°C. The suspension was then transferred in quadruplicate onto 96-well plates containing confluent MDCK cultures in 50 μl complete MEM medium. Prior to use, MDCK cells were seeded at $3x10^4$ cells per well in MDCK cell culture medium, grown until cells had reached confluence, washed with 300-350 μl PBS, pH 7.4 and finally 50 μl complete MEM medium was

added to each well. The inoculated cells were cultured for 3-4 days at 37°C and observed daily for the development of cytopathogenic effect (CPE). CPE was compared to the positive control.

The human anti-H3 HA and/or anti-H7 HA antibodies of Example 5 were subjected to the above-described VNA. Of these antibodies, all antibodies, except CR8040, CR8052 and CR8069, neutralized the A/Wisconsin/67/2005 H3N2 strain. The concentrations (in μg/ml) at which these antibodies protect MDCK cultures against CPE are given in Table 11.

Example 7

Cross-binding reactivity of anti-H3N2 IgGs

The H3N2 neutralizing IgG antibodies described above were validated in ELISA for binding specificity, *i.e.* binding to different HA antigens. For this purpose, baculovirus expressed recombinant H1 (A/New Caledonia/20/1999), H3 (A/Wisconsin/67/2005, A/New York/55/2004, A/Wyoming/3/2003) and H7 (A/Netherlands/219/2003) HA's (Protein Sciences, CT, USA) were coated to Maxisorp™ ELISA plates. After coating, the plates were washed three times with PBS containing 0.1% v/v Tween-20 and blocked in PBS containing 3% BSA or 2% ELK for 1 hr at room temperature. The plates were emptied, washed three times with PBS/0.1% Tween-20 and the IgG antibodies were added to the wells. Incubation was allowed to proceed for one hr, the plates were washed with PBS/0.1% Tween-20 and bound antibodies were detected (using OD 492nm measurement) using an anti-human IgG antibody conjugated to peroxidase. As a control, an unrelated IgG CR4098 was used.

From the selected H3N2 neutralizing antibodies, CR8001 shows heterosubtypic cross-binding to all the recombinant HA's tested, CR8020, CR8021, CR8041, CR8043 and CR8057 show heterosubtypic cross-binding to all 3 tested H3 HA's as well as the H7 HA. CR8003, CR8015, CR8016, CR8017, CR8018, CR8038, CR8039, CR8040, CR8049, CR8050, CR8052 and CR8069 show cross-binding to all 3 tested H3 HA's. One antibody, CR8019, shows binding to only 2 of the H3 HA's. See table 12.

Additionally, the selected H3N2 neutralizing antibodies were used to test heterosubtypic binding by FACS analysis. For this purpose, full-length recombinant influenza A subtypes H1 (A/New Caledonia/20/1999), H3 (A/Wisonsin/67/2005) and

H7 (A/Netherlands/219/2003) HA's were expressed on the surface of PER.C6 cells. The cells were incubated with IgG antibodies for 1 hr followed by three wash steps with PBS+0.1%BSA. Bound antibodies were detected using PE conjugated anti-human antibody. As a control, untransfected PER.C6 cells were used.

From the H3N2 neutralizing antibodies, CR8001 shows cross-binding activity to influenza A subtypes H1, H3 and H7 HA but not wild-type PER.C6 cells. In addition, CR8020 and CR8041 show strong binding to both H3 and H7 HA. CR8043 and CR8057 show strong binding to H3 HA and weak binding to H7 HA. CR8055 showed low levels of background staining on PER.C6 cells. The remaining 13 antibodies show binding to H3 transfected cells only See table 12.

Example 8

Cross-neutralizing activity of anti-H3N2 IgGs

In order to determine whether the selected IgGs were capable of blocking multiple influenza A strains, additional *in vitro* virus neutralization assays (VNA) were performed. The VNA were performed on MDCK cells (ATCC CCL-34). MDCK cells were cultured in MDCK cell culture medium (MEM medium supplemented with antibiotics, 20 mM Hepes and 0.15% (w/v) sodium bicarbonate (complete MEM medium), supplemented with 10% (v/v) fetal bovine serum). The H1N1 (A/New Caledonia/20/1999 A/Brisbane/59/2007 and A/Solomon Islands/IVR-145), H3N2 (A/Hong Kong/1/68, A/Johannesburg/33/94, A/Panama/2000/1999, A/Hiroshima/52/2005 and A/Wisconsin/67/2005), H7N3 (A/Mallard/Netherlands/12/2000) and H10 (A/Chick/Germany/N/49) strains which were used in the assay were all diluted to a titer of 5,7 $x10^3$ TCID50/ml (50% tissue culture infective dose per ml), with the titer calculated according to the method of Spearman and Karber. The IgG preparations (80 μg/ml) were serially 2-fold diluted (1:2 - 1:512) in complete MEM medium in quadruplicate wells. 25 μl of the respective IgG dilution was mixed with 25 μl of virus suspension (100 TCID50/25 μl) and incubated for one hr at 37°C. The suspension was then transferred in quadruplicate onto 96-well plates containing confluent MDCK cultures in 50 μl complete MEM medium. Prior to use, MDCK cells were seeded at $3x10^4$ cells per well in MDCK cell culture medium, grown until cells had reached confluence, washed with 300-350 μl PBS, pH 7.4 and finally 50 μl complete MEM medium was added to each well. The inoculated cells were cultured for 3-4 days at 37°C and

observed daily for the development of cytopathogenic effect (CPE). CPE was compared to the positive control.

From the panel of H3N2 neutralizing antibodies, CR8020 and CR8041 show heterosubtypic cross-neutralizing activity to all tested influenza A subtypes H3, H7 and H10 viruses but not H1 viruses. In addition, CR8043 shows cross-neutralization to all tested H3 and H10 virus strains. CR8039, CR8041, CR8043 and CR8057 show cross-neutralization of all tested H3 virus strains. An additional 13 antibodies show cross-neutralization to more than 1 of the tested H3 virus strains.See table 13.

Example 9

Anti-H3N2 antibodies bind to the pre-fusion conformation of HA

In order to determine whether the selected IgGs were capable of binding the pre- or post-fusion conformation of the HA molecule, an *in vitro* pH-shift experiment was performed.

For this purpose, full-length recombinant influenza A subtype H3 (A/Wisonsin/67/2005) HA was expressed on the surface of PER.C6 cells. To assay for specific reactivity at different structural HA conformations, 3×10^5 cells were treated with 10 µg/ml trypsin-EDTA in DMEM for 30 min at RT, washed and incubated for 5 min in acidified PBS (pH 4.9), washed and then incubated for 20 min in the presence of 20 mM DTT at RT. Cells were split at each step and untreated adherent cells were resuspended in 0.05% EDTA. Cell fractions of each treatment were incubated with anti-H3N2 IgGs CR8001, CR8020, CR8041, CR8043 and CR8057 for 30 min. Cells were then incubated for 30 min with phycoerythrin-conjugated anti-IgG (Southern Biotech). Stained cells were analysed using a FACS Calibur with CELLQuest Pro software (Becton Dickinson). FACS binding of IgG1 to surface expressed H3 rHA was measured after sequential treatment with trypsin (striped bars), pH 4.9 buffered medium (solid white bars) and DTT (crossed bars) and expressed as percentage binding to untreated rHA (solid black bars). See Figure 2. Antibodies CR8001, CR8020, CR8041 and CR8043 all show a marked decrease in binding after pH-shift indicating specificity for an epitope present only before the low pH induced conformational change of the HA molecule. Antibody CR8057 showed a decrease in binding only after DTT treatment indicating specificity for a conformation independent epitope available only when HA1 is present.

Example 10

Anti-H3N2 antibody CR8041 prevents cleavage of HA0

In order to determine whether the selected IgGs were capable of protecting the HA molecule from protease cleavage, an *in vitro* protease susceptibility assay was performed.

For this purpose, 7.5 µg recombinant soluble influenza A subtype H3 (A/Wisonsin/67/2005) HA (Protein Sciences, CT, USA) was subjected to different pH (4.9, 5.3 and 8.0) treatments for 1 hour at 37°C. After incubation, reactions were neutralized. The samples were digested overnight with 0.5 µg trypsin in the presence and absence of 7.5 µg CR8041 or CR8057 Fab fragments. Reactions were quenched by addition of SDS loading buffer. 3µl Nupage reducing agent (Invitrogen) was added to each sample. Samples were run on a 4-12% BisTris gel in 1x MOPS buffer. Protein bands were visualized by colloidal blue staining (see Figure 3). In the absence of Fab fragments, the H3 HA molecule is readily converted to its protease-susceptible post-fusion form at pH 4.9 or 5.3, but not at pH 8.0. In the presence of Fab fragment CR8057 the degradation of H3 HA and thus the conformational change at pH 4.9 is not inhibited. In contrast, the presence of Fab CR8041 not only prevents H3 HA conformational change and degradation at low pH, but also the pH independent cleavage of HA0 into HA1 and HA2. These results point towards an epitope for CR8041 on, or close to, the cleavage site. Competition experiments (results not shown) with the anti-H3N2 antibody panel indicate an overlapping epitope and a similar working mechanism for the CR8001, CR8020 and CR8043 antibodies.

Example 11

Mechanism of action of the binding molecules of the invention

The HA glycoprotein is a trimer in which each monomer consists of two disulphide - linked glycopolypeptides (named HA1 and HA2) that are produced during infection by proteolytic cleavage of a precursor (HA0). Cleavage is necessary for virus infectivity since it is required to prime the HA for membrane fusion, to allow conformational change.

Activation of the primed molecule occurs at low pH in endosomes, between pH5 and pH6, and requires extensive changes in HA structure. The 3-dimensional structure of the pre-fusion uncleaved (**I**), pre-fusion cleaved (**II**) and post-fusion HA (**III**) conformations are schematically shown in Figure 4.

In vitro, the conformational changes of the HA molecule can be mimicked using HA surface-expressed mammalian cells. First, the proteolytic cleavage can be triggered by adding trypsin to the cells. Second, the pre- to post-fusion conformational change can be achieved by lowering the pH. Additionally, the HA1 part of the molecule can be removed by adding a reducing agent like DTT. In this way and by addition of the antibodies at specific stages it is possible to investigate at what stage the antibody interferes with the infection process. Hereto, PER.C6® cells were transfected with an H3 HA expression construct harboring HA from A/Wisonsin/67/2005 and subjected to different treatments as described in example 10.

For this experiment, cells were first incubated with anti-H3 mAbs before trypsin cleavage and subsequently treated as described above (see Figure 5).

Binding of anti-H3 mAbs was detected with PE-conjugated anti-human antibody according to standard protocols. Fluorescence signals were measured by FACS analysis. 'Cells only' means the signal obtained after mAb binding to untreated cells and was set at 100%. As can be seen in Figure 5, the mAbs are still bound to HA following the different treatments. Since it was shown in example 10 above that the H3 mAbs CR8020, CR8041 and CR8043 only bind to the pre-fusion state (i.e. before the conformational shift due to lower pH), it was concluded that binding of the antibody in fact inhibits the trypsin cleavage (see also example 10), at least *in vitro*, and thus also the subsequent steps leading to the conformational change and fusion. Antibody CR8057 which binds the HA1 part of the HA molecule near the receptor attachment site is capable of binding to HA after conformational shift and, as expected, is lost when HA1 part is removed following disruption of the disulphide bonds between HA1 and HA2 domains by DTT treatment.

The inhibition of trypsin cleavage was subsequently confirmed in a different *in vitro* experiment. First, a time course experiment was done to determine how long H3 HA should be incubated with trypsin to achieve proper cleavage of HA0 in HA1 and HA2. Hereto, recombinant soluble H3 HA (A/Wisconsin/67/2005; Protein Sciences, CT, USA) was incubated in 4mM Tris.HCl buffer at pH8.0 containing 6.7 μg/ml Trypsin and 1% N-dodecyl-β-demaltosid. Trypsin digestion was stopped at several time-points by addition of 1%BSA. Samples were run on SDS-page gel (reduced) and blotted according to standard methods. HA0, HA1 and HA2 bands were detected using a rabbit anti-H3HA polyclonal antibody (Protein Sciences, CT, USA). Figure 6

shows that 2 hrs incubation is enough for near complete cleavage evidenced by appearance of the HA1 and HA2 bands on the reducing gel. Next, recombinant soluble H3 HA was incubated with either CR8020, CR8041, CR8043 or CR8057 and subsequently subjected to trypsin cleavage at pH8.0. Trypsin digestion was again stopped at several time-points by adding 1%BSA. Samples were run on SDS-page (reduced) and blotted. HA0, HA1 and HA2 bands were detected using an anti-H3 polyclonal antibody. The results show that all three mAbs CR8020, CR8041 and CR8043 prevent trypsin cleavage *in vitro* since incubation of the H3 HA bound to the antibody with trypsin results in protection of the HA0 form of HA on the gel (Figure 7). In contrast, incubation of H3 HA with a control mAb (CR8057) at the same conditions results in disappearance of the HA0 band. This experiment confirms the data discussed in example 10 for CR8041 and extends this observation to CR8020 and CR8043 antibodies. The binding molecules of the invention thus prevent at least trypsin cleavage of the HA0 molecule, at least *in vitro*. It is, however, noted that this does not exclude that additional inhibitory effects are also mediated by the CR8020, CR8041 and CR8043 mAbs that are more downstream in the process of infection and result in interference with the pH-induced conformational shift and/or fusion process. To investigate whether this could be the case the experiment discussed above was repeated but now the antibody CR8043, or the antibody CR8057 as a control, added to the cells expressing H3 HA only after trypsin cleavage. Following incubation the cells were subsequently incubated in low pH buffer as described in example 10 and treated with DTT as described. If the mechanism of action would be restricted to inhibition of trypsin cleavage it is expected that the mAb CR8043 loses binding after pH treatment since we have established in example 10 that the antibodies do not bind to the post-fusion conformation of HA. In contrast, as can be seen from Figure 8, mAb CR8043 binding is still detected after exposure to low pH and subsequent DTT treatment indicating that the pH-induced conformational shift is also inhibited by CR8043, at least *in vitro*. CR8057 which has been shown to bind to the HA1 region of HA behaves as expected and is no longer detectable when the HA1 part is lost following DTT treatment.

To investigate whether antibodies CR8020 and CR8041 are also capable of blocking the pH-induced conformational change of HA, the experiments discussed above were repeated. Now the antibodies CR8020, CR8041 and CR8043, or the antibody CR8057 as a control, were added to cells expressing either A/Hong Kong/1/1968,

A/Hong Kong/24/1985 or A/Wisconsin/67/2005 subtype H3 HA either after all treatments described in above, before low pH incubation or before trypsin cleavage. As shown earlier for A/Wisconsin/67/2005 H3 HA, the CR8020, CR8041 and CR8043 antibodies recognize a epitope present only before low pH treatment. This epitope is conserved in the three HAs used in this experiment as can be seen in fig 9c. If the mechanism of action would be restricted to inhibition of trypsin cleavage it is expected that the mAbs CR8020, CR8041 and CR8043 lose binding of already cleaved HA after pH treatment since we have established in example 10 that the antibodies do not bind to the post-fusion conformation of HA. In contrast, as can be seen from Figure 9b, mAb binding is still detected after exposure to low pH and subsequent DTT treatment on all three different H3 HAs indicating that the pH-induced conformational shift is also inhibited by CR8020, CR8041 anc CR8043, at least *in vitro*. CR8057 which has been shown to bind to the highly variable HA1 region of HA, shows no binding to A/Hong kong/1/1968 and A/Hong Kong/24/1985 HAs.

Example 12

In vitro generated escape mutants indicate that the position of the epitope coincides with a conserved sequence in H3 HA

To investigate to which region in HA CR8020, CR8041 and CR8043 bind it was attempted to generate escape mutants in *in vitro* cultures. A/Hong Kong/1/1968 viruses were passaged in MDCK cell cultures in the presence of limiting amounts of monoclonal antibodies. First, it was determined what concentration of antibody resulted in a 3 log reduction of virus infection following inoculation of MDCK cells with 100 TCID50 units mixed with different amounts of monoclonal antibody and incubation for 3 days. This concentration of antibody was added to the inoculum in serial passages and after each passage the virus was plaque titrated in the absence and presence of different amounts of antibody to determine whether the viruses are still sensitive to antibody-mediated neutralization. This procedure was followed for each of the mAbs CR8020, CR8041 and CR8043. From each culture escape viruses could be isolated by plaque assay and, of two isolates of each, viral RNA was extracted and used to determine the HA sequence. The observed mutated amino acids were as follows:

CR8020: D19N and Q27L in both analyzed plaques;

CR8041: G33E in two plaques;

CR8043: R25M in one and Q34R in the other plaque.

All three monoclonal antibodies show escape mutations in a similar domain in the HA2 part of the HA stem region adjacent to the fusion peptide. Comparison of amino acid sequences of H3N2 viruses present in the NCBI influenza database (http://www.ncbi.nlm.nih.gov/genomes/FLU/Database/select.cgi) in this region reveals a striking conservation of the sequence. Table 14 depicts the sequence variation in the HA2 region between amino acids W14 and K39 with the observed escape mutations highlighted. N= number of strains having a specific sequence. In addition, the year of isolation (years) is indicated as well as the strains tested positive in neutralization experiments with the H3 antibodies (Pa= A/Panama/2000/1999; Wis= A/Wisconsin/67/2005; Hs= A/Hiroshima/52/2005; HK= A/HongKong/1/1968). Of the 1363 H3 viruses present in the database that contained the mentioned HA2 sequence the majority (81%) had sequences that are present in virus strains that were shown to be neutralized. Of the remaining sequences most have amino acids that can be considered conserved changes. For the other mutations a functional neutralization test will be needed to establish whether the change affects the functionality of the antibody. Importantly, three amino acid changes that came up in the escape virus experiment (R25, G33 and Q34) do not occur in natural influenza sequences and the other two mutations appeared only in combination (D19 and Q27), a combination which is also not present in the natural sequences. This could mean that the mutations have a negative affect on the virus fitness. Altogether, it is concluded that the antibodies interact with an epitope on HA2 that is highly conserved between H3 subtype viruses confirming the broad neutralization capability of the monoclonal antibodies.

Example 13

Preparation of monoclonal antibodies for in vivo experiments

To enable characterization and the subsequent validation of the IgGs as potential therapeutic antibodies *in vivo*, they need to be manufactured and purified in sufficient quantities. The IgGs were produced in PER.C6® cells in a 25 L Wave-bag and the culture was harvested. From the clarified harvest, IgG was purified using Protein A affinity chromatography and a buffer exchange step. The monomer content of the purified buffer exchanged IgG is ~99% both before and after 0.2 μm sterile

filtration. Additional *in vitro* virus neutralization assays (VNA) were performed with the different antibody preparations obtained, as described above. The results are shown in Table 15.

Example 14

Prophylactic activity of human IgG monoclonal antibodies against lethal H3N2 challenge in vivo

MAbs CR8020, CR8041 and CR8043 were tested for prophylactic efficacy in a mouse lethal challenge model with influenza A/HK/1/68-MA20 (H3N2) virus in female 129X1/SvJ mice (Jackson Labs) (MA=mouse adapted). A/HK/1/68-MA20 virus was obtained from Prof. E.G. Brown, University of Ottawa, Ottawa, Ontario, Canada; Brown, E. G. et al. (2001). The virus was passaged once in embryonated chicken eggs before use in the mice experiments. All mice were acclimatised and maintained for a period of at least 4 days prior to the start of the experiment.

MAbs were dosed at 30, 10, 3 and 1 mg/kg intravenously in the tail vein (*vena coccygeus*) at day -1 before challenge, assuming an average weight of 18 g per mouse and a fixed dose volume of 0.2 mL. The mice (n=8 per group) were then challenged at day 0 with 25 LD50 A/HK/1/68-MA20 (H3N2) virus by intranasal inoculation. The actual dose of the virus administered was estimated by titrating a few replicate samples from the inoculum remaining after inoculation of the animals was completed. Virus titers (TCID50/mL) of the inoculum were determined on MDCK cells. The results showed that no inactivation of virus had unintentionally occurred during preparation or administration of the inoculum. Clinical signs and body weights were determined daily from day-1 before challenge until the end of the study at day 21. Clinical signs were scored with a scoring system (0=no clinical signs; 1=rough coat; 2=rough coat, less reactive, passive during handling; 3=rough coat, rolled up, laboured breathing, passive during handling; 4=rough coat, rolled up, laboured breathing, does not roll back on stomach when laid down on its back). At a score of 4 the animal was euthanized. To analyze mAb plasma levels at day 0 and determine the presence of hemagglutination inhibiting (HI) antibodies at day 21, blood samples were drawn from all mice on D0, just before challenge, and on D21 post infection.

The mAbs were tested in 2 separate experiments. In each experiment a negative control antibody (CR3014) group was taken along, dosed at 30 mg/kg. MAb CR8020 was tested in the first experiment, mAbs CR8041 and CR8043 in the second.

All mice were active and appeared healthy without showing signs of disease during the acclimatization period. Fig. 10 shows the survival rates of the mice, following mAb administration. A clear dose-response relationship was observed, with all groups dosed with CR8020, CR8041 or CR8043 at 30, 10 or 3 mg/kg showing 100% survival, whereas at 1 mg/kg CR8020 25% of the mice survived and none of the mice survived in the 1 mg/kg CR8041 and CR8043 groups. The two control mAb groups showed 0% survival. In the first experiment, administration of mAb CR8020 resulted in a statistically significant difference in survival time at all four concentrations tested, compared to the control group ($p<0.005$; Log Rank Test). In the second experiment, administration of mAbs CR8041 and CR8043 also resulted in a statistically significant difference in survival time at all four concentrations tested, compared to the control group ($p<0.001$ for both mAbs; Log Rank Test).

In Figure 11 the mean body weight change of the mice during the 21 day study period following mAb administration is shown. Like with the survival rates, there is a clear inverse relationship between the weight loss and dose of antibody used. When the concentration of antibody was increased, the weight loss decreased: mice in groups dosed with CR8020, CR8041 or CR8043 at 30, 10 or 3 mg/kg showed an increase in mean body weight of approximately 10-15% from day 0 -day 21, consistent with age-related weight gain, whereas in the 1 mg/kg groups and in the control mAb groups the mean body weight of the mice declined in the study period. Body weight changes were analysed in more detail with Area under the Curve (AUC) analysis. For the purpose of this analysis, the last observed body weight was carried forward to day 21 if a mouse died / was euthanized during follow-up of the study. Briefly, the weight per mouse at day 0 was used as baseline value and weight change from day 0 to day 21 was determined relative to baseline. The AUC was defined as the summation of the area above and the area below the baseline. Mean AUC values of the mAb dose groups were compared with the respective control groups using analysis of variance with Dunnet's adjustment for multiple comparisons (Table 16). The analysis showed that the mean AUC of the 3, 10 and 30 mg/kg groups from CR8020, CR8041 and CR8043 differed statistically significantly ($P < 0.001$) from that of the corresponding control groups (Table 16). Both for the CR8041- as well as for the CR8043 1 mg/kg dose groups a statistically significant difference was found when compared to the control group ($p=0.004$ and $p< 0.001$ respectively). However, due to the two surviving mice in the CR8020 1 mg/kg dose group an increase in

variation of body weight was observed and therefore no statistical significant difference could be demonstrated when comparing to the control group.

Additional analysis was performed to investigate a dose response in the reduction of weight loss by comparing mean AUC values per antibody concentration for each antibody using analysis of variance with Tukey's adjustment for multiple comparisons (Table 16). Both for mAbs CR8020 and CR8041 the body weight loss in the 1 mg/kg groups is statistically significantly higher ($p < 0.001$) than in the respective 3 mg/kg groups, whereas there is no statistically significant difference between the 3, 10 and 30 mg/kg groups ($p > 0.05$). For mAb CR8043 both the weight loss in the 1 mg/kg group was statistically significantly higher than in the 3 mg/kg group ($p < 0.001$) and that of the 3 mg/kg group was significantly higher than that of the 10 mg/kg group ($p<0.001$). The mean AUC of the 10 and 30 mg/kg groups of CR8043 did not significantly differ ($p=0.997$).

Median clinical scores of the mice are depicted in Fig. 12. The mice dosed with CR8020, CR8041 or CR8043 at 30 and 10 mg/kg did not show any clinical signs, as indicated by a median clinical score of 0 throughout the 21 day study period of the 2 studies. MAb 8020 also showed no clinical score in the 3 mg/kg dose group, whereas in the 3 mg/kg dose groups of mAb 8041 and 8043 increases in clinical score were observed to a median score of 1 and 3 respectively. In the 1 mg/kg dose groups of all three mAbs, clinical scores were increased reaching a median score of 4 in all groups. Mice observed with clinical score 4 were euthanized on the same day. The 2 surviving mice in the CR8020 1 mg/kg dose group became ill at day 7 of the study and showed a maximum clinical score of 1 and 3 respectively. Both mice recovered completely. Of the CR8041 and CR8043 3 mg/kg dose groups the body weight loss profile shows a similar pattern as the clinical score profile.

These results show that at least three human anti-H3N2 antibodies, identified and developed as disclosed herein (CR8020, CR8041 and CR8043) are each separately able to provide protection against a lethal dose of influenza H3N2 *in vivo*. A clear dose-response relationship between the amount of each antibody administered and survival rate was observed. The results show that anti-H3N2 IgG antibody CR8041 and 8043 were able to prevent clinical manifestation of H3N2 infection in mice when administered one day prior to infection at a dose of 10 mg/kg or higher. MAb CR8020 was able to prevent clinical manifestation of H3N2 infection in mice when administered one day prior to infection at a dose of 3 mg/kg or higher.

Example 15

Protective and therapeutic activity of human IgG monoclonal antibodies against lethal H3N2 challenge in vivo

A study was performed to test the therapeutic effect of the monoclonal antibodies as disclosed herein, exemplified by CR8020, in a post-infection model, against a lethal H3N2 A/HK/1/68-MA20 influenza virus challenge in vivo.

Mice (n=10 per group) were dosed with mAb CR8020 at 15 mg/kg intravenously in the tail vein (*vena coccygeus*) at day -1 before challenge (group 1; prophylaxis positive control) or at day 1, 2, 3, 4, 5 or 6 after challenge (groups 2-7), assuming an average weight of 18 g per mouse and a fixed dose volume of 0.2 mL. Group 8 received negative control mAb CR3014 (15 mg/kg) at day 1 after challenge. The mice were challenged at day 0 with 25 LD50 (2.8 log TCID50) A/HK/1/68-MA20 (H3N2) virus by intranasal inoculation. The virus batch and the type, and age of mice were the same as used in example 14. Clinical signs and body weights were determined daily from day-1 before challenge until the end of the study at day 21.

Figure 13A shows the survival rates of the mice, following intravenous administration of mAb CR8020 (15 mg/kg in all groups) or control mAb (15 mg/kg). When 15 mg/kg mAb CR8020 was administered at day 1 pre-challenge or days 1 or 2 post challenge, all animals survived the viral challenge, whereas the survival rate in the control mAb group was 0%. When 15 mg/kg mAb CR8020 was administered at day 3 or 4 after challenge, 50% and 10% survival was observed, respectively. The survival time of each of these groups was statistically significantly different compared to the control group (day 3 group: $p<0.001$ and day 4 group $p=0.002$; Log Rank Test). Groups treated with 15 mg/kg CR8020 at day 5 or 6 showed a survival rate of 0%. There was no statistically significant difference in survival time of the day 5 or 6 treated groups compared to the control group ($p=0.648$ and $p=0.342$, respectively; Log Rank Test).

In Figure 13B the mean body weight change relative to day 0 of the mice during the 21 day study period is shown. Like with the survival rates, there is a clear relationship between weight loss and time of 15 mg/kg mAb CR8020 administration: when treatment with 15 mg/kg mAb CR8020 is administered at later timepoints, the weight loss increased.

Body weight changes were statistically analyzed in more detail using Area under the Curve (AUC) analysis (Table 17). For area under the curve analysis the last observed body weight was carried forward to day 21 if a mouse died / was euthanized during follow-up of the study. Briefly, the weight per mouse at day 0 was used as baseline value and weight change from day 0 to day 21 was determined relative to baseline. The AUC was defined as the summation of the area above and the area below the baseline.

Median clinical scores of the mice are depicted in Figure 13C. Of the mice treated with 15 mg/kg CR8020 at day -1 pre-challenge, all survived and none showed any clinical signs during the observation period. Mice treated with 15 mg/kg CR8020 at day 1 post challenge showed a 100% survival, however, 4 out of 10 animals showed clinical signs, reaching a maximum clinical score between 1 and 3. Of the animals treated with 15 mg/kg CR8020 at day 2 post challenge all survived. However, 9 out of 10 animals showed clinical signs reaching a maximum clinical score of 2 or 3. Animals treated with 15 mg/kg CR8020 at day 3 post challenge showed a 50% survival. Of the survivors (n=5), all animals showed clinical signs with a maximum clinical score of 3. Off the animals treated with 15 mg/kg CR8020 at day 4 post challenge all but one mouse died. The surviving mouse showed clinical signs reaching a maximum clinical score of 2. All mice that survived across the treatment arms were free from symptoms at day 21.

Clinical scores were analyzed using the GENMOD procedure (SAS) to fit a model for repeated measures with mice as subject and data measured on an ordinal scale (Table 18). Since the curves do have different patterns the variable “day” was entered as a class variable in this model. From the groups treated with 15 mg/kg mAb CR8020 at day -1 before challenge and day 1 and 2 post challenge in which 100% of the mice survived, the median clinical score was significantly different from the control mAb group during most of the study period of 21 days ($p \leq 0.001$ for all 3 groups). From the groups treated with 15 mg/kg mAb CR8020 at day 3 or day 4 post challenge in which respectively 50% and 10% of the mice survived, the median clinical score was also significantly different from the control mAb group during most of the study period of 21 days ($p < 0.05$ for both groups). From the groups treated with 15 mg/kg mAb CR8020 at day 5 or day 6 post challenge the median clinical score was significantly different from the control mAb group at day 3 only ($p \leq 0.001$). This difference, although statistically significant, is not considered relevant.

In conclusion, therapy with 15 mg/kg of mAb CR8020 provides 100% protection up to day 2 after challenge in a lethal H3N2 mouse model. When administered at day 3 or day 4 after challenge, treatment with 15 mg/kg mAb CR8020 provides partial protection. When administered at day 5 or day 6 after challenge no protective effect of 15 mg/kg mAb CR8020 was observed in the lethal H3N2 mouse model.

These results show that a post-infection treatment with a monoclonal antibody directed against H3N2 influenza virus, as disclosed herein and exemplified by antibody CR8020, can rescue mammalian subjects, as showed herein in mice, after challenge with a lethal dose of H3N2 influenza virus. Even at a late stage, i.e. 4 days post-infection, the antibody is able to partially protect mice from lethal infection with influenza H3N2 virus. Strikingly, at day 21 post-infection, all surviving antibody-treated animals reached normal body weight levels and did not show any remaining clinical signs.

Example 16

Prophylactic activity of human IgG monoclonal antibodies against lethal H7N7 challenge in vivo

A study was performed to test the prophylactic effect of the monoclonal antibodies as disclosed herein, exemplified by CR8020, against a lethal challenge with H7N7 influenza virus in vivo. MAb CR8020 was tested for prophylactic efficacy in a mouse lethal challenge model with mouse adapted influenza A/Chicken/Netherlands/621557/2003 (H7N7) virus (Central Veterinary Institute (CVI), Lelystad, The Netherlands). The A/CH/NL/621557/03 (H7N7) virus was adapted to mice after 3 lung-to-lung passages. The mouse adapted H7N7 Passage 3 virus was propagated in embryonated chicken eggs in CVI's laboratory. All mice (Balb/c, female, age 6-8 weeks, n=8 per group) were acclimatised and maintained for a period of at least 4 days prior to the start of the experiment. MAb CR8020 was dosed at 30, 10, 3 or 1 mg/kg intravenously in the tail vein (*vena coccygeus*) at day -1 before challenge, assuming an average weight of 18 g per mouse and a fixed dose volume of 0.2 mL. A control group was taken along dosed with 30 mg/kg negative control mAb CR3014. The mice were then challenged at day 0 with 25 LD_{50} A/CH/NL/621557/03 (H7N7) virus by intranasal inoculation. The actual dose of the

virus administered was estimated by titrating a few replicate samples from the inoculum remaining after inoculation of the animals was completed. Virus titers ($TCID_{50}$/mL) of the inoculum were determined on MDCK cells. Clinical signs and body weights were determined daily from day-1 before challenge until the end of the study at day 21 in the same manner as described in example 14. To analyze mAb plasma levels at day 0 and determine the presence of hemagglutination inhibiting (HI) antibodies at day 21, blood samples were drawn from all mice on D0, just before challenge, and on D21 post infection.

All mice were active and appeared healthy without showing signs of disease during the acclimatization period. Fig. 14A shows the survival rates of the mice, following mAb administration. Mice dosed with 1 mg/kg mAb CR8020 or more showed a survival rate of 100%, whereas in the control mAb group 0% survived. In Figure 14B the mean body weight change of the mice during the 21 day study period following mAb administration is shown. In the mAb CR8020 3, 10 and 30 mg/kg groups the mice did not loose weight over the 21 day study period, whereas in the mAb CR8020 1 mg/kg and control mAb groups weight loss was observed, with the mean body weight of the mice in the mAb CR8020 1 mg/kg group recovering to base line level at day 21. Body weight changes were analyzed in more detail with Area under the Curve (AUC) analysis (Table 19). For area under the curve analysis the last observed body weight was carried forward to day 21 if a mouse died / was euthanized during follow-up of the study. Briefly, the weight per mouse at day 0 was used as baseline value and weight change from day 0 to day 21 was determined relative to baseline. The AUC was defined as the summation of the area above and the area below the baseline.

There is a clear inverse relationship between the weight loss and dose of antibody used. When the concentration of antibody was increased, the weight loss decreased. The mean difference in weight loss, as compared to the control mAb, was 47.44, 79.75, 86.71 and 80.48 g*day in the mAb CR8020 1, 3, 10 and 30 mg/kg groups, respectively. All differences were statistically significant ($p<0.001$).

Median clinical scores of the mice are depicted in Fig. 14C. All, except one or two, animals within each group showed clinical signs (score = 1) at day 1 post challenge. This is probably not related to the viral challenge, since a non-challenged group taken along in the study showed a similar effect at day 1 (data not shown).

Of the mice treated with 3, 10 or 30 mg/kg mAb CR8020 at day -1 pre-challenge, all survived and none of the animals showed any clinical signs during the observation period (from day 2 to day 21 post infection). Mice treated with 1 mg/kg mAb CR8020 at day -1 pre-challenge, showed a 100% survival rate, but 8 out of 8 mice showed clinical signs reaching a maximum clinical score of 3.

These results show that human anti-H3N2 antibody CR8020, identified and developed as disclosed herein (CR8020) is able to provide heterosubtypic protection against a lethal dose of influenza H7N7 *in vivo*. When administered one day prior to infection at a dose of 3 mg/kg or higher, mAb CR8020 was able to completely prevent clinical manifestation of H7N7 infection in mice. At a dose of 1 mg/kg CR8020 administered one day prior to infection, all mice survived the lethal challenge, and the body weight loss and clinical signs observed fully resolved at the end of the 21 day study period.

A second study was performed to assess and compare the prophylactic efficacy of mAb CR8020, CR8041 and CR8043 in the H7N7 mouse model. MAb CR8020, CR8041 and CR8043 (produced in PER.C6® cells) were tested for prophylactic efficacy in the mouse lethal challenge model with mouse adapted influenza A/Chicken/Netherlands/621557/2003 (H7N7) virus (Central Veterinary Institute (CVI), Lelystad, The Netherlands. Briefly, all mice (Balb/c, female, age 6-8 weeks, n=8 per group) were acclimatised and maintained for a period of at least 4 days prior to the start of the experiment. MAb CR8020 was dosed at 10, 3 or 1 mg/kg intravenously in the tail vein (vena coccygeus) at day -1 before challenge, assuming an average weight of 18 g per mouse and a fixed dose volume of 0.2 mL. Mabs CR8041 and CR8043 were dosed in the same manner at 30, 10, 3 or 1 mg/kg. A control group was taken along dosed with 30 mg/kg negative control mAb CR3014. After mAb administration, the mice were challenged at day 0 with 25 LD_{50} mouse adapted A/CH/NL/621557/03 (H7N7) virus by intranasal inoculation. Clinical signs and body weights were determined daily from day-1 before challenge until the end of the study at day 21.

In Fig. 15 the survival rates, the % bodyweight change and the clinical scores of the mice are depicted, following prophylactic administration of the mAbs. As shown in Fig.15A, 100% survival was observed in the groups receiving 3 or 10 mg/kg CR8020, 10 or 30 mg/kg CR8041 and in the group receiving 30 mg/kg CR8043. In the control mAb group the survival rate was 0%. Prophylactic administration of CR8020 at all three dose levels, and CR8041 at all four dose levels provided a

statistically significant improvement of survival time, compared to the control mAb group (log-rank, $p<0.002$). Prophylactic administration of 1 mg/kg of CR8043 did not result in a statistically significant improvement of survival time, compared to the control mAb group (log-rank, $p=0.692$). Increasing the CR8043 dose to 3 mg/kg or more, resulted in a statistically significant improvement of survival time, compared to the control mAb group (log-rank, $p\leq0.034$).

In a post hoc analysis, the survival times were compared of the lowest dose groups of mAbs CR8020, CR8041 and CR8043. Prophylactic administration of 1 mg/kg CR8020 resulted in a statistically significant improvement of survival time, compared to 1 mg/kg of CR8041 and 1 mg/kg of CR8043 (log-rank, respectively $p=0.029$ and $p<0.001$). In addition, prophylactic administration of 1 mg/kg CR8041 resulted in a statistically significant improvement of survival time when compared to 1 mg/kg CR8043 (log-rank, $p=0.004$).

In Fig. 15B the mean body weight change of the mice during the 21 day study period following prophylactic administration of the mAbs is shown. In the mAb CR8020 and mAb CR8041 1 mg/kg groups severe weight loss was observed comparable to that of the control mAb group. In the higher dose groups of mAb CR8020 and CR8041, weight loss during the 21 day study was limited or absent. In the groups dosed with mAb CR8043 severe weight loss was observed in all groups, with the mean body weight of the group dosed at 30 mg/kg recovering almost to base line level at day 21. Body weight changes were analyzed in more detail with Area under the Curve (AUC) analysis (Table 21). There is a clear inverse relationship between the weight loss and dose of antibody used. When the concentration of antibody was increased, the weight loss decreased. With 1 mg/kg of CR8020 there was no statistically significant reduction in weight loss compared to the control group ($p=0.356$). Increasing the dosing to 3 or 10 mg/kg resulted in a statistically significant reduction in weight loss, compared to the control group ($p<0.001$ in both cases). With 1 mg/kg of CR8041 there was no statistically significant reduction in weight loss compared to the control group ($p=1$).

Increasing the dosing to 3, 10 or 30 mg/kg CR8041 resulted in a statistically significant reduction in weight loss, compared to the control group ($p<0.001$ in all 3 cases). With 1, 3 or 10 mg/kg of CR8043 there was no statistically significant reduction in weight loss compared to the control group ($p=0.997$, 0.510 and 0.992 respectively). Increasing the dosing to 30 mg/kg resulted in a statistically significant

reduction in weight loss, compared to the control group ($p<0.001$). In an additonal analysis of the mean AUC of body weight change data, mAbs CR8020, CR8041 and CR8043 were compared using an univariate analysis of variance with antibody and doses included in the model as fixed factors. Since a dose of 30 mg/kg of CR8020 was not included in the study, the comparison was limited to antibody doses of 1, 3 and 10 mg/kg. Differences between antibodies were estimated by using marginal means with Sidak adjustment for multiple comparisons. Over the three doses of antibody considered, treatment with CR8020 resulted in a statistically significant improved reduction of weight loss compared to CR8041 and CR8043 (mean difference in marginal means of 23.73 and 68.29 g*day, respectively $p=0.013$ and $p<0.001$). In addition, treatment with CR8041 resulted in a statistically significant improved reduction of weight loss when compared to CR8043 (difference in marginal means of 44.56 g*day, $p<0.001$).

Median clinical scores of the mice are depicted in Fig15C. All mice, except one at day 0 (3 mg/kg CR8020 group) showed clinical signs (score = 1, rough coat)) from day 0 - day 3. This increase was not observed in the acclimatization period and at day -1. The cause of this increased clinical score is not precisely clear. Of the groups treated with 3 or 10 mg/kg mAb CR8020 at day -1 pre-challenge, the median clinical score returned to 0 at day 9 post challenge, whereas in the control group the median clinical score reached 4 at day 8, with all mice died or euthanized at day 9. The CR8020 1 mg/kg group showed a median clinical score of 3 from day 4-13, returning to score 0 at day 15. Of the groups treated with 3, 10 or 30 mg/kg mAb CR8041 at day -1 pre-challenge, the median clinical score returned to 0 at day 9, 10 or 12 post challenge, respectively. The CR8041 1 mg/kg group reached a median clinical score of 4 at day 10 after challenge. Of the groups treated with 1, 3 or 10 mg/kg CR8043 the median clinical score reached 4 at day 9, 9 or 12, respectively, whereas the median clinical score of the 30 mg/kg CR8043 group reached 3 from day 6—13 and returned to 0 at day 14.

The above results clearly show that human anti-H3N2 antibodies CR8020, CR8041 and CR8043 are able to provide heterosubtypic protection against a lethal dose of influenza H7N7 in vivo. Mab CR8020 was found to be the most potent of the three mAbs against the mouse adapted influenza A/CH/NL/621557/03 (H7N7) virus, based on the outcome of the post hoc analyses of survival times and body weigth change. At a dose of 3 or 10 mg/kg mAb CR8020 administered one day prior to

infection 100 % of the mice survived the lethal challenge and clinical manifestation of the H7N7 infection was strongly reduced. At a dose of 1 mg/kg CR8020 administered one day prior to infection, 75% of the mice survived the lethal challenge in this experiment, and the clinical signs of the surviving mice resolved completely at day 15 of the 21 day study period.

Example 17

Therapeutic activity of human IgG monoclonal antibodies against lethal H7N7 challenge in vivo

This study was performed to assess the therapeutic efficacy and window of mAb CR8020 in the H7N7 model. MAb CR8020 (produced in PER.C6® cells) was tested for therapeutic efficacy in the mouse lethal challenge model with mouse adapted influenza A/Chicken/Netherlands/621557/2003 (H7N7) virus (Central Veterinary Institute (CVI), Lelystad, The Netherlands). Briefly, all mice (Balb/c, female, age 6-8 weeks, n=8 per group) were acclimatised and maintained for a period of at least 4 days prior to the start of the experiment. MAb CR8020 was dosed at 15 mg/kg intravenously in the tail vein (vena coccygeus) at day -1 before challenge, (group 1; prophylaxis positive control) or at day 1, 2, 3, 4, 5 or 6 after challenge (groups 2-7), assuming an average weight of 18 g per mouse and a fixed dose volume of 0.2 mL. Group 8 received negative control mAb CR3014 (15 mg/kg) at day 1 after challenge. The mice were challenged at day 0 with 25 LD_{50} mouse adapted A/CH/NL/621557/03 (H7N7) virus by intranasal inoculation. Clinical signs and body weights were determined daily from day-1 before challenge until the end of the study at day 21.

Figure 16A shows the survival rates of the mice, following intravenous administration of mAb CR8020 (15 mg/kg in all groups) or control mAb (15 mg/kg). When 15 mg/kg mAb CR8020 was administered at day 1 pre-challenge or days 1 or 3 post challenge, all animals survived the viral challenge, whereas in the control mAb group the survival rate was 0%. When 15 mg/kg mAb CR8020 was administered at days 2 and 4, respectively 87.5% and 50% survival was observed. The survival time of these groups was statistically significantly different from that of the control mAb group (p=0.002 and p=0.014, respectively). Groups treated with 15 mg/kg CR8020 at day 5 and 6 experienced a survival rate of 0% and there was no statistically significant

difference in survival time of these groups compared to the control mAb group (p=0.837 and p=0.876, respectively).

In Figure 16B the mean body weight change relative to day 0 of the mice during the 21 day study period is shown. In general, mean body weight loss increases when mAb CR8020 is administered at later timepoints following challenge. However, the mean body weight curves of the mAb CR8020 day 2 and 3 treatment groups cross at day 10, due to the single non surviving mouse in the day 2 treatment group. Area under the curve analysis of the bodyweight changes shows a sharp transition in the mean weight loss between the treatments at days -1 to day 3 compared to treatment at days 4 to 6 (Table 22). Treatment with 15 mg/kg of CR8020 at day -1 pre-challenge or day 1, 2 or 3 post challenge resulted in a statistically significant reduction in weight loss compared to the control group ($p<0.001$ for all 4 groups). Treatment with 15 mg/kg of CR8020 at days 4, 5 or day 6 did not result in a statistically significant reduction in weight loss compared to the control group (p=0.566, p=0.979 and p=0.858, respectively).

Median clinical scores of the mice are depicted in Figure 16C. Of the animals treated with 15 mg/kg CR8020 at day -1 pre-challenge, all survived and none of the animals showed any clinical signs during the observation period. Animals treated at day 1 post challenge showed a 100% survival, however, 7 out of 8 animals showed clinical signs reaching a maximum clinical score of 1. The 8th animal reached a maximum clinical score of 3. Of the animals treated at day 2 post challenge all but one animal survived. The surviving animals (7 out of 8) showed clinical signs reaching a maximum clinical score of 1 (n=4) or 3 (n=3). Animals treated at day 3 post challenge showed a 100% survival and all animals showed clinical signs with a maximum clinical score of 3. Of the animals treated at day 4 post challenge, 50% survived the lethal challenge. The surviving animals showed clinical signs reaching a maximum clinical score of 3. Animals treated at day 5 or 6 post challenge did not survive. Clinical scores were analyzed using the GENMOD procedure (SAS) to fit a model to repeated measures with mice as subject and data measured on an ordinal scale (Table 23). From the groups treated with 15 mg/kg mAb CR8020 at day -1 before challenge and day 1, 2, 3 or 4 post challenge, the median clinical score was statistically significantly different from the control mAb group during most of the study period of 21 days (day 8-21; $p \leq 0.038$ for all 4 groups). From the group treated with 15 mg/kg mAb CR8020 at day 5 post challenge the median clinical score was

significantly different from the control mAb group at day 8 only ($p \leq 0.001$). This difference, although statistically significant, is not considered relevant. The median clinical score of the 15 mg/kg mAb CR8020 day 6 treatment group was not statistically different from the control group.

This study clearly shows that therapy with 15 mg/kg of mAb CR8020 provides 87.5-100% protection when administered up to day 3 after challenge in a lethal H7N7 mouse model. When administered at day 4 after challenge, treatment with 15 mg/kg mAb CR8020 provides partial protection. When administered at day 5 or day 6 after challenge no protective effect of 15 mg/kg mAb CR8020 was observed in the lethal H7N7 mouse model. In other words, when administered 4 days or more before death, CR8020 provided protection in this lethal mouse model.

Example 18

Cocktail of monoclonal antibodies that efficiently neutralizes multiple influenza subtypes from phylogenetic groups 1 and 2.

The seasonal influenza vaccine each year consists of two different preparations inducing immunity to influenza A strains, a representative for the circulating H1 subtype and a representative for the circulating H3 strain. The underlying reason for this is that influenza strains from the H1 and H3 subtype are so much different that the vaccines prepared from either type does not induce protection against the other subtype. Ideally, a broadly protective monoclonal antibody preparation to treat influenza would be effective against influenza strains from both the phylogenetic group 1 (H1) and group 2 (H3). However, again due to the sequence differences between the HA molecules such single antibody is hard to find. For example, the Fab28 antibody described in WO 2009/115972 binds and neutralizes H1 subtypes much better than H3 subtype viruses probably due to the less conservation of the epitope between the group 1 and group 2 viruses compared to viruses within a phylogenetic group. To reach the goal of a single product effective against multiple influenza subtypes from both phylogenetic groups one may thus have to combine two or more different antibodies in a cocktail. In order to be successful such preparation should consist of antibodies that do not interfere with each other.

Antibodies that efficiently neutralize viruses from H1, H5 and H9 subtypes have been described in WO2008/028946, with antibodies CR6261 and CR6323 as typical examples. The binding region (epitope) of CR6261 has been elucidated in

detail using co-crystallization of H1 or H5 HA molecules and CR6261 (see also PDB database entries 3GBM and 3GBM at http://www.pdb.org and Ekiert et al., 2009). To investigate whether the monoclonal antibodies of the present invention can be used in combination with the previously described CR6261 antibodies it was tested whether the antibodies were able to bind to subtypes from both phylogenetic groups 1 and 2. Hereto, Elisa and FACS binding experiments were done as described in Example 7 using HA molecules of H1 and H5 subtypes as well as H3 and H7 subtypes with CR6261, CR6323, CR8001, CR8020, CR8041 and CR8043. The results are summarized in table 20 and show that the antibodies that broadly neutralize viruses of group 1 do not bind to viruses of group 2 and vice versa. Since the antibodies do not interfere with each other it can be expected that the neutralizing potency of the antibodies against the respective subtypes will be maintained resulting in efficient neutralization of both group 1 and 2 subtypes.

Therefore a cocktail comprising CR6261 and/or CR6323 on the one hand and CR8020, CR8041, and/or CR8043 on the other hand will be active against viruses of at least both subtypes H1 and H3. Thus, efficient protection is possible to influenza subtypes from phylogenetic groups 1 and 2 using one preparation.

Example 19.

Binding kinetics of the binding molecules.

The affinities of papain cleaved Fab fragments of CR8020 and CR8043 were measured using the Octet RED system and streptavidin biosensors from ForteBio. Influenza hemagglutinin antigens of the H3 subtypes A/Wisconsin/67/2005 (Protein Science) and A/Brisbane/10/2007 (Protein Science) were biotinylated for immobilization to streptavidin biosensors (ForteBio). Fab binding experiments were repeated 5 times using a concentration range between 2.3-150 nM and 0.16-30 nM for CR8020 and CR8043, respectively, in kinetic buffer (ForteBio, 18.5032). The experimental set-up for affinity measurements on the Octet was as follows: Immobilization of the biotinylated hemagglutinin to streptavidin biosensors for 1800 second followed by association of the serial diluted Fabs CR8020 and CR8043 for 1200 second and subsequent dissociation in kinetic buffer for 1800 seconds. Binding data were analyzed with Octet Analysis software using the 1:1 model.

The affinity constant (K_d-value) of the binding molecules for HA of the H3 subtype are shown in Table 24.

Table 1: First round Vkappa, Vlambda and VH amplifications

Primer name	Primer nucleotide sequence	SEQ ID NO:
OK1 (HuVK1B)	GAC ATC CAG WTG ACC CAG TCT CC	192
OK2 (HuVK2)	GAT GTT GTG ATG ACT CAG TCT CC	193
OK3 (HuVK2B2)	GAT ATT GTG ATG ACC CAG ACT CC	194
OK4 (HuVK3B)	GAA ATT GTG WTG ACR CAG TCT CC	195
OK5 (HuVK5)	GAA ACG ACA CTC ACG CAG TCT CC	196
OK6 (HuVK6)	GAA ATT GTG CTG ACT CAG TCT CC	197
OCK (HuCK)	ACA CTC TCC CCT GTT GAA GCT CTT	198
OL1 (HuVL1A)*	CAG TCT GTG CTG ACT CAG CCA CC	199
OL1 (HuVL1B)*	CAG TCT GTG YTG ACG CAG CCG CC	200
OL1 (HuVL1C)*	CAG TCT GTC GTG ACG CAG CCG CC	201
OL2 (HuVL2B)	CAG TCT GCC CTG ACT CAG CC	202
OL3 (HuVL3A)	TCC TAT GWG CTG ACT CAG CCA CC	203
OL4 (HuVL3B)	TCT TCT GAG CTG ACT CAG GAC CC	204
OL5 (HuVL4B)	CAG CYT GTG CTG ACT CAA TC	205
OL6 (HuVL5)	CAG GCT GTG CTG ACT CAG CCG TC	206
OL7 (HuVL6)	AAT TTT ATG CTG ACT CAG CCC CA	207
OL8 (HuVL7/8)	CAG RCT GTG GTG ACY CAG GAG CC	208
OL9 (HuVL9)#	CWG CCT GTG CTG ACT CAG CCM CC	209
OL9 (HuVL10)#	CAG GCA GGG CTG ACT CAG	210
OCL (HuCL2)X	TGA ACA TTC TGT AGG GGC CAC TG	211
OCL (HuCL7)X	AGA GCA TTC TGC AGG GGC CAC TG	212
OH1(HuVH1B7A)+	CAG RTG CAG CTG GTG CAR TCT GG	213
OH1 (HuVH1C)+	SAG GTC CAG CTG GTR CAG TCT GG	214
OH2 (HuVH2B)	CAG RTC ACC TTG AAG GAG TCT GG	215
OH3 (HuVH3A)	GAG GTG CAG CTG GTG GAG	216
OH4 (HuVH3C)	GAG GTG CAG CTG GTG GAG WCY GG	217
OH5 (HuVH4B)	CAG GTG CAG CTA CAG CAG TGG GG	218
OH6 (HuVH4C)	CAG STG CAG CTG CAG GAG TCS GG	219
OH7 (HuVH6A)	CAG GTA CAG CTG CAG CAG TCA GG	220
OCM (HuCIgM)	TGG AAG AGG CAC GTT CTT TTC TTT	221

* Mix in 1:1:1 ratio
\# Mix in 1:1 ratio
X Mix in 1:1 ratio
\+ Mix in 1:1 ratio

Table 2: Second round Vkappa, Vlambda and VH amplifications

Primer name	Primer nucleotide sequence	SEQ ID NO
OK1S (HuVK1B-SAL)	TGA GCA CAC AGG TCG ACG GAC ATC CAG WTG ACC CAG TCT CC	222
OK2S (HuVK2-SAL)	TGA GCA CAC AGG TCG ACG GAT GTT GTG ATG ACT CAG TCT CC	223
OK3S (HuVK2B2-SAL)	TGA GCA CAC AGG TCG ACG GAT ATT GTG ATG ACC CAG ACT CC	224
OK4S (HuVK3B-SAL)	TGA GCA CAC AGG TCG ACG GAA ATT GTG WTG ACR CAG TCT CC	225
OK5S (HuVK5-SAL)	TGA GCA CAC AGG TCG ACG GAA ACG ACA CTC ACG CAG TCT CC	226
OK6S (HuVK6-SAL)	TGA GCA CAC AGG TCG ACG GAA ATT GTG CTG ACT CAG TCT CC	227
OJK1 (HuJK1-NOT)	GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT TTC CAC CTT GGT CCC	228
OJK2 (HuJK2-NOT)	GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAG CTT GGT CCC	229
OJK3 (HuJK3-NOT)	GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT ATC CAC TTT GGT CCC	230
OJK4 (HuJK4-NOT)	GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC	231
OJK5 (HuJK5-NOT)	GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT AAT CTC CAG TCG TGT CCC	232
OL1S (HuVL1A-SAL)*	TGA GCA CAC AGG TCG ACG CAG TCT GTG CTG ACT CAG CCA CC	233
OL1S (HuVL1B-SAL)*	TGA GCA CAC AGG TCG ACG CAG TCT GTG YTG ACG CAG CCG CC	234
OL1S (HuVL1C-SAL)*	TGA GCA CAC AGG TCG ACG CAG TCT GTC GTG ACG CAG CCG CC	235
OL2S (HuVL2B-SAL)	TGA GCA CAC AGG TCG ACG CAG TCT GCC CTG ACT CAG CC	236
OL3S (HuVL3A-SAL)	TGA GCA CAC AGG TCG ACG TCC TAT GWG CTG ACT CAG CCA CC	237
OL4S (HuVL3B-SAL)	TGA GCA CAC AGG TCG ACG TCT TCT GAG CTG ACT CAG GAC CC	238
OL5S (HuVL4B-SAL)	TGA GCA CAC AGG TCG ACG CAG CYT GTG CTG ACT CAA TC	239
OL6S (HuVL5-SAL)	TGA GCA CAC AGG TCG ACG CAG GCT GTG CTG ACT CAG CCG TC	240
OL7S (HuVL6-SAL)	TGA GCA CAC AGG TCG ACG AAT TTT ATG CTG ACT CAG CCC CA	241
OL8S (HuVL7/8-SAL)	TGA GCA CAC AGG TCG ACG CAG RCT GTG GTG ACY CAG GAG CC	242
OL9S (HuVL9-SAL)#	TGA GCA CAC AGG TCG ACG CWG CCT GTG CTG ACT CAG CCM CC	243
OL9S (HuVL10-SAL)#	TGA GCA CAC AGG TCG ACG CAG GCA GGG CTG ACT CAG	244
OJL1 (HuJL1-NOT)	GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT GAC CTT GGT CCC	245
OJL2 (HuJL2/3-NOT)	GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC	246
OJL3 (HuJL7-NOT)	GAG TCA TTC TCG ACT TGC GGC CGC ACC GAG GAC GGT CAG CTG GGT GCC	247
OH1S (HuVH1B-SFI)+	GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG RTG CAG CTG GTG CAR TCT GG	248
OH1S (HuVH1C-SFI)+	GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC SAG GTC CAG CTG GTR CAG TCT GG	249

OH2S (HuVH2B-SFI)	GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG RTC ACC TTG AAG GAG TCT GG	250
OH3S (HuVH3A-SFI)	GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG	251
OH4S (HuVH3C-SFI)	GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG WCY GG	252
OH5S (HuVH4B-SFI)	GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTA CAG CAG TGG GG	253
OH6S (HuVH4C-SFI)	GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG STG CAG CTG CAG GAG TCS GG	254
OH7S (HuVH6A-SFI)	GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG	255
OJH1 (HuJH1/2-XHO)	GAG TCA TTC TCG ACT CGA GAC RGT GAC CAG GGT GCC	256
OJH2 (HuJH3-XHO)	GAG TCA TTC TCG ACT CGA GAC GGT GAC CAT TGT CCC	257
OJH3 (HuJH4/5-XHO)	GAG TCA TTC TCG ACT CGA GAC GGT GAC CAG GGT TCC	258
OJH4 (HuJH6-XHO)	GAG TCA TTC TCG ACT CGA GAC GGT GAC CGT GGT CCC	259

* Mix in 1:1:1 ratio
# Mix in 1:1 ratio
+ Mix in 1:1 ratio

Table 3. Second round VL regions amplification overview

Template	5′ primer	3′ primer	Product	Share in PK/PL(%)	Pool	Share in VL (%)
K1	OK1S	OJK1	K1J1	25	PK1	30
	OK1S	OJK2	K1J2	25		
	OK1S	OJK3	K1J3	10		
	OK1S	OJK4	K1J4	25		
	OK1S	OJK5	K1J5	15		
K2	OK2S	OJK1	K2J1	25	PK2	4
	OK2S	OJK2	K2J2	25		
	OK2S	OJK3	K2J3	10		
	OK2S	OJK4	K2J4	25		
	OK2S	OJK5	K2J5	15		
K3	OK3S	OJK1	K3J1	25	PK3	1
	OK3S	OJK2	K3J2	25		
	OK3S	OJK3	K3J3	10		
	OK3S	OJK4	K3J4	25		
	OK3S	OJK5	K3J5	15		
K4	OK4S	OJK1	K4J1	25	PK4	19
	OK4S	OJK2	K4J2	25		
	OK4S	OJK3	K4J3	10		
	OK4S	OJK4	K4J4	25		
	OK4S	OJK5	K4J5	15		
K5	OK5S	OJK1	K5J1	25	PK5	1
	OK5S	OJK2	K5J2	25		
	OK5S	OJK3	K5J3	10		
	OK5S	OJK4	K5J4	25		
	OK5S	OJK5	K5J5	15		
K6	OK6S	OJK1	K6J1	25	PK6	5
	OK6S	OJK2	K6J2	25		
	OK6S	OJK3	K6J3	10		
	OK6S	OJK4	K6J4	25		
	OK6S	OJK5	K6J5	15		
L1	OL1S	OJL1	L1J1	30	PL1	14
	OL1S	OJL2	L1J2	60		
	OL1S	OJL3	L1J3	10		
L2	OL2S	OJL1	L2J1	30	PL2	10
	OL2S	OJL2	L2J2	60		
	OL2S	OJL3	L2J3	10		
L3	OL3S	OJL1	L3J1	30	PL3	10
	OL3S	OJL2	L3J2	60		
	OL3S	OJL3	L3J3	10		
L4	OL4S	OJL1	L4J1	30	PL4	1
	OL4S	OJL2	L4J2	60		
	OL4S	OJL3	L4J3	10		
L5	OL5S	OJL1	L5J1	30	PL5	1
	OL5S	OJL2	L5J2	60		
	OL5S	OJL3	L5J3	10		
L6	OL6S	OJL1	L6J1	30	PL6	1
	OL6S	OJL2	L6J2	60		
	OL6S	OJL3	L6J3	10		

L7	OL7S	OJL1	L7J1	30	PL7	1
	OL7S	OJL2	L7J2	60		
	OL7S	OJL3	L7J3	10		
L8	OL8S	OJL1	L8J1	30	PL8	1
	OL8S	OJL2	L8J2	60		
	OL8S	OJL3	L8J3	10		
L9	OL9S	OJL1	L9J1	30	PL9	1
	OL9S	OJL2	L9J2	60		
	OL9S	OJL3	L9J3	10		
					VL	100%

Table 4. Second round VH regions amplification overview

Template	5′ primer	3′ primer	Product	Share in PK/PL (%)	Pool	Share in VH (%)
H1	OH1S	OJH1	H1J1	10	PH1	25
	OH1S	OJH2	H1J2	10		
	OH1S	OJH3	H1J3	60		
	OH1S	OJH4	H1J4	20		
H2	OH2S	OJH1	H2J1	10	PH2	2
	OH2S	OJH2	H2J2	10		
	OH2S	OJH3	H2J3	60		
	OH2S	OJH4	H2J4	20		
H3	OH3S	OJH1	H3J1	10	PH3	25
	OH3S	OJH2	H3J2	10		
	OH3S	OJH3	H3J3	60		
	OH3S	OJH4	H3J4	20		
H4	OH4S	OJH1	H4J1	10	PH4	25
	OH4S	OJH2	H4J2	10		
	OH4S	OJH3	H4J3	60		
	OH4S	OJH4	H4J4	20		
H5	OH5S	OJH1	H5J1	10	PH5	2
	OH5S	OJH2	H5J2	10		
	OH5S	OJH3	H5J3	60		
	OH5S	OJH4	H5J4	20		
H6	OH6S	OJH1	H6J1	10	PH6	20
	OH6S	OJH2	H6J2	10		
	OH6S	OJH3	H6J3	60		
	OH6S	OJH4	H6J4	20		
H7	OH7S	OJH1	H7J1	10	PH7	1
	OH7S	OJH2	H7J2	10		
	OH7S	OJH3	H7J3	60		
	OH7S	OJH4	H7J4	20		
					VH	100%

Table 5: Characteristics of the individual IgM memory B cell libraries.

IgM memory libraries						
Donor	Cells		Libraries			
	Total PBL ($x10^6$)	% memory B cells	Size ($x10^6$)	% Insert frequency	% ORF	% Unique
Individual 1			3	96	74	98
Individual 2	72.5	1.7	5	98	79	98
Individual 3	67.5	1.4	3	96	79	98
Individual 4	132.5	2.3	6	98	69	99

Table 6: Cross-binding activity of single-chain phage antibodies to HA molecules of different HA subtypes as measured by ELISA (ELISA titer; OD 492 nm). X= not determined; H3= HA of H3 subtype; H7= HA of H7 subtype; HB= HA of influenza virus B.

SC #	H3	H7	HB
sc08-001	0.885	2.451	x
sc08-003	1.320	0.222	x
sc08-006	0.511	0.227	x
sc08-007	0.074	2.365	x
sc08-009	0.095	1.130	x
sc08-010	0.165	1.242	x
sc08-011	0.090	1.802	x
sc08-013	0.078	1.400	x
sc08-014	0.239	0.834	x
sc08-015	0.727	0.165	x
sc08-016	1.112	0.164	x
sc08-017	1.158	0.285	x
sc08-018	0.711	0.221	x

Table 7: Cross-binding activity of PEG/NACl-precipitated and filter-sterilized phage antibodies to HA molecules of different HA subtypes as measured by ELISA (OD 492 nm).H1= HA of H1 subtype, H3= HA of H3 subtype; H5=HA of H5 subtype; H7= HA of H7 subtype; B(O)= HA of influenza virus B/Ohio/01/2005.

SC #	H1	H3	H5	H7	B(O)
sc08-001	+	+	-	+	-
sc08-003	-	+	-	-	-
sc08-006	-	+	-	-	-
sc08-007	-	-	-	+	-
sc08-009	-	-	-	+	-
sc08-010	-	-	-	+	-
sc08-011	-	-	-	+	-
sc08-013	-	-	-	+	-
sc08-014	+	+	-	+	-
sc08-015	-	+	-	-	-
sc08-016	-	+	-	-	-
sc08-017	-	+	-	-	-
sc08-018	-	+	-	-	-

Table 8: FACS analysis of PEG/NACl-precipitated and filter-sterilized phage antibodies (expressed as MFI=mean fluorescence intensity). PER.C6=untransfected PER.C6 cells (control); mH1, mH3, mH5, mH7, mHB= membrane bound HA of the subtypes H1, H3, H5, H7 and influenza B subtypes respectively.

SC #	PER.C6	mH1	mH3	mH5	mH7	mHB
sc08-001	2	27	68	5	62	x
sc08-003	5	9	77	7	7	x
sc08-006	2	6	69	5	6	x
sc08-007	1	5	4	4	73	x
sc08-009	11	12	11	10	15	x
sc08-010	2	4	3	4	60	x
sc08-011	1	3	4	4	73	x
sc08-013	2	5	3	7	61	x
sc08-014	10	26	82	17	32	x
sc08-015	3	7	79	7	6	x
sc08-016	1	7	82	5	5	x
sc08-017	1	6	81	5	5	x
sc08-018	2	6	74	6	7	x

Table 9: Data of the CDR regions of the *HA* specific immunoglobulins (SEQ ID NO).

IgG#	Vh locus	HCDR1	HCDR2	HCDR3	Vl locus	LCDR1	LCDR2	LCDR3
CR8001	3-53	SNYVS (81)	LIYTGGTTYYADSVKG (82)	VSALRFLQWPNYAMDV (83)	1-4	SGTRSDVGGHNYVS (84)	EVSHRPS (85)	SSYTGEGPLGV (86)
CR8003	3-7	SYWMS (87)	NMKQDGSEKYYVDSVKG (88)	GSCDDSWTGCHDAFDI (89)	2-14	GGNNIGSKSVH (90)	DSARPS (91)	QVWESGSDLRLL (92)
CR8015	3-7	SYWMS (87)	NMKQDGSEKYYVDSVKG (88)	GSCDDSWTGCHDAFDI (89)	2-14	GGDNIGRKSVH (93)	DNSDRPS (94)	HVWGSSRDHYV (95)
CR8016	3-7	SYWMS (87)	NMKQDGSEKYYVDSVKG (88)	GSCDDSWTGCHDAFDI (89)	1-13	TGSSSNIGAGYDVH (96)	GNN (97)RPS	QSYDSSLSVYV (98)
CR8017	3-7	SYWMS (87)	NMKQDGSEKYYVDSVKG (88)	GSCDDSWTGCHDAFDI (89)	2-13	QGDSLRSYYAS (99)	AKTNRPS (100)	NSRDSSGNHVV (101)
CR8018	3-7	SYWMS (87)	NMKQDGSEKYYVDSVKG (88)	GSCDDSWTGCHDAFDI (89)	1-4	TGTSSDVGGYNYVS (102)	EVSHRPS (85)	SSYTGEGPLGV (86)
CR8019	3-23	TSAMS (103)	GISGSGATTYYAGSVKG (104)	DTSLFEYDTSGFTAPGNAFDI (105)	O12	RASQSISGYLN (106)	GASTLQS (107)	QQTYTSPPYA (108)
CR8020	1-18	RFGVS (109)	WISAYNGDTYYAQKFQA (110)	EPPLFYSSWSLDN (111)	A27	ARASQSVSMNYLA (112)	GASRRAT (113)	QQYGTSPRT (114)
CR8021	3-23	AYAMN (115)	AIGGSGGSTYYADSVKG (116)	GRDWTGGYFFDS (117)	B3	KSSQSIFYSSNNKNYLT (118)	WASTRES (119)	QQYYSIPYT (120)
CR8038	3-23	GYAMS (121)	DIGGSGGGTYYADSVKG (122)	SSSWDRAYFFDS (123)	B3	KSSQSVLYSSIHKNYLA (124)	WASTRES (119)	QQYYRSPPT (125)
CR8039	4-59	SYYWS (126)	YIYYRGGTSYNPSLKS (127)	KDWGSAAGSVWYFDL (128)	1-2	TGTSSDVGGYNYVS (129)	EVSKRPS (130)	SSYAGSNNLI (131)
CR8040	3-33	SYGMH (132)	FIWYDGSNKHYADSMKG (133)	DGGYSTWEWYFDL (134)	A26	RASQGIGSNLH (135)	YASQSIT (136)	HQSSSLPLT (137)
CR8041	1-18	SFGLS (138)	WISAYNGEIKYAQKFQG (139)	EPPLYFSSWSLDF (140)	A27	ARASQSVSSNYLA (141)	GASRRAT (142)	QQYDSSPRT (143)
CR8043	1-03	AYSMH (144)	WINTAIGNTQYSQKFQD (145)	GASWDARGWSGY (146)	B3	KSSQSVFSSSTNKNYLA (147)	WSSTRES (148)	HQYYTAPWT (149)
CR8049	2-26	NTRMGVS (150)	HIFSNDETSYRTSLKR (151)	IGSGYESSAYSTWLDP (152)	2-14	EGDTIGSKSVH (153)	NDRDRPS (154)	QVWESGGDQTV (155)
CR8050	4-34	DHYWS (156)	EVVHSGDTNYTPSLRN (157)	GRNVAVVGAIQRHYDY (158)	A27	RASQSVSRNYLA (159)	GASSRAT (160)	QHYGSVLVA (161)
CR8052	4-61	SGTYYWS (162)	DISYSGSTNYNPSLKS (163)	AMAAYNYDRGGYNDYYYMDV (164)	O12	RASQGINTYLN (165)	AASTLQS (166)	QQSYSTAIT (167)
CR8055	3-33	TYGMH (168)	FIWYDGSNKHYQDSVKG (169)	DGGYSTWEWYFDL (170)	A26	RASRSIGSDLH (171)	FASQSMS (172)	HQSSSLPLT (137)
CR8057	3-53	VIFMS (173)	IIYIDDSTYYADSVKG (174)	ESGDFGDQTGPYHYYAMDV (175)	2-14	TGSSGDIGGYNAVS (176)	EVTSRPS (177)	CSFADSNILI (178)
CR8069	3-43	DYTMH (179)	LISWDGGMSNYADSVKG (180)	DIRPRMPARHFMDV (181)	L2	RASQNVNYNLA (182)	VASTRAT (183)	QQYNNWPPAIT (184)

Table 10. Data of the HA-specific IgGs. SEQ ID NO's of the nucleotide and amino acid sequences of the variable regions of the heavy and light chains

Name IgG	SEQ ID NO of nucleotide sequence heavy chain variable region	SEQ ID NO of amino acid sequence heavy chain variable region	SEQ ID NO of nucleotide sequence light chain variable region	SEQ ID NO of amino acid sequence light chain variable region
CR8001	1	2	3	4
CR8003	5	6	7	8
CR8015	9	10	11	12
CR8016	13	14	15	16
CR8017	17	18	19	20
CR8018	21	22	23	24
CR8019	25	26	27	28
CR8020	29	30	31	32
CR8021	33	34	35	36
CR8038	37	38	39	40
CR8039	41	42	43	44
CR8040	45	46	47	48
CR8041	49	50	51	52
CR8043	53	54	55	56
CR8049	57	58	59	60
CR8050	61	62	63	64
CR8052	65	66	67	68
CR8055	69	70	71	72
CR8057	73	74	75	76
CR8069	77	78	79	80

Table 11: In vitro neutralization of influenza virus H3N2 by selected IgGs

Neutralization titer SK50 (µg/ml)	
IgG #	**A/Wisconsin/67/2005**
CR8001	11.95
CR8003	5.31
CR8015	23.78
CR8016	1.77
CR8017	2.82
CR8018	6.03
CR8019	1.98
CR8020	8.45
CR8021	1.77
CR8038	3.54
CR8039	1.8
CR8040	>40
CR8041	3.99
CR8043	1.49
CR8049	3.26
CR8050	1.77
CR8052	>40
CR8055	1.07
CR8057	0.011
CR8069	ND

Table 12: Cross-binding reactivity of anti-H3N2 IgGs. NCal.=A/New Caledonia/20/1999 (H1N1); Wisc.=A/Wisconsin/67/2005 (H3N2); NY.=A/New York/55/2004 (H3N2), Wyo.=A/Wyoming/3/2003 (H3N2); Neth.=A/Netherlands/219/2003 (H7N7); ND=not done.

	IgG Elisa binding (titration)					Facs binding, [IgG]=5µg/ml, MFI			
IgG #	H1 NCal.	H3 Wisc	H3 NY	H3 Wyo	H7 Neth	PerC6	H1	H3	H7
CR8001	+	+	+	+	+	4	100	763	106
CR8003	-	+	+	+	-	3	3	657	5
CR8015	-	+	+	+	-	3	4	600	4
CR8016	-	+	+	+	-	3	3	840	5
CR8017	-	+	+	+	-	3	3	558	4
CR8018	-	+	+	+	-	3	3	348	4
CR8019	-	+	-	+	-	3	4	685	6
CR8020	-	+	+	+	+	4	3	657	140
CR8021	-	+	+	+	+	4	4	678	4
CR8038	-	+	+	+	-	ND	ND	ND	ND
CR8039	-	+	+	+	-	4	4	503	4
CR8040	-	+	+	+	-	4	5	446	4
CR8041	-	+	+	+	+	4	4	364	120
CR8043	-	+	+	+	+	4	4	646	11
CR8049	-	+	+	+	-	3	3	542	4
CR8050	-	+	+	+	-	6	8	282	6
CR8052	-	+	+	+	-	4	4	364	5
CR8055	-	-	-	-	-	21	31	433	26
CR8057	-	+	+	+	low	7	8	943	15
CR8069	-	+	+	+	-	4	6	447	5

Table 13: Cross-neutralizing activity of anti-H3N2 IgGs; ND=not done

Neutralization titer SK50 (µg/ml)										
	H1			H3					H7	H10
IgG #	A/New Caledonia/20/1999	A/Brisbane/59/1007	A/Solomon islands/IVR-145	A/Wisconsin/67/2005	A/Hiroshima/52/2005	A/Panama/2000/1999	A/Johannesburg/33/1994	A/Hong Kong/1/1968	A/Mallard/Netherlands/12/2000	A/Chick/Germany/N/49
CR8001	>40	>40	>40	11.95	13.02	>40	6.51	7.07	>40	>40
CR8003	>40	>40	>40	5.31	4.27	>40	>40	ND	>40	>40
CR8015	>40	>40	>40	23.78	28.28	>40	>40	ND	>40	>40
CR8016	>40	>40	>40	1.77	8.84	28.28	>40	ND	>40	>40
CR8017	>40	>40	>40	2.82	13.55	>40	>40	ND	>40	>40
CR8018	>40	>40	>40	6.03	8.45	>40	>40	ND	>40	>40
CR8019	>40	>40	>40	1.98	0.88	>40	0.88	ND	>40	>40
CR8020	>40	>40	>40	8.45	11.95	7.74	7.07	1.77	0.028	17.68
CR8021	ND	>40	>40	1.77	2.5	>40	3.54	14.14	>40	>40
CR8038	ND	ND	ND	3.54	7.07	>40	5.95	ND	>40	>40
CR8039	>40	>40	>40	1.8	3.26	4.6	1.33	2.97	>40	>40
CR8040	>40	>40	>40	>40	>40	>40	6.77	ND	>40	>40
CR8041	>40	>40	>40	3.99	4.75	2.99	1.69	1.05	1.105	25
CR8043	>40	>40	>40	1.49	3.54	10.15	2.66	4.2	>40	14.87
CR8049	>40	>40	>40	3.26	3.54	>40	>40	ND	>40	>40
CR8050	ND	ND	ND	1.77	ND	6.5	1.49	ND	>40	>40
CR8052	>40	>40	>40	>40	>40	21.89	>40	ND	>40	>40
CR8055	>40	>40	>40	1.07	1.15	>40	3.38	ND	>40	>40
CR8057	>40	>40	>40	0.011	0.0068	0.022	2.17	2.17	>40	>40
CR8069	>40	>40	>40	ND	3.54	11.89	3.54	11.89	>40	>40

Table 14: Sequence conservation around the binding region of the H3 mAbs CR8020, CR8041 and CR8043

	HA2 position																									N	Years	Tested strains
	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39			
Consensus	W	E	G	M	V	D	G	W	Y	G	F	R	H	Q	N	S	E	G	T	G	Q	A	A	D	L	K			
Group_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	655	1972-2008	Pa
Group_2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	380	2004-2008	Hs-Wi
Group_3	-	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	127	1999-2004	
Group_4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	-	-	-	-	-	-	-	91	2007-2009	
Group_5	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	69	1968-1997	HK
Group_6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	10	2007	
Group_7	-	-	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-	-	6	1999-2004	
Group_8	-	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	4	2002-2007	
Group_9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	3	2004	
Group_10	-	-	-	-	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1999	
Group_11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	-	-	-	-	-	F	-	2	2009	
Group_12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	2	2003-2004	
Group_13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	F	-	1	2006	
Group_14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-	1	2007	
Group_15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	I	-	-	-	-	-	-	-	1	2007	
Group_16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	R	-	-	-	-	-	-	-	1	2008	
Group_17	-	-	-	-	-	-	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2003	
Group_18	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2001	
Group_19	-	-	-	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1999	
Group_20	-	-	-	-	I	-	-	-	-	-	-	-	-	R	-	-	-	-	-	-	-	-	-	-	-	-	1	1975	
Group_21	-	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-	-	-	R	-	-	-	-	-	-	-	1	2008	
Group_22	-	-	-	-	M	-	-	-	-	-	-	-	-	H	-	-	-	-	-	-	-	-	-	-	-	-	1	2002	
Group_23	-	K	-	-	-	-	-	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2002	

Table 15: Neutralization titers on various Influenza A strains

	H1	H3						H7		H10
IgG#	3 H1N1 strains (1999-2007)	A/Wisconsin/67/2005	A/Hiroshima/52/2005	A/Panama/2000/1999	A/Johannesburg/33/1994	A/Hong Kong/1/1968	A/HK/1/68-M20	A/HK/1/68-M20c	A/Mallard/Netherlands/12/2000	A/Netherlands/219/2003	A/Chicken/Germany/N/49
CR8001	>40	11.9	13.0	>40	6.5	7.1	5.26	ttd	>40	39.8	>40
CR8020	>40	3.5	3.5	5.0	2.0	1.8	1.8	1.8	2.5	27.6	6.6
CR8041	>40	3.3	3.5	5.0	1.6	1.7	1.8	1.7	38.0	258.0	37.2
CR8043	>40	1.6	1.8	4.2	1.2	0.8	1.8	1.2	>40	>40	6.6
CR8057	>40	0.005	0.003	0.01	>40	>40	>40	>40	>40	-	>250

All SK50 titers in ug/ml; Mouse-adapted strains; Ma 'pandemic' H7 strain

Table 16. Mean area under the curve of body weight change from baseline at day 0.

Study	Group	Mean AUC (g*day)	SD (g*day)	p-value[a] (mAb vs control)	p-value[b]
1	30 mg/kg control	-82.86	14.15		
	1 mg/kg CR8020	-63.21	30.91	0.09	
	3 mg/kg CR8020	16.95	8.20	<0.001	<0.001 (1 vs 3 mg/kg)
	10 mg/kg CR8020	31.44	9.09	<0.001	0.454 (3 vs 10 mg/kg)
	30 mg/kg CR8020	25.62	12.94	<0.001	ns
2	30 mg/kg control	-86.51	8.83		
	1 mg/kg CR8041	-68.26	11.41	0.004	
	3 mg/kg CR8041	19.51	13.82	<0.001	<0.001 (1 vs 3 mg/kg)
	10 mg/kg CR8041	35.23	11.06	<0.001	0.061 (3 vs 10 mg/kg)
	30 mg/kg CR8041	28.21	7.89	<0.001	ns
	1 mg/kg CR8043	-66.19	8.74	<0.001	
	3 mg/kg CR8043	8.48	11.81	<0.001	<0.001 (1 vs 3 mg/kg)
	10 mg/kg CR8043	31.57	7.90	<0.001	<0.001 (3 vs 10 mg/kg)
	30 mg/kg CR8043	27.72	6.61	<0.001	0.997 (10 vs 30 mg/kg)

[a]Mean AUC values of the mAb dose groups were compared to the control Ab groups using analysis of variance with Dunnet's adjustment for multiple comparisons.
[b]Mean AUC values per antibody concentration were compared for each antibody using analysis of variance with Tukey's adjustment for multiple comparisons.
ns= not statistically significant

Table 17. Mean area under the curve of body weight change from baseline at day 0.

Group	Mean AUC (g*day)	SD (g*day)	p-value[a]
15 mg/kg CR8020 at day -1	33.44	10.06	<0.001
15 mg/kg CR8020 at day 1	10.70	16.23	<0.001
15 mg/kg CR8020 at day 2	-15.23	11.60	<0.001
15 mg/kg CR8020 at day 3	-65.45	35.90	0.003
15 mg/kg CR8020 at day 4	-85.95	23.14	0.742
15 mg/kg CR8020 at day 5	-100.88	12.78	0.986
15 mg/kg CR8020 at day 6	-84.91	12.28	0.653
Control mAb at day 1	-95.76	11.55	

[a]Mean AUC values of the 15 mg/kg mAb CR8020 dosed groups were compared to the control mAb group using analysis of variance with Dunnet's adjustment for multiple comparisons in the post-hoc analysis.Prophylactic treatment with 15 mg/kg mAb CR8020 resulted in a statistically significant reduction in weight loss compared to the control group ($p<0.001$). Therapeutic treatment at day 1, day 2 or day 3 with 15 mg/kg mAb CR8020 also resulted in a statistically significant reduction in weight loss compared to the control group ($p<0.001$, $p<0.001$ and $p=0.003$, respectively). Treatment at days 4, day 5 or day 6 with 15 mg/kg mAb CR8020 did not result in a statistically significant reduction in weight loss compared to the control group ($p> 0.05$ for all three groups).

Table 18 Median clinical scores.
The interval with significant difference between clinical scores compared to the control mAb group are listed (e.g. between 15 mg/kg at day -1 and the control group the difference in clinical scores is significant from day 4 onwards).

	Relative to control	
Group	Interval (day(s)	p
15 mg/kg CR8020 at day -1	4-21	≤0.001
15 mg/kg CR8020 at day 1	2-21	≤0.001
15 mg/kg CR8020 at day 2	3, 5-21	≤0.001
15 mg/kg CR8020 at day 3	3, 5-21	≤ 0.012
15 mg/kg CR8020 at day 4	3, 5-21	≤0.034
15 mg/kg CR8020 at day 5	3	≤0.001
15 mg/kg CR8020 at day 6	3	≤0.001

Table 19. Mean area under the curve of body weight change from baseline at day 0.

Group	Mean AUC (g*day)	SD (g*day)	p-value[a] (mAb CR8020 vs control)
30 mg/kg control	-93.06	10.88	
1 mg/kg CR8020	-45.61	15.05	<0.001
3 mg/kg CR8020	-13.31	9.51	<0.001
10 mg/kg CR8020	-6.35	12.40	<0.001
30 mg/kg CR8020	-12.59	7.35	<0.001

[a]Mean AUC values of the mAb CR8020 dosed groups were compared to the control mAb group using analysis of variance with Dunnet's adjustment for multiple comparisons in the post-hoc analysis.

Table 20: Summary of binding and neutralization properties of monoclonal antibodies specific for influenza virus HA.

	H1		H3	
	Binding	VNA	Binding	VNA
CR6261	+	+	-	-
CR6323	+	+	-	-
CR8001	+	-	+	+
CR8020	-	-	+	+
CR8041	-	-	+	+
CR8043	-	-	+	+

Table 21. Mean area under the curve of body weight change from baseline at day 0.

Group	**Mean AUC (g*day)**	**SD (g*day)**	**p-value (mAb vs control)[a]**
30 mg/kg control	-101.38	11.67	
1 mg/kg CR8020	-82.58	34.71	0.356
3 mg/kg CR8020	-5.70	23.97	<0.001
10 mg/kg CR8020	2.13	13.13	<0.001
1 mg/kg CR8041	-105.05	17.04	1
3 mg/kg CR8041	-32.22	30.87	<0.001
10 mg/kg CR8041	-20.06	17.92	<0.001
30 mg/kg CR8041	-10.01	10.11	<0.001
1 mg/kg CR8043	-107.75	11.04	0.997
3 mg/kg CR8043	-117.88	5.91	0.510
10 mg/kg CR8043	-94.00	23.23	0.992
30 mg/kg CR8043	-56.82	17.55	<0.001

[a]Mean AUC values of the mAb CR8020 dosed groups were compared to the control mAb group using analysis of variance with Dunnet's adjustment for multiple comparisons in the post-hoc analysis.

Table 22. Mean area under the curve of body weight change from baseline at day 0.

Group	**Mean AUC (g*day)**	**SD (g*day)**	**p-value (mAb vs control)[a]**
15 mg/kg CR8020 at day -1	-7.68	8.17	< 0.001
15 mg/kg CR8020 at day 1	-20.43	8.41	< 0.001
15 mg/kg CR8020 at day 2	-38.18	37.35	< 0.001
15 mg/kg CR8020 at day 3	-28.27	9.63	< 0.001
15 mg/kg CR8020 at day 4	-99.11	37.90	0.566
15 mg/kg CR8020 at day 5	-93.62	10.29	0.979
15 mg/kg CR8020 at day 6	-94.06	7.65	0.858
Control antibody at day 1	-93.33	10.58	

[a]Mean AUC values were compared using the RobustReg procedure (SAS) which allocates less weight to outliers.

Table 23. Median clinical scores.

	Relative to control	
Group	Interval (day(s)	p
15 mg/kg CR8020 at day -1	4-21	≤0.001
15 mg/kg CR8020 at day 1	2, 5-21	≤ 0.012
15 mg/kg CR8020 at day 2	6-21	≤ 0.038
15 mg/kg CR8020 at day 3	7-21	≤ 0.035
15 mg/kg CR8020 at day 4	5, 6, 8-21	≤ 0.016
15 mg/kg CR8020 at day 5	8	<0.001
15 mg/kg CR8020 at day 6	-	≥ 0.449

Table 24. Binding kinetics

A/Wisconsin

Fab	kon (1/Ms)	Kdis (1/s)	KD (nM)
CRF8020	2.03E+05	2.08E-03	11.2
CRF8043	4.08E+05	9.86E-05	0.3

A/Brisbane

Fab	kon (1/Ms)	Kdis (1/s)	KD (nM)
CRF8020	1.81E+05	1.43E-03	8.9
CRF8043	3.12E+05	8.69E-05	0.3

Nucleotide and amino acid sequence of the heavy and light chain variable regions:

>SC08-001 VH DNA (SEQ ID NO: 1)
GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCCTGATCCAGCCGGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTG
GATTCACCGTCAGTAGCAACTACGTGAGCTGGGTCCGCCAGGCCCCAGGGAAGGGGCTGGAGTGGCTCTCACTTAT
TTACACGGGTGGTACCACATACTACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACTCCAAGAAT
ACGGTGTTTCTTCAAATGAACAGCCTGAGAGCCGAGGACGCGGCCATGTATTACTGTGCGAGAGTGTCAGCATTAC
GGTTTTTGCAGTGGCCAAACTACGCGATGGACGTC

>SC08-001 VH PROTEIN (SEQ ID NO: 2)
EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYVSWVRQAPGKGLEWLSLIYTGGTTYYADSVKGRFTISRDNSKN
TVFLQMNSLRAEDAAMYYCARVSALRFLQWPNYAMDV

>SC08-001 VL DNA (SEQ ID NO: 3)
CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACGGTCGATCACCATCTCCTGCTCTGGAACCC
GCAGTGACGTTGGTGGTCATAATTATGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCCAAACTCATGATTTA
TGAGGTCAGTCATCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAGCACGGCCTCCCTGACC
ATCTCTGGCCTCCAGTCTGAGGACGAGGCTGATTATTACTGCAGCTCTTATACAGGTGAAGGCCCCCTAGGAGTG

>SC08-001 VL PROTEIN (SEQ ID NO: 4)
QSALTQPASVSGSPGRSITISCSGTRSDVGGHNYVSWYQQHPGKAPKLMIYEVSHRPSGVSNRFSGSKSGSTASLT
ISGLQSEDEADYYCSSYTGEGPLGV

>SC08-003 VH DNA (SEQ ID NO: 5)
GAGGTGCAGCTGGTGGAGACCGGGGGAGACTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTG
AATTCAGCTTCAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAAGGGCTGGAGTGGGTGGCCAACAT
GAAGCAAGATGGAAGTGAGAAGTACTATGTGGACTCTGTGAAGGGCCGGTTCACCATCTCCAGAGACAACGCCAAG
AACTCATTATATCTGCAAATGAACAGCCTGAGAGGCGAGGACACGGCTGTGTATTACTGTGCGAGGGGTTCCTGTG
ACGATTCTTGGACTGGTTGTCATGATGCTTTTGACATC

>SC08-003 VH PROTEIN (SEQ ID NO: 6)
EVQLVETGGDLVQPGGSLRLSCSASEFSFSSYWMSWVRQAPGKGLEWVANMKQDGSEKYYVDSVKGRFTISRDNAK
NSLYLQMNSLRGEDTAVYYCARGSCDDSWTGCHDAFDI

>SC08-003 VL DNA (SEQ ID NO: 7)
GTGTTGACGCAGCCGCCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATTGCCTGTGGGGGAAACAACATTG
GGAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCCAGGCCCCTGTGCTGGTCGTCTATGATGATAGCGCCCG
GCCCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAATTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAG
GCCGGGGATGAAGCCGACTATTACTGTCAGGTGTGGGAGAGTGGTAGTGATCTACGACTGCTT

>SC08-003 VL PROTEIN (SEQ ID NO: 8)
VLTQPPSVSVAPGQTARIACGGNNIGSKSVHWYQQKPGQAPVLVVYDDSARPSGIPERFSGSNSGNTATLTISRVE
AGDEADYYCQVWESGSDLRLL

>SC08-015 VH DNA (SEQ ID NO: 9)
CAGGTGCAGCTGCAGGAGTCGGGGGGGAGACTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTG
AATTCAGCTTCAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAAGGGCTGGAGTGGGTGGCCAACAT
GAAGCAAGATGGAAGTGAGAAGTACTATGTGGACTCTGTGAAGGGCCGGTTCACCATCTCCAGAGACAACGCCAAG
AACTCATTATATCTGCAAATGAACAGCCTGAGAGGCGAGGACACGGCTGTGTATTACTGTGCGAGGGGTTCCTGTG
ACGATTCTTGGACTGGTTGTCATGATGCTTTTGACATC

>SC08-015 VH PROTEIN (SEQ ID NO: 10)
QVQLQESGGDLVQPGGSLRLSCSASEFSFSSYWMSWVRQAPGKGLEWVANMKQDGSEKYYVDSVKGRFTISRDNAK
NSLYLQMNSLRGEDTAVYYCARGSCDDSWTGCHDAFDI

>SC08-015 VL DNA (SEQ ID NO: 11)
GTGTTGACGCAGCCGCCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAAGATTACCTGTGGGGGAGACAACATTG
GAAGAAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCCTGGCCCCTGTGCTGGTCGTCAATGATAATAGCGACCG
GCCCTCAGGGATCCCTGCGCGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAA
GCCGGGGATGAGGCCGACTATTACTGTCACGTGTGGGGTAGTAGTCGTGACCATTATGTC

>SC08-015 VL PROTEIN (SEQ ID NO: 12)
VLTQPPSVSVAPGQTAKITCGGDNIGRKSVHWYQQKPGLAPVLVVNDNSDRPSGIPARFSGSNSGNTATLTISRVE
AGDEADYYCHVWGSSRDHYV

>SC08-016 VH DNA (SEQ ID NO: 13)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTG
AATTCAGCTTCAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAAGGGCTGGAGTGGGTGGCCAACAT
GAAGCAAGATGGAAGTGAGAAGTACTATGTGGACTCTGTGAAGGGCCGGTTCACCATCTCCAGAGACAACGCCAAG
AACTCATTATATCTGCAAATGAACAGCCTGAGAGGCGAGGACACGGCTGTGTATTACTGTGCGAGGGGTTCCTGTG
ACGATTCTTGGACTGGTTGTCATGATGCTTTTGACATC

>SC08-016 VH PROTEIN (SEQ ID NO: 14)
EVQLVESGGDLVQPGGSLRLSCSASEFSFSSYWMSWVRQAPGKGLEWVANMKQDGSEKYYVDSVKGRFTISRDNAK
NSLYLQMNSLRGEDTAVYYCARGSCDDSWTGCHDAFDI

>SC08-016 VL DNA (SEQ ID NO: 15)
CAGTCTGTCGTGACGCAGCCGCCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGTCACCATCTCCTGCACTGGGAGCA
GCTCCAACATCGGGGCAGGTTATGATGTACACTGGTACCAGCAGCTTCCAGGAACAGCCCCCAAACTCCTCATCTA
TGGTAACAACAATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGATCCAGGTCTGGCCCTTTAGCCCTCCTGGCC
ATCACTGGGCTCCAGGCTGAGGATGAGGCTGATTATTACTGCCAGTCCTATGACAGCAGCCTGAGTGTTTATGTC

>SC08-016 VL PROTEIN (SEQ ID NO: 16)
QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNNNRPSGVPDRFSGSRSGPLALLA
ITGLQAEDEADYYCQSYDSSLSVYV

>SC08-017 VH DNA (SEQ ID NO: 17)
GAGGTGCAGCTGGTGGAGACTGGGGGAGACTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTG
AATTCAGCTTCAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAAGGGCTGGAGTGGGTGGCCAACAT
GAAGCAAGATGGAAGTGAGAAGTACTATGTGGACTCTGTGAAGGGCCGGTTCACCATCTCCAGAGACAACGCCAAG
AACTCATTATATCTGCAAATGAACAGCCTGAGAGGCGAGGACACGGCTGTGTATTACTGTGCGAGGGGTTCCTGTG
ACGATTCTTGGACTGGTTGTCATGATGCTTTTGACATC

>SC08-017 VH PROTEIN (SEQ ID NO: 18)
EVQLVETGGDLVQPGGSLRLSCSASEFSFSSYWMSWVRQAPGKGLEWVANMKQDGSEKYYVDSVKGRFTISRDNAK
NSLYLQMNSLRGEDTAVYYCARGSCDDSWTGCHDAFDI

>SC08-017 VL DNA (SEQ ID NO: 19)
TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAGACA
GCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCCCTGTACTTGTCATCTATGCTAAAAC
CAACCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCACCTCAGGAAACACTGCTTCCTTGACCATCACTGGG
GCTCAGGCGGAGGATGAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTA

>SC08-017 VL PROTEIN (SEQ ID NO: 20)
SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYAKTNRPSGIPDRFSGSTSGNTASLTITG
AQAEDEADYYCNSRDSSGNHVV

>SC08-018 VH DNA (SEQ ID NO: 21)
GAGGTGCAGCTGGTGGAGACTGGGGGAGACTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTG
AATTCAGCTTCAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAAGGGCTGGAGTGGGTGGCCAACAT
GAAGCAAGATGGAAGTGAGAAGTACTATGTGGACTCTGTGAAGGGCCGGTTCACCATCTCCAGAGACAACGCCAAG
AACTCATTATATCTGCAAATGAACAGCCTGAGAGGCGAGGACACGGCTGTGTATTACTGTGCGAGGGGTTCCTGTG
ACGATTCTTGGACTGGTTGTCATGATGCTTTTGATATC

>SC08-018 VH PROTEIN (SEQ ID NO: 22)

EVQLVETGGDLVQPGGSLRLSCSASEFSFSSYWMSWVRQAPGKGLEWVANMKQDGSEKYYVDSVKGRFTISRDNAK
NSLYLQMNSLRGEDTAVYYCARGSCDDSWTGCHDAFDI

>SC08-018 VL DNA (SEQ ID NO: 23)
CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGAACCA
GCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCCAAACTCATGATTTA
TGAGGTCAGTCATCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAGCACGGCCTCCCTGACC
ATCTCTGGCCTCCAGTCTGAGGACGAGGCTGATTATTACTGCAGCTCTTATACAGGTGAAGGCCCCCTAGGAGTG

>SC08-018 VL PROTEIN (SEQ ID NO: 24)
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYEVSHRPSGVSNRFSGSKSGSTASLT
ISGLQSEDEADYYCSSYTGEGPLGV

>SC08-019 VH DNA (SEQ ID NO: 25)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGGAGCCTCTG
GAATCAGCGTTAGCACTTCTGCCATGAGCTGGGTCCGCCAGGTTCCAGGGAAGGGGCTGGAGTGGGTCTCAGGTAT
TAGTGGTAGTGGTGCTACCACATACTACGCAGGCTCCGTGAAGGGTCGATTCACCATCTCCAGAGACAAATCCAAG
AACACACTGCATCTGCAAATGAGCAGACTGAGAGCCGAGGACACGGCCATTTACTACTGTGCGAAAGATACCTCCT
TGTTTGAGTATGATACAAGTGGTTTTACGGCTCCCGGCAATGCTTTTGATATC

>SC08-019 VH PROTEIN (SEQ ID NO: 26)
EVQLVESGGGLVQPGGSLRLSCGASGISVSTSAMSWVRQVPGKGLEWVSGISGSGATTYYAGSVKGRFTISRDKSK
NTLHLQMSRLRAEDTAIYYCAKDTSLFEYDTSGFTAPGNAFDI

>SC08-019 VL DNA (SEQ ID NO: 27)
GACATCCAGWTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGATGACAGAGTCACCATCACTTGCCGGGCAA
GTCAGAGCATTAGCGGCTATTTAAATTGGTATCAACAGAAACCAGGGAAAGCCCCTAACCTCCTGATCTATGGTGC
ATCCACTTTGCAGAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCACC
AGTCTGCAACCTGAAGACTATGCAACTTACTACTGTCAACAGACTTACACCTCCCCTCCGTACGCT

>SC08-019 VL PROTEIN (SEQ ID NO: 28)
DIQXTQSPSSLSASVDDRVTITCRASQSISGYLNWYQQKPGKAPNLLIYGASTLQSGVPSRFSGSGSGTDFTLTIT
SLQPEDYATYYCQQTYTSPPYA

>SC08-020 VH DNA (SEQ ID NO: 29)
CAGGTACAGCTGCAGCAGTCAGGAGCTGAGGTGAAGACCCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCCTCTG
GATACACCTTTACCAGGTTTGGTGTCAGCTGGATACGACAGGCCCCTGGACAAGGGCTTGAGTGGATTGGATGGAT
CAGCGCTTACAATGGTGACACATACTATGCACAGAAGTTCCAGGCCAGAGTCACCATGACCACAGACACATCCACG
ACCACAGCCTACATGGAGATGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGAACCCCCCC
TTTTTTACAGCAGCTGGTCTCTTGACAAC

>SC08-020 VH PROTEIN (SEQ ID NO: 30)
QVQLQQSGAEVKTPGASVKVSCKASGYTFTRFGVSWIRQAPGQGLEWIGWISAYNGDTYYAQKFQARVTMTTDTST
TTAYMEMRSLRSDDTAVYYCAREPPLFYSSWSLDN

>SC08-020 VL DNA (SEQ ID NO: 31)
GAAATTGTGWTGACRCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA
GTCAGAGTGTTAGCATGAACTACTTAGCCTGGTTCCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGG
TGCGTCCCGCAGGGCCACTGGCATCCCCGACAGGATCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATC
AGCAGACTGGAGCCTGCAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTACCTCACCTCGGACG

>SC08-020 VL PROTEIN (SEQ ID NO: 32)
EIVXTQSPGTLSLSPGERATLSCRASQSVSMNYLAWFQQKPGQAPRLLIYGASRRATGIPDRISGSGSGTDFTLTI
SRLEPADFAVYYCQQYGTSPRT

>SC08-021 VH DNA (SEQ ID NO: 33)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGATACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTG
GATTCACCTTTAGCGCCTATGCCATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTAT
TGGTGGTAGTGGCGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAACTCCAAG

AAGATCCTGTATCTGCAAATGAACGGCCTGAGAGCCGAGGACACGGCCATATATTACTGTGCGAAAGGCCGGGATT
GGACTGGGGGTTACTTCTTTGACTCC

>SC08-021 VH PROTEIN (SEQ ID NO: 34)
EVQLVESGGGLIQPGGSLRLSCAASGFTFSAYAMNWVRQAPGKGLEWVSAIGGSGGSTYYADSVKGRFTISRDNSK
KILYLQMNGLRAEDTAIYYCAKGRDWTGGYFFDS

>SC08-021 VL DNA (SEQ ID NO: 35)
GACATCCAGWTGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTCCA
GCCAGAGTATTTTCTACAGCTCCAACAATAAGAACTACTTAACTTGGTACCAGCAGAAACCAGGACAGCCTCCTAA
GCTGCTCATTTACTGGGCATCTACCCGGGAATCCGGAGTCCCTGACCGATTCAGTGGCAGCGGGTCTGGGACAGAT
TTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTATTACTGTCAGCAATACTATAGTATTCCCT
ACACT

>SC08-021 VL PROTEIN (SEQ ID NO: 36)
DIQXTQSPDSLAVSLGERATINCKSSQSIFYSSNNKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTD
FTLTISSLQAEDVAVYYCQQYYSIPYT

>SC08-038 VH DNA (SEQ ID NO: 37)
GAGGTGCAGCTGGTGGACTCTGGGGGAGGCTTGGTACAGCCGGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTG
GATTCGCCTTTAGCGGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGATAT
TGGTGGTAGTGGTGGTGGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATGCCAAG
AACACGCTGTATCTGCAAATGAATAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAAGCAGTAGCT
GGGACCGGGCCTACTTCTTTGACTCC

>SC08-038 VH PROTEIN (SEQ ID NO: 38)
VQLVDSGGGLVQPGGSLRLSCAASGFAFSGYAMSWVRQAPGKGLEWVSDIGGSGGGTYYADSVKGRFTISRDNAKN
TLYLQMNSLRAEDTAVYYCAKSSSWDRAYFFDS

>SC08-038 VL DNA (SEQ ID NO: 39)
GATATTGTGATGACCCAGACTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTCCA
GCCAGAGTGTTTTATACAGCTCCATCCATAAGAACTACTTAGCCTGGTACCAGCAAAAACCAGGACAGCCTCCTAA
GCTGCTCATTTACTGGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATTCAGTGGCAGCGGGTCTGGGACAGAT
TTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTATTACTGTCAGCAATATTATAGATCTCCTC
CAACT

>SC08-038 VL PROTEIN (SEQ ID NO: 40)
DIVMTQTPDSLAVSLGERATINCKSSQSVLYSSIHKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTD
FTLTISSLQAEDVAVYYCQQYYRSPPT

>SC08-039 VH DNA (SEQ ID NO: 41)
CAGCTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACGTGCACTGTCTCTG
GCGGCTCCATCGGTAGTTACTACTGGAGCTGGATACGGCAGCCCCCAGGGAAGGGACTGGAGTGGATTGGATATAT
CTATTACCGTGGGGGTACCAGTTACAACCCCTCCCTCAAGAGTCGAGTCACCATATCAGTCGACACGTCCAAGAGC
CAGTTCACCTTGAAGCTGAACTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGAAAGGACTGGGGAT
CAGCGGCCGGAAGTGTCTGGTACTTCGATCTC

>SC08-039 VH PROTEIN (SEQ ID NO: 42)
QLQLQESGPGLVKPSETLSLTCTVSGGSIGSYYWSWIRQPPGKGLEWIGYIYYRGGTSYNPSLKSRVTISVDTSKS
QFTLKLNSVTAADTAVYYCARKDWGSAAGSVWYFDL

>SC08-039 VL DNA (SEQ ID NO: 43)
CAGTCTGCCCTGACTCAGCCTCCCTCCGCGTCCGGGTCTCCTGGACAGTCAGTCACCATCTCCTGCACTGGAACCA
GCAGTGACGTTGGTGGTTATAATTATGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCCAAACTCATGATTCG
TGAGGTCAGTAAGCGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGTTCCAAGTCTGGCAACACGGCCTCCCTGACC
GTCTCTGGGCTCCAGGCTGAGGATGAGGCTGAATACTACTGCAGCTCGTATGCAGGCAGCAACAATCTGATA

>SC08-039 VL PROTEIN (SEQ ID NO: 44)
QSALTQPPSASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIREVSKRPSGVPDRFSGSKSGNTASLT
VSGLQAEDEAEYYCSSYAGSNNLI

>SC08-040 VH DNA (SEQ ID NO: 45)
GAGGTGCAGCTGGTGGAGTCAGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTG
GATTCGCTTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGACTGGAGTGGGTGACCTTTAT
ATGGTATGATGGAAGTAATAAACACTATGCAGACTCCATGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAG
AACACGCTGTATCTGCAAATGAGCAGCCTGAGAGCCGAGGACACGGCTGTTTATTACTGTGCGAGAGATGGGGGAT
ATAGCACCTGGGAATGGTACTTCGATCTC

>SC08-040 VH PROTEIN (SEQ ID NO: 46)
EVQLVESGGGVVQPGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLEWVTFIWYDGSNKHYADSMKGRFTISRDNSK
NTLYLQMSSLRAEDTAVYYCARDGGYSTWEWYFDL

>SC08-040 VL DNA (SEQ ID NO: 47)
GAAATTGTGCTGACTCAGTCTCCGGACTTTCAGTCTGTGACTCCAAAGGAGAGAGTCACCATCACCTGCCGGGCCA
GTCAGGGCATTGGCAGTAACTTACACTGGTACCAGCAGAAACCAGATCAGTCTCCAAAGCTCCTCATCAAGTATGC
TTCCCAGTCCATCACAGGGGTCCCCTCGAGGTTCAGTGGCAGGGGATCTGGGACAGATTTCACCCTCACCATCAAT
AGCCTGGAAGTTGAAGATGCTGCAGTGTATTACTGTCATCAGAGTAGTAGTTTACCGCTCACT

>SC08-040 VL PROTEIN (SEQ ID NO: 48)
EIVLTQSPDFQSVTPKERVTITCRASQGIGSNLHWYQQKPDQSPKLLIKYASQSITGVPSRFSGRGSGTDFTLTIN
SLEVEDAAVYYCHQSSSLPLT

>SC08-041 VH DNA (SEQ ID NO: 49)
CAGGTGCAGCTGGTGCAGTCTGGCGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCCAGGCTTCGG
GTTACACCTTTACCTCCTTTGGTCTCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCCTGAGTGGATGGGATGGAT
CAGCGCTTACAATGGTGAAATAAAGTATGCACAGAAGTTCCAGGGCAGAGTCTCCATGACCACAGACACATCAACG
AGGACAGCCTACATGGAGGTGCGGAGCCTCAGACCTGACGACACGGCCGTATACTACTGTGCGAGAGAGCCCCCCC
TGTATTTCAGTAGCTGGTCTCTCGACTTC

>SC08-041 VH PROTEIN (SEQ ID NO: 50)
QVQLVQSGAEVKKPGASVKVSCQASGYTFTSFGLSWVRQAPGQGPEWMGWISAYNGEIKYAQKFQGRVSMTTDTST
RTAYMEVRSLRPDDTAVYYCAREPPLYFSSWSLDF

>SC08-041 VL DNA (SEQ ID NO: 51)
GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA
GTCAGAGTGTTAGCAGCAACTACTTAGCCTGGTTCCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGG
TGCATCAAGGAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATC
AGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGATAGCTCACCTCGGACG

>SC08-041 VL PROTEIN (SEQ ID NO: 52)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSNYLAWFQQKPGQAPRLLIYGASRRATGIPDRFSGSGSGTDFTLTI
SRLEPEDFAVYYCQQYDSSPRT

>SC08-043 VH DNA (SEQ ID NO: 53)
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGCTTTCCTGCAAGGCTTCTG
GATACACCTTCACTGCCTATTCTATGCATTGGGTGCGCCAGGCCCCCGGACAAAGCCTTGAGTGGTTGGGATGGAT
CAACACTGCCATCGGTAACACACAATATTCACAGAAGTTCCAGGACAGAGTCACCATTACCAGGGACACATCTGCG
CGCACATCGTACATGGAACTGAGCAGCCTGAGATCTGGAGACACGGCTGTCTATTTCTGTGCGAGAGGGGCCTCTT
GGGACGCCCGTGGGTGGTCTGGCTAC

>SC08-043 VH PROTEIN (SEQ ID NO: 54)

QVQLVQSGAEVKKPGASVKLSCKASGYTFTAYSMHWVRQAPGQSLEWLGWINTAIGNTQYSQKFQDRVTITRDTSA
RTSYMELSSLRSGDTAVYFCARGASWDARGWSGY

>SC08-043 VL DNA (SEQ ID NO: 55)
GACATCCAGWTGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTCCA
GCCAGAGTGTTTTTTCCAGCTCCACCAATAAGAACTACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTCCTAA
GGTGCTAATTTACTGGTCATCTACCCGGGAATCCGGGGTCCCTGACCGATTCAGTGCCAGCGGGTCTGGGACAGAT
TTCACTCTCACCATCAGCAGCCTGCAGGCTGCAGATGTGGCAGTTTATTACTGTCACCAATATTATACTGCTCCGT
GGACG

>SC08-043 VL PROTEIN (SEQ ID NO: 56)
DIQXTQSPDSLAVSLGERATINCKSSQSVFSSSTNKNYLAWYQQKPGQPPKVLIYWSSTRESGVPDRFSASGSGTD
FTLTISSLQAADVAVYYCHQYYTAPWT

>SC08-049 VH DNA (SEQ ID NO: 57)
CAGGTCACCTTGAAGGAGTCTGGTCCTGTACTGGTGAAGCCCAAAGAGACCCTCACGCTGACCTGCACCGTCTCTG
GGTTCTCACTCAGCAACACTAGAATGGGTGTGAGTTGGATCCGTCAGCCCCCAGGGAAGGCCCTGGAGTGGCTTGC
GCACATCTTTTCGAACGACGAAACATCCTACAGGACATCTCTGAAGAGGAGGCTCACCATCTCCCAGGACATCTCC
AAAAGTCAGGTGGTCCTTTCTATGACCAACGTGGACCCTGCAGACACAGCCACATATTTTTGTGCACGGATCGGGT
CTGGCTATGAGAGTAGTGCTTACTCCACCTGGCTCGACCCC

>SC08-049 VH PROTEIN (SEQ ID NO: 58)
QVTLKESGPVLVKPKETLTLTCTVSGFSLSNTRMGVSWIRQPPGKALEWLAHIFSNDETSYRTSLKRRLTISQDIS
KSQVVLSMTNVDPADTATYFCARIGSGYESSAYSTWLDP

>SC08-049 VL DNA (SEQ ID NO: 59)
CAGTCTGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGGCAGACGGCCAGGCTCACCTGTGAGGGAGACA
CAATTGGCAGTAAAAGTGTGCACTGGTACCAGCAGAGGCCAGGCCAGGCCCCTGTGTTGGTCGTCTATAATGATCG
CGACCGGCCCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAACTCTGGGCGCACGGCCACCCTGACCATCAGCAGG
GTCGAAGCCGGGGATGAGGCCGACTATTTCTGTCAGGTGTGGGAGAGTGGAGGTGATCAGACTGTC

>SC08-049 VL PROTEIN (SEQ ID NO: 60)
QSVLTQPPSVSVAPGQTARLTCEGDTIGSKSVHWYQQRPGQAPVLVVYNDRDRPSGIPERFSGSNSGRTATLTISR
VEAGDEADYFCQVWESGGDQTV

>SC08-050 VH DNA (SEQ ID NO: 61)
CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTATTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGCTGTGTATG
GTGGGTCGTTCACTGATCACTACTGGAGCTGGATCCGCCAGTCCCCAGGGAAGGGGCTGGAGTGGATTGGTGAAGT
CGTTCATAGTGGAGACACCAACTACACCCCGTCCCTCAGAAATCGAGTTTCCATATCGGTCGACTCGTCCAAGAAT
CAGTTCTCCCTGAGGCTGGGGTCTGTGACCGCCGCGGACACGGCTGTCTATTACTGTGCGAGAGGCAGGAATGTTG
CGGTAGTTGGTGCTATTCAGAGGCACTATGACTAC

>SC08-050 VH PROTEIN (SEQ ID NO: 62)
QVQLQQWGAGLLKPSETLSLTCAVYGGSFTDHYWSWIRQSPGKGLEWIGEVVHSGDTNYTPSLRNRVSISVDSSKN
QFSLRLGSVTAADTAVYYCARGRNVAVVGAIQRHYDY

>SC08-050 VL DNA (SEQ ID NO: 63)
GAAATTGTGATGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA
GTCAGAGTGTTAGCAGAAACTACTTAGCCTGGTACCAGCAGAAGCCTGGCCTGGCTCCCAGGCTCCTCATCTCTGG
TGCATCGAGCAGGGCCACTGGCGTCCCAGACAGGTTCAGTGGCAGGGGGTCTGACACAGACTTCACTCTCACCATC
AGCAGACTGGAGCCTGAAGATTTTGCCGTGTATTACTGTCAGCACTATGGTTCGGTCCTTGTAGCT

>SC08-050 VL PROTEIN (SEQ ID NO: 64)
EIVMTQSPGTLSLSPGERATLSCRASQSVSRNYLAWYQQKPGLAPRLLISGASSRATGVPDRFSGRGSDTDFTLTI
SRLEPEDFAVYYCQHYGSVLVA

>SC08-052 VH DNA (SEQ ID NO: 65)
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTG
GTGGCTCCGTCAGCAGTGGTACTTACTACTGGAGCTGGATCCGGCAGCCCCCAGGGAAGGGACTGGAGTGGATTGG
GGATATCTCTTACAGTGGGAGCACCAACTACAACCCCTCCCTCAAGAGTCGAGTCACCATTTCTAGAGACACGTCC
AAGAACCTGGTCTCCCTGAAGCTGACCTCTGTGACCGCTGCGGACACGGCCGTGCATTACTGTGCGAGAGCGATGG
CGGCTTATAATTATGACAGGGGTGGTTATAACGACTACTACTACATGGACGTC

>SC08-052 VH PROTEIN (SEQ ID NO: 66)
QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGTYYWSWIRQPPGKGLEWIGDISYSGSTNYNPSLKSRVTISRDTS
KNLVSLKLTSVTAADTAVHYCARAMAAYNYDRGGYNDYYYMDV

>SC08-052 VL DNA (SEQ ID NO: 67)
GACATCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTCGGAGACAGAGTCACCATCACTTGCCGGGCAA
GTCAGGGCATTAACACCTATTTAAATTGGTATCAGCAAAAACCAGGGAAGGCCCCTAAGGTCCTGATCTTTGCTGC
ATCCACTTTGCAAAGTGGAGTCCCATCAAGGTTCAGTGGCAGTGGTTCTGGGACAGAATTCACTCTCAACATCAAC
AATCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACTGCGATCACT

>SC08-052 VL PROTEIN (SEQ ID NO: 68)
DIQLTQSPSSLSASVGDRVTITCRASQGINTYLNWYQQKPGKAPKVLIFAASTLQSGVPSRFSGSGSGTEFTLNIN
NLQPEDFATYYCQQSYSTAIT

>SC08-055 VH DNA (SEQ ID NO: 69)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCGGCGTCTG
GATTCAGCTTCACCACCTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCCTTTAT
TTGGTATGATGGAAGTAACAAACACTATCAAGACTCCGTGAAGGGCCGATTCACCATCTCCAAGGACAATTCCAAC
AACATGTTGTATCTGCAAATGGACAGCCTGAGAGTCGCCGACACGGCTGTTTATTACTGTGTGAGAGATGGGGGAT
ATAGCACCTGGGAATGGTACTTCGATCTC

>SC08-055 VH PROTEIN (SEQ ID NO: 70)
EVQLVESGGGVVQPGRSLRLSCAASGFSFTTYGMHWVRQAPGKGLEWVAFIWYDGSNKHYQDSVKGRFTISKDNSN
NMLYLQMDSLRVADTAVYYCVRDGGYSTWEWYFDL

>SC08-055 VL DNA (SEQ ID NO: 71)
GAAATTGTGCTGACTCAGTCTCCAGACTTTCAGTCTGTGGCTCCAAAGGAGAAAGTCACCATCACCTGCCGGGCCA
GTCGGAGCATTGGTAGTGACTTGCACTGGTTTCAGCAGAGGCCAGATCAGTCTCCAAAGCTCCTCATCAAGTTTGC
TTCCCAGTCCATGTCAGGGGTCCCCTCGAGGTTCAGTGGCAGTGGGTCTGGGAGAGATTTCACCCTCACCATCAGT
AGCCTGGAGGCTGAAGATGCTGCTACGTATTACTGTCATCAGAGTAGTAGTTTACCGCTCACT

>SC08-055 VL PROTEIN (SEQ ID NO: 72)
EIVLTQSPDFQSVAPKEKVTITCRASRSIGSDLHWFQQRPDQSPKLLIKFASQSMSGVPSRFSGSGSGRDFTLTIS
SLEAEDAATYYCHQSSSLPLT

>SC08-057 VH DNA (SEQ ID NO: 73)
GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTCCAACCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTG
GGTTCACCGACAGTGTCATCTTCATGAGTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGTGTCTCAATTAT
TTATATCGATGATTCCACATACTACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGACACAATTCCATGGGC
ACAGTGTTTCTTGAAATGAACAGCCTGAGACCTGACGACACGGCCGTCTATTACTGTGCGACAGAGAGCGGAGACT
TTGGTGACCAAACGGGTCCCTATCATTACTACGCTATGGACGTC

>SC08-057 VH PROTEIN (SEQ ID NO: 74)
EVQLVESGGGLVQPGGSLRLSCAASGFTDSVIFMSWVRQAPGKGLECVSIIYIDDSTYYADSVKGRFTISRHNSMG
TVFLEMNSLRPDDTAVYYCATESGDFGDQTGPYHYYAMDV

>SC08-057 VL DNA (SEQ ID NO: 75)
CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGAAGCA
GCGGTGACATTGGTGGTTATAACGCTGTCTCCTGGTACCAACACCACCCAGGCAAAGCCCCCAAACTGATGATTTA
TGAGGTCACTAGTCGGCCCTCAGGGGTTTCCGATCGCTTCTCTGCGTCCAGGTCTGGCGACACGGCCTCCCTGACT
GTCTCTGGTCTCCAGGCTGAGGACGAGGCTCACTATTACTGCTGCTCATTTGCAGACAGCAACATTTTGATT

>SC08-057 VL PROTEIN (SEQ ID NO: 76)
QSALTQPASVSGSPGQSITISCTGSSGDIGGYNAVSWYQHHPGKAPKLMIYEVTSRPSGVSDRFSASRSGDTASLT
VSGLQAEDEAHYYCCSFADSNILI

>SC08-069 VH DNA (SEQ ID NO: 77)
GAGGTGCAGCTGGTGGAGACTGGGGGAGTCGTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTG
GCTTCACGTTTGAGGATTATACCATGCACTGGGTCCGTCAAGTTCCGGGGAAGGGTCTGGAGTGGGTCGCGCTCAT
TAGTTGGGATGGCGGTATGTCAAACTATGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACAGCAAA
AACTCTCTGTATCTGCAAGTGAGCAGTCTGAGAAGTGAAGACACCGCCCTGTATTACTGTGCAAAAGATATACGAC
CCCGTATGCCAGCTCGTCACTTTATGGACGTC

>SC08-069 VH PROTEIN (SEQ ID NO: 78)
EVQLVETGGVVVQPGGSLRLSCAASGFTFEDYTMHWVRQVPGKGLEWVALISWDGGMSNYADSVKGRFTISRDNSK
NSLYLQVSSLRSEDTALYYCAKDIRPRMPARHFMDV

>SC08-069 VL DNA (SEQ ID NO: 79)
GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCGGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA
GTCAGAATGTCAACTACAACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGTTGC
ATCCACCAGGGCCACTGGTATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTTCACTCTCACCATCAGC
AGTCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATAACTGGCCTCCGGCGATCACT

>SC08-069 VL PROTEIN (SEQ ID NO: 80)
EIVLTQSPATLSVSPGERATLSCRASQNVNYNLAWYQQKPGQAPRLLIYVASTRATGIPDRFSGSGSGTEFTLTIS
SLQSEDFAVYYCQQYNNWPPAIT

>CR6261 HC DNA (SEQ ID NO: 185)

```
gaggtgcagc tggtggagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaagtc      60
tcttgcaagg cttctggagg ccccttccgc agctatgcta tcagctgggt gcgacaggcc     120
cctggacaag ggcctgagtg gatgggaggg atcatcccta tttttggtac aacaaaatac     180
gcaccgaagt tccagggcag agtcacgatt accgcggacg atttcgcggg cacagtttac     240
atggagctga gcagcctgcg atctgaggac acggccatgt actactgtgc gaaacatatg     300
gggtaccagg tgcgcgaaac tatggacgtc tggggcaaag ggaccacggt caccgtctcg     360
agtgctagca ccaagggccc cagcgtgttc cccctggccc ccagcagcaa gagcaccagc     420
ggcggcacag ccgccctggg ctgcctggtg aaggactact tccccgagcc cgtgaccgtg     480
agctggaaca gcggcgcctt gaccagcggc gtgcacacct tccccgccgt gctgcagagc     540
agcggcctgt acagcctgag cagcgtggtg accgtgccca gcagcagcct gggcacccag     600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa acgcgtggag     660
cccaagagct gcgacaagac ccacacctgc ccccccctgcc ctgcccccga gctgctgggc     720
ggaccctccg tgttcctgtt ccccccccaag cccaaggaca ccctcatgat cagccggacc     780
cccgaggtga cctgcgtggt ggtggacgtg agccacgagg accccgaggt gaagttcaac     840
tggtacgtgg acggcgtgga ggtgcacaac gccaagacca agccccggga ggagcagtac     900
aacagcacct accgggtggt gagcgtgctc accgtgctgc accaggactg gctgaacggc     960
aaggagtaca agtgcaaggt gagcaacaag gccctgcctg ccccccatcga gaagaccatc    1020
agcaaggcca agggccagcc ccgggagccc caggtgtaca ccctgccccc cagccgggag    1080
gagatgacca agaaccaggt gtccctcacc tgtctggtga agggcttcta ccccagcgac    1140
atcgccgtgg agtgggagag caacggccag cccgagaaca actacaagac caccccccct    1200
gtgctggaca gcgacggcag cttcttcctg tacagcaagc tcaccgtgga caagagccgg    1260
tggcagcagg gcaacgtgtt cagctgcagc gtgatgcacg aggccctgca caaccactac    1320
acccagaaga gcctgagcct gagccccggc aag                                 1353
```

>CR6261 HC PROTEIN (SEQ ID NO: 186)

E V Q L V E S G A E V K K P G S S V K V S C K A S G G P F R S Y A I S W V R
Q A P G Q G P E W M G G I I P I F G T T K Y A P K F Q G R V T I T A D D F A
G T V Y M E L S S L R S E D T A M Y Y C A K H M G Y Q V R E T M D V W G K G
T T V T V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D
Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V
T V P S S S L G T Q T Y I C N V N H K P S N T K V D K R V E P K S C D K T H
T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V
V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y
R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K
A K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D
I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K
S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G K

>CR6261 LC DNA (SEQ ID NO: 187)

```
cagtctgtgt tgacgcagcc gccctcagtg tctgcggccc caggacagaa ggtcaccatc      60
tcctgctctg gaagcagctc caacattggg aatgattatg tatcctggta ccagcagctc     120
ccaggaacag cccccaaact cctcatttat gacaataata agcgaccctc agggattcct     180
gaccgattct ctggctccaa gtctggcacg tcagccaccc tgggcatcac cggactccag     240
actggggacg aggccaacta ttactgcgca acatgggatc gccgcccgac tgcttatgtt     300
gtcttcggcg gagggaccaa gctgaccgtc ctaggtgcgg ccgcaggcca gcccaaggcc     360
gctcccagcg tgaccctgtt ccccccctcc tccgaggagc tgcaggccaa caaggccacc     420
ctggtgtgcc tcatcagcga cttctaccct ggcgccgtga ccgtggcctg gaaggccgac     480
agcagccccg tgaaggccgg cgtggagacc accaccccca gcaagcagag caacaacaag     540
tacgccgcca gcagctacct gagcctcacc cccgagcagt ggaagagcca ccggagctac     600
agctgccagg tgacccacga gggcagcacc gtggagaaga ccgtggcccc caccgagtgc     660
agc                                                                   663
```

>CR6261 LC PROTEIN (SEQ ID NO: 188)

Q S V L T Q P P S V S A A P G Q K V T I S C S G S S S N I G N D Y V S W Y Q
Q L P G T A P K L L I Y D N N K R P S G I P D R F S G S K S G T S A T L G I
T G L Q T G D E A N Y Y C A T W D R R P T A Y V V F G G G T K L T V L G A A
A G Q P K A A P S V T L F P P S S E E L Q A N K A T L V C L I S D F Y P G A
V T V A W K A D S S P V K A G V E T T T P S K Q S N N K Y A A S S Y L S L T
P E Q W K S H R S Y S C Q V T H E G S T V E K T V A P T E C S

Vector pIg-C911-HCgamma1 (SEQ ID NO:190)

```
tcgacggatc gggagatctc ccgatcccct atggtgcact ctcagtacaa tctgctctga      60
tgccgcatag ttaagccagt atctgctccc tgcttgtgtg ttggaggtcg ctgagtagtg     120
cgcgagcaaa atttaagcta caacaaggca aggcttgacc gacaattgca tgaagaatct     180
gcttagggtt aggcgttttg cgctgcttcg ctaggtggtc aatattggcc attagccata     240
ttattcattg gttatatagc ataaatcaat attggctatt ggccattgca tacgttgtat     300
ccatatcata atatgtacat ttatattggc tcatgtccaa cattaccgcc atgttgacat     360
tgattattga ctagttatta atagtaatca attacggggt cattagttca tagcccatat     420
atggagttcc gcgttacata acttacggta aatggcccgc ctggctgacc gcccaacgac     480
ccccgcccat tgacgtcaat aatgacgtat gttcccatag taacgccaat agggactttc     540
cattgacgtc aatgggtgga gtatttacgg taaactgccc acttggcagt acatcaagtg     600
tatcatatgc caagtacgcc ccctattgac gtcaatgacg gtaaatggcc cgcctggcat     660
tatgcccagt acatgacctt atgggacttt cctacttggc agtacatcta cgtattagtc     720
atcgctatta ccatggtgat gcggttttgg cagtacatca atgggcgtgg atagcggttt     780
gactcacggg gatttccaag tctccacccc attgacgtca atgggagttt gttttggcac     840
caaaatcaac gggactttcc aaaatgtcgt aacaactccg ccccattgac gcaaatgggc     900
ggtaggcgtg tacggtggga ggtctatata agcagagctc gtttagtgaa ccgtcagatc     960
gcctggagac gccatccacg ctgttttgac ctccatagaa gacaccggga ccgatccagc    1020
ctccgcggcc gggaacggtg cattggaagc tggcctggat atcctgactc tcttaggtag    1080
ccttgcagaa gttggtcgtg aggcactggg caggtaagta tcaaggttac aagacaggtt    1140
taaggagatc aatagaaact gggcttgtcg agacagagaa gactcttgcg tttctgatag    1200
gcacctattg gtcttactga catccacttt gcctttctct ccacaggtgt ccactcccag    1260
ttcaattaca gctcgccacc atgggatgga gctgtatcat cctcttcttg gtactgctgc    1320
```

tggcccagcc ggccagtgac cttgaccggt gcaccacttt tgatgatgtt caagctccta 1380
attacactca acatacttca tctatgaggg gggtttacta tcctgatgaa atttttagat 1440
cggacactct ttatttaact caggatttat ttcttccatt ttattctaat gttacagggt 1500
ttcatactat taatcatacg tttggcaacc ctgtcatacc ttttaaggat ggtatttatt 1560
ttgctgccac agagaaatca aatgttgtcc gtggttgggt ttttggttct accatgaaca 1620
acaagtcaca gtcggtgatt attattaaca attctactaa tgttgttata cgagcatgta 1680
actttgaatt gtgtgacaac ccttttcttg ctgtttctaa acccatgggt acacagacac 1740
atactatgat attcgataat gcatttaatt gcactttcga gtacatatct gatgcctttt 1800
cgcttgatgt ttcagaaaag tcaggtaatt ttaaacactt acgagagttt gtgtttaaaa 1860
ataaagatgg gtttctctat gtttataagg gctatcaacc tatagatgta gttcgtgatc 1920
taccttctgg ttttaacact ttgaaaccta tttttaagtt gcctcttggt attaacatta 1980
caaattttag agccattctt acagcctttt cacctgctca agacatttgg ggcacgtcag 2040
ctgcagccta ttttgttggc tatttaaagc caactacatt tatgctcaag tatgatgaaa 2100
atggtacaat cacagatgct gttgattgtt ctcaaaatcc acttgctgaa ctcaaatgct 2160
ctgttaagag ctttgagatt gacaaaggaa tttaccagac ctctaatttc agggttgttc 2220
cctcaggaga tgttgtgaga ttccctaata ttacaaactt gtgtcctttt ggagaggttt 2280
ttaatgctac taaattccct tctgtctatg catgggagag aaaaaaaatt tctaattgtg 2340
ttgctgatta ctctgtgctc tacaactcaa catttttttc aacctttaag tgctatggcg 2400
tttctgccac taagttgaat gatctttgct tctccaatgt ctatgcagat tcttttgtag 2460
tcaagggaga tgatgtaaga caaatagcgc caggacaaac tggtgttatt gctgattata 2520
attataaatt gccagatgat ttcatgggtt gtgtccttgc ttggaatact aggaacattg 2580
atgctacttc aactggtaat tataattata aatataggta tcttagacat ggcaagctta 2640
ggccctttga gagagacata tctaatgtgc ctttctcccc tgatggcaaa ccttgcaccc 2700
cacctgctct taattgttat tggccattaa atgattatgg tttttacacc actactggca 2760
ttggctacca accttacaga gttgtagtac tttcttttga acttttaaat gcaccggcca 2820
cggtttgtgg accaaaatta tccactgacc ttattaagaa ccagtgtgtc aattttaatt 2880
ttaatggact cactggtact ggtgtgttaa ctccttcttc aaagagattt caaccatttc 2940
aacaatttgg ccgtgatgtt tctgatttca ctgattccgt tcgagatcct aaaacatctg 3000
aaatattaga catttcacct tgctcttttg ggggtgtaag tgtaattaca cctggaacaa 3060
atgcttcatc tgaagttgct gttctatatc aagatgttaa ctgcactgat gtttctacag 3120
caattcatgc agatcaactc acaccagctt ggcgcatata ttctactgga aacaatgtat 3180
tccagactca ggcaggctgt cttataggag ctgagcatgt cgacacttct tatgagtgcg 3240
acattcctat tggagctggc atttgtgcta gttaccatac agtttcttta ttacgtagta 3300
ctagccaaaa atctattgtg gcttatacta tgtctttagg tgctgatagt tcaattgctt 3360
actctaataa caccattgct atacctacta acttttcaat tagcattact acagaagtaa 3420
tgcctgtttc tatggctaaa acctccgtag attgtaatat gtacatctgc ggagattcta 3480
ctgaatgtgc taatttgctt ctccaatatg gtagcttttg cacacaacta aatcgtgcac 3540
tctcaggtat tgctgctgaa caggatcgca acacacgtga agtgttcgct caagtcaaac 3600
aaatgtacaa aaccccaact ttgaaatatt ttggtggttt taatttttca caaatattac 3660
ctgaccctct aaagccaact aagaggtctt ttattgagga cttgctcttt aataaggtga 3720
cactcgctga tgctggcttc atgaagcaat atggcgaatg cctaggtgat attaatgcta 3780
gagatctcat ttgtgcgcag aagttcaatg gacttacagt gttgccacct ctgctcactg 3840
atgatatgat tgctgcctac actgctgctc tagttagtgg tactgccact gctggatgga 3900
catttggtgc tggcgctgct cttcaaatac cttttgctat gcaaatggca tataggttca 3960
atggcattgg agttacccaa aatgttctct atgagaacca aaaacaaatc gccaaccaat 4020
ttaacaaggc gattagtcaa attcaagaat cacttacaac aacatcaact gcattgggca 4080
agctgcaaga cgttgttaac cagaatgctc aagcattaaa cacacttgtt aaacaactta 4140
gctctaattt tggtgcaatt tcaagtgtgc taaatgatat cctttcgcga cttgataaag 4200
tcgaggcgga ggtacaaatt gacaggttaa ttacaggcag acttcaaagc cttcaaacct 4260
atgtaacaca acaactaatc agggctgctg aaatcagggc ttctgctaat cttgctgcta 4320
ctaaaatgtc tgagtgtgtt cttggacaat caaaaagagt tgacttttgt ggaaagggct 4380
accaccttat gtccttccca caagcagccc cgcatggtgt tgtcttccta catgtcacgt 4440
atgtgccatc ccaggagagg aacttcacca cagcgccagc aatttgtcat gaaggcaaag 4500
catacttccc tcgtgaaggt gtttttgtgt ttaatggcac ttcttggttt attacacaga 4560
ggaacttctt ttctccacaa ataattacta cagacaatac atttgtctca ggaaattgtg 4620
atgtcgttat tggcatcatt aacaacacag tttatgatcc tctgcaacct gagcttgact 4680
cattcaaaga agagctggac aagtacttca aaaatcatac atcaccagat gttgattttg 4740
gcgacatttc aggcattaac gcttctgtcg tcaacattca aaaagaaatt gaccgcctca 4800
atgaggtcgc taaaaattta aatgaatcac tcattgacct tcaagaactg ggaaaatatg 4860
agcaatatat taaatggcct ctcgacgaac aaaaactcat ctcagaagag gatctgaatg 4920

ctgtgggcca ggacacgcag gaggtcatcg tggtgccaca ctccttgccc tttaaggtgg 4980
tggtgatctc agccatcctg gccctggtgg tgctcaccat catctccctt atcatcctca 5040
tcatgctttg gcagaagaag ccacgttagg cggccgctcg agtgctagca ccaagggccc 5100
cagcgtgttc cccctggccc ccagcagcaa gagcaccagc ggcggcacag ccgccctggg 5160
ctgcctggtg aaggactact tccccgagcc cgtgaccgtg agctggaaca gcggcgcctt 5220
gaccagcggc gtgcacacct tccccgccgt gctgcagagc agcggcctgt acagcctgag 5280
cagcgtggtg accgtgccca gcagcagcct gggcacccag acctacatct gcaacgtgaa 5340
ccacaagccc agcaacacca aggtggacaa acgcgtggag cccaagagct gcgacaagac 5400
ccacacctgc ccccccctgcc ctgcccccga gctgctgggc ggaccctccg tgttcctgtt 5460
ccccccccaag cccaaggaca ccctcatgat cagccggacc cccgaggtga cctgcgtggt 5520
ggtggacgtg agccacgagg accccgaggt gaagttcaac tggtacgtgg acggcgtgga 5580
ggtgcacaac gccaagacca agccccggga ggagcagtac aacagcacct accgggtggt 5640
gagcgtgctc accgtgctgc accaggactg gctgaacggc aaggagtaca agtgcaaggt 5700
gagcaacaag gccctgcctg cccccatcga gaagaccatc agcaaggcca agggccagcc 5760
ccgggagccc caggtgtaca ccctgccccc cagccgggag gagatgacca agaaccaggt 5820
gtccctcacc tgtctggtga agggcttcta ccccagcgac atcgccgtgg agtgggagag 5880
caacggccag cccgagaaca actacaagac cacccccccct gtgctggaca gcgacggcag 5940
cttcttcctg tacagcaagc tcaccgtgga caagagccgg tggcagcagg gcaacgtgtt 6000
cagctgcagc gtgatgcacg aggccctgca caaccactac acccagaaga gcctgagcct 6060
gagccccggc aagtgataat ctagagggcc cgtttaaacc cgctgatcag cctcgactgt 6120
gccttctagt tgccagccat ctgttgtttg cccctccccc gtgccttcct tgaccctgga 6180
aggtgccact cccactgtcc tttcctaata aaatgaggaa attgcatcgc attgtctgag 6240
taggtgtcat tctattctgg ggggtggggt ggggcaggac agcaaggggg aggattggga 6300
agacaatagc aggcatgctg gggatgcggt gggctctatg gcttctgagg cggaaagaac 6360
cagctggggc tctagggggt atccccacgc gccctgtagc ggcgcattaa gcgcggcggg 6420
tgtggtggtt acgcgcagcg tgaccgctac acttgccagc gccctagcgc ccgctccttt 6480
cgctttcttc ccttcctttc tcgccacgtt cgccggcttt ccccgtcaag ctctaaatcg 6540
ggggctccct ttagggttcc gatttagtgc tttacggcac ctcgacccca aaaaacttga 6600
ttagggtgat ggttcacgta gtgggccatc gccctgatag acggtttttc gccctttgac 6660
gttggagtcc acgttcttta atagtggact cttgttccaa actggaacaa cactcaaccc 6720
tatctcggtc tattcttttg atttataagg gattttgccg atttcggcct attggttaaa 6780
aaatgagctg atttaacaaa aatttaacgc gaattaattc tgtggaatgt gtgtcagtta 6840
gggtgtggaa agtccccagg ctccccagca ggcagaagta tgcaaagcat gcatctcaat 6900
tagtcagcaa ccaggtgtgg aaagtcccca ggctccccag caggcagaag tatgcaaagc 6960
atgcatctca attagtcagc aaccatagtc ccgccctaa ctccgcccat cccgccccta 7020
actccgccca gttccgccca ttctccgccc catggctgac taatttttt tatttatgca 7080
gaggccgagg ccgcctctgc ctctgagcta ttccagaagt agtgaggagg ctttttgga 7140
ggcctaggct tttgcaaaaa gctcccggga gcttgtatat ccattttcgg atctgatcaa 7200
gagacaggat gaggatcgtt tcgcatgatt gaacaagatg gattgcacgc aggttctccg 7260
gccgcttggg tggagaggct attcggctat gactgggcac aacagacaat cggctgctct 7320
gatgccgccg tgttccggct gtcagcgcag gggcgcccgg ttctttttgt caagaccgac 7380
ctgtccggtg ccctgaatga actgcaggac gaggcagcgc ggctatcgtg gctggccacg 7440
acgggcgttc cttgcgcagc tgtgctcgac gttgtcactg aagcgggaag ggactggctg 7500
ctattgggcg aagtgccggg gcaggatctc ctgtcatctc accttgctcc tgccgagaaa 7560
gtatccatca tggctgatgc aatgcggcgg ctgcatacgc ttgatccggc tacctgccca 7620
ttcgaccacc aagcgaaaca tcgcatcgag cgagcacgta ctcggatgga agccggtctt 7680
gtcgatcagg atgatctgga cgaagagcat caggggctcg cgccagccga actgttcgcc 7740
aggctcaagg cgcgcatgcc cgacggcgag gatctcgtcg tgacccatgg cgatgcctgc 7800
ttgccgaata tcatggtgga aaatggccgc ttttctggat tcatcgactg tggccggctg 7860
ggtgtggcgg accgctatca ggacatagcg ttggctaccc gtgatattgc tgaagagctt 7920
ggcggcgaat gggctgaccg cttcctcgtg ctttacggta tcgccgctcc cgattcgcag 7980
cgcatcgcct tctatcgcct tcttgacgag ttcttctgag cgggactctg gggttcgaaa 8040
tgaccgacca agcgacgccc aacctgccat cacgagattt cgattccacc gccgccttct 8100
atgaaaggtt gggcttcgga atcgttttcc gggacgccgg ctggatgatc ctccagcgcg 8160
gggatctcat gctggagttc ttcgcccacc ccaacttgtt tattgcagct tataatggtt 8220
acaaataaag caatagcatc acaaatttca caaataaagc attttttttca ctgcattcta 8280
gttgtggttt gtccaaactc atcaatgtat cttatcatgt ctgtataccg tcgacctcta 8340
gctagagctt ggcgtaatca tggtcatagc tgtttcctgt gtgaaattgt tatccgctca 8400
caattccaca caacatacga gccggaagca taaagtgtaa agcctggggt gcctaatgag 8460
tgagctaact cacattaatt gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt 8520

cgtgccagct gcattaatga atcggccaac gcgcggggag aggcggtttg cgtattgggc 8580
gctcttccgc ttcctcgctc actgactcgc tgcgctcggt cgttcggctg cggcgagcgg 8640
tatcagctca ctcaaaggcg gtaatacggt tatccacaga atcaggggat aacgcaggaa 8700
agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg 8760
cgtttttcca taggctccgc cccctgacg agcatcacaa aaatcgacgc tcaagtcaga 8820
ggtggcgaaa cccgacagga ctataaagat accaggcgtt tccccctgga agctccctcg 8880
tgcgctctcc tgttccgacc ctgccgctta ccggatacct gtccgccttt ctcccttcgg 8940
gaagcgtggc gctttctcat agctcacgct gtaggtatct cagttcggtg taggtcgttc 9000
gctccaagct gggctgtgtg cacgaacccc ccgttcagcc cgaccgctgc gccttatccg 9060
gtaactatcg tcttgagtcc aacccggtaa gacacgactt atcgccactg gcagcagcca 9120
ctggtaacag gattagcaga gcgaggtatg taggcggtgc tacagagttc ttgaagtggt 9180
ggcctaacta cggctacact agaagaacag tatttggtat ctgcgctctg ctgaagccag 9240
ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa acaaaccacc gctggtagcg 9300
gtttttttgt ttgcaagcag cagattacgc gcagaaaaaa aggatctcaa gaagatcctt 9360
tgatcttttc tacggggtct gacgctcagt ggaacgaaaa ctcacgttaa gggattttgg 9420
tcatgagatt atcaaaaagg atcttcacct agatcctttt aaattaaaaa tgaagtttta 9480
aatcaatcta aagtatatat gagtaaactt ggtctgacag ttaccaatgc ttaatcagtg 9540
aggcacctat ctcagcgatc tgtctatttc gttcatccat agttgcctga ctccccgtcg 9600
tgtagataac tacgatacgg gagggcttac catctggccc cagtgctgca atgataccgc 9660
gagacccacg ctcaccggct ccagatttat cagcaataaa ccagccagcc ggaagggccg 9720
agcgcagaag tggtcctgca actttatccg cctccatcca gtctattaat tgttgccggg 9780
aagctagagt aagtagttcg ccagttaata gtttgcgcaa cgttgttgcc attgctacag 9840
gcatcgtggt gtcacgctcg tcgtttggta tggcttcatt cagctccggt tcccaacgat 9900
caaggcgagt tacatgatcc cccatgttgt gcaaaaaagc ggttagctcc ttcggtcctc 9960
cgatcgttgt cagaagtaag ttggccgcag tgttatcact catggttatg gcagcactgc 10020
ataattctct tactgtcatg ccatccgtaa gatgcttttc tgtgactggt gagtactcaa 10080
ccaagtcatt ctgagaatag tgtatgcggc gaccgagttg ctcttgcccg gcgtcaatac 10140
gggataatac cgcgccacat agcagaactt taaaagtgct catcattgga aaacgttctt 10200
cggggcgaaa actctcaagg atcttaccgc tgttgagatc cagttcgatg taacccactc 10260
gtgcacccaa ctgatcttca gcatctttta ctttcaccag cgtttctggg tgagcaaaaa 10320
caggaaggca aaatgccgca aaaaagggaa taagggcgac acggaaatgt tgaatactca 10380
tactcttcct ttttcaatat tattgaagca tttatcaggg ttattgtctc atgagcggat 10440
acatatttga atgtatttag aaaaataaac aaatagggggt tccgcgcaca tttccccgaa 10500
aagtgccacc tgacg 10515

Vector pIg-C909-Ckappa (SEQ ID NO:191)

tcgacggatc gggagatctc ccgatcccct atggtgcact ctcagtacaa tctgctctga 60
tgccgcatag ttaagccagt atctgctccc tgcttgtgtg ttggaggtcg ctgagtagtg 120
cgcgagcaaa atttaagcta caacaaggca aggcttgacc gacaattgtt aattaacatg 180
aagaatctgc ttagggttag gcgttttgcg ctgcttcgct aggtggtcaa tattggccat 240
tagccatatt attcattggt tatatagcat aaatcaatat tggctattgg ccattgcata 300
cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca ttaccgccat 360
gttgacattg attattgact agttattaat agtaatcaat tacggggtca ttagttcata 420
gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc 480
ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag 540
ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac 600
atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg 660
cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg 720
tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat gggcgtggat 780
agcggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt 840
tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc ccattgacgc 900
aaatgggcgg taggcgtgta cggtgggagg tctatataag cagagctcgt ttagtgaacc 960
gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga caccgggacc 1020
gatccagcct ccgcggccgg gaacggtgca ttggaatcga tgactctctt aggtagcctt 1080
gcagaagttg gtcgtgaggc actgggcagg taagtatcaa ggttacaaga caggtttaag 1140
gagatcaata gaaactgggc ttgtcgagac agagaagact cttgcgtttc tgataggcac 1200
ctattggtct tactgacatc cactttgcct ttctctccac aggtgtccac tcccagttca 1260
attacagctc gccaccatgc ggctgcccgc ccagctgctg ggccttctca tgctgtgggt 1320
gcccgcctcg agatctatcg atgcatgcca tggtaccaag cttgccacca tgagcagcag 1380

ctcttggctg ctgctgagcc tggtggccgt gacagccgcc cagagcacca tcgaggagca 1440
ggccaagacc ttcctggaca agttcaacca cgaggccgag gacctgttct accagagcag 1500
cctggccagc tggaactaca acaccaacat caccgaggag aacgtgcaga acatgaacaa 1560
cgccggcgac aagtggagcg ccttcctgaa ggagcagagc acactggccc agatgtaccc 1620
cctgcaggag atccagaacc tgaccgtgaa gctgcagctg caggccctgc agcagaacgg 1680
cagcagcgtg ctgagcgagg acaagagcaa gcggctgaac accatcctga acaccatgtc 1740
caccatctac agcaccggca aagtgtgcaa ccccgacaac ccccaggagt gcctgctgct 1800
ggagcccggc ctgaacgaga tcatggccaa cagcctggac tacaacgagc ggctgtgggc 1860
ctgggagagc tggcggagcg aagtgggcaa gcagctgcgg cccctgtacg aggagtacgt 1920
ggtgctgaag aacgagatgg ccagggccaa ccactacgag gactacggcg actactggag 1980
aggcgactac gaagtgaacg gcgtggacgg ctacgactac agcagaggcc agctgatcga 2040

ggacgtggag cacaccttcg aggagatcaa gcctctgtac gagcacctgc acgcctacgt 2100
gcgggccaag ctgatgaacg cctaccccag ctacatcagc cccatcggct gcctgcccgc 2160
ccacctgctg ggcgacatgt ggggccggtt ctggaccaac ctgtacagcc tgaccgtgcc 2220
cttcggccag aagcccaaca tcgacgtgac cgacgccatg gtggaccagg cctgggacgc 2280
ccagcggatc ttcaaggagg ccgagaagtt cttcgtgagc gtgggcctgc ccaacatgac 2340
ccagggcttt tgggagaaca gcatgctgac cgaccccggc aatgtgcaga aggccgtgtg 2400
ccaccccacc gcctgggacc tgggcaaggg cgacttccgg atcctgatgt gcaccaaagt 2460
gaccatggac gacttcctga ccgcccacca cgagatgggc cacatccagt acgacatggc 2520
ctacgccgcc cagcccttcc tgctgcggaa cggcgccaac gagggctttc acgaggccgt 2580
gggcgagatc atgagcctga gcgccgccac ccccaagcac ctgaagagca tcggcctgct 2640
gagccccgac ttccaggagg acaacgagac cgagatcaac ttcctgctga agcaggccct 2700
gaccatcgtg ggcaccctgc ccttcaccta catgctggag aagtggcggt ggatggtgtt 2760
taagggcgag atccccaagg accagtggat gaagaagtgg tgggagatga agcgggagat 2820
cgtgggcgtg gtggagcccg tgccccacga cgagacctac tgcgaccccg ccagcctgtt 2880
ccacgtgagc aacgactact ccttcatccg gtactacacc cggaccctgt accagttcca 2940
gttccaggag gccctgtgcc aggccgccaa gcacgagggc cccctgcaca agtgcgacat 3000
cagcaacagc accgaggccg gacagaaact gttcaacatg ctgcggctgg gcaagagcga 3060
gccctggacc ctggccctgg agaatgtggt gggcgccaag aacatgaatg tgcgccccct 3120
gctgaactac ttcgagcccc tgttcacctg gctgaaggac cagaacaaga acagcttcgt 3180
gggctggagc accgactgga gcccctacgc cgaccagagc atcaaagtgc ggatcagcct 3240
gaagagcgcc ctgggcgaca aggcctacga gtggaacgac aacgagatgt acctgttccg 3300
gagcagcgtg gcctatgcca tgcggcagta cttcctgaaa gtgaagaacc agatgatcct 3360
gttcggcgag gaggacgtga gagtggccaa cctgaagccc cggatcagct tcaacttctt 3420
cgtgaccgcc cccaagaacg tgagcgacat catcccccgg accgaagtgg agaaggccat 3480
ccggatgagc cggagccgga tcaacgacgc cttccggctg aacgacaact ccctggagtt 3540
cctgggcatc cagcccaccc tgggccctcc caaccagccc cccgtgagca tctggctgat 3600
cgtgtttggc gtggtgatgg gcgtgatcgt ggtgggaatc gtgatcctga tcttcaccgg 3660
catccgggac cggaagaaga agaacaaggc ccggagcggc gagaacccct acgccagcat 3720
cgatatcagc aagggcgaga acaaccccgg cttccagaac accgacgacg tgcagaccag 3780
cttctgataa tctagaacga gctcgaattc gaagcttctg cagacgcgtc gacgtcatat 3840
ggatccgata tcgccgtggc ggccgcaccc agcgtgttca tcttcccccc ctccgacgag 3900
cagctgaaga gcggcaccgc cagcgtggtg tgcctgctga acaacttcta cccccgggag 3960
gccaaggtgc agtggaaggt ggacaacgcc ctgcagagcg gcaacagcca ggagagcgtg 4020
accgagcagg acagcaagga ctccacctac agcctgagca gcaccctcac cctgagcaag 4080
gccgactacg agaagcacaa ggtgtacgcc tgcgaggtga cccaccaggg cctgagcagc 4140
cccgtgacca agagcttcaa ccggggcgag tgttaataga cttaagttta aaccgctgat 4200
cagcctcgac tgtgccttct agttgccagc catctgttgt ttgcccctcc cccgtgcctt 4260
ccttgaccct ggaaggtgcc actcccactg tcctttcctа ataaaatgag gaaattgcat 4320
cgcattgtct gagtaggtgt cattctattc tgggggggtgg ggtggggcag gacagcaagg 4380
gggaggattg ggaagacaat agcaggcatg ctggggatgc ggtgggctct atggcttctg 4440
aggcggaaag aaccagctgg ggctctaggg ggtatcccca cgcgccctgt agcggcgcat 4500
taagcgcggc gggtgtggtg gttacgcgca gcgtgaccgc tacacttgcc agcgccctag 4560
cgcccgctcc tttcgctttc ttcccttcct ttctcgccac gttcgccggc tttccccgtc 4620
aagctctaaa tcgggggctc cctttagggt tccgatttag tgctttacgg cacctcgacc 4680
ccaaaaaact tgattagggt gatggttcac gtagtgggcc atcgccctga tagacggttt 4740
ttcgcccttt gacgttggag tccacgttct ttaatagtgg actcttgttc caaactggaa 4800
caacactcaa ccctatctcg gtctattctt ttgatttata agggattttg gccatttcgg 4860
cctattggtt aaaaaatgag ctgatttaac aaaaatttaa cgcgaattaa ttctgtggaa 4920

tgtgtgtcag ttagggtgtg gaaagtcccc aggctcccca gcaggcagaa gtatgcaaag 4980
catgcatctc aattagtcag caaccaggtg tggaaagtcc ccaggctccc cagcaggcag 5040
aagtatgcaa agcatgcatc tcaattagtc agcaaccata gtcccgcccc taactccgcc 5100
catcccgccc ctaactccgc ccagttccgc ccattctccg ccccatggct gactaatttt 5160
ttttatttat gcagaggccg aggccgcctc tgcctctgag ctattccaga agtagtgagg 5220
aggctttttt ggaggcctag gcttttgcaa aaagctcccg ggagcttgta tatccatttt 5280
cggatctgat cagcacgtga tgaaaaagcc tgaactcacc gcgacgtctg tcgagaagtt 5340
tctgatcgaa aagttcgaca gcgtctccga cctgatgcag ctctcggagg gcgaagaatc 5400
tcgtgctttc agcttcgatg taggagggcg tggatatgtc ctgcgggtaa atagctgcgc 5460
cgatggtttc tacaaagatc gttatgttta tcggcacttt gcatcggccg cgctcccgat 5520
tccggaagtg cttgacattg gggaattcag cgagagcctg acctattgca tctcccgccg 5580

tgcacagggt gtcacgttgc aagacctgcc tgaaaccgaa ctgcccgctg ttctgcagcc 5640
ggtcgcggag gccatggatg cgatcgctgc ggccgatctt agccagacga gcgggttcgg 5700
cccattcgga ccacaaggaa tcggtcaata cactacatgg cgtgatttca tatgcgcgat 5760
tgctgatccc catgtgtatc actggcaaac tgtgatggac gacaccgtca gtgcgtccgt 5820
cgcgcaggct ctcgatgagc tgatgctttg ggccgaggac tgccccgaag tccggcacct 5880
cgtgcacgcg gatttcggct ccaacaatgt cctgacggac aatggccgca taacagcggt 5940
cattgactgg agcgaggcga tgttcgggga ttcccaatac gaggtcgcca acatcttctt 6000
ctggaggccg tggttggctt gtatggagca gcagacgcgc tacttcgagc ggaggcatcc 6060
ggagcttgca ggatcgccgc ggctccgggc gtatatgctc cgcattggtc ttgaccaact 6120
ctatcagagc ttggttgacg gcaatttcga tgatgcagct tgggcgcagg gtcgatgcga 6180
cgcaatcgtc cgatccggag ccgggactgt cgggcgtaca caaatcgccc gcagaagcgc 6240
ggccgtctgg accgatggct gtgtagaagt actcgccgat agtggaaacc gacgccccag 6300
cactcgtccg agggcaaagg aatagcacgt gctacgagat ttcgattcca ccgccgcctt 6360
ctatgaaagg ttgggcttcg gaatcgtttt ccgggacgcc ggctggatga tcctccagcg 6420
cggggatctc atgctggagt tcttcgccca ccccaacttg tttattgcag cttataatgg 6480
ttacaaataa agcaatagca tcacaaattt cacaaataaa gcatttttt cactgcattc 6540
tagttgtggt ttgtccaaac tcatcaatgt atcttatcat gtctgtatac cgtcgacctc 6600
tagctagagc ttggcgtaat catggtcata gctgtttcct gtgtgaaatt gttatccgct 6660
cacaattcca cacaacatac gagccggaag cataaagtgt aaagcctggg gtgcctaatg 6720
agtgagctaa ctcacattaa ttgcgttgcg ctcactgccc gctttccagt cgggaaacct 6780
gtcgtgccag ctgcattaat gaatcggcca acgcgcgggg agaggcggtt tgcgtattgg 6840
gcgctcttcc gcttcctcgc tcactgactc gctgcgctcg gtcgttcggc tgcggcgagc 6900
ggtatcagct cactcaaagg cggtaatacg gttatccaca gaatcagggg ataacgcagg 6960
aaagaacatg tgagcaaaag gccagcaaaa ggccaggaac cgtaaaaagg ccgcgttgct 7020
ggcgtttttc cataggctcc gccccctga cgagcatcac aaaaatcgac gctcaagtca 7080
gaggtggcga aacccgacag gactataaag ataccaggcg tttccccctg gaagctccct 7140
cgtgcgctct cctgttccga ccctgccgct taccggatac ctgtccgcct ttctcccttc 7200
gggaagcgtg gcgctttctc atagctcacg ctgtaggtat ctcagttcgg tgtaggtcgt 7260
tcgctccaag ctgggctgtg tgcacgaacc ccccgttcag cccgaccgct gcgccttatc 7320
cggtaactat cgtcttgagt ccaacccggt aagacacgac ttatcgccac tggcagcagc 7380
cactggtaac aggattagca gagcgaggta tgtaggcggt gctacagagt tcttgaagtg 7440
gtggcctaac tacggctaca ctagaagaac agtatttggt atctgcgctc tgctgaagcc 7500
agttaccttc ggaaaaagag ttggtagctc ttgatccggc aaacaaacca ccgctggtag 7560
cggtttttt gtttgcaagc agcagattac gcgcagaaaa aaaggatctc aagaagatcc 7620
tttgatcttt tctacggggt ctgacgctca gtggaacgaa aactcacgtt aagggatttt 7680
ggtcatgaga ttatcaaaaa ggatcttcac ctagatcctt ttaaattaaa aatgaagttt 7740
taaatcaatc taaagtatat atgagtaaac ttggtctgac agttaccaat gcttaatcag 7800
tgaggcacct atctcagcga tctgtctatt tcgttcatcc atagttgcct gactccccgt 7860
cgtgtagata actacgatac gggagggctt accatctggc cccagtgctg caatgatacc 7920
gcgagaccca cgctcaccgg ctccagattt atcagcaata aaccagccag ccggaagggc 7980
cgagcgcaga agtggtcctg caactttatc cgcctccatc cagtctatta attgttgccg 8040
ggaagctaga gtaagtagtt cgccagttaa tagtttgcgc aacgttgttg ccattgctac 8100
aggcatcgtg gtgtcacgct cgtcgtttgg tatggcttca ttcagctccg gttcccaacg 8160
atcaaggcga gttacatgat cccccatgtt gtgcaaaaaa gcggttagct ccttcggtcc 8220
tccgatcgtt gtcagaagta agttggccgc agtgttatca ctcatggtta tggcagcact 8280
gcataattct cttactgtca tgccatccgt aagatgcttt tctgtgactg gtgagtactc 8340
aaccaagtca ttctgagaat agtgtatgcg gcgaccgagt tgctcttgcc cggcgtcaat 8400
acgggataat accgcgccac atagcagaac tttaaaagtg ctcatcattg gaaaacgttc 8460

ttcggggcga aaactctcaa ggatcttacc gctgttgaga tccagttcga tgtaacccac 8520
tcgtgcaccc aactgatctt cagcatcttt tactttcacc agcgtttctg ggtgagcaaa 8580
aacaggaagg caaaatgccg caaaaaaggg aataagggcg acacggaaat gttgaatact 8640
catactcttc ctttttcaat attattgaag catttatcag ggttattgtc tcatgagcgg 8700
atacatattt gaatgtattt agaaaaataa acaaataggg gttccgcgca catttccccg 8760
aaaagtgcca cctgacg 8777

Vector pIg-C910-Clambda (SEQ ID NO:192)

tcgacggatc gggagatctc ccgatcccct atggtgcact ctcagtacaa tctgctctga 60
tgccgcatag ttaagccagt atctgctccc tgcttgtgtg ttggaggtcg ctgagtagtg 120
cgcgagcaaa atttaagcta caacaaggca aggcttgacc gacaattgtt aattaacatg 180
aagaatctgc ttagggttag gcgttttgcg ctgcttcgct aggtggtcaa tattggccat 240
tagccatatt attcattggt tatatagcat aaatcaatat tggctattgg ccattgcata 300
cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca ttaccgccat 360
gttgacattg attattgact agttattaat agtaatcaat tacggggtca ttagttcata 420
gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc 480
ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag 540
ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac 600
atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg 660
cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg 720
tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat gggcgtggat 780
agcggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt 840
tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc ccattgacgc 900
aaatgggcgg taggcgtgta cggtgggagg tctatataag cagagctcgt ttagtgaacc 960
gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga caccgggacc 1020
gatccagcct ccgcggccgg gaacggtgca ttggaatcga tgactctctt aggtagcctt 1080
gcagaagttg gtcgtgaggc actgggcagg taagtatcaa ggttacaaga caggtttaag 1140
gagatcaata gaaactgggc ttgtcgagac agagaagact cttgcgtttc tgataggcac 1200
ctattggtct tactgacatc cactttgcct ttctctccac aggtgtccac tcccagttca 1260
attacagctc gccaccatgc ggttctccgc tcagctgctg ggccttctgg tgctgtggat 1320
tcccggcgtc tcgagatcta tcgatgcatg ccatggtacc aagcttgcca ccatgagcag 1380
cagctcttgg ctgctgctga gcctggtggc cgtgacagcc gcccagagca ccatcgagga 1440
gcaggccaag accttcctgg acaagttcaa ccacgaggcc gaggacctgt tctaccagag 1500
cagcctggcc agctggaact acaacaccaa catcaccgag gagaacgtgc agaacatgaa 1560
caacgccggc gacaagtgga gcgccttcct gaaggagcag agcacactgg cccagatgta 1620
ccccctgcag gagatccaga acctgaccgt gaagctgcag ctgcaggccc tgcagcagaa 1680
cggcagcagc gtgctgagcg aggacaagag caagcggctg aacaccatcc tgaacaccat 1740
gtccaccatc tacagcaccg gcaaagtgtg caaccccgac aacccccagg agtgcctgct 1800
gctggagccc ggcctgaacg agatcatggc caacagcctg gactacaacg agcggctgtg 1860
ggcctgggag agctggcgga gcgaagtggg caagcagctg cggcccctgt acgaggagta 1920
cgtggtgctg aagaacgaga tggccagggc caaccactac gaggactacg gcgactactg 1980
gagaggcgac tacgaagtga acggcgtgga cggctacgac tacagcagag gccagctgat 2040
cgaggacgtg gagcacacct tcgaggagat caagcctctg tacgagcacc tgcacgccta 2100
cgtgcgggcc aagctgatga acgcctaccc cagctacatc agccccatcg gctgcctgcc 2160
cgcccacctg ctgggcgaca tgtggggccg gttctggacc aacctgtaca gcctgaccgt 2220
gcccttcggc cagaagccca acatcgacgt gaccgacgcc atggtggacc aggcctggga 2280
cgcccagcgg atcttcaagg aggccgagaa gttcttcgtg agcgtgggcc tgcccaacat 2340
gacccagggc ttttgggaga acagcatgct gaccgacccc ggcaatgtgc agaaggccgt 2400
gtgccacccc accgcctggg acctgggcaa gggcgacttc cggatcctga tgtgcaccaa 2460
agtgaccatg gacgacttcc tgaccgccca ccacgagatg ggccacatcc agtacgacat 2520
ggcctacgcc gcccagccct tcctgctgcg gaacggcgcc aacgagggct ttcacgaggc 2580
cgtgggcgag atcatgagcc tgagcgccgc caccccaag cacctgaaga gcatcggcct 2640
gctgagcccc gacttccagg aggacaacga gaccgagatc aacttcctgc tgaagcaggc 2700
cctgaccatc gtgggcaccc tgcccttcac ctacatgctg gagaagtggc ggtggatggt 2760
gtttaagggc gagatcccca aggaccagtg gatgaagaag tggtgggaga tgaagcggga 2820
gatcgtgggc gtggtggagc ccgtgcccca cgacgagacc tactgcgacc ccgccagcct 2880
gttccacgtg agcaacgact actccttcat ccggtactac acccggaccc tgtaccagtt 2940
ccagttccag gaggccctgt gccaggccgc caagcacgag ggcccctgc acaagtgcga 3000
catcagcaac agcaccgagg ccggacagaa actgttcaac atgctgcggc tgggcaagag 3060
cgagccctgg accctggccc tggagaatgt ggtgggcgcc aagaacatga atgtgcgccc 3120

cctgctgaac tacttcgagc ccctgttcac ctggctgaag gaccagaaca agaacagctt 3180
cgtgggctgg agcaccgact ggagccccta cgccgaccag agcatcaaag tgcggatcag 3240
cctgaagagc gccctgggcg acaaggccta cgagtggaac gacaacgaga tgtacctgtt 3300
ccggagcagc gtggcctatg ccatgcggca gtacttcctg aaagtgaaga accagatgat 3360
cctgttcggc gaggaggacg tgagagtggc caacctgaag ccccggatca gcttcaactt 3420
cttcgtgacc gcccccaaga acgtgagcga catcatcccc cggaccgaag tggagaaggc 3480
catccggatg agccggagcc ggatcaacga cgccttccgg ctgaacgaca actccctgga 3540
gttcctgggc atccagccca ccctgggccc tcccaaccag ccccccgtga gcatctggct 3600
gatcgtgttt ggcgtggtga tgggcgtgat cgtggtggga atcgtgatcc tgatcttcac 3660
cggcatccgg gaccggaaga agaagaacaa ggcccggagc ggcgagaacc cctacgccag 3720
catcgatatc agcaagggcg agaacaaccc cggcttccag aacaccgacg acgtgcagac 3780
cagcttctga taatctagaa cgagctcgaa ttcgaagctt ctgcagacgc gtcgacgtca 3840
tatggatccg atatcgccgt ggcggccgca ggccagccca aggccgctcc cagcgtgacc 3900
ctgttccccc cctcctccga ggagctgcag gccaacaagg ccaccctggt gtgcctcatc 3960
agcgacttct accctggcgc cgtgaccgtg gcctggaagg ccgacagcag ccccgtgaag 4020
gccggcgtgg agaccaccac ccccagcaag cagagcaaca acaagtacgc cgccagcagc 4080
tacctgagcc tcacccccga gcagtggaag agccaccgga gctacagctg ccaggtgacc 4140
cacgagggca gcaccgtgga gaagaccgtg gcccccaccg agtgcagcta atagacttaa 4200
gtttaaaccg ctgatcagcc tcgactgtgc cttctagttg ccagccatct gttgtttgcc 4260
cctcccccgt gccttccttg accctggaag gtgccactcc cactgtcctt tcctaataaa 4320
atgaggaaat tgcatcgcat tgtctgagta ggtgtcattc tattctgggg ggtggggtgg 4380
ggcaggacag caagggggag gattgggaag acaatagcag gcatgctggg gatgcggtgg 4440
gctctatggc ttctgaggcg gaaagaacca gctggggctc taggggtat ccccacgcgc 4500
cctgtagcgg cgcattaagc gcggcgggtg tggtggttac gcgcagcgtg accgctacac 4560
ttgccagcgc cctagcgccc gctcctttcg ctttcttccc ttcctttctc gccacgttcg 4620
ccggctttcc ccgtcaagct ctaaatcggg ggctcccttt agggttccga tttagtgctt 4680
tacggcacct cgaccccaaa aaacttgatt agggtgatgg ttcacgtagt gggccatcgc 4740
cctgatagac ggtttttcgc cctttgacgt tggagtccac gttctttaat agtggactct 4800
tgttccaaac tggaacaaca ctcaacccta tctcggtcta ttcttttgat ttataaggga 4860
ttttggccat ttcggcctat tggttaaaaa atgagctgat ttaacaaaaa tttaacgcga 4920
attaattctg tggaatgtgt gtcagttagg gtgtggaaag tccccaggct ccccagcagg 4980
cagaagtatg caaagcatgc atctcaatta gtcagcaacc aggtgtggaa agtccccagg 5040
ctccccagca ggcagaagta tgcaaagcat gcatctcaat tagtcagcaa ccatagtccc 5100
gcccctaact ccgcccatcc cgcccctaac tccgcccagt tccgcccatt ctccgcccca 5160
tggctgacta attttttta tttatgcaga ggccgaggcc gcctctgcct ctgagctatt 5220
ccagaagtag tgaggaggct tttttggagg cctaggcttt tgcaaaaagc tcccgggagc 5280
ttgtatatcc attttcggat ctgatcagca cgtgatgaaa aagcctgaac tcaccgcgac 5340
gtctgtcgag aagtttctga tcgaaaagtt cgacagcgtc tccgacctga tgcagctctc 5400
ggagggcgaa gaatctcgtg ctttcagctt cgatgtagga gggcgtggat atgtcctgcg 5460
ggtaaatagc tgcgccgatg gtttctacaa agatcgttat gtttatcggc actttgcatc 5520
ggccgcgctc ccgattccgg aagtgcttga cattggggaa ttcagcgaga gcctgaccta 5580
ttgcatctcc cgccgtgcac agggtgtcac gttgcaagac ctgcctgaaa ccgaactgcc 5640
cgctgttctg cagccggtcg cggaggccat ggatgcgatc gctgcggccg atcttagcca 5700
gacgagcggg ttcggcccat tcggaccgca aggaatcggt caatacacta catggcgtga 5760
tttcatatgc gcgattgctg atccccatgt gtatcactgg caaactgtga tggacgacac 5820
cgtcagtgcg tccgtcgcgc aggctctcga tgagctgatg ctttgggccg aggactgccc 5880
cgaagtccgg cacctcgtgc acgcggattt cggctccaac aatgtcctga cggacaatgg 5940
ccgcataaca gcggtcattg actggagcga ggcgatgttc ggggattccc aatacgaggt 6000
cgccaacatc ttcttctgga ggccgtggtt ggcttgtatg gagcagcaga cgcgctactt 6060
cgagcggagg catccggagc ttgcaggatc gccgcggctc cgggcgtata tgctccgcat 6120
tggtcttgac caactctatc agagcttggt tgacggcaat ttcgatgatg cagcttgggc 6180
gcagggtcga tgcgacgcaa tcgtccgatc cggagccggg actgtcgggc gtacacaaat 6240
cgcccgcaga agcgcggccg tctggaccga tggctgtgta gaagtactcg ccgatagtgg 6300
aaaccgacgc cccagcactc gtccgagggc aaaggaatag cacgtgctac gagatttcga 6360
ttccaccgcc gccttctatg aaaggttggg cttcggaatc gttttccggg acgccggctg 6420
gatgatcctc cagcgcgggg atctcatgct ggagttcttc gcccacccca acttgtttat 6480
tgcagcttat aatggttaca aataaagcaa tagcatcaca aatttcacaa ataaagcatt 6540
tttttcactg cattctagtt gtggtttgtc caaactcatc aatgtatctt atcatgtctg 6600
tataccgtcg acctctagct agagcttggc gtaatcatgg tcatagctgt ttcctgtgtg 6660
aaattgttat ccgctcacaa ttccacacaa catacgagcc ggaagcataa agtgtaaagc 6720

ctggggtgcc taatgagtga gctaactcac attaattgcg ttgcgctcac tgcccgcttt 6780
ccagtcggga aacctgtcgt gccagctgca ttaatgaatc ggccaacgcg cggggagagg 6840
cggtttgcgt attgggcgct cttccgcttc ctcgctcact gactcgctgc gctcggtcgt 6900
tcggctgcgg cgagcggtat cagctcactc aaaggcggta tacggttat ccacagaatc 6960
aggggataac gcaggaaaga acatgtgagc aaaaggccag caaaaggcca ggaaccgtaa 7020
aaaggccgcg ttgctggcgt ttttccatag gctccgcccc cctgacgagc atcacaaaaa 7080
tcgacgctca agtcagaggt ggcgaaaccc gacaggacta taaagatacc aggcgtttcc 7140
ccctggaagc tccctcgtgc gctctcctgt tccgaccctg ccgcttaccg gatacctgtc 7200
cgcctttctc ccttcgggaa gcgtggcgct ttctcatagc tcacgctgta ggtatctcag 7260
ttcggtgtag gtcgttcgct ccaagctggg ctgtgtgcac gaacccccgg ttcagcccga 7320
ccgctgcgcc ttatccggta actatcgtct tgagtccaac ccggtaagac acgacttatc 7380
gccactggca gcagccactg gtaacaggat tagcagagcg aggtatgtag gcggtgctac 7440
agagttcttg aagtggtggc ctaactacgg ctacactaga agaacagtat ttggtatctg 7500
cgctctgctg aagccagtta ccttcggaaa aagagttggt agctcttgat ccggcaaaca 7560
aaccaccgct ggtagcggtt tttttgtttg caagcagcag attacgcgca gaaaaaaagg 7620
atctcaagaa gatcctttga tcttttctac ggggtctgac gctcagtgga acgaaaactc 7680
acgttaaggg attttggtca tgagattatc aaaaaggatc ttcacctaga tccttttaaa 7740
ttaaaaatga agttttaaat caatctaaag tatatatgag taaacttggt ctgacagtta 7800
ccaatgctta atcagtgagg cacctatctc agcgatctgt ctatttcgtt catccatagt 7860
tgcctgactc cccgtcgtgt agataactac gatacgggag ggcttaccat ctggccccag 7920
tgctgcaatg ataccgcgag acccacgctc accggctcca gatttatcag caataaacca 7980
gccagccgga agggccgagc gcagaagtgg tcctgcaact ttatccgcct ccatccagtc 8040
tattaattgt tgccgggaag ctagagtaag tagttcgcca gttaatagtt tgcgcaacgt 8100
tgttgccatt gctacaggca tcgtggtgtc acgctcgtcg tttggtatgg cttcattcag 8160
ctccggttcc caacgatcaa ggcgagttac atgatccccc atgttgtgca aaaaagcggt 8220
tagctccttc ggtcctccga tcgttgtcag aagtaagttg gccgcagtgt tatcactcat 8280
ggttatggca gcactgcata attctcttac tgtcatgcca tccgtaagat gcttttctgt 8340
gactggtgag tactcaacca agtcattctg agaatagtgt atgcggcgac cgagttgctc 8400
ttgcccggcg tcaatacggg ataataccgc gccacatagc agaactttaa aagtgctcat 8460
cattggaaaa cgttcttcgg ggcgaaaact ctcaaggatc ttaccgctgt tgagatccag 8520
ttcgatgtaa cccactcgtg cacccaactg atcttcagca tcttttactt tcaccagcgt 8580
ttctgggtga gcaaaaacag gaaggcaaaa tgccgcaaaa aagggaataa gggcgacacg 8640
gaaatgttga atactcatac tcttcctttt tcaatattat tgaagcattt atcagggtta 8700
ttgtctcatg agcggataca tatttgaatg tatttagaaa aataaacaaa taggggttcc 8760
gcgcacattt ccccgaaaag tgccacctga cg 8792

Sequence of immature HA from A/Hong Kong/1/1968 (SEQ ID NO:193)

MKTIIALSYIFCLALGQDLPGNDNSTATLCLGHHAVPNGTLVKTITDDQIEVTNATELV
QSSSTGKICNNPHRILDGIDCTLIDALLGDPHCDVFQNETWDLFVERSKAFSNCYPYDV
PDYASLRSLVASSGTLEFITEGFTWTGVTQNGGSNACKRGPG
SGFFSRLNWLTKSGSTYPVLNVTMPNNDNFDKLYIWGVHHPSTNQEQTSLYVQASGR
VTVSTRRSQQTIIPNIGSRPWVRGLSSRISIYWTIVKPGDVLVINSNGNLIAPRGYFKMRT
GKSSIMRSDAPIDTCISECITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKLATGMRN
VPEKQTRGLFGAIAGFIENGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGK
LNRVIEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLT
DSEMNKLFEKTRRQLRENAEDMGNGCFKIYHKCDNACIESIRNGTYDHDVYRDEALN
NRFQIKGVELKSGYKDWILWISFAISCFLLCVVLLGFIMWACQRGNIRCNICI

REFERENCES

Air MA (1981), Sequence relationships among the hemagglutinin genes of 12 subtypes of influenza A virus. Proc Natl Acad Sci USA 78(12):7639-7643.

Boel E et al. (2000), Functional human monoclonal antibodies of all isotypes constructed from phage display library-derived single-chain Fv antibody fragments. J Immunol Methods 239:153-166.

Brown EG et al. (2001), Pattern of mutation in the genome of influenza A virus on adaptation to increased virulence in the mouse lung: Identification of functional themes. PNAS 98:6883-6888.

Burton DR and Barbas CF (1994), Human antibodies from combinatorial libraries. Adv Immunol. 57:191-280.

Chou TC and P Talalay (1984), Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22:27-55.

De Kruif J et al. (1995a), Rapid selection of cell subpopulation-specific human monoclonal antibodies from a synthetic phage antibody library. Proc Natl Acad Sci USA 92:3938.

De Kruif J et al. (1995b), Selection and application of human single-chain Fv antibody fragments from a semi-synthetic phage antibody display library with designed CDR3 regions. J Mol Biol 248:97-105.

Ekiert et al. (2009), Antibody recognition of a highly conserved influenza virus epitope. Science 324:246-251.

Fouchier AM et al. (2005), Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J Virol 79(5):2814-2822.

Gocník M. et al. (2007), Antibodies specific to the HA2 glycopolypeptide of influenza A virus haemagglutinin with fusion-inhibition activity contribute to the protection of kice against lethal infection, J Gen Virol 88: 951-955.

Huls G et al. (1999), Antitumor immune effector mechanisms recruited by phage display-derived fully human IgG1 and IgA1 monoclonal antibodies. Cancer Res 59:5778-5784.

Östberg L and Pursch E (1983), Human x (Mouse x Human) hybridomas stably producing human antibodies. Hybridoma 2(4): 361-367.

Slootstra JW et al. (1996), Structural aspects of antibody-antigen interaction revealed through small random peptide libraries. Mol Divers 1:87-96.

Stropkovská A et al. (2009), Broadly cross-reactive monoclonal antibodies against HA2 glycopeptide of influenza A virus hemagglutinin of H3 subtype reduce replication of influenza A viruses of human and avian origin. Acta Virologica 53: 15-20.

The World Health Organization Global Influenza Program Surveillance Network (2005), Evolution of H5N1 Avian Influenza Viruses in Asia. Emerg Infect Dis 11:1515-1521.

Varečková E. et al. (2003a), Inhibition of fusion activity of influenza A haemagglutinin mediated by HA2-specific monoclonal antibodies. Arch Virol 148: 469-486.

Varečková E. et al. (2003b), A monoclonal antibody specific to the HA2 glycoprotein of influenza A virus haemagglutinin that inhibits its fusion activity reduces replication of the virus. Acta Virologica 47: 229-236.

Wang T. et al. (2010), Boradly protective monoclonal antibodies against H3 influenza viruses following sequential immunization with different hemagglutinins. PLoS Pathogens 6(2): 1-9.

CLAIMS

1. An isolated human binding molecule capable of specifically recognizing and binding to an epitope in influenza haemagglutinin protein (HA), having neutralizing activity against influenza viruses comprising HA of the H3 subtype, such as H3N2, and having cross-neutralizing activity against at least an influenza virus comprising HA of the H7 subtype, and/or an influenza virus comprising HA of the H10 subtype, wherein the binding molecule binds to an epitope comprising the amino acid at position 19, 25, 27, 33 and/or 34 of the HA2 polypeptide of the H3 HA protein, wherein the binding molecule is selected from the group consisting of:
   a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:109, a heavy chain CDR2 region of SEQ ID NO:110, and a heavy chain CDR3 region of SEQ ID NO:111; and a light chain CDR1 region of SEQ ID NO: 112, a light chain CDR2 region of SEQ ID NO: 113 and a light chain CDR3 region of 114,
   a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:138, a heavy chain CDR2 region of SEQ ID NO:139, and a heavy chain CDR3 region of SEQ ID NO:140, and a light chain CDR1 region of SEQ ID NO: 141, a light chain CDR2 region of SEQ ID NO: 142 and a light chain CDR3 region of 143,
   a binding molecule comprising a heavy chain CDR1 region of SEQ ID NO:144, a heavy chain CDR2 region of SEQ ID NO:145, and a heavy chain CDR3 region of SEQ ID NO:146; and a light chain CDR1 region of SEQ ID NO: 147, a light chain CDR2 region of SEQ ID NO: 148 and a light chain CDR3 region of 149.

2. An isolated binding molecule according to claim 1, capable of specifically recognizing and binding to an epitope in influenza haemagglutinin protein (HA), having neutralizing activity against influenza viruses comprising HA of the H3 subtype, such as H3N2, characterized in that said binding molecule has at least neutralizing activity against one or more of the H3N2 strains selected from the group consisting of A/Wisconsin/67/2005, A/Hiroshima/52/2005, A/Panama/2007/99, and A/Johannesburg/33/94.

3. A binding molecule according to claim 2, wherein said binding molecule further has neutralizing activity against the H3N2 strain A/Hong Kong/1/68.

4. A binding molecule according to any one of claims 1-3, wherein the binding molecule is capable of preventing *in vitro* the trypsin cleavage of the H3 HA precursor molecule HA0 in HA1 and HA2.

5. A binding molecule according to any one of claims 1-4, wherein the binding molecule is capable of preventing the conformational change of the H3 HA protein required for fusion of the viral membrane with the endosomal membrane of an infected cell.

6. A binding molecule according to any one of claims 1-5, wherein the binding molecule is not capable of binding to and neutralizing influenza virus A comprising HA of the H1 subtype, such as H1N1.

7. A binding molecule according to any one of claims 1-6, wherein said binding molecule is a human monoclonal antibody.

8. A binding molecule according to any one of claims 1-7 for use as a medicament.

9. A binding molecule according to any one of claims 1- 8 for use as a medicament in the diagnostic, therapeutic and/or prophylactic treatment of influenza infection caused by influenza virus comprising HA of the H3 subtype, such as H3N2.

10. An immunoconjugate comprising a binding molecule according to any one of the claims 1-9, the immunoconjugate further comprising at least one tag.

11. A pharmaceutical composition comprising a binding molecule according to any one of claims 1-9, and/or an immunoconjugate according to claim 10 and a pharmaceutically acceptable excipient.

12. Use of a binding molecule according to any one of claims 1-9, and/or an immunoconjugate according to claim 10 in the preparation of a medicament for the diagnosis, prophylaxis, and/or treatment of an influenza virus infection.

13. Use according to claim 12 characterized in that of influenza infection is caused by influenza virus comprising HA of the H3 subtype H3, such as H3N2.

14. A pharmaceutical composition according to claim 11 comprising at least one additional binding molecule.

15. A pharmaceutical composition according to claim 14 characterized in that the additional binding molecule is capable of neutralizing influenza virus comprising HA of the H1 and H5 subtype, such as H1N1 and H5N1, or a functional variant thereof.

16. A nucleic acid molecule encoding a binding molecule according to any one of the claims 1-9.

17. A vector comprising at least one nucleic acid molecule according to claim 16.

18. A host comprising at least one vector according to claim 17.

19. A host according to claim 18, wherein the host is a human cell.

20. A method of producing a binding molecule according to any one of claims 1-9, wherein the method comprises the steps of:
culturing a host according to claim 18 or 19 under conditions conducive to the expression of the binding molecule or functional variant, and optionally,
recovering the expressed binding molecule or functional variant.