TITLE OF THE INVENTION

Human binding molecules capable of binding to and neutralizing influenza B viruses and uses thereof

FIELD OF THE INVENTION

The invention relates to medicine. The invention in particular relates to human binding molecules, e.g. monoclonal antibodies or antigen-binding fragments thereof, capable of binding to and neutralizing influenza B viruses, in particular neutralizing binding molecules binding to and neutralizing influenza B viruses from both the B/Yamagata and/or B/Victoria lineage. In addition, the invention relates to the diagnosis, prophylaxis and/or treatment of infections caused by an influenza B virus, in particular of infections caused by influenza B viruses from the B/Yamagata and B/Victoria lineages.

BACKGROUND OF THE INVENTION

Influenza infection (also referred to as "influenza" or "the flu") 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)). There are 3 types of influenza flu virus (types A, B and C) responsible for infectious pathologies in humans and animals. Currently, the type A and type B viruses are the agents responsible for the influenza epidemics and pandemics observed in humans.

Current approaches to dealing with annual influenza epidemics include annual vaccination, preferably generating heterotypic cross-protection. However, 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. Alternatively, antiviral drugs, such as oseltamivir (Tamiflu®) can be effective for prevention and treatment of influenza infection. The number of influenza virus strains showing resistance against antiviral drugs such as oseltamivir is, however, increasing.

An alternative approach is the development of antibody-based prophylactic or therapeutic means to neutralize various seasonal influenza viruses.

Broadly cross-neutralizing antibodies recognizing epitopes in the conserved stem-region of HA of influenza A viruses of phylogenetic group 1 (such as influenza viruses comprising HA of the H1 or H5 subtype) have recently been disclosed (e.g. CR6261, see WO2008/028946), as well as cross-neutralizing antibodies recognizing a highly conserved epitope in the stem-region of HA of influenza A viruses of phylogenetic group 2, such as influenza viruses comprising HA of the H3 and/or H7 subtypes (e.g. CR8020, CR8043; see WO 2010/130636). More recently, antibodies capable of binding to and neutralizing influenza A viruses of both phylogenetic group 1 and group 2, as well as influenza B viruses were discovered (e.g. CR9114, described in application no. EP11173953.8).

To date, less attention has been paid to influenza B viruses. This may be due to the fact that – primarily being restricted to humans as host - influenza B viruses lack the large animal reservoirs that are key to the emergence of pandemic influenza A strains. However, the cumulative impact of annual epidemics during interpandemic periods exceeds that of pandemics and although the morbidity and mortality rates attributable to influenza B are lower than those of e.g. H3N2 viruses, they are higher than those of H1N1 viruses (Thompson (2003), Thompson (2004).

The evolution of influenza B viruses is characterized by co-circulation of antigenically and genetically distinct lineages for extended periods of time. Two lineages, represented by the prototype viruses B/Victoria/2/87 (Victoria lineage) and B/Yamagata/16/88 (Yamagata lineage), are currently distinguished (Kanegae (1990), Rota (1990)). B/Yamagata was the major lineage circulating until the 1980s, when B/Victoria lineage viruses appeared. Since then, drift variants of both influenza B lineages have been co-circulating globally, with both lineages concurrently circulating in recent influenza seasons.

Given the fact that influenza B viruses are the major cause of seasonal influenza epidemics every 2 – 4 years, and in view of the severity of the respiratory illness caused by certain influenza B viruses, as well has the high economic impact of the seasonal epidemics, there is an ongoing need for alternative and effective means for the prevention

and treatment influenza B subtypes. There is thus a need for binding molecules, preferably broadly neutralizing human binding molecules, capable of cross-neutralizing influenza B viruses.

SUMMARY OF THE INVENTION

The invention provides binding molecules, in particular human binding molecules, capable of specifically binding to and neutralizing influenza B virus strains from both the B/Yamagata and B/Victoria lineages. The binding molecules do not bind to influenza A virus subtypes.

The invention also pertains to immunoconjugates and/or pharmaceutical compositions comprising the binding molecules, as well as to nucleic acid molecules encoding at least the binding region of the human binding molecules.

The binding molecules, immunoconjugates and/or nucleic acid molecules of the invention are suitable for use as a universal prophylactic, diagnostic and/or treatment agent for influenza B viruses, irrespective of the causative influenza B virus subtype.

DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic epitope map based on competition experiments. Anti-influenza B antibodies identified in the invention cluster into 4 groups based on binding/competition to influenza B HA.

FIG. 2 shows the results of an immunofluorescence entry assay designed to analyze the ability of the binding molecules to block receptor binding and internalization of the influenza virus. **A**. Inhibition of viral entry by immune-fluorescence read-out; **B**. Infection of MDCK cells with B/Florida/04/2006.

FIG. 3 shows inhibition of viral egress by the binding molecule of the invention.

FIG. 4 shows the results of scanning EM of influenza B infected cells.

FIG. 5 shows in vivo protection by CR8033 against lethal influenza B infection (B/Florida/04/2006 and B/Malaysia/2506/2004) in mice.

DESCRIPTION OF THE INVENTION

Definitions of terms as used in the present 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. HA. 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 nucleic acid molecule or 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 nucleic acid molecule or binding molecule. A functional variant of a binding molecule according to the invention is capable of competing for binding to the binding partner, i.e. the influenza virus, 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 also other classifications of amino acid residue families than the one used above can 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" in relation to influenza A viruses refers to influenza A virus variants that are characterized by various combinations of the hemagglutinin (H) and neuramidase (N) viral surface proteins. Influenza A virus subtypes may be referred to by their H number, such as for example "influenza virus comprising HA of the H1 or H3 subtype", or "H1 influenza virus" "H3 influenza virus", or by a combination of an H number and an N number, such as for example "influenza virus subtype H3N2" or "H3N2". The term influenza virus "subtype" specifically includes all individual influenza virus "strains" within each subtype, which usually result from mutations and show different pathogenic profiles. Such strains may also be referred to as various "isolates" of a viral subtype. Accordingly, as used herein, the terms "strains" and "isolates" may be used interchangeably.

The term "neutralizing" as used herein in relation to the binding molecule of the invention refers to a binding molecule that inhibits an influenza virus from replication, in vitro and/or within a subject, 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 attachment of the virus to the target cell, or by inhibiting viral egress from infected cells, 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 B viruses from both the B/Yamagata and the B/Victoria lineage, and/or different influenza B virus strains within these lineages.

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 molecule of the invention and include B-cells that originally express these binding molecule 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 germ line 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.

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 other binding partners. 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 or compound 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 analogue, 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 used dosages and concentrations, and is compatible with other ingredients of the formulation comprising the drug, agent or binding molecule. Pharmaceutically acceptable excipients are widely applied and known 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 an influenza B virus. Ameloriation as used in herein may refer to the reduction of visible or perceptible disease symptoms, viremia, 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 as well as those in which infection with influenza virus is to be prevented. Subjects partially or totally recovered from infection with influenza virus might also be in need of treatment. Prevention encompasses inhibiting or reducing the spread of influenza virus or inhibiting or reducing the onset, development or progression of one or more of the symptoms associated with infection with influenza virus.

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.

DETAILED DESCRIPTION

In a first aspect the present invention provides binding molecules capable of specifically binding to hemagglutinin (HA) of influenza B virus strains of the B/Yamagata and B/Victoria lineage, and capable of neutralizing said influenza B virus strains of the B/Yamagata and/or B/Victoria lineage. According to the invention, the binding molecules do not bind to HA of influenza A viruses. The binding molecules are capable of neutralizing influenza B viruses both *in vitro* and *in vivo*.

Preferably, the binding molecules are human binding molecules. In a preferred embodiment, the binding molecules are human antibodies, or antigen-binding fragments thereof.

In certain embodiments, the binding molecules bind to a different epitope as compared to the epitope of CR9114 (as described in the co-pending application EP11173953.8), comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 116, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 117. CR9114 has been shown to be capable of binding to

and *in vivo* neutralizing influenza A viruses of both phylogenetic group 1 and 2, as well as influenza B viruses.

In certain embodiments, the binding molecules bind to the head region of the HA protein of influenza B viruses, in particular to the head region of HA1 of influenza B viruses.

In certain embodiments, the binding molecules block the cellular receptor binding of influenza B viruses of the B/Yamagata lineage and/or the B/Victoria lineage.

In certain embodiments, the binding molecules do not block the cellular receptor binding of influenza viruses of the B/Yamagata lineage and/or the B/Victoria lineage.

In certain embodiments, the binding molecules block egress of influenza B viruses, in particular of influenza virus strains of both the B/Victoria and the B/Yamagata lineage, from infected cells.

In certain embodiments, the isolated binding molecules are capable of specifically binding to the hemagglutinin protein (HA) of an influenza B virus and capable of neutralizing influenza B virus strains of both the B/Victoria/2/87 lineage and the B/Yamagata/16/88 lineage, wherein the binding molecules do not bind to the HA protein of influenza A virus subtypes, and comprise a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 71 or a sequence of amino acids having at least or at least about 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity thereto.

In certain embodiments, the binding molecules comprise a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73, or a sequence of amino acids having at least or at least about 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity thereto.

In an embodiment, the binding molecule comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 71 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 73.

The invention also provides binding molecules capable of specifically binding to the hemagglutinin protein (HA) of an influenza B virus and capable of neutralizing influenza B virus strains of both the B/Victoria/2/87 lineage and the B/Yamagata/16/88 lineage, wherein the binding molecules do not bind to the HA protein of influenza A

virus subtypes, and wherein the binding molecules comprise a heavy chain CDR1, comprising the sequence of amino acid residues set forth in SEQ ID NO:1; a heavy chain CDR2, comprising the sequence of amino acid residues set forth in SEQ ID NO:2, and a heavy chain CDR3, comprising the sequence of amino acid residues set forth in SEQ ID NO:3. According to the invention, CDR regions are according to Kabat *et al.* (1991) as described in Sequences of Proteins of Immunological Interest.

In certain embodiments, the binding molecules comprise a light chain CDR1, comprising the sequence of amino acid residues set forth in SEQ ID NO: 4, a light chain CDR2, comprising the sequence of amino acid residues set forth in SEQ ID NO: 5, and a light chain CDR3, comprising the sequence of amino acid residues set forth in SEQ ID NO: 6.

In certain embodiments, the binding molecule comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 2, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 3, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 5, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6.

The invention further provides binding molecules that immunospecifically bind to the same epitope on an influenza B virus HA protein as a binding molecule, comprising a heavy chain variable sequence comprising the amino acid sequence of SEQ ID NO: 71 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 73.

In certain embodiments, the binding molecule comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 15, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 16, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 17, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 18, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:19.

In certain embodiments, the binding molecule comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a heavy chain CDR2 comprising

the amino acid sequence of SEQ ID NO: 27, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 24, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:30.

The invention also provides binding molecules, capable of specifically binding to the hemagglutinin protein (HA) and capable of neutralizing influenza B virus strains of the B/Victoria lineage, in particular the influenza B virus strain B/Malaysia/2506/2004, when the amino acid on position 168 of HA of the influenza B virus, in particular the influenza B virus strain B/Malaysia/2506/2004, is proline (P), and is not capable of neutralizing influenza B virus strains of the B/Victoria lineage, in particular B/Malaysia/2506/2004, when the amino acid on position 168 of the HA of the influenza B virus, in particular B/Malaysia/2506/2004, is glutamine (Q).

In certain embodiments, the invention provides binding molecules, capable of specifically binding to the hemagglutinin protein (HA) and capable of neutralizing influenza B virus strains of the B/Yamagata lineage, in particular the influenza B virus strain B/Florida/04/2006, when the amino acid on position 38 of HA of the influenza B virus, in particular the influenza B virus strain B/Florida/04/200, is lysine (K), and is also capable of neutralizing influenza B virus strains of the B/Yamagata lineage, in particular B/Florida/04/2006, when the amino acid on position 38 of HA of the influenza B virus, in particular B/Florida/04/2006, is glutamic acid (E).

The invention further provides binding molecules that are capable of specifically binding to the hemagglutinin protein (HA) of an influenza B virus and capable of neutralizing influenza B virus strains of both the B/Victoria/2/87 lineage and the B/Yamagata/16/88 lineage, and do not bind to the HA protein of influenza A virus subtypes, and comprise a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 75, or a sequence of amino acids having at least or at least about 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity thereto.

In certain embodiments, the binding molecules comprise a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 77, or a sequence of

amino acids having at least or at least about 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity thereto.

In certain embodiments, the binding molecules comprise heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 75 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77.

In certain embodiments, the binding molecule comprises a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 78 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 79.

In certain embodiments, the invention provides binding molecules capable of specifically binding to the hemagglutinin protein (HA) of an influenza B virus and capable of neutralizing influenza B virus strains of both the B/Victoria/2/87 lineage and the B/Yamagata/16/88 lineage, wherein the binding molecules do not bind to the HA protein of influenza A virus subtypes, and wherein the binding molecules comprise a heavy chain CDR1, comprising the sequence of amino acid residues set forth in SEQ ID NO: 7; a heavy chain CDR2, comprising the sequence of amino acid residues set forth in SEQ ID NO: 8, and a heavy chain CDR3, comprising the sequence of amino acid residues set forth in SEQ ID NO:9.

In certain embodiments, the binding molecules comprise a light chain CDR1, comprising the sequence of amino acid residues set forth in SEQ ID NO: 10, a light chain CDR2, comprising the sequence of amino acid residues set forth in SEQ ID NO: 11, and a light chain CDR3, comprising the sequence of amino acid residues set forth in SEQ ID NO: 12 or 13.

In certain embodiments, the binding molecule comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 7, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 8, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:12.

In certain embodiments, the binding molecule comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 7, a heavy chain CDR2 comprising

the amino acid sequence of SEQ ID NO: 8, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:13.

The invention further provides binding molecules that immunospecifically bind to the same epitope on an influenza B virus HA protein as a binding molecule, comprising a heavy chain variable sequence comprising the amino acid sequence of SEQ ID NO: 75 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77.

In certain embodiments, the binding molecule comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 20, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 21, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 22, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 24, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:25.

In certain embodiment, the binding molecule comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 31, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 32, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 33, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 5, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:34.

In certain embodiments, the invention provides binding molecules, capable of specifically binding to the hemagglutinin protein (HA) and capable of neutralizing influenza B virus strains of the B/Victoria lineage, in particular the influenza B virus strain B/Malaysia/2506/2004, when the amino acid on position 168 of HA of the influenza B virus, in particular the influenza B virus strain B/Malaysia/2506/2004, is proline (P), and also capable of neutralizing influenza B virus strains of the B/Victoria lineage, in particular B/Malaysia/2506/2004, when the amino acid on position 168 of the HA of the influenza B virus, in particular B/Malaysia/2506/2004, is glutamine (Q).

In certain embodiments, the invention provides binding molecules, capable of specifically binding to the hemagglutinin protein (HA) and capable of neutralizing influenza B virus strains of the B/Yamagata lineage, in particular the influenza B virus strain B/Florida/04/2006, when the amino acid on position 38 of HA of the influenza B virus, in particular the influenza B virus strain B/Florida/04/200, is lysine (K), and not capable of neutralizing influenza B virus strains of the B/Yamagata lineage, in particular B/Florida/04/2006, when the amino acid on position 38 of HA of the influenza B virus, in particular B/Florida/04/2006, is glutamic acid (E).

The present invention further provides binding molecules, capable of specifically binding to the hemagglutinin protein (HA) of an influenza B virus and capable of neutralizing influenza B virus strains of both the B/Victoria/2/87 lineage and the B/Yamagata/16/88 lineage, wherein the binding molecules do not bind to the HA protein of influenza A virus subtypes, and wherein the binding molecules comprise a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 113 or a sequence of amino acids having at least or at least about 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity thereto.

In certain embodiments, the binding molecules comprise a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 115, or a sequence of amino acids having at least or at least about 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity thereto.

In an embodiment, the binding molecule comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 113 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 115.

In certain embodiments, the invention provides binding molecules capable of specifically binding to the hemagglutinin protein (HA) of an influenza B virus and capable of neutralizing influenza B virus strains of both the B/Victoria/2/87 lineage and the B/Yamagata/16/88 lineage, wherein the binding molecules do not bind to the HA protein of influenza A virus subtypes, and wherein the binding molecules comprise a heavy chain CDR1, comprising the sequence of amino acid residues set forth in SEQ ID NO:54; a heavy chain CDR2, comprising the sequence of amino acid residues set forth in

SEQ ID NO:55 and a heavy chain CDR3, comprising the sequence of amino acid residues set forth in SEQ ID NO:56.

In certain embodiments, the binding molecules comprise a light chain CDR1, comprising the sequence of amino acid residues set forth in SEQ ID NO: 57, a light chain CDR2, comprising the sequence of amino acid residues set forth in SEQ ID NO: 5, and a light chain CDR3, comprising the sequence of amino acid residues set forth in SEQ ID NO: 58.

In certain embodiment, the binding molecule comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 54, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 56, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 57 a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 5, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:58.

The invention further provides binding molecules that immunospecifically bind to the same epitope on an influenza B virus HA protein as a binding molecule, comprising a heavy chain variable sequence comprising the amino acid sequence of SEQ ID NO: 113 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 115.

Influenza B viruses, like influenza A 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 both influenza A and B viruses 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 which 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.

In certain embodiments, the binding molecules are capable of specifically binding to the HA1 subunit of the HA protein, in particular to the head region of the HA1 subunit. The binding molecules may be capable of specifically binding to linear or structural and/or conformational epitopes on the HA1 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.

The binding molecules of the invention may be capable of specifically binding to influenza B viruses that are viable, living and/or infective or that are in inactivated/attenuated form. Methods for inactivating/attenuating virus, e.g. influenza viruses 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 B viruses, such as *inter alia* a preparation of one or more proteins and/or (poly)peptides, derived from subtypes of influenza B viruses or one or more recombinantly produced proteins and/or polypeptides of influenza B viruses. The nucleotide and/or amino acid sequence of proteins of various influenza B 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 can be intact immunoglobulin molecules such as monoclonal antibodies, or the binding molecules can be antigen-binding

fragments thereof, including, but not limited to, heavy and light chain variable regions, 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 strains or a fragment thereof. In a preferred embodiment the binding molecules of the invention are human monoclonal antibodies, and/or antigen-binding fragments thereof. The binding molecules may also be nanobodies, alphabodies, affibodies, FN3-domain scaffolds and other scaffolds based on domains in (human) repeat proteins, like Adnectins, Anticalins, Darpins, etc, or other scaffolds comprising epitope binding sequences.

In certain embodiments, the binding molecules are intact antibodies comprising complete heavy and light chain variable regions as well as complete heavy and light chain constant regions.

In certain embodiments, the binding molecules have complement-dependent cytotoxic activity (CDC) and/or antibody-dependent cell-mediated cytotoxic (ADCC) activity.

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, and one or more other binding molecules that bind to influenza and have influenza virus inhibiting effect. In other words, the binding molecules can be used in combination, *e.g.*, as a pharmaceutical composition comprising two or more binding molecules, 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 may also comprise at least one binding molecule according to the invention and 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 infection

Typically, binding molecule according to the invention can bind to its binding partners, i.e. an influenza B virus of the B/Yamagata and/or B/Victoria lineage, and/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).

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.

Typically, the binding molecules according to the invention have a neutralizing activity of 50 µg/ml or less, preferably 20 µg/ml or less, more preferably a neutralizing activity of 10 µg/ml or less, even more preferably 5 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 or a fragment thereof in soluble form such as for instance in a sample or in suspension or may bind to influenza viruses or fragments 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 in purified/isolated or non-purified/non-isolated form.

As discussed above, the present invention in certain embodiments provides isolated human binding molecules that are able to recognize and bind to an epitope in the influenza haemagglutinin protein (HA) of influenza B viruses, wherein said binding

molecules have neutralizing activity against influenza B viruses of both the B/Yamagata and/or B/Victoria lineages, both *in vitro* and *in vivo*. According to the invention, it has been shown that binding molecules of the present invention cross-neutralize influenza virus subtypes belonging to both phylogenetic lineages. 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 can also determine whether they are able to neutralize influenza viruses of different subtypes in vitro and/or *in vivo*.

Another aspect of the invention includes functional variants of the binding molecule as defined above. Molecules are considered to be functional variants of a binding molecule according to the invention, if the variant binding molecules are capable of competing for immunospecifically binding to an influenza virus or a fragment thereof with the "parental" or "reference" binding molecules. In other words, molecules are considered to be functional variants of a binding molecule according to the invention when the functional variants are still capable of binding to the same or overlapping epitope of the influenza virus 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. 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 the influenza virus or a fragment thereof. For instance, functional variants according to the invention may have increased or decreased binding affinities for an influenza virus or a fragment thereof compared to the parental binding molecules. In certain embodiments, 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 80% to about 99%, preferably at least about 70% to about 99%, more preferably at least about 80% to about 99%, even more preferably at least about 90% to about 99%, most preferably at least about 95% to about 99%, in particular at least about 97% to about 99% amino acid sequence identity and/or 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 certain embodiments the functional variants of the invention have neutralizing activity against influenza B viruses. The neutralizing activity may either be identical, or be higher or lower compared to the parental binding molecules. As used in this application, when the term (human) binding molecule is used, this also encompasses functional variants of the (human) binding molecule. Assays for verifying if a variant 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 certain embodiments, the functional variants are binding molecules comprising a heavy chain variable sequence comprising one or more amino acid mutations, such as one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen amino acid mutations, as compared to SEQ ID NO: 71 and/or a light chain variable region comprising one or more amino acid mutations, such as one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen amino acid mutations as compared to SEQ ID NO: 73.

In certain embodiments, the functional variants are binding molecules comprising a heavy chain variable sequence comprising one or more amino acid mutations, such as one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen amino acid mutations, as compared to SEQ ID NO: 75 and/or a light chain variable region comprising one or more amino acid mutations, such as one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen amino acid mutations as compared to SEQ ID NO: 77.

In certain embodiments, the functional variants are binding molecules comprising a heavy chain variable sequence comprising one or more amino acid mutations, such as one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen amino acid mutations, as compared to SEQ ID NO: 113 and/or a light chain variable region comprising one or more amino acid mutations, such as one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen amino acid mutations as compared to SEQ ID NO: 115.

In certain embodiments, a binding molecule according to the invention is selected from the group consisting of binding molecules comprising:

(a) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 2, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 3, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 17, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 18, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 60;

(b) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 61, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 2, and a heavy chain

CDR3 comprising the amino acid sequence of SEQ ID NO: 3, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 62, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 18, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 63;

(c) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 2, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 3, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 64, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 65, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 66; and

(d) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 59, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 2, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 3, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 68, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 69;

(e) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 36 and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 37, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 39;

(f) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 40, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 42, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 43, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 5, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 44;

(g) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 45, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 46, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 47, and a light chain CDR1

comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 49; and

(h) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 45, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 50, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 47, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 51, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 52, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 53.

In certain embodiments, the binding molecule is selected from the group consisting of:

a) a binding molecule comprising a heavy chain variable region of SEQ ID NO: 119 and a light chain variable region of SEQ ID NO: 121;

b) a binding molecule comprising a heavy chain variable region of SEQ ID NO: 123 and a light chain variable region of SEQ ID NO: 125;

c) a binding molecule comprising a heavy chain variable region of SEQ ID NO: 127 and a light chain variable region of SEQ ID NO: 129;

d) a binding molecule comprising a heavy chain variable region of SEQ ID NO: 131 and a light chain variable region of SEQ ID NO: 133;

e) a binding molecule comprising a heavy chain variable region of SEQ ID NO: 77 and a light chain variable region of SEQ ID NO: 79;

f) a binding molecule comprising a heavy chain variable region of SEQ ID NO: 101 and a light chain variable region of SEQ ID NO: 103;

g) a binding molecule comprising a heavy chain variable region of SEQ ID NO: 105 and a light chain variable region of SEQ ID NO: 107; and

h) a binding molecule comprising a heavy chain variable region of SEQ ID NO: 109 and a light chain variable region of SEQ ID NO: 111.

In certain embodiments, the binding molecules according to the invention are for a use as a medicament, and preferably for use in the therapeutic and/or prophylactic treatment of influenza infection caused by influenza B viruses. The influenza virus that causes the influenza infection and that can be treated using the binding molecules of the

present invention may be an influenza B virus of the B/Yamagata and/or B/Victoria lineage.

The present invention also relates to pharmaceutical compositions comprising at least one binding molecule according to the invention, and at least a pharmaceutically acceptable excipient.

In yet another embodiment the invention relates to the use of a binding molecule according to the invention in the preparation of a medicament for the prophylaxis, and/or treatment of an influenza virus infection.

The influenza virus infections that can be prevented and/or treated using the binding molecules of the invention may 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.

In yet a further aspect, the invention provides 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 further embodiments, 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 or to monitor the development or progression of an influenza virus 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 viruses or fragments 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 HA 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.

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 nucleic acid molecules 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 aspect 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.

A method of producing a binding molecule according to the invention is an additional aspect 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 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 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 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 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 certain embodiments 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, such as another antibody directed against the HA protein or against other antigenic structures present on influenza viruses, such as M2, and/or a binding molecules neutralizing one or more other pathogens. In another embodiment the additional binding molecule may be formulated for simultaneous separate or sequential administration.

In certain embodiments, the binding molecules exhibit synergistic neutralizing activity, when used in combination. As used herein, the term "synergistic" means that the combined effects of the binding molecules when used in combination are 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. 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 binding molecule. The non-neutralizing and neutralizing binding molecules may also act synergistically in neutralizing influenza virus.

In certain embodiments, the pharmaceutical composition may comprise at least one binding molecule according to the invention and at least one further binding molecule, preferably a further influenza virus neutralizing binding molecule. The binding molecules in the pharmaceutical composition preferably are capable of reacting with influenza viruses of different subtypes. The binding molecules may be of high affinity and 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.

In certain embodiments, 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 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 viruses 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 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, 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, 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 physicochemical 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 or diagnostic agent. So, methods of diagnosis, treatment and/or prevention of an influenza virus infection using the binding molecules, immunoconjugates, compositions, or pharmaceutical compositions of the invention are another aspect 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 infection caused by an influenza B virus. They are suitable for treatment of yet untreated patients suffering from an influenza virus infection and patients who have been or are treated for an influenza virus 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 (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 influenza 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, $\leq$

1 year old), hospitalized patients and already infected 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 0.01-100 mg/kg body weight, preferably 0.1-50 mg/kg body weight, preferably 0.01-15 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 infection, in particular an influenza virus infection caused by influenza B viruses.

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 an aspect 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 influenza virus. The invention thus further pertains to a method of detecting influenza B subtype influenza virus in a sample, wherein the method comprises the steps of

(a) assaying the level of influenza B virus antigen in a biological sample using a binding molecule according to the invention and/or an immunoconjugate according to the invention; and

(b) comparing the assayed level of influenza B virus antigen with a control level

whereby an increase in the assayed level of influenza B virus antigen compared to the control level of the influenza B virus antigen is indicative of an influenza B virus infection.

The biological sample may be a biological sample including, but not limited to blood, serum, stool, sputum, nasopharyngal 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 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 in a concentration 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.

The invention further provides methods of treating or preventing an influenza B virus infection in a subject, comprising administering to the subject a therapeutically or prophylactically effective amount of the binding molecules, immunoconjugates and/or pharmaceutical compositions of the invention. In certain embodiments, the subject is a mammal, preferably a human.

Furthermore, binding molecules of the invention can be used to identify specific binding structures of influenza virus. 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 can be screened for peptides capable of binding to the binding molecules of the invention.

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. Single chain Fv (scFv) phage display libraries were obtained as described in WO 2008/028946, which is incorporated by reference herein. The final library was checked for insert frequency with a colony PCR using a primer set flanking the inserted VH-VL regions (100-150 single colonies). Typically, more than 95% of the colonies showed a correct length insert (see Table 1). 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 1). The frequency of mutations in the V genes was also analysed. About 95% of the sequences 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.

A two-round PCR amplification approach was applied, using the primer sets shown in WO 2008/028946, to isolate the immunoglobulin VH and VL regions from the respective donor repertoire.

First round amplification on the respective cDNA yielded seven, six and nine products of about 650 base pairs for VH, Vkappa and Vlambda regions, respectively. For IgM memory B cell VH region amplification, the OCM constant primer (IgM constant heavy chain specific) was used in combination with OH1 to OH7. The thermal cycling program for first round amplifications was: 2 min 96°C (denaturation step), 35 cycles of 30 sec 96°C/ 30 sec 60°C/ 50 sec 72°C, 10 min 72°C final elongation and 6°C refrigeration. The products were loaded on and isolated from a 1% agarose gel using gel-extraction columns (Macherey-Nagel, MN) and eluted in 50 µl 5 mM Tris-HCl pH 8.0. Ten percent of first round products (5 µl) were subjected to second round amplification. 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/ 50 sec 72°C, 10 min 72°C final elongation and 6°C refrigeration. The second round products (~350 base pairs) were first loaded on gel and extracted from the agarose as above. Then the fragments were pooled according to natural occurrence of J segments found in immunoglobulin gene products, resulting in seven, six and nine pools for respectively the VH, Vkappa and Vlambda variable regions as shown in Table 1 and 2.

To obtain a normalized distribution of immunoglobulin sequences in the immune library the six Vkappa and nine Vlambda light chain pools were mixed according to the percentages mentioned in Table 1. This single final VL pool (5 µg) was digested with SalI and NotI restriction enzymes, loaded on and isolated from a 1.5% agarose gel (~350 base pairs) using MN-extraction columns and ligated in SalI-NotI cut PDV-C06 vector

(~5000 base pairs) as follows: 500ng PDV-C06 vector, 70 ng VL insert, 5 µl 10X ligation buffer (NEB), 2.5 T4 DNA Ligase (400 U/µl) (NEB), and ultrapure water was added up to a total volume of 50µl (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 and the pellet was air dried for several min and then dissolved in 50 µl buffer containing 10 mM Tris-HCl, pH 8.0. 2 µl ligation mixture was used for the transformation of 40 µl TG-1 electro-competent cells (Agilent) 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 750 µl SOC medium (Invitrogen) containing 5% (w/v) glucose (Sigma) at 37°C and transferred to a 15 ml round bottom culture tube. Another 750 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 and a 1 to 10.000 dilution were 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 ten 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 12 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 maxiprep columns (MN) 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 2), 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 2, 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), with the exception of the number of 240 mm plates used. For the final library twenty plates were used, resulting in approximately $2x10^7$ cfu. The final library was checked for insert frequency with a colony PCR using a primer set flanking the inserted VH-VL regions (100-150 single colonies). Typically, more than 95% of the colonies showed a correct length insert (see Table 3). 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 3). The frequency of mutations in the V genes was also analysed. About 95% of the sequences 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 B.

Selection was performed using the antibody phage display libraries against recombinant hemagglutinin (HA) of influenza B (B/Ohio/01/2005, B/Florida/04/2006 and B/Brisbane/60/2008). HA antigens were diluted in PBS (5.0 μg/ml), added to MaxiSorp™ Nunc-Immuno Tubes (Nunc), 2 ml per tube, and incubated overnight at 4°C on a rotating wheel. The immunotubes were emptied and washed three times with 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

the phage display library (350-500 µl, amplified using CT helper phage (see WO 02/103012)) were blocked in blocking buffer (optionally: 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 in 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 was 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 (usually done with clones which are positive in ELISA).

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 influenza B HA (B/Ohio/01/2005, B/Malaysia/2506/2004, B/Jilin/20/2003, B/Brisbane/60/2008 and B/Florida/04/2006) (Protein Sciences, CT, USA) was coated (0.5µg/ml) 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 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 hour; the plates were washed five times with PBS/0.1% Tween-20. 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 immune libraries, fourteen unique single-chain phage antibodies specific for both Yamagata-like and Victoria-like influenza B HA were obtained (sc08-031, sc08-032, sc08-033, sc08-034, sc08-035, sc08-059, sc10-023, sc10-032, sc10-049, sc10-051, sc11-035, sc11-036, sc11-038 and sc11-039). See Table 4.

These fourteen phage antibodies were used for construction of fully human immunoglobulins for further characterization (see Example 4).

EXAMPLE 4

Construction of fully human immunoglobulin molecules (human monoclonal antibodies) from the selected single chain Fvs

From the selected specific single-chain phage antibodies (scFv) clones, plasmid DNA was obtained and nucleotide sequences were determined using standard sequencing techniques. The VH and VL gene identity of the scFvs was determined (see Table 5) using the IMGT/V-QUEST search page (Brochet, et al. (2008)).

The heavy chain variable region (VH) of the scFvs was cloned by restriction digestion (SfiI/XhoI) for expression in the IgG expression vector pIg-C911-HCgamma1, which was digested with the same enzymes. The light variable region (VL) was also cloned into its IgG designated expression vector pIG-C909-Ckappa , or pIg-C910-Clambda using SalI/NotI for the insert fragment and XhoI/NotI for the target vector, as described previously in WO 2008/028946.

To remove a potential de-amidation site in one of the antibodies (CR8059), a single amino acid mutant antibody (CR8071) was generated by assembly PCR. Two overlapping PCR fragments that each contained the desired mutation were generated. These fragments were mixed in equimolar ratios and served as template in a second round PCR to obtain the full length LC sequence. Nucleotide sequences for all constructs were verified using standard sequencing techniques. The resulting expression constructs encoding the human IgG1 heavy and light chains were transiently expressed together in HEK293T cells. After one week, the supernatants containing human IgG1 antibodies were obtained and processed using standard purification procedures. The human IgG1 antibodies were titrated in a concentration range of between 10 to 0.003 µg/ml against influenza B HA antigen (data not shown). An unrelated antibody was included as a control antibody.

The amino acid sequence of the CDRs of both, heavy and light chain, of the selected immunoglobulin molecules is given in Table 5. The nucleotide sequence and amino acid sequence of the heavy and light chain variable regions are given below.

EXAMPLE 5

Cross-binding reactivity of anti-influenza B IgGs

The selected anti-influenza B antibodies were used to test breadth of binding by FACS analysis. For this purpose, full-length recombinant influenza B expression vectors coding for HA (B/Mississippi/04/2008, B/Houston/B60/1997, B/Nashville/45/1991, B/Florida/01/2009, B/Mississippi/07/2008 and B/Ohio/01/2005) were transfected into PER.C6® cells using lipofectamin (Invitrogen) in a 1 to 5 ratio. 48 hour after transfection, the PER.C6® cells expressing the Influenza B HA on the surface were analysed by FACS (CantoII, BD bioscience). Hereto the cells were incubated with IgG antibodies for 1 hour followed by three sequential wash steps with PBS containing 0.1%BSA. Bound antibodies were detected using a PE-conjugated secondary anti-human antibody which was also incubated for 1 hour. As a negative control, untransfected PER.C6® cells were used and incubated with the secondary antibody. The FACS results showed that the influenza B binding antibodies CR8033, CR8059, CR8071, CR10032 and CR10051 showed binding to all six tested influenza B HAs (Table 6).

EXAMPLE 6

Competition for binding to HA of cross-reactive anti-influenza B IgGs

The anti-influenza B IgG antibodies described above were validated for competition for epitopes on influenza B HA. Hereto, B/Brisbane/60/2008, B/Florida/04/2006 and B/Jillin/20/2003 were labeled with biotin using the EZ-link Sulpho-NHS-LC-LC-biotin kit (Pierce). 1 µl of the 10 mM biotin solution was added to 110 µg of recombinant HA, which is a six-fold molar excess of biotin, and incubated for 30 to 40 minutes at room temperature. The free unincorporated biotin was removed using an Amicon Ultra centrifugal filter (0.5 ml, 10K Ultracel-10K membrane; Millipore, cat#: UFC501096). Hereto the sample (300 µl) was loaded on the column and spun for 10 minutes at 14000 RPM in an Eppendorf tabletop centrifuge (20800 rcf). The flow trough

was discarded and 0.4 ml DPBS buffer was loaded on the column and spun again. This step was repeated two times. The labeled sample was recovered by turning the column upside down into a new collector tube; then 200 µl DPBS was loaded and spun for 1 minute at 1000 rpm in a table top centrifuge. The HA concentration was measured using a Nanodrop ND-1000 apparatus (Thermo Scientific).

The actual competition experiment was done on an Octet-QK bio-layer interferometry instrument (ForteBio) according to the settings in Table 7 using streptavidin-coated biosensors (ForteBio, cat# 18-5019) that were pre-wetted for 30 minutes in kinetic buffer at room temperature. When the second antibody was able to bind the Influenza B HA in the presence of the first, this was considered as non-competing (see Table 8). As controls, stem-binding antibody CR9114 (as described in co-pending application EP11173953.8) and non-binding antibody CR8057 (as described in WO2010/130636) were used.

Antibodies CR10023 and CR10049 compete for binding CR8033. Antibodies CR10032 and CR10051 compete for binding with CR8059. Antibody CR10049 competes for binding with CR10032. None of the tested antibodies compete with stem-binding antibody CR9114. These results indicate the presence of at least three to four different epitopes on the influenza B HA (Fig. 1).

EXAMPLE 7

Cross-neutralizing activity of IgGs

In order to determine whether the selected IgGs were capable of blocking multiple influenza B strains, in vitro virus neutralization assays (VNAs) were performed. The VNAs were performed on MDCK cells (ATCC CCL-34) that were cultured in MDCK cell culture medium (MEM medium supplemented with 20 mM Hepes and 0.15% (w/v) sodium bicarbonate (complete MEM medium), supplemented with 10% (v/v) fetal bovine serum). The influenza B Yamagata-like (B/Harbin/7/1994 and B/Florida/04/2006) and Victoria-like (B/Malaysia/2506/2004 and B/Brisbane/60/2008) strains 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 (100 µg/ml) were serially 2-fold diluted (1:2 - 1:512) in complete MEM

medium in quadruplicate wells. 50µl of the respective IgG dilution was mixed with 50µl of virus suspension (100 TCID50/35µl) and incubated for one hr at 37°C. The suspension was then transferred in quadruplicate into 96-well plates containing confluent MDCK cultures in 100µl complete MEM medium. Prior to use, MDCK cells were seeded at $2x10^4$ 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 100µl 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.

CR8032, CR8033, CR8034, CR8035, CR8059, CR8071, CR10023, CR10032, CR10049, CR10051, CR11035, CR11036, CR11038 and CR11039 all showed cross-neutralizing activity to representative strains of both, Yamagata and Victoria-like influenza B virus strains. See Table 9.

EXAMPLE 8

Receptor binding blocking activity of IgGs

In order to determine whether the selected IgGs were capable of blocking the receptor mediated binding of influenza B strains to host cells, haemagglutination inhibition (HI) assays were performed. The influenza B Yamagata-like (B/Harbin/7/1994 and B/Florida/04/2006) and Victoria-like (B/Malaysia/2506/2004 and B/Brisbane/60/2008) virus strains were diluted to 8 HA units, as determined in an HAU assay, and combined with an equal volume of serially diluted IgG and incubated for 1 hr at room temperature. An equal volume of 0.5% Turkey red blood cells (TRBC) was added to the wells and incubation continued for 30 min. Button formation was scored as evidence of hemagglutination.

CR8059, CR8071, CR10032, CR10051 and CR11036 did not show HI activity to any of the tested influenza B virus strains (>10 µg/ml for CR11036, > 50 µg/ml for the other antibodies), indicating that they do not block the receptor binding. Antibodies CR8033 and CR10023 show HI activity to representative strains of only the Yamagata-, but not the Victoria-like influenza B virus strains. Antibody CR11035 shows HI activity

to a representative strain of only the Victoria-, but not the Yamagata-like influenza B virus strains. Antibodies CR10049, CR11038 and CR11039 show HI activity to representative strains of both Yamagata and Victoria-like influenza B virus strains. See Table 10.

Alternatively, an immunofluorescence entry assay was designed to analyze the ability of a given antibody to block receptor binding and internalization of the virus. Therefore, the virus was pre-incubated with the antibody in serial, two-fold dilution steps before being added to a confluent monolayer of MDCK cells plated in a 96-well dish in infection medium (DMEM+200mM glutamine) for two to three hours. The inoculum was subsequently removed and replaced with antibody at indicated concentrations for 16 – 18 hrs at 37 °C, 5% CO2. After this time, the supernatants were removed and the plates were fixed in 80% acetone for subsequent immunofluorescence detection by labeling infected cells using a mouse monoclonal anti-NP primary antibody (Santa Cruz, sc-52027) and an Alexa488-coupled anti-mouse secondary antibody (Invitrogen A11017) followed by DAPI labeling of cellular nuclei (see Fig. 2a). As was seen with the HI assay, antibody CR8033 specifically blocked the viral entry of Yamagata-like virus B/Florida/04/2006 but not Victoria-like virus B/Malaysia/2506/2004. Antibody CR8059 did not block the entry of the tested influenza B viruses. Some of the plates were subsequently analyzed using a BD Pathway 855 bioimager. To assess the level of entry inhibition, the fluorescence intensities per given well above a defined background and amount of infected cells (using DAPI stain to define a cell) was analyzed using BD Pathway imaging analysis tools. The percentage of infected cells treated with indicated dilutions of antibody compared to infected cells treated with a non-binding control antibody is displayed in Fig. 2b.

EXAMPLE 9

Egress inhibition of anti-HA IgGs

To investigate the mechanism of action of the antibody, an egress assay was designed to analyze the amount of virus particles released into the supernatant 18 hrs post infection under antibody treatment conditions. The detection (or absence) of an anti-HA

signal after gel electrophoresis followed by Western blot of such supernatants is taken as indication for the presence (or absence) of released virus particles.

Four hours prior to the experiment, 40,000 MDCK cells per well were seeded in DMEM/glutamine into 96-well plates. The amount of virus needed to achieve 90-100% infection was titrated in a separate experiment. The required amount of virus was added to the cells and incubated at 37 °C, 5% CO2. After three hours, the supernatants were removed and cells were washed thrice with PBS to remove non-internalized virus particles. Cells were replenished with infection medium containing mAbs (serial dilution starting at 20µg/ml). After 16 – 18 hrs at 37 °C, 5% CO2, the supernatants were harvested and the remaining cells were lysed (Tris HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% (v/v) Triton-X). Samples were subjected to SDS-PAGE/Western blot to analyze the amount of virions released into the supernatant measured by developing the WB using a rabbit polyclonal anti-HA staining (Protein Sciences) followed by an HRP-coupled anti-rabbit F(ab')2-fragment (Jackson Immuno Research Laboratories, 111-036-047). As shown in Fig 3, both antibodies CR8033 and CR8059 inhibit the release of viral particles in a concentration dependent manner. Further experiments have shown that at least CR8071 and CR10051 also inhibit the release of viral particles.

Proper infection of the cells was checked by fixing identically-treated wells with 80% acetone. The amount of infection was assessed using immunofluorescence labeling using a mouse monoclononal anti-NP primary antibody (Santa Cruz, sc-52027) and an Alexa488-coupled anti-mouse secondary antibody (Invitrogen A11017). The plates were subsequently analyzed using a BD Pathway 855 bioimager (results not shown).

EXAMPLE 10

Scanning electron microscopy of influenza B infected cells

MDCK cells were seeded on glass coverslips one day prior to the experiment. The next day, cells were infected with different amounts of virus to determine the amount that yielded 90 – 100% infected cells after 18 hrs post infection. Three hours after the initial infection, the supernatants were removed; cells were washed thrice with PBS, before media containing the indicated concentration of antibodies were added. After an additional 15 – 18 hrs, the cell culture medium was removed and cells were fixed in 2.5%

glutaraldehyde buffer and stored at 4 °C until further analysis. The samples were subjected to further chemical fixation using glutaraldehyde (GA) and/or osmium tetroxid (OsO4). Prior to SEM imaging, the specimens were subjected to acetone dehydration and critical-point-drying. Finally, the cells were be mounted on alumina stubs and coated with thin layer of carbon and examined in a Zeiss Ultra 55 SEM microscope.

The surface of influenza B infected MDCK cells is covered with electron dense spherical particles (Fig. 4b), in contrast to uninfected controls (Fig. 4a). Incubation with antibody CR8059 does not prevent the formation of these spherical particles (Fig. 4c) whereas incubation with antibody CR8033 greatly diminishes the formation of particles (Fig. 4d). In contrast to CR8059 incubated cells, budding virions can not readily be detected on CR8033 incubated cells (Fig. 4e and f).

EXAMPLE 11

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

A study was performed to test the prophylactic effect of the monoclonal antibodies CR8033 and CR8071 against a lethal challenge with two influenza B viruses *in vivo*. MAbs CR8033 and CR8071 were tested for prophylactic efficacy in a mouse lethal challenge model with mouse adapted influenza B/Florida/04/2006 virus. The B/Florida/04/2006 virus was adapted to mice after 5 lung-to-lung passages. The mouse adapted influenza B passage 5 virus was propagated in embryonated chicken eggs. All mice (Balb/c, female, age 6-8 weeks, n=8 per group) were acclimatized and maintained for a period of at least four days prior to the start of the experiment. MAbs CR8033 and CR8071 were dosed at 0.06, 0.2, 0.6, 1.7, and 5 mg/kg intravenously into 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 vehicle control. The mice were then challenged at day 0 with 25 LD_{50} mouse adapted B/Florida/04/2006 influenza B virus by intranasal inoculation. Fig 5 shows the survival rates of the mice, following mAb administration. Mice dosed with dosages as low as 0.2 mg/kg for CR8033 and 0.6 mg/kg for CR8071 showed significantly higher survival rates than the vehicle treated control animals.

Alternatively, mAbs CR8033 and CR8071 were tested for prophylactic efficacy in a mouse lethal challenge model with mouse adapted influenza B/Malaysia/2506/2004 virus. The B/Malaysia/2506/2004 virus was adapted to mice after 4 lung-to-lung passages. The mouse adapted influenza B passage 4 virus was propagated in embryonated chicken eggs. All mice (Balb/c, female, age 6-8 weeks, n=8 per group) were acclimatized and maintained for a period of at least four days prior to the start of the experiment. MAbs CR8033 and CR8071 were dosed at 0.06, 0.2, 0.6, 1.7 and 5 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 vehicle control. The mice were then challenged at day 0 with 25 LD_{50} mouse adapted B/Malaysia/2506/2004 influenza B virus by intranasal inoculation. Fig. 5 shows the survival rates of the mice, following mAb administration. Mice dosed with dosages as low as 0.2 mg/kg for CR8033 and 0.6 mg/kg for CR8071 showed significantly higher survival rates than the vehicle treated control animals.

These results show that human anti-influenza antibodies CR8033 and CR8071, identified and developed as disclosed herein, are able to provide *in vivo* protection against a lethal dose of influenza B viruses of both the B/Yamagata and the B/Victoria lineages when administered one day prior to infection at a dose equal to or higher than 0.2 or 0.6 mg/kg, respectively.

Table 1: 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		
	OL5S	OJL1	L5J1	30		

L5	OL5S	OJL2	L5J2	60	PL5	1
	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 2: 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 3: Characteristics of the individual IgM memory B cell libraries.

Library	Cells Used	Library size	Intact Orf
MEM-05-M08	540.000 IgM memory cells Facs sorted from 1 donor	5.9E+07	
MEM-05-M09	775.000 IgM memory cells Facs sorted from 1 donor	2.35E+07	
MEM-05-M10	700.000 IgM memory cells Facs sorted from 1 donor	1.7E+07	
Flu-PBMC-09-M02	1E+07 total PBMC's from 1 donor	1.0E+07	75%
Flu-Bcell-09-M03	280.000 Macs sorted B cells from 1 donor	2.0E+07	76%
Flu-MEM-09-M08	800.000 IgM memory cells Facs sorted from 1 donor	2.4E+07	85%
Flu-PBMC-10-M03	3E+07 total PBMC's from 3 donors (1E+07 PBMC's per donor)	2.8E+07	82%
Flu-PBMC-10-M04	3E+07 total PBMC's from 3 donors (1E+07 PBMC's per donor)	3.1E+07	87%
Flu-PBMC-10-M05	3E+07 total PBMC's from 3 donors (1E+07 PBMC's per donor)	3.3E+07	89%
Flu-PBMC-11-G01	4E+07 total PBMC's from 4 donors (1E+07 PBMC's per donor)	>1E+07	82%

Table 4: Binding of scFv-phages to recombinant Influenza B HA

	Yamagata		Victoria		
	B/Jilin/20/03	B/Florida/04/06	B/Malaysia/2506/04	B/ohio/01/05	B/Brisbane/60/08
sc08-031	++	nt	++	++	nt
sc08-032	++	nt	++	++	nt
sc08-033	++	++	++	+++	++
sc08-034	++	nt	++	++	nt
sc08-035	++	nt	++	++	nt
sc08-059	++	++	++	++	++
sc10-023	++	++	+	++	+
sc10-032	+++	+++	++	+++	+++
sc10-049	+++	+++	+++	+++	+++
sc10-051	+++	+++	+++	+++	+++
sc11-035	nt	+++	nt	++	++
sc11-036	nt	+++	nt	+++	+++
sc11-038	nt	++	nt	++	+++
sc11-039	nt	+++	nt	++	+++

+++	strong binding
++	binding
+	weak binding
-	no binding
nt	not tested

Table 5A: Amino acid sequences of HC CDRs of selected antibodies

CR #	VH locus	CDR1-HC (SEQ ID NO)	CDR2-HC(SEQ ID NO)	CDR3-HC (SEQ ID NO)
CR8033	IGHV3-9*01	GFSFDEYT (1)	INWKGNFM (2)	AKDRLESSAMDILEGGTFDI (3)
CR8059	IGHV1-18*01	GYIFTESG (7)	ISGYSGDT (8)	ARDVQYSGSYLGAYYFDY (9)
CR8071	IGHV1-18*01	GYIFTESG (7)	ISGYSGDT (8)	ARDVQYSGSYLGAYYFDY (9)
CR10023	IGHV3-9*01	GFTFDDYA (14)	INWVSTTM (15)	AKDRLESAAIDILEGGTFDI (16)
CR10032	IGHV4-39*02	GGSINSSPYK (20)	FYYDGST (21)	AAYCSSISCHAYYDYMNV (22)
CR10049	IGHV3-23*04	GFTFSSYA (26)	LSDESTT (27)	AEDLGTVMDSYYYGMNV (28)
CR10051	IGHV1-46*01	GDTFTNYH (31)	INPSGGDT (32)	ATDESPGLLTGLRDYWYYYGMDV (33)
CR11024	IGHV1-2*02	GYSFTGYY (35)	INPISGDT (36)	ARVAGEDWFGDLDY (37)
CR11035	IGHV1-18*01	GYAFNGYG (40)	INTYKVNT (41)	ARDWGGPFGNAFDF (42)
CR11036	IGHV1-46*01	GYAFTSYY (45)	MNLHGGST (46)	ARESPDSSGYPGYYGMDV (47)
CR11038	IGHV1-46*01	GYAFTSYY (45)	MNPHGGST (50)	ARESPDSSGYPGYYGMDV (47)
CR11039	GHV1-18*01	GYAFTGYG (54)	INTYKFNT (55)	ARDWAGPFGNAFDV (56)
CR08031	IGHV3-9*01	GFTFDEYI (59)	INWKGNFM (2)	AKDRLESSAMDILEGGTFDI (3)
CR08032	IGHV3-9*01	GFSFDEYI (61)	INWKGNFM (2)	AKDRLESSAMDILEGGTFDI (3)
CR08034	IGHV3-9*01	GFTFDEYI (59)	INWKGNFM (2)	AKDRLESSAMDILEGGTFDI (3)
CR08035	IGHV3-9*01	GFTFDEYI (59)	INWKGNFM (2)	AKDRLESSAMDILEGGTFDI (3)

Table 5B Amino acid sequences of LC CDRs of selected antibodies

CR #	VL locus	CDR1-LC (SEQ ID NO)	CDR2-LC(SEQ ID NO)	CDR3-LC (SEQ ID NO)
CR8033	IGKV3-20*01	QSVSSSY (4)	GAS (5)	QQYGSSPWT (6)
CR8059	IGLV1-47*01	SSNIGTNY (10)	RSY (11)	ATWDDSLNGWV (12)
CR8071	IGLV1-47*01	SSNIGTNY (10)	RSY (11)	ATWDDSLDGWV (13)
CR10023	IGLV2-8*01	SSDVGGYNY (17)	DVS (18)	SSYASGSTYV (19)
CR10032	IGKV2-28*01	QSLRHENGYNY (23)	LGS (24)	MQALTQTLT (25)
CR10049	IGKV2-28*01	QSLLHSNGLNY (29)	LGS (24)	MQALQTPFT (30)
CR10051	IGKV3-20*01	QSVSSSY (4)	GAS (5)	QQYGSSPLCS (34)
CR11024	IGKV3-20*01	QSVSSSY (4)	GTS (38)	QQYGSSPRT (39)
CR11035	IGKV1-39*01	QSVGSY (43)	GAS (5)	QQSYSTPRT (44)
CR11036	IGKV3-20*01	QSVSSDF (48)	GTS (38)	QQYGSSTWT (49)
CR11038	IGLV1-44*01	RSNIGSNP (51)	TND (52)	AAWDDSLKGWV (53)
CR11039	GHV1-18*01	QDISDY (57)	GAS (5)	QQYGNLPPT (58)
CR08031	IGLV2-14*01	SSDVGGYNY (17)	DVS (18)	SSYTSSSTHV (60)
CR08032	IGLV2-14*01	RRDVGDYKY (62)	DVS (18)	SSYTTSNTRV (63)
CR08034	IGKV1-17*01	QGIRND (64)	AAS (65)	QQANTYPLT (66)
CR08035	IGLV3-19*01	SLRSYY (67)	GKN (68)	DSRDSSGTHYV (69)

Table 6: Binding of purified IgG to cell expressed Influenza B HA

	Yamagata			Victoria		
	Nasvile/45/91	Mississippi/04/08	Houston/B0/97	Mississippi/07/08	Florida/01/09	Ohio/01/05
CR8031	++	NT	NT	NT	-	NT
CR8032	+++	+	NT	++	-	NT
CR8033	++	++	++	++	++	++
CR8034	++	NT	NT	NT	-	NT
CR8035	+++	+	+++	+	-	++
CR8059	+++	+++	+++	+++	+++	+++
CR8071	+++	+++	+++	+++	+++	+++
CR10023	+++	-	+++	-	-	-
CR10032	+++	+++	+++	+++	+++	+++
CR10049	+	-	+	-	-	+
CR10051	++	++	++	++	++	++

+++ strong binding
++ binding
+ weak binding
- no binding

Table 7: Plate layout octet competition experiment

	Step				
	Base line 1	Loading HA	Baseline2	Association 1st IgG (15 ug/ml)	Association / competition 2nd IgG (15 ug/ml)
Duration (seconds)	60	1200	60	700	700
Row A	Kinetic buffer	A Biotine labelled influenza B HA 10 ug/ml	Kinetic buffer	CR8033	CR8033 In 2nd measurement CR8059 etc
Row B				CR8059	
Row C				CR10023	
Row D				CR10032	
Row E				CR10049	
Row F				CR10051	
Row G				CR9114*	
Row I				CR8057*	

*control antibodies (CR9114: binding, CR8057 non-binding)

Table 8: Competition experiment on influenza B HA

	CR8033	CR8059	CR10023	CR10032	CR10049	CR10051	CR9114	CR8057
CR8033	X	N	Y	N	Y	N	N	N
CR8059	N	X	N	Y	N	Y	N	N
CR10023	Y	N	X	Y	Y	N	N	N
CR10032	N	Y	Y	X	Y	Y	N	N
CR10049	Y	N	Y	N	X	N	N	N
CR10051	N	Y	N	Y	N	X	N	N
CR9114	N	N	N	N	N	N	X	N
CR8057	-	-	-	-	-	-	-	

Y: competition; N: no competition; X: self competition; -: No binding

Table 9: Virus neutralization assays on influenza B virus strains

VNA	Yamagata		Victoria	
titres in µg/ml	B/Harbin/7/1994	B/Florida/04/2006	B/Malaysia/2506/2004	B/Brisbane/60/2008
CR8031	1.24	0.88	>50*	NT
CR8032	0.69	0.69	3.88*	NT
CR8033	0.03	0.02	0.88*	5.95
CR8034	0.29	0.12	2.31*	NT
CR8035	0.66	0.66	4.46*	NT
CR8059	2.39	3.23	17.68*	4.55
CR8071	2.34	2.12	14.87*	3.72
CR10023	0.26	≤0.55	12.5*	7.07
CR10032	12.5	21.02	53.03*	35.36
CR10049	4.42	1.3	25*	35.36
CR10051	0.28	0.63	1.77*	1.51
CR11035	0.16	≤0.06	0.53*	0.93
CR11036	0.02	≤0.06	0.22*	≤0.14
CR11038	0.05	≤0.06	2.1*	0.55
CR11039	0.02	≤0.06	0.06*	≤0.14

NT: not tested

* assay was done with 25TCIDs

Table 10: Heamagglutination inhibition assay on influenza B virus strains

HI	Yamagata		Victoria	
titres in µg/ml	B/Harbin/7/1994	B/Florida/04/2006	B/Malaysia/2506/2004	B/Brisbane/60/2008
CR8033	0.39	0.22	>50	>50
CR8059	>50	>50	>50	>50
CR8071	>50	>50	>50	>50
CR10023	1.1	1.56	>50	>50
CR10032	>50	>50	>50	>50
CR10049	>50	1.1	4.42	35.36
CR10051	>50	>50	>50	>50
CR11035	NT	>10	NT	0.26
CR11036	NT	>10	NT	>10
CR11038	NT	1.25	NT	0.31
CR11039	NT	0.63	NT	0.44

NT: not tested

SEQUENCES

>SC08-033 VH DNA (SEQ ID NO: 70)
GAGGTGCAGCTGGTGGAGACTGGGGGAGGCCTGGTACAGCCTGGCAGGTCCCTGAGACTGTCCTGTGCAGCC
TCTGGATTCAGCTTTGATGAGTACACCATGCATTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTC
GCAGGTATTAATTGGAAAGGTAATTTCATGGGTTATGCGGACTCTGTCCAGGGCCGATTCACCATCTCCAGA
GACAACGGCAAGAACTCCCTCTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATTACTGT
GCAAAAGACCGGCTGGAGAGTTCAGCTATGGACATTCTAGAAGGGGGTACTTTTGATATCTGGGGCCAAGGG
ACAATGGTCACC

>SC08-033 VH PROTEIN (SEQ ID NO: 71)
EVQLVETGGGLVQPGRSLRLSCAASGFSFDEYTMHWVRQAPGKGLEWVAGINWKGNFMGYADSVQGRFTISR
DNGKNSLYLQMNSLRAEDTALYYCAKDRLESSAMDILEGGTFDIWGQGTMVT

>SC08-033 VL DNA (SEQ ID NO: 72)
GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGG
GCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTC
ATCTATGGTGCATCCACCAGGGCCACTGGTATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTC
ACTCTCACCATCAGCAGACTGGAGCCTGAAGATCTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCG
TGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC

>SC08-033 VL PROTEIN (SEQ ID NO: 73)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTDF
TLTISRLEPEDLAVYYCQQYGSSPWTFGQGTKVEIK

>SC08-059 VH DNA (SEQ ID NO: 74)
GAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAGGGTCTCCTGCAGGGCC
TCTGGTTACATCTTTACCGAATCTGGTATCACCTGGGTGCGCCAGGCCCCTGGACAAGGGCTTGAGTGGATG
GGATGGATCAGCGGTTACAGTGGTGACACAAAATATGCACAGAAACTCCAGGGCAGAGTCACCATGACCAAA
GACACATCCACGACCACAGCCTACATGGAATTGAGGAGCCTGAGATATGACGACACGGCCGTATATTACTGT
GCGAGAGACGTCCAGTACAGTGGGAGTTATTTGGGCGCCTACTACTTTGACTATTGGAGCCCGGGAACCCTG
GTCACCGTCTCGAGC

>SC08-059 VH PROTEIN (SEQ ID NO: 75)
EVQLVQSGAEVKKPGASVRVSCRASGYIFTESGITWVRQAPGQGLEWMGWISGYSGDTKYAQKLQGRVTMTK
DTSTTTAYMELRSLRYDDTAVYYCARDVQYSGSYLGAYYFDYWSPGTLVTVSS

>SC08-059 VL DNA (SEQ ID NO: 76)
TCCTATGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCTCTTGTTCTGGA
AGCAGCTCCAACATCGGAACTAATTATGTATACTGGTACCAGCAGTTCCCAGGAACGGCCCCCAAACTCCTC
ATCTATAGGAGTTATCAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCTCCTCAGCC
TCCCTGGCCATCAGTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGTGCAACATGGGATGACAGCCTG
AATGGTTGGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAG

>SC08-059 VL PROTEIN (SEQ ID NO: 77
SYVLTQPPSASGTPGQRVTISCSGSSSNIGTNYVYWYQQFPGTAPKLLIYRSYQRPSGVPDRFSGSKSGSSA
SLAISGLQSEDEADYYCATWDDSLNGWVFGGGTKLTVL

>CR08071 VH PROTEIN (SEQ ID NO: 78
QVQLVQSGAEVKKPGASVRVSCRASGYIFTESGITWVRQAPGQGLEWMGWISGYSGDTKYAQKLQGRVTMTK
DTSTTTAYMELRSLRYDDTAVYYCARDVQYSGSYLGAYYFDYWSPGTLVTVSS

>CR08071 VL PROTEIN (SEQ ID NO: 79)
QSVLTQPPSASGTPGQRVTISCSGSSSNIGTNYVYWYQQFPGTAPKLLIYRSYQRPSGVPDRFSGSKSGSSA
SLAISGLQSEDEADYYCATWDDSLDGWVFGGGTKLTVLRK

>SC10-051 VH DNA (SEQ ID NO: 80)
GAGGTCCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTAGAACTTTCCTGCAAGGCA
TCTGGAGACACCTTCACCAACTACCATATACACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATG
GGAATAATCAATCCTAGTGGTGGTGACACAGACTACTCACAGAAGTTCCAGGGCAGAGTCACCCTGACCAGG
GACAGGTCCACAAACACATTCTATATGAAGTTGGCCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GCGACAGATGAGAGTCCCGGACTTTTGACTGGCCTTCGGGATTACTGGTACTACTACGGTATGGACGTCTGG
GGCCAGGGGACCACGGTCACCGTCTCGAG

>SC10-051 VH PROTEIN (SEQ ID NO: 81)
EVQLVQSGAEVKKPGASVELSCKASGDTFTNYHIHWVRQAPGQGLEWMGIINPSGGDTDYSQKFQGRVTLTR
DRSTNTFYMKLASLRSEDTAVYYCATDESPGLLTGLRDYWYYYGMDVWGQGTTVTVS

>SC10-051 VL DNA (SEQ ID NO: 82)
GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGG
GCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTC
ATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTC
ACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCT
CTGTGCAGTTTTGGCCAGGGGACCAAGCTGGAGATCAAAC

>SC10-051 VL PROTEIN (SEQ ID NO: 83)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDF
TLTISRLEPEDFAVYYCQQYGSSPLCSFGQGTKLEIK

>SC10-049 VH DNA (SEQ ID NO: 84)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCC
TCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTC
TCACGTCTTAGTGATGAAAGTACCACATACTATGCAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGAC
AATTCCAAGAACACACTGTATCTGCAGATGAACAGCCTGAAAGCCGACGACACGGCCATATATTACTGTGCG
GAGGATCTGGGGACGGTGATGGACTCCTACTACTACGGTATGAACGTCTGGGGCCCAGGGACCACGGTCACC
GTCTCGAG

> SC10-049 VH PROTEIN (SEQ ID NO: 85)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSRLSDESTTYYADSVKGRFTISRD
NSKNTLYLQMNSLKADDTAIYYCAEDLGTVMDSYYYGMNVWGPGTTVTVS

>SC10-049 VL DNA (SEQ ID NO: 86)
GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCCATCTCCTGCAGG
TCTAGTCAGAGCCTCCTGCATAGTAATGGACTCAATTATTTGGATTGGTACCTGCAGAAGCCAGGGCAGTCT
CCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCCGGGGTCCCTGACAGGTTCAGTGGCAGTGGATCA
GGCACAGATTTTACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCT
CTACAAACTCCTTTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAAC

>SC10-049 VH PROTEIN (SEQ ID NO: 87)
DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGLNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGS
GTDFTLKISRVEAEDVGVYYCMQALQTPFTFGGGTKVEIK

>SC10-023 VH DNA (SEQ ID NO: 88)
GAGGTGCAGCTGGTGGAGACTGGGGGAGGCTTGGTACAGCCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCC
TCTGGATTCACCTTTGATGATTATGCCATGCATTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTC
TCAGGTATTAATTGGGTTAGTACTACCATGGGCTATGCGGACTCTGTGAAGGGCCGATTCACCATCTCCAGA
GACAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATTACTGT
GCAAAAGATAGGCTGGAGAGTGCAGCTATAGACATTCTAGAAGGGGGTACTTTTGATATCAGGGGCCAAGGG
ACAATGGTCACCGTCTCGAGCG

>SC10-023 VH PROTEIN (SEQ ID NO: 89)
EVQLVETGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGINWVSTTMGYADSVKGRFTISR
DNAKNSLYLQMNSLRAEDTALYYCAKDRLESAAIDILEGGTFDIRGQGTMVTVSS

>SC10-023 VL DNA (SEQ ID NO: 90)
CAGTCTGCCCTGACTCAGCCTCCCTCCGCGTCCGGCTCTCCTGGACAGTCAGTCACCATCTCCTGCACTGGA
ACCAGCAGTGATGTTGGTGGTTATAACTATGTCTCCTGGTACCAACAGCACCCAGGCAAAGCCCCCAAACTC
ATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGGTCCAAGTCTGGCAACACG
GCCTCCCTGACCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGAATATTACTGCAGCTCATATGCAAGCGGC
AGCACTTATGTCTTCGGAACTGGGACCAAGGTCACCGTCCTAG

>SC10-023 VL PROTEIN (SEQ ID NO: 91)
QSALTQPPSASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVPDRFSGSKSGNT
ASLTISGLQAEDEAEYYCSSYASGSTYVFGTGTKVTVL

>SC10-032 VH DNA (SEQ ID NO: 92)
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGGCACCCTGTCCCTCACCTGCAATGTC
TCTGGTGGCTCCATCAACAGTAGTCCCTATAAGTGGGCCTGGATCCGCCAGTCCCCAGGGAAGGGGCTGGAG
TGGATTGGGACTTTCTATTATGATGGGAGCACCGACTACAACCCGTCCCTCCAGAGTCGACTCACCATTTCC
GGAGACATGTCCAGTAACCACTTCTCCTTGAGGCTGAGGTCTGTGACCGCCGCAGACACGGCTGTGTATTAC
TGTGCGGCCTATTGTAGTAGTATAAGCTGCCATGCCTATTACGACTACATGAACGTCTGGGGCAAAGGGACC
ACGGTCACCGTCTCGAGC

>SC10-032 VH PROTEIN (SEQ ID NO: 93)
QVQLQESGPGLVKPSGTLSLTCNVSGGSINSSPYKWAWIRQSPGKGLEWIGTFYYDGSTDYNPSLQSRLTIS
GDMSSNHFSLRLRSVTAADTAVYYCAAYCSSISCHAYYDYMNVWGKGTTVTVSS

>SC10-032 VL DNA (SEQ ID NO: 94)
GAAATTGTGCTGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCCATCTCCTGCAGG
TCTAGTCAGAGCCTCCGACATGAGAATGGATACAACTATTTGGATTGGTACCTGCAGAAGCCAGGGCAGTCT
CCACAGCTCCTGATGTATTTGGGTTCTGTTCGGGCCTCCGGGGTCCCTGACAGGTTCAGTGGCAGTGGATCA
GGCACAGATTTTACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCT
CTACAAACGCTCACTTTCGGCGGAGGGACCAAGCTGGAGATCAAAC

>SC10-032 VL PROTEIN (SEQ ID NO: 95)
EIVLTQSPLSLPVTPGEPASISCRSSQSLRHENGYNYLDWYLQKPGQSPQLLMYLGSVRASGVPDRFSGSGS
GTDFTLKISRVEAEDVGVYYCMQALQTLTFGGGTKLEIK

>SC11-024 VH DNA (SEQ ID NO: 96)
GAGGTGCAGCTGGTGCAGTCTGGGGCTGAAATTAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCT
TCTGGATACAGCTTCACCGGCTACTATATGCACTGGGTGCGACAGGCCCCTGGACAAGGACCTGAGTGGATG
GGGCGGATCAACCCTATCAGTGGTGACACAAACTATGCACAGAGGTTTCAGGGCAGGGTCACCTTGACCAGG
GACAGGTCCACCAGCACAGCCTACATGGAGCTGAGCGGGCTGAAATCTGACGACACGGCCGTATATTTCTGT
GCGAGAGTCGCGGGTGAAGATTGGTTCGGGGATCTTGACTATTGGGGCCAGGGAACCCTGGTCACCGTCTCG
AGCG

>SC11-24 VH PROTEIN (SEQ ID NO: 97)
EVQLVQSGAEIKKPGASVKVSCKASGYSFTGYYMHWVRQAPGQGPEWMGRINPISGDTNYAQRFQGRVTLTR
DRSTSTAYMELSGLKSDDTAVYFCARVAGEDWFGDLDYWGQGTLVTVSS

>SC11-024 VL DNA (SEQ ID NO: 98)
GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGG
GCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTC
ATCTTTGGAACATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTC

ACTCTCACCATCAGCAGGCTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCT
CGGACGTTCGGCCAAGGGACCAAGGTGGAGATCAAAC

>SC11-024 VL PROTEIN (SEQ ID NO: 99)
EIVLTQSPATLSVSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIFGTSSRATGIPDRFSGSGSGTDF
TLTISRLEPEDFAVYYCQQYGSSPRTFGQGTKVEIK

>SC11-035 VH DNA (SEQ ID NO: 100)
CAGGTGCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGACC
TCTGGTTACGCCTTTAACGGCTACGGTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGGTG
GCATGGATCAACACTTACAAAGTTAACACACATTATGCACAGAATCTCCGGGGCAGGGTCACCGTGAGCATA
GACACATCCACGACCACAGCCTATATGGAACTGAGGAGCCTGAGATCTGACGACACGGCCGTCTATTACTGT
GCGAGAGACTGGGGTGGGCCGTTTGGGAACGCTTTTGATTTCTGGGGCCAAGGGACAATGGTCACCGTCTCG
AGCG

>SC11-035 VH PROTEIN (SEQ ID NO: 101)
QVQLVQSGAEVKKPGSSVKVSCKTSGYAFNGYGISWVRQAPGQGLEWVAWINTYKVNTHYAQNLRGRVTVSI
DTSTTTAYMELRSLRSDDTAVYYCARDWGGPFGNAFDFWGQGTMVTVSS

>SC11-035 VL DNA (SEQ ID NO: 102)
GACATCCAGATGACCCAGTCTCCATCCTCCCTGGCTGCATCTATAGGAGACAGTGTCACCATCACTTGCCGG
GCAAGTCAGAGCGTTGGCTCTTACTTAAATTGGTATCAGCAAAAACCAGGGAAAGCCCCTAAGTTGTTGATC
TATGGTGCATCCAATGTGCAAAGTGGGGTCCCATCAAGGTTTAGTGGCAGTGAGTCTGGGACAGAGTCCACA
CTCACCATCAACAATCTGCAGCCTGAAGATTCTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTAGA
ACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC

>SC11-035 VL PROTEIN (SEQ ID NO: 103)
DIQMTQSPSSLAASIGDSVTITCRASQSVGSYLNWYQQKPGKAPKLLIYGASNVQSGVPSRFSGSESGTEST
LTINNLQPEDSATYYCQQSYSTPRTFGQGTKVEIK

>SC11-036 VH DNA (SEQ ID NO: 104)
GAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGACGGTTTCCTGCAAGGCA
TCTGGATACGCCTTCACCAGCTACTATTTACACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATG
GGGATAATGAATCTTCATGGTGGTAGCACAACCTACGCACAGAAGTTCCAGGGCAGAGTCACCATGACCAGG
GACACGTCCACGAGGACAGTTTACATGGAGCTGAGCGGCCTGAGATCTGAGGACTCGGCCGTATATTACTGT
GCCCGAGAGAGTCCCGATAGCAGTGGTTATCCTGGCTACTACGGTATGGACGTCTGGGGCCAGGGGACCACG
GTCACCGTCTCGAGC

>SC11-036 VH PROTEIN (SEQ ID NO: 105)
EVQLVQSGAEVKKPGASVTVSCKASGYAFTSYYLHWVRQAPGQGLEWMGIMNLHGGSTTYAQKFQGRVTMTR
DTSTRTVYMELSGLRSEDSAVYYCARESPDSSGYPGYYGMDVWGQGTTVTVSS

>SC11-036 VL DNA (SEQ ID NO: 106)
GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGG
GCCAGTCAGAGTGTTAGCAGCGACTTCTTCGCCTGGTACCAGCAGAAACGTGGCCAGACTCCCACCCTCCTC
ATCTATGGTACATCCACCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTC
ACACTCAGCGTCGCCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCGACG
TGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC

>SC11-036 VL PROTEIN (SEQ ID NO: 107)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSDFFAWYQQKRGQTPTLLIYGTSTRATGIPDRFSGSGSGTDF
TLSVARLEPEDFAVYYCQQYGSSTWTFGQGTKVEIK

>SC11-038 VH DNA (SEQ ID NO: 108)
GAGGTGCAGCTGGTGGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCA
TCTGGATACGCCTTCACCAGCTACTATTTGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATG
GGGATAATGAACCCTCATGGTGGTAGCACAACCTACGCACAGAAGTTCCAGGGCAGAGTCACCATGACCAGG

GACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GCCCGAGAGAGTCCCGATAGTAGTGGTTATCCTGGCTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG
GTCACCGTCTCGAGC

>SC11-038 VH PROTEIN (SEQ ID NO: 109)
EVQLVESGAEVKKPGASVKVSCKASGYAFTSYYLHWVRQAPGQGLEWMGIMNPHGGSTTYAQKFQGRVTMTR
DTSTSTVYMELSSLRSEDTAVYYCARESPDSSGYPGYYGMDVWGQGTTVTVSS

>SC11-038 VL DNA (SEQ ID NO: 110)
TCCTATGAGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATGTCTTGTTCTGGA
AGCAGATCCAACATCGGATCTAATCCTGTAAGCTGGTTCCAGCAACTCCCGGGAATGGTCCCCAAACTCCTC
ATCTATACTAATGATCAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCCCCTCAGCC
TCCCTGGCCATCAGTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTG
AAAGGTTGGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAG

>SC11-038 VL PROTEIN (SEQ ID NO: 111)
SYELTQPPSASGTPGQRVTMSCSGSRSNIGSNPVSWFQQLPGMVPKLLIYTNDQRPSGVPDRFSGSKSGPSA
SLAISGLQSEDEADYYCAAWDDSLKGWVFGGGTKLTVL

>SC11-039 VH DNA (SEQ ID NO: 112)
GAGGTCCAGCTGGTACAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTGTGAAGATCTCCTGTAAGACT
TCTGGTTACGCCTTTACCGGCTACGGTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGCCTTGAGTGGATG
GGATGGATCAACACTTACAAATTTAACACAAATTATGCACAGAACCTGCAGGGCAGAGTCACCATGACCATA
GACACATCCACGAGCGCAGCCTACATGGAGCTGAGGAGCCTGAGATATGAGGACACGGCCGTATATTTCTGT
GCGAGAGACTGGGCTGGGCCGTTTGGGAATGCTTTTGATGTCTGGGGCCAGGGGACAATGGTCACCGTCTCG
AGCG

>SC11-039 VH PROTEIN (SEQ ID NO: 113)
EVQLVQSGAEVKKPGESVKISCKTSGYAFTGYGISWVRQAPGQGLEWMGWINTYKFNTNYAQNLQGRVTMTI
DTSTSAAYMELRSLRYEDTAVYFCARDWAGPFGNAFDVWGQGTMVTVSS

>SC11-039 VL DNA (SEQ ID NO: 114)
ACATCCAGATGACCCAGTCTCCATCTTCCCTGTCTGCATCTATAGGAGACAGAGTCGCCATCACTTGCCAGG
CGAGTCAGGACATTAGCGACTATTTAAATTGGTATCAGCAACAACCAGGGAAAGCCCCTAAGCTCCTGCTCT
ACGGTGCATCCAATTTGGAAACAGGGGTCCCATCAAGGTTCAGTGGAAGTGGATCTGGGACAGATTTTACTT
TCACCATCAGCAGCCTGCAGCCTGAAGACATTGCAACATATTATTGTCAACAGTATGGTAATCTCCCTCCGA
CTTTCGGCGGGGGGACCAAGCTGGAGATCAAAC

>SC11-039 VL PROTEIN (SEQ ID NO: 115)
IQMTQSPSSLSASIGDRVAITCQASQDISDYLNWYQQQPGKAPKLLLYGASNLETGVPSRFSGSGSGTDFTF
TISSLQPEDIATYYCQQYGNLPPTFGGGTKLEIK

>SC09-114 VH PROTEIN (SEQ ID NO: 116)
QVQLVQSGAEVKKPGSSVKVSCKSSGGTSNNYAISWVRQAPGQGLDWMGGISPIFGSTAYAQKFQGRVTISA
DIFSNTAYMELNSLTSEDTAVYFCARHGNYYYYSGMDVWGQGTTVTVSS

>SC09-114 VL PROTEIN (SEQ ID NO: 117)
SYVLTQPPAVSGTPGQRVTISCSGSDSNIGRRSVNWYQQFPGTAPKLLIYSNDQRPSVVPDRFSGSKSGTSA
SLAISGLQSEDEAEYYCAAWDDSLKGAVFGGGTQLTVL

>SC08-031 VH DNA (SEQ ID NO: 118)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTACAGCCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCC
TCTGGATTCACCTTTGATGAGTATATCATGCATTGGGTCCGGCAAGCTCCCGGGAAGGGCCCGGAATGGGTC
GCAGGTATTAATTGGAAAGGTAATTTCATGGGTTATGCGGACTCTGTCCAGGGCCGATTCACCATCTCCAGA
GACAACGCCAAGAACTCCCTCTATCTGCAAATGAACAGTCTGAGAGCTGACGACACGGCCTTATATTACTGT
GCAAAAGACCGGCTGGAGAGTTCAGCTATGGACATTCTAGAAGGGGGTACTTTTGATATCTGGGGCCAAGGG
ACAATGGTCACC

>SC08-031 VH PROTEIN (SEQ ID NO:119)
EVQLVESGGGLVQPGRSLRLSCAASGFTFDEYIMHWVRQAPGKGPEWVAGINWKGNFMGYADSVQGRFTISR
DNAKNSLYLQMNSLRADDTALYYCAKDRLESSAMDILEGGTFDIWGQGTMVT

>SC08-031 VL DNA (SEQ ID NO: 120)
CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGA
ACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTACCAACAGCACCCAGGCAAAGCCCCCAAACTC
ATGATTTATGATGTCAGTAGTCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCGACACG
GCCTCCCTGAGCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAGCTCATATACAAGCAGC
AGCACTCATGTCTTCGGAACTGGGACCAAGGTCACCGTCCTAG

>SC08-031 VL PROTEIN (SEQ ID NO:121)
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSSRPSGVSNRFSGSKSGDT
ASLSISGLQAEDEADYYCSSYTSSSTHVFGTGTKVTVL

>SC08-032 VH DNA (SEQ ID NO: 122)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTACAGCCTGGCAGGTCCCTGAGACTGTCCTGTGCAGCC
TCTGGATTCAGCTTTGATGAGTACATCATGCATTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTC
GCAGGTATTAATTGGAAAGGTAATTTCATGGGTTATGCGGACTCTGTCCAGGGCCGATTCACCATCTCCAGA
GACAACGGCAAGAACTCCCTCTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATTACTGT
GCAAAAGACCGGCTGGAGAGTTCAGCTATGGACATTCTAGAAGGGGGTACTTTTGATATCTGGGGCCAAGGG
ACAATGGTCACCGTCTCGAGC

>SC08-032 VH PROTEIN (SEQ ID NO:123)
EVQLVESGGGLVQPGRSLRLSCAASGFSFDEYIMHWVRQAPGKGLEWVAGINWKGNFMGYADSVQGRFTISR
DNGKNSLYLQMNSLRAEDTALYYCAKDRLESSAMDILEGGTFDIWGQGTMVTVSS

>SC08-032 VL DNA (SEQ ID NO: 124)
CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGA
ACCCGCAGGGACGTTGGTGATTATAAGTATGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCCAAACTC
ATGATTTATGATGTCAGTAATCGGCCCTCAGGGGTCTCTAATCGCTTCTCTGGCTCCAAGTCTGGCACCACG
GCCTCCCTGACCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTATTGCAGTTCATACACAACCAGC
AACACTCGGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAG

>SC08-032 VL PROTEIN (SEQ ID NO:125)
QSALTQPASVSGSPGQSITISCTGTRRDVGDYKYVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSGTT
ASLTISGLQAEDEADYYCSSYTTSNTRVFGGGTKLTVL

>SC08-034 VH DNA (SEQ ID NO: 126)
GAGGTGCAGCTGGTGGAGACTGGGGGAGGCCTGGTACAGCCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCC
TCTGGATTCACCTTTGATGAGTATATCATGCATTGGGTCCGGCAAGCTCCCGGGAAGGGCCCGGAATGGGTC
GCAGGTATTAATTGGAAAGGTAATTTCATGGGTTATGCGGACTCTGTCCAGGGCCGATTCACCATCTCCAGA
GACAACGCCAAGAACTCCCTCTATCTGCAAATGAACAGTCTGAGAGCTGACGACACGGCCTTATATTACTGT
GCAAAAGACCGGCTGGAGAGTTCAGCTATGGACATTCTAGAAGGGGGTACTTTTGATATCTGGGGCCAAGGG
ACAATGGTCACCGTCTCGAGC

>SC08-034 VH PROTEIN (SEQ ID NO: 127)
EVQLVETGGGLVQPGRSLRLSCAASGFTFDEYIMHWVRQAPGKGPEWVAGINWKGNFMGYADSVQGRFTISR
DNAKNSLYLQMNSLRADDTALYYCAKDRLESSAMDILEGGTFDIWGQGTMVTVSS

>SC08-034 VL DNA (SEQ ID NO: 128)
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTGGGAGACAGAGTCACCATCACTTGCCGG
GCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATC
TATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACT
CTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTATTGTCAACAGGCTAACACTTATCCACTC
ACTTTCGGCGGAGGGACCAAGCTGGAGATCAAAC

>SC08-034 VL PROTEIN (SEQ ID NO:129)
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQANTYPLTFGGGTKLEIK

>SC08-035 VH DNA (SEQ ID NO: 130)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTACAGCCTGGCAGGTCCCTGAGACTCTCCTGTGCAGCC
TCTGGATTCACCTTTGATGAGTATATCATGCATTGGGTCCGGCAAGCTCCCGGGAAGGGCCCGGAATGGGTC
GCAGGTATTAATTGGAAAGGTAATTTCATGGGTTATGCGGACTCTGTCCAGGGCCGATTCACCATCTCCAGA
GACAACGCCAAGAACTCCCTCTATCTGCAAATGAACAGTCTGAGAGCTGACGACACGGCCTTATATTACTGT
GCAAAAGACCGGCTGGAGAGTTCAGCTATGGACATTCTAGAAGGGGGTACTTTTGATATCTGGGGCCAAGGG
ACAATGGTCACCGTCTCGAGC

>SC08-035 VH PROTEIN (SEQ ID NO: 131)
EVQLVESGGGLVQPGRSLRLSCAASGFTFDEYIMHWVRQAPGKGPEWVAGINWKGNFMGYADSVQGRFTISR
DNAKNSLYLQMNSLRADDTALYYCAKDRLESSAMDILEGGTFDIWGQGTMVTVSS

>SC08-035 VL DNA (SEQ ID NO: 132)
TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGA
GACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCCCTGTACTTGTCATCTAT
GGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCAGCTCAAGAAACACAGCTTCCTTG
ACCATCACTGGGGCTCAGGCGGAAGATGAGGCTGACTATTATTGTGACTCCCGGGACAGCAGTGGAACCCAT
TATGTCTTCGGAGGTGGGACCAAGGTCACCGTCCTAG

>SC08-035 VL PROTEIN (SEQ ID NO: 133)
SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSRNTASL
TITGAQAEDEADYYCDSRDSSGTHYVFGGGTKVTVL

REFERENCES

Brochet et al., Nucl. Acids Res. 36, W503-508 (2008).

De Kruif J et al., Proc. Natl. Acad. Sci. USA 92:3938 (1995).

Kanegae et al., J. Virol. 64: 2860-2865 (1990).

Kubota-Koketsu et al., Biochem. Biophys. Res. Comm. 387: 180-185 (2009).

Rota et al., J. Gen. Virol. 73: 2737-2742 (1992).

Thompson et al. JAMA 289(2): 179-186 (2003).

Thompson et al., JAMA 292(11): 1333-1340 (2004).

Wrammert et al., Nature 453: 667-672 (2008).

CLAIMS

1. A binding molecule capable of specifically binding to hemagglutinin (HA) of influenza B virus strains of the B/Yamagata and B/Victoria lineage, and capable of neutralizing said influenza B virus strains of the B/Yamagata and/or B/Victoria lineage, wherein the binding molecule does not bind to HA of influenza A viruses, and wherein the binding molecule comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 7, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 8, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:12 or SEQ ID NO: 13.

2. A binding molecule according to claim 1, wherein the binding molecule binds to the head region of the HA protein of influenza B viruses, in particular to the head region of HA1of influenza B viruses.

3. A binding molecule according to claim 1 or 2, wherein the binding molecule comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 75 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77.

4. A binding molecule according to claim 1 or 2, wherein the binding molecule comprises a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 78 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 79.

5. A binding molecule according to any one of the claims 1-4, wherein the binding molecule inhibits egress of influenza B virus from infected cells.

6. A binding molecule according to any one of the preceding claims, wherein the binding molecule is a human monoclonal antibody, or an antigen-binding fragment thereof.

7. An immunoconjugate, comprising at least one binding molecule according to any one of the preceding claims and further comprising at least one tag.

8. A nucleic acid molecule encoding a binding molecule according to any one of claims 1-6.

9. A binding molecule according to any one of claims 1-6, an immunoconjugate according to claim 7 and/or a nucleic acid molecule according to claim 8 for use as a medicament, preferably for use in the diagnosis, prophylaxis and/or treatment of an influenza infection caused by an influenza B virus.

10. A functional variant of a binding molecule according to any one of the claims 1-6.

11.A pharmaceutical composition comprising a binding molecule according to any of the claims 1-6, and/or an immunoconjugate according to claim 7, and a pharmaceutically acceptable carrier or excipient.

12. A method of detecting an influenza B virus infection comprising:

(a) assaying the level of influenza B virus antigen in a biological sample using a binding molecule according to claim 1-6, and/or an immunoconjugate according to claim 7; and

(b) comparing the assayed level of influenza B virus antigen with a control level whereby an increase in the assayed level of influenza B virus antigen compared to the control level of the influenza B virus antigen is indicative of an influenza B virus infection.