

PAN-NEURAMINIDASE INHIBITING ANTIBODIES

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT

[0001] This invention was made with Government support under HHSN272201400008C and AI141990 awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

[0002] The present invention relates to antibodies or antigen-binding fragments that are selective for neuraminidase of influenza viruses. Notably, the antibodies or antigen-binding fragments are selective for neuraminidases of both Influenza A and Influenza B viruses. Also provided are pharmaceutical compositions and methods of treating influenza using the antibodies or antigen-binding fragments provided herein.

BACKGROUND

[0003] Seasonal influenza virus infections cause significant morbidity and mortality every year around the globe. In addition, pandemics occur in irregular intervals and can claim millions of lives. Current seasonal influenza vaccines induce narrow, strain-specific immune responses and have variable effectiveness depending on how well they match circulating strains (1). These vaccines also do not protect against new pandemic viruses. Therefore, there is an urgent need to develop broadly protective or universal influenza virus vaccines (2). Current vaccines are designed to induce antibody responses against the major surface glycoprotein of the virus, the hemagglutinin (HA) (3). Antibodies to HA, specifically to its globular head domain, can block virus binding to its sialic acid receptor, but such activity is often restricted to the vaccine strain and lacks breadth. Antibodies to the HA stalk have been shown to have much greater breadth within and across influenza A groups 1 and 2, and rarely also to influenza B viruses (4-7).

[0004] The second major surface glycoprotein of the virus is neuraminidase (NA), which is a receptor destroying enzyme that cleaves terminal sialic acid from N-linked glycans (8). This activity is important for releasing incoming viruses trapped by glycans of natural defense proteins on mucosal surfaces and for releasing of nascent viruses budding from infected cells. Anti-NA antibodies can block this activity by directly binding to the enzymatic site of NA or by

sterically hindering interactions between NA and its substrate (9, 10). Anti-NA monoclonal antibodies (mAbs) and NA vaccination have been shown to protect against influenza virus lethal challenge in animal models (9-15). Furthermore, NA vaccination in guinea pigs can prevent virus transmission (16). Most importantly, anti-NA antibody titers have been shown to independently correlate with protection from infection in field studies as well as in human challenge studies (17-19). Human influenza viruses can mutate the dominant antigenic sites in HA at a high rate, due to immune pressure, error rate of the polymerase and the high plasticity of its globular head domain (20). NA, on the other hand, has been shown to exhibit a slower drift that is discordant with that of HA (21, 22). Therefore, antibody responses against NA typically show broader cross-reactivity, but this breadth has been assumed to be limited to the respective subtype (N1-N9 for influenza A viruses) (8).

[0005] There is an urgent need for more effective antiviral therapies for influenza.

BRIEF SUMMARY

[0006] The present invention relates to an antibody or antigen-binding fragment thereof, comprising a) an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 70% identity to SEQ ID NOs: 1, 2, 3, 7, 10, 13, 14, 40, 42 or 44; b) an immunoglobulin light chain variable region comprising an amino acid sequence having at least about 70% identity to any one of SEQ ID NOs: 4, 5, 6, 8, 9, 11, 12, 15, 16, 41, 43, and 45; or c) a combination thereof.

[0007] The invention also relates to an antibody or antigen-binding fragment thereof that specifically binds a neuraminidase of an influenza virus, comprising a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7, a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14 or a combination of any thereof; (b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4, 8, 11 or 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9, a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16, or a combination of any thereof; or (c) a combination thereof.

[0008] Also provided are an antibody or antigen-binding fragment that binds a neuraminidase of an influenza virus wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising at least about 70% of SEQ ID NO: 40, 42 or 44.

[0009] Further provided is an antibody or antigen-binding fragment that binds a neuraminidase of an influenza virus, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 70% of SEQ ID NO: 41, 43, or 45.

[0010] Also provided are an antibody or antigen-binding fragment that that is structurally similar to any of the antibodies or antigen binding fragments described herein.

[0011] Also provided are an antibody or antigen binding fragment having specific affinity for an N1 neuraminidase of an influenza virus, and wherein the antibody or antigen binding fragment binds to an active site residue of the N1 neuraminidase comprising at least about 70% of SEQ ID NO: 46 and containing at least one residue selected from R118, E119, L134, D151, R152, R156, W178, I222, R224, E276, E277, R371, and Y406 according to the amino acid numbering of SEQ ID NO: 46.

[0012] Further provided are nucleic acids comprising a nucleotide sequence encoding an immunoglobulin light chain variable region and/or an immunoglobulin heavy chain variable region of any antibody or antigen binding fragment provided herein. Also provided are expression vectors comprising the nucleic acids, host cells comprising the expression vectors and methods of producing the antibodies and antigen binding fragments herein.

[0013] Also provided are influenza vaccines comprising (a) an antibody or any antigen-binding fragments described herein and/or (b) a polypeptide or a nucleic acid encoding the polypeptide, the polypeptide comprising an amino acid sequence comprising at least about 70% identity to an epitope targeted by any antibody or antigen-binding fragment described herein.

[0014] Also disclosed are pharmaceutical compositions comprising any of the antibodies or antigen binding fragments disclosed herein and a pharmaceutically acceptable carrier.

[0015] Also provided are methods of preventing or treating influenza in a subject in need thereof, the methods comprising administering any antibody or antigen binding fragment described herein, any nucleic acid comprising a nucleotide sequence encoding at least a portion of an antibody or antigen binding fragment herein, any expression vector described herein, any vaccine described herein, or any composition comprising at least one of the antibodies disclosed herein to the subject.

[0016] Other objects and features will be in part apparent and in part pointed out hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1A shows a phylogenetic tree of influenza A and B virus NAs. The reactivity breadth of the three mAbs is indicated. The scale bar represents a 6% change in amino acids. The tree was built using amino acid sequences in ClustalOmega and visualized in FigTree.

[0018] FIG. 1B shows alignment of the amino-acid sequences of each mAb to the inferred unmutated common ancestor (UCA).

[0019] FIG. 1C shows a heat map of antibody binding to recombinant protein in ELISA. The minimal binding concentration is indicated.

[0020] FIG. 1D shows a heat map of antibody activity in ELLA NI assays. The IC₅₀ is indicated.

[0021] FIG. 2A shows NA representative inhibition curves in an ELLA assay (large substrate, steric hindrance sensitive) against H3N2 strain A/Hong Kong/4801/2014.

[0022] FIG. 2B shows the activity of the same mAbs against A/Hong Kong/4801/2014 in an NA star assay (small substrate, steric hindrance insensitive).

[0023] FIG. 2C shows activity of the Fab of mAb 1G01 in an ELLA assay against A/Hong Kong/4801/2014.

[0024] FIG. 2D shows a germline (GL) version of the three mAbs is also active against H3N2 strain A/Hong Kong/4801/2014.

[0025] FIG. 2E shows three mAbs are active in a microneutralization assay against H3N2 strain A/Hong Kong/4801/2014.

[0026] FIG. 2F shows ADCC reporter bioassay activity of the three mAbs. MAb CR9114 which has known ADCC reporter activity against H3N2 was used as positive control.

[0027] FIG. 3A shows the crystal structure of 1G04 with Hunan N9 NA at 3.45 Å resolution. Top left: the NA tetramer with one Fab bound to each protomer of the NA tetramer. Bottom left: the NA protomer with one Fab. Top right: the epitope on the NA. Bottom right: the Fab paratope on the NA. For left panels, one NA-Fab protomer is colored with NA in light grey and Fab light chain (L-chain) in light grey and Fab heavy chain (H-chain) in dark grey. The NA and Fab in the right panels are in dark grey and light grey, respectively. N-linked glycans are shown in stick representation with grey carbon atoms. Calcium ions are shown as black spheres. The molecular surface depicting the epitope is colored in black/dark grey.

[0028] FIG. 3B shows the crystal structure of 1E01 with Japan57 N2 NA at 2.45 Å resolution. Top left: the NA tetramer with one Fab bound to each protomer of the NA tetramer.

Bottom left: the NA protomer with one Fab. Top right: the epitope on the NA. Bottom right: the Fab paratope on the NA. For left panels, one NA-Fab protomer is colored with NA in light grey and Fab light chain (L-chain) in light grey and Fab heavy chain (H-chain) in dark grey. The NA and Fab in the right panels are in dark grey and light grey, respectively. N-linked glycans are shown in stick representation with grey carbon atoms. Calcium ions are shown as black spheres. The molecular surface depicting the epitope is colored in black/dark grey.

[0029] FIG. 3C shows the crystal structure of 1G01 with CA04 N1 NA at 3.27 Å resolution. Top left: the NA tetramer with one Fab bound to each protomer of the NA tetramer. Bottom left: the NA protomer with one Fab. Top right: the epitope on the NA. Bottom right: the Fab paratope on the NA. For left panels, one NA-Fab protomer is colored with NA in light grey and Fab light chain (L-chain) in light grey and Fab heavy chain (H-chain) in dark grey. The NA and Fab in the right panels are in dark grey and light grey, respectively. N-linked glycans are shown in stick representation with grey carbon atoms. Calcium ions are shown as black spheres. The molecular surface depicting the epitope is colored in black/dark grey.

[0030] FIG. 4A shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 2 neuraminidase (N2). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0031] FIG. 4B shows lung titers of animals treated prophylactically with mAbs (as described for FIG. 4A) on day 3 and day 6 post infection. Three mice per group were used.

[0032] FIG. 4C shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 2 neuraminidase (N2). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0033] FIG. 4D shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 2 neuraminidase (N3). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0034] FIG. 4E shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 2 neuraminidase (N6). Animals were treated with 5

mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0035] FIG. 4F shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 2 neuraminidase (N7). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0036] FIG. 4G shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 2 neuraminidase (N9). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0037] FIG. 4H shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 1 neuraminidase (N1). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0038] FIG. 4I shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 1 neuraminidase (N1). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0039] FIG. 4J shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 1 neuraminidase (N4). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0040] FIG. 4K shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 1 neuraminidase (N5). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0041] FIG. 4L shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 1 neuraminidase (N8). Animals were treated with 5 mg/kg of mAbs intraperitoneally 2 hours before intranasal virus challenge. Five animals per

group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0042] FIG. 4M shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing an influenza B virus neuraminidase. Animals were treated with 5 mg/kg of mAbs. Percent weight loss is shown and percent survival is indicated in the respective figure legends intraperitoneally 2 hours before intranasal virus challenge. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0043] FIG. 4N shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 2 neuraminidase (N2). Animals were treated with 5 mg/kg of mAb 48 hours post infection. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0044] FIG. 4O shows the efficacy of the three mAbs in a mouse model against challenge with a virus expressing a group 2 neuraminidase (N2). Animals were treated with 5 mg/kg of mAbs 72 hours post infection. Five animals per group were used. Percent weight loss is shown and percent survival is indicated in the figure legend.

[0045] FIG. 5 shows the general structure of an IgG antibody.

[0046] FIG. 6A shows the inhibitory effect of mAbs 1G04, 1E01 and 1G01 against (A) pH1N1 A/Singapore/GP1908/2015 in a small substrate NA-Star assay. Inhibition curves are shown and the dotted line represents 50% inhibition of NA activity. An irrelevant human IgG1 mAb was used as negative control antibody.

[0047] FIG. 6B shows the inhibitory effect of mAbs 1G04, 1E01 and 1G01 against H5N1 A/Indonesia/5/2005 in a small substrate NA-Star assay. Inhibition curves are shown and the dotted line represents 50% inhibition of NA activity. An irrelevant human IgG1 mAb was used as negative control antibody.

[0048] FIG. 6C shows the inhibitory effect of mAbs 1G04, 1E01 and 1G01 against H7N9 A/Shanghai/2/2013 in a small substrate NA-Star assay. Inhibition curves are shown and the dotted line represents 50% inhibition of NA activity. An irrelevant human IgG1 mAb was used as negative control antibody.

[0049] FIG. 6D shows the inhibitory effect of mAbs 1G04, 1E01 and 1G01 against B/Malaysia/2506/2004 in a small substrate NA-Star assay. Inhibition curves are shown and the dotted line represents 50% inhibition of NA activity. An irrelevant human IgG1 mAb was used as negative control antibody.

[0050] FIG. 7A shows percent inhibition of the wild-type virus (E119) by mAbs 1G04, 1E01 and 1G01 shown as inhibition curves.

[0051] FIG. 7B shows the inhibition of an oseltamivir-resistant H3N2 virus (E119V). Technical duplicates were performed.

[0052] FIG. 8A shows the minimal neutralizing concentration of mAbs against H3N2 A/Philippines/2/1982 virus. An irrelevant human IgG1 mAb was used as negative control antibody. The antibodies were run in technical duplicates.

[0053] FIG. 8B shows the minimal neutralizing concentration of mAbs against H9N2 A/chicken/Hong Kong/G9/1997 virus. An irrelevant human IgG1 mAb was used as negative control antibody. The antibodies were run in technical duplicates.

[0054] FIG. 8C shows the minimal neutralizing concentration of mAbs against H7N2 A/feline/New York/16-040082-1/2016 virus. An irrelevant human IgG1 mAb was used as negative control antibody. The antibodies were run in technical duplicates.

[0055] FIG. 9A shows the area under the curve values for 1G04, 1E01 and 1G01 as measured in ADCC assays testing pH1N1 A/Singapore/GP1908/2015 virus. Human mAb CR9114 was used as positive control and an irrelevant human IgG mAb as negative control antibody. All mAbs were run in technical duplicates.

[0056] FIG. 9B shows the area under the curve values for 1G04, 1E01 and 1G01 as measured in ADCC assays testing H5N1 A/Indonesia/5/2005 virus. Human mAb CR9114 was used as positive control and an irrelevant human IgG mAb as negative control antibody. All mAbs were run in technical duplicates.

[0057] FIG. 9C shows the area under the curve values for 1G04, 1E01 and 1G01 as measured in ADCC assays testing H7N9 A/Shanghai/2/2013 virus. Human mAb CR9114 was used as positive control and an irrelevant human IgG mAb as negative control antibody. All mAbs were run in technical duplicates.

[0058] FIG. 9D shows the area under the curve values for 1G04, 1E01 and 1G01 as measured in ADCC assays testing B/Malaysia/2506/2004 virus. Human mAb CR9114 was used as positive control and an irrelevant human IgG mAb as negative control antibody. All mAbs were run in technical duplicates.

[0059] FIG. 10 shows the interaction of 1G04 Fab CDRs with H9N9 NA from the crystal structure at 3.45 Å resolution. 1G04 light chain is shown in light grey carbon atoms (to the left in each panel labeled CDRL1 or CDRL2), and heavy chain in grey carbon atoms (to the left in each panel labeled CDRH1 and CDRH2, or in the center of panel labeled CDR H3). The

NA side-chains are shown in dark grey carbon atoms (to the right in panels labeled CDRL1, CDRL2, CDRH1, and CDRH2 or towards the perimeter in the panel labeled CDRH3).

Hydrogen bonds or salt bridges are in black dotted lines.

[0060] FIG. 11. Interaction of 1E01 Fab CDRs with Japan57 N2 NA from the crystal structure at 2.45 Å resolution. 1E01 light chain is shown in light grey carbon atoms (to the left in each panel labeled CDRL1 or CDRL2), and heavy chain in grey carbon atoms (to the left in each panel labeled CDRH1 and CDRH2, or in the center of panel labeled CDR H3). The NA side-chains are shown in dark grey carbon atoms (to the right in panels labeled CDRL1, CDRL2, CDRH1, and CDRH2 or towards the perimeter in the panel labeled CDRH3). Hydrogen bonds or salt bridges are in black dotted lines.

[0061] FIG. 12. Interaction of 1G01 Fab CDRs with CA04 N1 NA from the crystal structure at 3.27 Å resolution. 1G01 light chain is shown in light grey carbon atoms (to the left in each panel labeled CDRL1 or CDRL2), and heavy chain in grey carbon atoms (to the left in each panel labeled CDRH1 and CDRH2, or in the center of panel labeled CDR H3). The NA side-chains are shown in dark grey carbon atoms (to the right in panels labeled CDRL1, CDRL2, CDRH1, and CDRH2 or towards the perimeter in the panel labeled CDRH3). Hydrogen bonds or salt bridges are in black dotted lines. 1G01 Fab recognizes one NA protomer except for Y67 from FR L3 that interacts with residue W456 from a neighboring NA protomer.

DETAILED DESCRIPTION

[0062] The present invention relates to novel antibodies and antigen-binding fragments that show broad specificity to neuraminidases on a variety of influenza viruses. The antibodies and antigen-binding fragments herein advantageously can bind any known neuraminidase (and are not limited by type). In various embodiments, the antibodies and antigen-binding fragments comprise a) an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 70% identity to SEQ ID NOs: 1, 2, 3, 7, 10, 13, 14, 40, 42 or 44; b) an immunoglobulin light chain variable region comprising an amino acid sequence having at least about 70% identity to any one of SEQ ID NOs: 4, 5, 6, 8, 9, 11, 12, 15, 16, 41, 43, and 45; or c) a combination thereof. In additional embodiments, the antibodies and antigen-binding fragments can comprise an immunoglobulin heavy chain variable region comprising at least one complementary determining region (CDR) having an amino acid sequence comprising any one of SEQ ID NOs: 1, 2, 3, 7, 10, 13, and 14 and/or an immunoglobulin light chain variable region comprising at least one complementary determining region (CDR) having an amino acid

sequence comprising any one of SEQ ID NOs: 4, 5, 6, 8, 9, 11, 12, 15, and 16. In various embodiments, the antibodies and antigen-binding fragments comprise an immunoglobulin heavy chain variable region comprising at least about 70% of SEQ ID NO: 40, 42 or 44. In various embodiments, the antibodies and antigen-binding fragments comprise an immunoglobulin light chain variable region comprising at least about 70% of SEQ ID NO: 41, 43, or 45. Specific light and heavy chains of the antibodies of the present invention are described in more detail herein.

Definitions

[0063] As used herein, the term "antigen-binding fragment" means any antigen-binding fragment of an antibody, including an intact antibody or antigen-binding fragment that has been modified, engineered or chemically conjugated. Examples of antibodies that have been modified or engineered are chimeric antibodies, humanized antibodies, and multispecific antibodies (e.g., bispecific antibodies. 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.). 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 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, N.Y.

[0064] The term "complementarity determining regions" (CDR) as used herein means sequences within the variable regions of antibodies 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.

[0065] "Influenza A virus" as used herein refers to a type of influenza virus that can be further characterized into different "subtypes" that are characterized by various combinations of the hemagglutinin (H) and neuraminidase (N) viral surface proteins. There are 18 different hemagglutinin subtypes and 9 different neuraminidase subtypes (H1 through H18 and N1 through N9) of Influenza A viruses. Influenza A neuraminidases are further divided into subtypes (Group 1 and Group 2). Group 1 contains N1, N4, N5, and N8 and Group 2 contains N2, N3, N6, N7, and N9. According to the present invention influenza A virus subtypes may be referred to by their H number, such as for example "influenza virus comprising HA of the H1 or H5 subtype", or "H1 influenza virus" "H5 influenza virus", or by referring to their N number, such as for example "influenza virus comprising NA of the N1 or N2 subtype", or by a combination of a H number and an N number, such as for example "influenza virus subtype "H5N1 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 current nomenclature for human influenza virus strains or isolates includes the geographical location of the first isolation, strain number and year of isolation, usually with the antigenic description of HA and NA given in brackets, e.g. A/Moscow/10/00 (H3N2). Non-human strains also include the host of origin in the nomenclature.

[0066] "Influenza B virus" as used herein, refers to a second category (type) of influenza virus. Unlike influenza A, influenza B viruses are not divided into subtypes but can be broken down into lineages and strains (e.g., B/Yamagata and B/Victoria). However, influenza B viruses do contain hemagglutinin and neuraminidase proteins which are classified herein as "Type B hemagglutinin" and Type B neuraminidase", respectively.

[0067] 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 are 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 antibodies of the invention and include B-cells that originally express these antibodies and which cells have been modified to over-express the binding molecule by immortalization, amplification, enhancement of expression etc.

[0068] Amino acid sequence identity percent (%) is understood as the percentage of nucleotide or amino acid residues that are identical with nucleotide or amino acid residues in a candidate sequence in comparison to a reference sequence when the two sequences are aligned. To determine percent identity, sequences are aligned and if necessary, gaps are introduced to achieve the maximum percent sequence identity. Sequence alignment procedures to determine percent identity are well known to those of skill in the art. Often publicly available computer software such as BLAST, BLAST2, ALIGN2 or Megalign (DNASTAR) software is used to align sequences. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. When sequences are aligned, the percent sequence identity of a given sequence A to, with, or against a given sequence B (which can alternatively be phrased as a given sequence A that has or comprises a certain percent sequence identity to, with, or against a given sequence B) can be calculated as: percent sequence identity = $(X/Y) \times 100$, where X is the number of residues scored as identical matches by the sequence alignment program's or algorithm's alignment of A and B and Y is the total number of residues in B. If the length of sequence A is not equal to the length of sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

[0069] 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.

[0070] By “pharmaceutically acceptable excipient” is meant any inert substance that is combined with an active molecule such as a drug, agent, or antibody 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.

[0071] The term “specifically binding”, as used herein, in reference to the interaction of 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. An antibody 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. Antibodies or fragments thereof that immunospecifically bind to an antigen may be cross-reactive with related antigens, carrying the same epitope. Preferably, antibodies or fragments thereof that immunospecifically bind to an antigen do not cross-react with other antigens.

[0072] The term “neutralizing” as used herein in relation to the antibodies of the invention refers to antibodies that inhibit an influenza virus from replication, in vitro and/or in vivo, regardless of the mechanism by which neutralization is achieved, or assay that is used to measure the neutralization activity.

[0073] The term “therapeutically effective amount” refers to an amount of the antibodies as defined herein that is effective for preventing, ameliorating and/or treating a condition resulting from infection with an influenza A virus. Amelioration as used in herein may refer to the reduction of visible or perceptible disease symptoms, viremia, or any other measurable manifestation of influenza infection.

[0074] 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.

[0075] 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.

[0076] The term "structurally similar" as it relates to a polypeptide (e.g., an antibody or antigen-binding fragment thereof) refers to a polypeptide or protein that has one or more conservative substitutions and/or chemical modifications relative to the reference polypeptide but that retains the overall secondary, tertiary and/or quaternary structure of the reference polypeptide or protein. A polypeptide or protein "structurally similar" to another polypeptide or protein would be expected to have similar binding affinity to the reference protein's binding target.

[0077] Generally, conservative substitutions can be made at any position so long as the required activity is retained. So-called conservative exchanges can be carried out in which the amino acid which is replaced has a similar property as the original amino acid, for example the exchange of Glu by Asp, Gln by Asn, Val by Ile, Leu by Ile, and Ser by Thr. For example, amino acids with similar properties can be Aliphatic amino acids (e.g., Glycine, Alanine, Valine, Leucine, Isoleucine); Hydroxyl or sulfur/selenium-containing amino acids (e.g., Serine, Cysteine, Selenocysteine, Threonine, Methionine); Cyclic amino acids (e.g., Proline); Aromatic amino acids (e.g., Phenylalanine, Tyrosine, Tryptophan); Basic amino acids (e.g., Histidine, Lysine, Arginine); or Acidic and their Amide (e.g., Aspartate, Glutamate, Asparagine, Glutamine). Deletion is the replacement of an amino acid by a direct bond. Positions for deletions include the termini of a polypeptide and linkages between individual protein domains. Insertions are introductions of amino acids into the polypeptide chain, a direct bond formally being replaced by one or more amino acids. Amino acid sequence can be modulated with the help of art-known computer simulation programs that can produce a polypeptide with, for example, improved activity or altered regulation. On the basis of this artificially generated polypeptide sequences, a corresponding nucleic acid molecule coding for such a modulated polypeptide can be synthesized in-vitro using the specific codon-usage of the desired host cell.

Neuraminidase Specificity and Antibody Properties

[0078] The present invention is based on the discovery of highly active heterosubtypic anti-NA antibodies that can inhibit influenza A as well as B virus. Earlier antibody characterization and vaccination studies suggested that, while protection can be very broad within a subtype, immunity to NA is not heterosubtypic (compared to anti-HA stalk antibodies that typically bind across different subtypes) (9-15). Attempts to raise heterosubtypic anti-NA mAbs in rabbits against a conserved, linear peptide yielded mAbs with low NI activity and little protective effect (28, 29). A small number of human anti-NA mAbs have recently been reported that did show some cross-reactive binding but did not have any anti-viral activity in vitro or in vivo (10). In contrast, the inventive monoclonal antibodies reported here are highly active and mediate strong protection in vivo.

[0079] Therefore, in various embodiments, the antibody or antigen-binding fragment can selectively bind to a neuraminidase of an influenza virus. The antibodies and antigen binding fragments described herein can have important applications, both for therapeutic and prophylactic treatment of influenza infections.

Therapeutic use to manage acute infections

[0080] Neuraminidase is a validated drug target and several small molecules that inhibit its activity are influenza therapeutics. Like the three mAbs reported here, these small molecules target the active site of the NA. Therefore, and because of the extensive breadth of these mAbs, they could potentially be used as antivirals for treatment of seasonal, pandemic and zoonotic influenza virus infection in humans. While small molecules certainly have advantages, the therapeutic window of these drugs is restricted to 48 hours post-onset of symptoms. In the mouse model, our mAbs showed solid protection when administered as late as 72 hours following a lethal influenza virus challenge, suggesting that they might have a longer therapeutic window. The basis for this effect might be their strong NI activity combined with effector function and potentially modulation of the immune response to infection (30).

Vaccines for prophylactic treatment

[0081] In addition to the potential of these mAbs to serve as therapeutics, they might also be useful for antibody-guided vaccine design. Currently licensed influenza virus vaccines, including inactivated and live attenuated vaccines, are poor inducers of anti-NA immunity (8). However, recombinant NA-based vaccines as well as NA virus-like particles have been shown to induce high titers of anti-NA antibodies that can broadly protect in lethal mouse and ferret challenge models (13-15). Furthermore, recombinant NA-based vaccines have been shown to

inhibit influenza virus transmission in the guinea pig model (16). While broad protection within a subtype was observed in these studies, heterosubtypic immunity was not detected although it was explicitly tested in the mouse model (13). However, sequential prime-boost regimens with recombinant NA vaccines that include different subtypes might result in the induction of 1G01-like antibodies. Furthermore, scaffolded/stabilized constructs that represent the 1G01 epitope might be able to induce similar heterosubtypic antibodies as well. One complication with the development of these vaccines might be that many species that are used as animal models for influenza virus vaccines do not have antibodies with long CDR H3 regions as featured by the three reported mAbs. Therefore, mice with a humanized immunoglobulin locus might be a better model for testing these vaccines.

[0082] In summary, three mAbs were synthesized that are clonally related and bind to the influenza virus NA by inserting a long CDR H3 into the enzymatic active site, taking up the space usually occupied by sialic acid. All three mAbs show broad binding with 1G01 inhibiting all influenza A virus NA subtypes and influenza B virus NA, making these mAbs promising candidates for therapeutic development. These antibodies are highly active inhibitors of NA activity in vitro and provide broad protection from mortality and morbidity in vivo. The discovery of these mAbs raises the hope that similar antibodies can be induced in the population if the right vaccination regimen is given. Knowledge about the binding mode and epitope of these mAbs may then guide the development of NA-based universal influenza virus vaccines.

Antibody Structure and Sequences Thereof

[0083] The general structure of an IgG antibody is shown in FIG. 5. Briefly, there are two major subunits: the heavy chain and the light chain connected via disulfide bonds. Each heavy chain and light chain is further divided into a variable or a constant region. The variable regions interact most directly with the antigen and further comprise three hyper variable regions (complementary determining domains, CDRs). Thus, a single antibody comprising 2 heavy chains and 2 light chains comprises a total of 12 CDRs (3 for each heavy chain and each light chain). However, each of the variable regions, particularly the CDRs, possess some degree of affinity for the antigen and maximum affinity can be achieved with a single heavy chain coupled to a single light chain. For this reason, a typical IgG antibody is considered divalent and can potentially target two different antigens simultaneously depending on the identity of the heavy and light chains. The variable region of the antibody (both the heavy and light chains) is collectively known as the Fab fragment and can be cleaved from the constant region (known as

the Fc portion) to form an antigen-binding fragment. In addition, as noted each of the CDRs possess some degree of affinity for the antigen, and can each be considered an antigen-binding fragment. An antibody fragment can have an equivalent binding affinity for the target as the parent antibody. Both divalent and monovalent antibody fragments are included in the present invention.

[0084] Therefore, in various embodiments, the antibody or antibody binding fragment comprises a heavy chain variable region (or fragment thereof) and/or a light chain variable region (or fragment thereof). The heavy chain variable region comprises three complementary defining regions (CDRs) classified as CDR_{H1}, CDR_{H2}, and CDR_{H3}. Likewise, the light chain variable region comprises three complementarity determining regions (CDRs) classified as CDR_{L1}, CDR_{L2}, and CDR_{L3}. Suitable CDR sequences that may be incorporated in the antibodies and antibody fragments of the present invention are described in Table 1 along with sequences that correspond to a hypothetical "common ancestor" similar to all of them and the antibodies from which they originate. Amino acid substitutions in the sequences relative to the "common ancestor" sequence are identified by bold and underlined letters.

Table 1: Illustrative CDR sequences for Anti-NA antibodies

CDR Name	SEQ ID NO:	Sequence	Origin
CDR _{H1}	SEQ ID NO: 1	GFTFDDYGM	Common Ancestor mAB 1 (1G04) mAB 2 (1E04)
	SEQ ID NO: 13	GF <u>K</u> FDDY <u>A</u> M	mAB 3 (1G01)
CDR _{H2}	SEQ ID NO: 2	INWNGGSTG	Common Ancestor
	SEQ ID NO: 7	<u>L</u> NWNG <u>D</u> <u>I</u> <u>T</u> <u>A</u>	mAB 1 (1G04) mAB 2 (1E04) mAB 3 (1G01)
CDR _{H3}	SEQ ID NO: 3	TWGEYTTREEPIHSWY	Common Ancestor mAB 1 (1G04)
	SEQ ID NO: 10	TWGD <u>Y</u> TT <u>G</u> <u>E</u> <u>E</u> <u>I</u> NSWY	mAB 2 (1E04)
	SEQ ID NO: 14	<u>S</u> WG <u>D</u> <u>Y</u> <u>T</u> <u>R</u> <u>G</u> <u>P</u> <u>E</u> <u>P</u> <u>K</u> <u>I</u> <u>T</u> WY	mAB 3 (1G01)
CDR _{L1}	SEQ ID NO: 4	GISSYL	Common Ancestor

CDR Name	SEQ ID NO:	Sequence	Origin
	SEQ ID NO: 8	<u>D</u> ISS <u>F</u> L	mAB 1 (1G04)
	SEQ ID NO: 11	<u>D</u> ISSYL	mAB 2 (1E04)
	SEQ ID NO: 15	G <u>D</u> G <u>Y</u> L	mAB 3 (1G01)
CDR _{L2}	SEQ ID NO: 5	STL	Common Ancestor
	SEQ ID NO: 9	S <u>L</u> L	mAB 1 (1G04) mAB 2 (1E04) mAB 3 (1G01)
CDR _{L3}	SEQ ID NO: 6	LNSYPLETF	Common Ancestor mAB 1 (1G04)
	SEQ ID NO: 12	<u>L</u> KSYPLETF	mAB 2 (1E04)
	SEQ ID NO: 16	<u>L</u> <u>D</u> SYPLETF	mAB 3 (1G01)

[0085] The CDRs are spaced out along the light and heavy chains and are flanked by four relatively conserved regions known as framework regions (FRs). Thus, the heavy chain variable region comprises four framework regions (FRs) classified as FR_{H1}, FR_{H2}, FR_{H3}, and FR_{H4} and the light chain variable region comprises four framework regions (FRs) classified as FR_{L1}, FR_{L2}, FR_{L3}, and FR_{L4}. Representative sequences for the framework regions in the antibodies described herein are shown in Table 2 below. As above, a hypothetical 'common ancestor' is also included and amino acid substitutions relative to the "common ancestor" sequences are shown in bold and underline.

Table 2: Illustrative FR sequences for Anti-NA antibodies

FR Name	SEQ ID NO:	Sequence	Origin
FR _{H1}	SEQ ID NO: 17	EVQLVESGGGVVRPGGSLRLSCAAS	Common Ancestor
	SEQ ID NO: 25	EVQLVESGGG <u>R</u> VVRPGGSLRLSCAAS	mAB 1 (1G04) mAB 2 (1E04)
	SEQ ID NO: 34	EVQLVESGGG <u>RAL</u> RPGGSLRLSCAAS	mAB 3 (1G01)
FR _{H2}	SEQ ID NO: 18	SWVRQAPGKGLEWVSG	Common Ancestor
	SEQ ID NO: 26	SWVRQAPGKGLE <u>F</u> VSG	mAB 1 (1G04)
	SEQ ID NO: 30	SWVRQ <u>P</u> PGKGLE <u>F</u> VSG	mAB 2 (1E04)

FR Name	SEQ ID NO:	Sequence	Origin
	SEQ ID NO: 35	SWVRQ <u>V</u> PGKGLE <u>F</u> VSG	mAB 3 (1G01)
FR _{H3}	SEQ ID NO: 19	YADSVKGRETISRDNAKNSLYLQMNSLR AEDTALYHCAVR	Common Ancestor
	SEQ ID NO: 27	<u>F</u> TDSVKGRETISRDNAK <u>S</u> SLYLQMNSLRA <u>D</u> DTA <u>F</u> <u>Y</u> YCARVR	mAB 1 (1G04)
	SEQ ID NO: 31	<u>F</u> TDSVKGRETISRDN <u>V</u> K <u>S</u> SLYLQMNSLRA <u>D</u> DTA <u>F</u> <u>Y</u> YCARVR	mAB 2 (1E04)
	SEQ ID NO: 36	Y <u>T</u> DSVKGRE <u>T</u> SRDNAKNSLYL <u>H</u> I <u>N</u> S <u>P</u> K <u>P</u> EDTALY <u>Y</u> CART <u>S</u>	mAB 3 (1G01)
FR _{H4}	SEQ ID NO: 20	FDLWGRGTLTVTSS	Common Ancestor mAB 1 (1G04) mAB 2 (1E04) mAB 3 (1G01)
FR _{L1}	SEQ ID NO: 21	DIQLTQSPSFLSASVGDRVTITCRASQ	Common Ancestor mAB 1 (1G04) mAB 2 (1E04)
	SEQ ID NO: 37	DIQLTQSPSFLSASVGDR <u>I</u> TITCRASQ	mAB 3 (1G01)
FR _{L2}	SEQ ID NO: 22	AWYQQKPGKAPKLLIYAA	Common Ancestor
	SEQ ID NO: 28	AWYQQKPG <u>N</u> APK <u>V</u> LIYAA	mAB 1 (1G04)
	SEQ ID NO: 32	AWYQQKPG <u>N</u> APKLLIYAA	mAB 2 (1E04)
	SEQ ID NO: 38	AWYQQ <u>R</u> PGKAP <u>N</u> LLIYAA	mAB 3 (1G01)
FR _{L3}	SEQ ID NO: 23	SGVPSRFSGSGSGTEFTLTISLQPEDFAT YYCQQ	Common Ancestor
	SEQ ID NO: 29	SGVPSRFSGSGSGT <u>F</u> FTLTISLQPEDFAT YYCQQ	mAB 1 (1G04)
	SEQ ID NO: 33	SGVPSRFS <u>A</u> FGSGTEFTLTISLQPEDFAT YYCQ <u>H</u>	mAB 2 (1E04)
	SEQ ID NO: 39	SGVPSRFSGSG <u>Y</u> GTEFTLTISLQPEDFAT YYCQ <u>H</u>	mAB 3 (1G01)

FR Name	SEQ ID NO:	Sequence	Origin
FR _{L4}	SEQ ID NO: 24	GPGTKVDIK	Common Ancestor mAB 1 (1G04) mAB 2 (1E04) mAB 3 (1G01)

[0086] Any of the CDR_H regions may be combined with one or more of the FR_H sequences described above to form a heavy chain variable region. In various embodiments, suitable heavy chain variable regions can comprise any one of SEQ ID NOs: 40, 42, or 44. Moreover, since many conservative substitutions may be envisioned by one of ordinary skill in the art, the antibody or antibody binding fragment can comprise a heavy chain variable region comprising at least about 70% sequence identity to any one of SEQ ID NOs: 40, 42 and 44. For example, the antibody or antibody binding fragment can comprise a heavy chain variable region comprising at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5% , or at least about 99.9% sequence identity to any one of SEQ ID NOs: 40, 42 and 44. Likewise, any of the CDR_L regions may be combined with one or more of the FR_L sequences described above to form a light chain variable region. In various embodiments, suitable light chain variable regions can comprise any one of SEQ ID NOs: 41, 43 or 45. Moreover, since many conservative substitutions may be envisioned by one of ordinary skill in the art without affecting the activity of the antibody, the antibody or antibody binding fragment can comprise a light chain variable region comprising at least about 70% sequence identity of any one of SEQ ID NOs: 41, 43 and 45. For example the antibody or antibody binding fragment can comprise a light chain variable region comprising at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5% or at least about 99.9% sequence identity to any one of SEQ ID NOs: 41, 43 and 45.

[0087] For ease of reference, sequences for SEQ IDs 40-45 are described in Table 3 below. In the table, CDR sequences within each chain are underlined and point mutations relative to a "common ancestor" are bold and italicized.

Table 3: Illustrative Heavy Chain or Light Chain Variable Regions for Anti-NA Antibodies

Chain Type	SEQ ID NO:	SEQUENCE
Heavy Chain Variable Region	SEQ ID NO: 40	EVQLVESGGGRVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEF VSGLNWNGDITAF ~~T~~ DSVKGRE ~~T~~ SRDNAKSSLYLQMNSLRADDTAFYY CARVRTWGEYTTREEPIHSWY FDLWGRGTLTVSS
	SEQ ID NO: 42	EVQLVESGGGRVVRPGGSLRLSCAASGFTFDDYGMSWVRQPPGKGLEF VSGLNWNGDITAF ~~T~~ DSVKGRE ~~T~~ SRDNV ~~K~~ SSLYLQMNSLRADDTAFYY CARVRTWGDYTTGEEI ~~I~~ NSWYFDLWGRGTLTVSS
	SEQ ID NO: 44	EVQLVESGGGRALRPGGSLRLSCAASGFKFDDYAMSWVRQVPGKGLEF VSGLNWNGDITAY ~~T~~ DSVKGRE ~~T~~ SRDNAKNSLYLH ~~I~~ NSPKPEDTALYY CARTSSWGDYTRGPEPKITWYFDLWGRGTLTVSS
Light Chain variable region	SEQ ID NO: 41	DIQLTQSPSFLSASVGDRVTITCRASQDISSFLAWYQQKPGNAPKVLIIY AASLLSGVPSRFSGSGSGTFFTLTISSLQPEDFATYYCQQLNSYPLETFGP GTKVDIK
	SEQ ID NO: 43	DIQLTQSPSFLSASVGDRVTITCRASQDISSYLA ~~W~~ YQQKPGNAPKLLIIYA ASLLSGVPSRFSAF ~~G~~ SGTEFTLTISSLQPEDFATYYCQH ~~L~~ KSYPLETFGPG TKVDIK
	SEQ ID NO: 45	DIQLTQSPSFLSASVGDRVTITCRASQGDGYLA ~~W~~ YQQRPGKAPNLLIIYA ASLLSGVPSRFSGSGY ~~G~~ GTEFTLTISSLQPEDFATYYCQH ~~L~~ DSYPLETFGPG TKVDIK

[0088] As may be envisioned by one of ordinary skill in the art, the various CDR sequences and FR sequences may be combined in various ways to form new antibodies. Specific combinations of the CDR sequences within or exclusive of the full heavy or light chain variable regions of Table 3, are described in more detail below.

[0089] In various embodiments, the antibody or antigen-binding fragment thereof comprises a) an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about

98%, at least about 99%, at least about 99.5%, or at least about 99.9% identity to SEQ ID NOs: 1, 2, 3, 7, 10, 13, 14, 40, 42 or 44; b) an immunoglobulin light chain variable region comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5% or at least about 99.9% identity to any one of SEQ ID NOs: 4, 5, 6, 8, 9, 11, 12, 15, 16, 41, 43, and 45; or c) a combination thereof.

[0090] In various embodiments, the antibody or antigen-binding fragment thereof comprises an immunoglobulin heavy chain comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7, a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14, or a combination of any thereof.

[0091] In further embodiments, the antibody or antigen-binding fragment thereof can comprise an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4, 8, 11 or 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9, a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16, or a combination of any thereof.

[0092] In some embodiments, the antibody or antigen-binding fragment comprises: a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7, or a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14; and (b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4, 8, 11 or 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9, or a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16.

[0093] In some embodiments, the immunoglobulin heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14.

[0094] In still further embodiments, the immunoglobulin heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13.

[0095] For example, the heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1.

[0096] For example, the heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 13.

[0097] For example, the heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2.

[0098] In further embodiments, the immunoglobulin heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7.

[0099] In still further embodiments, the immunoglobulin heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14.

[0100] For example, the heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3.

[0101] For example, the heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 10.

[0102] For example, the heavy chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 14.

[0103] For example, the immunoglobulin heavy chain variable region of the antibody or antigen-binding fragment can comprise

a) a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3 or SEQ ID NO: 10; or

b) a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 14.

[0104] In further embodiments, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4, 8, 11 or 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16.

[0105] In some embodiments, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L1} having an amino acid sequence comprising any one of SEQ ID NOs: 4, 8, 11 or 15.

[0106] For example, the immunoglobulin light chain variable region of the antibody or

antigen-binding fragment can comprise a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4.

[0107] For example, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 8.

[0108] As another example, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 11.

[0109] As a further example, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 15.

[0110] In some embodiments, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9.

[0111] For example, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5.

[0112] For example, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9.

[0113] In further embodiments, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16.

[0114] For example, immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6.

[0115] For example, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 12.

[0116] As another example, the immunoglobulin light chain variable region of the antibody or antigen-binding fragment can comprise a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 16.

[0117] For example, in various embodiments, the immunoglobulin light chain variable

region of the antibody or antigen-binding fragment can comprise:

a) a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 8, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6; or

b) a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 11, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 12; or

c) a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 16.

[0118] Accordingly, in various embodiments, the antibody or antigen binding fragment can comprise: (a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14; and (b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4, 8, 11 or 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16.

[0119] An illustrative antibody of the present invention can comprise a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3; and b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 8, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6.

[0120] A second illustrative antibody of the present invention can comprise a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 10; and b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 11, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 12.

[0121] A third illustrative antibody of the present invention can comprise a) an

immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 14; and b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 16.

[0122] In various embodiments, the antibody or antigen-binding fragment thereof comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.9% identity to SEQ ID NOs: 1, 2, 3, 7, 10, 13, 14, 40, 42 or 44. In various embodiments, the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising at least about 70% identity to SEQ ID NO: 40, 42 or 44. For example, in various embodiments, the antibody or antigen-binding fragment can comprise an immunoglobulin heavy chain variable region comprising at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% at least about 99.5%, or at least about 99.9% identity to SEQ ID NOs: 40, 42 or 44.

[0123] In some embodiments, the immunoglobulin heavy chain variable region comprises at least about 70% identity to SEQ ID NOs: 40, 42 or 44 and further comprises at least one of SEQ ID NO: 1, 3, 7, 10, 13 or 14.

[0124] In some embodiments, the immunoglobulin heavy chain variable region comprises at least about 70% identity to SEQ ID NOs: 40, 42 or 44 and further comprises at least one of SEQ ID NO: 1, 3, or 7. For example, the immunoglobulin heavy chain variable region can comprise SEQ ID NO: 1, 3 and 7.

[0125] In some embodiments, immunoglobulin heavy chain variable region comprises at least about 70% identity to SEQ ID NO: 40, 42 or 44 and further comprises at least one of SEQ ID NOs: 1, 7 or 10. For example, the immunoglobulin heavy chain variable region can comprise SEQ ID NOs: 1, 7, and 10.

[0126] In some embodiments, the immunoglobulin heavy chain variable region comprises at least about 70% identity to SEQ ID NO: 40, 42 or 44 and further comprises at least one of SEQ ID NOs: 7, 13, or 14. For example, the immunoglobulin heavy chain variable region can comprise SEQ ID NOs: 7, 13 and 14.

[0127] In some embodiments, the immunoglobulin heavy chain variable region comprises at least about 70% identity to SEQ ID NO: 40, 42 or 44 and further comprises a CDR_{H1} comprising SEQ ID NO: 1 or 13.

[0128] In some embodiments, the immunoglobulin heavy chain variable region comprises at least about 70% identity to SEQ ID NO: 40, 42 or 44 and further comprises a CDR_{H2} comprising SEQ ID NO: 2 or 7.

[0129] In some embodiments, the immunoglobulin heavy chain variable region comprises at least about 70% identity to SEQ ID NO: 40, 42 or 44 and further comprises a CDR_{H3} comprising SEQ ID NO: 3, 10 or 14.

[0130] In various embodiments, the antibody or antigen-binding fragment thereof comprises an immunoglobulin light chain variable region comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.9% identity to SEQ ID NOs: 4, 5, 6, 8, 9, 11, 12, 15, 16, 41, 43, or 45. In various embodiments, the antibody or antigen binding fragment can comprise an immunoglobulin light chain variable region comprising at least about 70% identity to any one of SEQ ID NOs: 41, 43, or 45. For example, in various embodiments, the antibody or antigen-binding fragment can comprise an immunoglobulin light chain variable region comprising at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.9% of SEQ ID NOs: 41, 43, or 45.

[0131] In some embodiments, the immunoglobulin light chain variable region comprises at least about 70% sequence identity to any one of SEQ ID NOs: 41, 43, or 45 and further comprises at least one of SEQ ID NOs: 4, 6, 8, 9, 11, 12, 15, or 16.

[0132] In some embodiments, the immunoglobulin light chain variable region comprises at least about 70% sequence identity to any one of SEQ ID NOs: 41, 43, or 45 and further comprises at least one of SEQ ID NO: 6, 8 or 9. For example, the immunoglobulin light chain variable region comprises SEQ ID NO: 6, 8, and 9.

[0133] In some embodiments, the immunoglobulin light chain variable region comprises at least about 70% sequence identity to any one of SEQ ID NOs: 41, 43, or 45 and further comprises at least one of SEQ ID NOs: 9, 11, or 12. For example, the immunoglobulin light chain variable region can comprise SEQ ID NOs: 9, 11, and 12.

[0134] In some embodiments, the immunoglobulin light chain variable region comprises

at least about 70% sequence identity to any one of SEQ ID NOs: 41, 43, or 45 and further comprises at least one of SEQ ID NOs: 9, 15 or 16. For example, the immunoglobulin light chain variable region can comprise SEQ ID NOs: 9, 15 and 16.

[0135] In some embodiments, the immunoglobulin light chain variable region comprises at least about 70% sequence identity to any one of SEQ ID NOs: 41, 43, or 45 and further comprises a CDR_{L1} comprising any one of SEQ ID NO: 4, 8, 11 or 15.

[0136] In some embodiments, the immunoglobulin light chain variable region comprises at least about 70% sequence identity to any one of SEQ ID NOs: 41, 43, or 45 and further comprises a CDR_{L2} comprising SEQ ID NO: 5 or 9.

[0137] In some embodiments, the immunoglobulin light chain variable region comprises at least about 70% sequence identity to any one of SEQ ID NOs: 41, 43, or 45 and further comprises a CDR_{L3} comprising any one of SEQ ID NOs: 6, 12 or 16.

[0138] In some embodiments, the antibody or antigen binding fragment can comprise an immunoglobulin heavy chain variable region comprising at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.9% sequence identity to any one of SEQ ID NOs: 40, 42 and 44 and an immunoglobulin light chain variable region comprising at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.9% sequence identity to any one of SEQ ID NOs: 41, 43, and 45.

[0139] In some embodiments, the antibody or antibody-binding fragment can comprise an immunoglobulin heavy chain variable region comprising any one of SEQ ID NO: 40, 42 and 44 and an immunoglobulin light chain variable region comprising any one of SEQ ID NO 41, 43 and 45.

[0140] For example, in some embodiments the antibody or antibody-binding fragment can comprise an immunoglobulin heavy chain variable region comprising SEQ ID NO: 40 and an immunoglobulin light chain variable region comprising SEQ ID NO 41, 43 and 45.

[0141] In some embodiments, the antibody or antibody-binding fragment can comprise an immunoglobulin heavy chain variable region comprising SEQ ID NO: 42 and an immunoglobulin light chain variable region comprising SEQ ID NO 41, 43 and 45.

[0142] In further embodiments, the antibody or antibody-binding fragment can comprise an immunoglobulin heavy chain variable region comprising SEQ ID NO: 44 and an

immunoglobulin light chain variable region comprising SEQ ID NO 41, 43 and 45.

[0143] In other embodiments, the antibody or antibody binding fragment comprises an immunoglobulin heavy chain variable region comprising SEQ ID NO: 40, 42 or 44 and an immunoglobulin light chain variable region comprising SEQ ID NO 41.

[0144] In some embodiments, the antibody or antibody binding fragment comprises an immunoglobulin heavy chain variable region comprising SEQ ID NO: 40, 42 or 44 and an immunoglobulin light chain variable region comprising SEQ ID NO 43.

[0145] In some embodiments, the antibody or antibody binding fragment comprises an immunoglobulin heavy chain variable region comprising SEQ ID NO: 40, 42 or 44 and an immunoglobulin light chain variable region comprising SEQ ID NO 45.

[0146] An illustrative antibody or antibody binding fragment provided herein may comprise an immunoglobulin heavy chain variable region comprising SEQ ID NO: 40 and an immunoglobulin light chain variable region comprising SEQ ID NO: 41.

[0147] A second illustrative antibody or antibody binding fragment provided herein may comprise an immunoglobulin heavy chain variable region comprising SEQ ID NO: 42 and an immunoglobulin light chain variable region comprising SEQ ID NO: 43.

[0148] A third illustrative antibody or antibody binding fragment provided herein may comprise an immunoglobulin heavy chain variable region comprising SEQ ID NO: 44 and an immunoglobulin light chain variable region comprising SEQ ID NO: 45.

Derivatives and Synthetically Synthesized Antibodies or Binding Moieties.

[0149] Also provided are peptides, polypeptides and/or proteins derived from any of the antibodies or antibody binding fragments described herein. Generally, as used herein, the derivatives provided here are substantially similar to the antibodies or antibody binding fragments described herein. For example, they may contain one or more conservative substitutions in their amino acid sequences or may contain a chemical modification. The derivatives and modified peptides/polypeptides/proteins all are considered "structurally similar" which means they retain the structure of the parent molecule and are expected to interact with the antigen in the same way as the parent molecule.

[0150] A class of synthetically derived antibodies or antigen-binding moieties can be generated by conservatively mutating residues on the parent molecule to generate a peptide, polypeptide or protein maintaining the same activity as the parent molecule. Representative conservative substitutions are known in the art and are also summarized here.

[0151] Generally, conservative substitutions can be made at any position so long as the required activity is retained. So-called conservative exchanges can be carried out in which the amino acid which is replaced has a similar property as the original amino acid, for example the exchange of Glu by Asp, Gln by Asn, Val by Ile, Leu by Ile, and Ser by Thr. For example, amino acids with similar properties can be Aliphatic amino acids (e.g., Glycine, Alanine, Valine, Leucine, Isoleucine); Hydroxyl or sulfur/selenium-containing amino acids (e.g., Serine, Cysteine, Selenocysteine, Threonine, Methionine); Cyclic amino acids (e.g., Proline); Aromatic amino acids (e.g., Phenylalanine, Tyrosine, Tryptophan); Basic amino acids (e.g., Histidine, Lysine, Arginine); or Acidic and their Amide (e.g., Aspartate, Glutamate, Asparagine, Glutamine). Deletion is the replacement of an amino acid by a direct bond. Positions for deletions include the termini of a polypeptide and linkages between individual protein domains. Insertions are introductions of amino acids into the polypeptide chain, a direct bond formally being replaced by one or more amino acids. Amino acid sequence can be modulated with the help of art-known computer simulation programs that can produce a polypeptide with, for example, improved activity or altered regulation. On the basis of this artificially generated polypeptide sequences, a corresponding nucleic acid molecule coding for such a modulated polypeptide can be synthesized in-vitro using the specific codon-usage of the desired host cell.

[0152] A second way to generate a functional peptide/polypeptide or protein based on the sequences provided herein is through the use of computational, "in-silico" design. For example, computationally designed antibodies or antigen-binding fragments may be designed using standard methods of the art. For example, see Strauch EM et al., (Nat Biotechnol. 2017 Jul;35(7):667-671), Fleishman SJ et al., (Science. 2011 May 13;332(6031):816-21), and Koday MT et al., (PLoS Pathog. 2016 Feb 4;12(2):e1005409), each incorporated by reference in their entirety.

[0153] In various embodiments, an antibody or antibody binding fragment thereof is provided that is structurally similar to any of the antibodies described herein. For example, the antibody or antigen binding fragment can be structurally similar to a single CDR loop. For example, the antibody or antigen binding fragment can be structurally similar to a CDR_{H3} loop, e.g., a loop comprising SEQ ID NOs: 3, 10, 14 or any combination thereof.

[0154] Also provided are antibodies or antigen-binding fragments that comprise a polypeptide that has a tertiary structure that is structurally similar to a polypeptide having an amino acid sequence of any one of SEQ ID NOs: 1 to 45. Specifically, the polypeptide may have a tertiary structure that is structurally similar to a single CDR_{H3} loop comprising any one of SEQ

ID NOs: 3, 10, and 14.

Binding and Function of the Antibodies and Antigen-Binding Fragments

[0155] The antibodies and antigen binding fragments thereof described herein can bind to an influenza virus. In various embodiments, the antibody or antigen binding fragment can bind to a neuraminidase of an influenza virus. In various embodiments, the neuraminidase is an influenza type A neuraminidase. In various embodiments, the neuraminidase is any one of N1 to N9 type neuraminidases. As described above, type A influenza viruses are classified based on their hemagglutinin and neuraminidases. Therefore, in various embodiments, the antibody or antigen binding fragment has a binding affinity for a neuraminidase expressed by an HXN1, HXN2, HXN3, HXN4, HXN5, HXN6, HXN7, HXN8, or HXN9 where "X" is any integer between 1 and 18. In various embodiments, the neuraminidase is a Type B neuraminidase. An advantage of the present invention is the general affinity these antibodies and/or antigen binding fragment have to all neuraminidases (i.e., they are not selective only to type A neuraminidases at the exclusion of Type B). In various embodiments, the neuraminidase may be expressed on the surface of the influenza virus. Further, the antibodies and antigen binding fragments herein may have a certain affinity for a specific epitope on the neuraminidase. The epitope may comprise, for example, an active site of an N1 neuraminidase, such as provided herein as SEQ ID NO: 46.

[0156] In various embodiments, the antibody or antigen binding fragment interacts with at least one active site residue of the neuraminidase. For example, the antibody or antigen binding fragment can interact with at least one active site residue of an N1 residue. A representative amino acid sequence (SEQ ID NO: 46) of an active site of the N1 neuraminidase (CA04N1) is shown in the table below.

N1 Neuraminidase Sequence CA04N1 Strain: <i>A/California/04/2009</i> SEQ ID NO: 46	MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLG NQNIETCNQSVITYENNTWVNQTYVNISNTNFAAGQS VVSVKLAGNSSLCPVSGWAIYSKDNSVRIGSKGDVFVI REPFISCSPLECRTFFLTQGALLNDKHSNGTIKDRSPYRT LMSCPIGEVPSPYNSRFESVAWSASACHDGINWLTIGIS GPDNGAVAVLKYNHIITDTIKSWRNNILRTQESECACV NGSCFTVMTDGPSTNGQASYKIFRIEKGKIVKSVEMNAP NYHYEECSCTPDSSEITCVCARDNWHGSRNPWVSFNQNL EYQIGYICSGIFGDNPRPNDKTGSCGPVSSNGANGVKGF SFKYGNVWIGRTKSISSRNGFEMIWDPNWGTGTDNNF SIKQDIVGINEWSGYSFVQHPELTGLDCIRPCFWVELI RGRPKENTIWTSGSSISFCGVNSDTVGSWPDGAELPFT IDK*
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[0157] In various embodiments, the antibody or antibody binding fragment described herein can interact with one or more residue selected from the group consisting of: R118, E119, D151, R152, I222, R224, E276, E277, R371, and Y406 according to the amino acid numbering of SEQ ID NO: 46. Preferably, when the antibody or antibody-binding fragment interacts with one or more of these residues, it comprises a CDRH3 region comprising SEQ ID NO: 3.

[0158] In various embodiments, the antibody or antibody binding fragment described herein can interact with one or more residue selected from the group consisting of: R118, E119, D151, R152, I222, R224, E277, R371, and Y406 according to the amino acid numbering of SEQ ID NO: 46. Preferably, when the antibody or antibody-binding fragment interacts with one or more of these residues, it comprises a CDRH3 region comprising SEQ ID NO: 10.

[0159] In various embodiments, the antibody or antibody binding fragment described herein can interact with one or more residue selected from the group consisting of: R118, E119, L134, D151, R152, R156, W178, I222, R224, E276, E277, R371, and Y406 according to the amino acid numbering of SEQ ID NO: 46. Preferably when the antibody or antibody-binding fragment interacts with one or more of these residues it comprises a CDRH3 region of SEQ ID NO: 14.

[0160] Therefore an antibody or antigen-binding fragment having specific affinity for an N1 neuraminidase of an influenza virus is provided, wherein the antibody or antigen binding fragment binds to an active site residue of the N1 neuraminidase comprising at least about 70% of SEQ ID NO: 46 and containing at least one residue selected from R118, E119, L134, D151, R152, R156, W178, I222, R224, E276, E277, R371, and Y406 according to the amino acid numbering of SEQ ID NO: 46.

[0161] The binding of the antibody or antigen-binding fragment can neutralize or inhibit the ability of the neuraminidase to do its normal function which is to cleave sialic acid receptors to facilitate the release of viral particles from infected cells. In various embodiments, the antibodies and/or binding fragment inhibit the function of the neuraminidase to cleave its substrate with an IC₅₀ of about 0.0001 µg/ml to about 30 µg/ml. For example, the antibody or antigen-binding fragment can have an IC₅₀ of about 0.001 µg/ml to about 30 µg/ml. The inhibitory function of the antibody or antigen binding fragment can be determined by measuring, for example, the ability of the neuraminidase to cleave its substrate (sialic acid) in the presence or absence of the antibody or antigen-binding fragment.

Humanized, Monoclonal and IgG antibodies

[0162] In various embodiments, the antibody or antigen binding fragment described herein is humanized. "Humanized" antibodies are generally chimeric or mutant monoclonal antibodies from mouse, rat, hamster, rabbit or other species, bearing human constant and/or variable region domains or specific changes. Techniques for generating a so-called "humanized" antibody are well known to those of skill in the art.

[0163] In various embodiments, the antibody or antigen binding fragment described herein is a monoclonal antibody. As used herein, the term "monoclonal antibodies" refer to antibodies or antigen binding fragments that bind to the same epitope of an antigen.

[0164] In various embodiments, the antibody or antigen binding fragment described herein is an IgG type antibody.

[0165] In various embodiments, the antibody or antigen binding fragment comprises a hinge region or Fc region that contains at least one amino acid substitution, deletion, or insertion relative to the sequence of a wild-type hinge region or a wild-type Fc region. For example, in some embodiments, the antibody or antigen binding fragment comprises an Fc region that contains at least one amino acid substitution, deletion, or insertion relative to the sequence of a wild-type Fc region. In various embodiments, the substitution, deletion, or insertion prevents or reduces recycling of the antibody or antigen-binding fragment.

[0166] In various embodiments, the antibody or antigen binding fragment comprises a heavy chain variable region and/or a light chain variable region having an amino acid sequence comprising at least one amino acid substitution, insertion, or deletion as compared to any of SEQ ID NOs: 40 – 46.

Antibody Production

[0167] Methods for producing antibodies of the invention are known in the art. For example, DNA molecules encoding light chain variable regions and/or heavy chain variable regions can be chemically synthesized. Synthetic DNA molecules can be ligated to other appropriate nucleotide sequences, including, e.g., constant region coding sequences, and expression control sequences, to produce conventional gene expression constructs encoding the desired antibody. Production of defined gene constructs is within routine skill in the art.

[0168] Nucleic acids encoding desired antibodies can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Illustrative host cells are E. coli cells, Chinese hamster ovary (CHO)

cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and myeloma cells that do not otherwise produce IgG protein. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the immunoglobulin light and/or heavy chain variable regions.

[0169] Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, e.g., Trp or Tac, and a prokaryotic signal sequence. The expressed secreted protein accumulates in refractile or inclusion bodies, and can be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the proteins refolded and cleaved by methods known in the art.

[0170] If the engineered gene is to be expressed in eukaryotic host cells, e.g., CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, a poly A sequence, and a stop codon, and, optionally, may contain enhancers, and various introns. This expression vector optionally contains sequences encoding all or part of a constant region, enabling an entire, or a part of, a heavy or light chain to be expressed. The gene construct can be introduced into eukaryotic host cells using conventional techniques. The host cells express VL or VH fragments, VL-VH heterodimers, VH-VL or VL-VH single chain polypeptides, complete heavy or light immunoglobulin chains, or portions thereof, each of which may be attached to a moiety having another function (e.g., cytotoxicity). In some embodiments, a host cell is transfected with a single vector expressing a polypeptide expressing an entire, or part of, a heavy chain (e.g., a heavy chain variable region) or a light chain (e.g., a light chain variable region). In other embodiments, a host cell is transfected with a single vector encoding (a) a polypeptide comprising a heavy chain variable region and a polypeptide comprising a light chain variable region, or (b) an entire immunoglobulin heavy chain and an entire immunoglobulin light chain. In still other embodiments, a host cell is co-transfected with more than one expression vector (e.g., one expression vector encoding a polypeptide comprising an entire, or part of, a heavy chain or heavy chain variable region, and another expression vector encoding a polypeptide comprising an entire, or part of, a light chain or light chain variable region).

[0171] A polypeptide comprising an immunoglobulin heavy chain variable region or light chain variable region can be produced by growing (culturing) a host cell transfected with an expression vector encoding such variable region, under conditions that permit expression of

the polypeptide. Following expression, the polypeptide can be harvested and purified or isolated using techniques known in the art, e.g., using affinity tags such as glutathione-S-transferase (GST) and histidine tags.

[0172] A monoclonal antibody that binds a neuraminidase protein, or an antigen-binding fragment of the antibody, can be produced by growing (culturing) a host cell transfected with: (a) an expression vector that encodes a complete or partial immunoglobulin heavy chain, and a separate expression vector that encodes a complete or partial immunoglobulin light chain; or (b) a single expression vector that encodes both chains (e.g., complete or partial heavy and light chains), under conditions that permit expression of both chains. The intact antibody (or antigen-binding fragment of the antibody) can be harvested and purified or isolated using techniques known in the art, e.g., Protein A, Protein G, affinity tags such as glutathione-S-transferase (GST) and histidine tags. It is within ordinary skill in the art to express the heavy chain and the light chain from a single expression vector or from two separate expression vectors.

[0173] Therefore, in various embodiments, a nucleic acid is provided, the nucleic acid comprising a nucleotide sequence encoding the antibody or antigen-binding fragment described herein. 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.

[0174] The nucleic acid can comprise, for example, a nucleotide sequence comprising any one of SEQ ID NOs: 47 – 52 as described in the following Table. For example, the nucleic acid can comprise any one of SEQ ID NOs: 47 – 49. For example, the nucleic acid can comprise any one of SEQ ID NOs: 50 – 52.

Name (SEQ ID NO:)	Sequence
1G01_H <i>Homo sapiens</i> [clone=1G01H] Human heavy chain clone 1G01 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 47)	GAGGTGCAGCTGGTGGAGTCTGGGGG ACGTGCGCTACGGCCTGGGGGGTCCCT GAGACTCTCCTGTGCAGCCTCTGGATT CAAGTTTGATGATTATGCCATGAGCTG GGTCCGCCAGGTTCCAGGGAAGGGAC TGGAGTTTGTCTCTGGTCTTAATTGGA ATGGTGACATCACAGCTTATACAGACT CTGTGAAGGGCCGATTCACTGTCTCCA GAGACAACGCCAAGAACTCCCTGTATC TGCACATCAACAGTCCGAAGCCCGAG GACACGGCCCTCTATTATTGTGCGAGA ACTAGCTCATGGGGCGATTATACAAGA

Name (SEQ ID NO:)	Sequence
	GGGCCTGAGCCAAAGATTACCTGGTAC TTCGATCTCTGGGGCCGGGGCACTCTG GTCACCGTCTCCTCAG
1G04_H <i>H. sapiens</i> [clone=1G04H] Human heavy chain clone 1G04 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 48)	GAGGTGCAGCTGGTGGAGTCTGGGGG ACGTGTGGTACGGCCGGGGGGATCCCT GCGACTCTCCTGTGCAGCCTCTGGATT CACCTTTGATGACTATGGCATGAGCTG GGTCCGCCAAGCTCCAGGGAAGGGGC TGGAAATTTGTCTCTGGTCTTAACTGGA ATGGAGATATCACAGCTTTCACAGACT CTGTGAAGGGCCGATTACCATCTCCA GAGACAACGCCAAGAGCTCCCTGTATC TGCAAATGAACAGTCTGAGAGCCGAC GACACGGCCTTCTATTACTGTGCGAGA GTGCGGACGTGGGGCGAGTATACAAC AAGAGAGGAGCCAATTCACAGCTGGT ACTTCGATCTCTGGGGCCGTGGCACCC TGGTCACCGTCTCCTCAG
1E01_H <i>H. sapiens</i> [clone=1E01H] Human heavy chain clone 1E01 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 49)	GAGGTGCAGCTGGTGGAGTCTGGGGG ACGTGTGGTACGGCCGGGGGGTCCCT GAGACTCTCCTGTGCCGCCTCTGGATT CACGTTTGATGATTATGGCATGAGTTG GGTACGCCAACCTCCAGGGAAGGGGC TGGAGTTTGTCTCTGGTCTTAACTGGA ATGGAGATATCACAGCTTTTACAGACT CTGTGAAGGGCCGATTACCATCTCCA GAGACAACGTCAAGAGTTCCCTGTATT TGCAAATGAACAGTCTGAGAGCCGAC GACACGGCCTTCTATTACTGTGCGAGA GTACGGACATGGGGCGACTATACAAC AGGAGAGGAGATAATCAACAGCTGGT ACTTCGATCTCTGGGGCCGTGGCACCC TGGTCACCGTCTCCTCAG
1E01_L <i>H. sapiens</i> [clone=1E01L] Human Light chain clone 1E01 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 50)	GACATCCAGTTGACCCAGTCTCCATCC TTCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGTCAG GACATTAGCAGCTATTTAGCCTGGTAT CAGCAAAAACCAGGGAATGCCCTTAA GCTCCTGATCTATGCTGCATCCCTTTTG CAAAGTGGGGTCCCATCAAGGTTACAGC GCCTTTGGATCTGGGACAGAATTCAC CTCACAATCAGCAGCCTGCAGCCTGAA GATTTTGCAACTTATTACTGTCAACAC CTTAAGAGTTACCCCTGTTCACTTTC GGCCCTGGGACCAAAGTGGATATCAA AC

Name (SEQ ID NO:)	Sequence
1G01_L <i>H. sapiens</i> [clone=1G01L] Human Light chain clone 1G01 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 51)	GACATCCAGTTGACCCAGTCTCCATCC TTCCTGTCTGCATCTGTTGGAGACAGA ATAACCATCACTTGCCGGGCCAGTCAG GGCATTGATGGTTATTTAGCCTGGTAT CAGCAAAGACCAGGGAAGCCCCTAA CCTCCTGATCTATGCTGCATCCCTTTG CAAAGTGGGGTCCCATCAAGGTTACG GGCAGTGGATATGGGACAGAATTCAC TCTACAATCAGCAGCCTGCAGCCTGA GGACTTTGCAACTTATTACTGTCAACA TCTTGACAGTTACCCCCTGTTCACTTTC GGCCCTGGGACCAAAGTGGATATCAA AC
1G04_L <i>H. sapiens</i> [clone=1G04L] Human Light chain clone 1G04 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 52)	GACATCCAGTTGACCCAGTCTCCATCC TTCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGTCAG GACATTAGCAGTTTTTTAGCCTGGTAT CAGCAAAAACCAGGGAATGCCCTAA GGTCTGATCTATGCTGCGTCCCTTTTG CAGAGTGGGGTCCCATCAAGGTTACG GGCAGTGGATCTGGGACAGATTTCACT CTACAATCAGCAGCCTGCAACCTGAA GATTTGCAACTTATTACTGTCAACAG CTTAATAGTTACCCCCTGTTCACTTTCG GCCCTGGGACCAAAGTGGATATCAAA C

[0175] In various embodiments, the nucleic acid comprises a nucleotide sequence encoding an immunoglobulin heavy chain variable region of the antibody or antigen-binding fragment described herein. In various embodiments, the nucleic acid comprises a nucleotide sequence encoding an immunoglobulin light chain variable region of the antibody or antigen-binding fragment described herein. In some embodiments, the nucleic acids encode one or more complementary determining regions (CDR) having the amino acid sequences described herein. As described above, a single nucleic acid may be provided that encodes more than one protein product (e.g., the immunoglobulin light chain and the immunoglobulin heavy chain). Alternatively, two or more separate nucleic acids may be provided each encoding one component of the antibody and/or antigen binding fragment (e.g., the light chain or the heavy chain).

[0176] In various embodiments, an expression vector is provided comprising one or more of the nucleic acids described herein. Vectors can be derived from plasmids such as: F, F1,

RP1, Col, pBR322, TOL, Ti, etc; cosmids; phages such as lambda, lambdoid, M13, Mu, P1, P22, Q β , T-even, T-odd, T2, T4, T7 etc; or plant viruses. Vectors can be used for cloning and/or 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. 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.

[0177] The expression vector may be transfected into a host cell to induce the translation and expression of the nucleic acid into the heavy chain variable region and/or the light chain variable region. Therefore, a host cell is provided comprising any expression vector described herein. 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, HEK293 and HEK293T cells.

[0178] Further a method is provided for producing an antibody or antigen-binding fragment that binds a neuraminidase of an influenza virus, the method comprising growing a host cell as described herein under conditions so that the host cell expresses a polypeptide or polypeptides comprising the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region, thereby producing the antibody or antigen-binding fragment and purifying the antibody or antigen-binding fragment.

Pharmaceutical Compositions

[0179] Also provided are pharmaceutical compositions comprising at least one antibody or antigen-binding fragment described herein and a pharmaceutically acceptable carrier.

[0180] Pharmaceutical compositions containing one or more of the antibodies or antigen-binding fragments described herein can be formulated in any conventional manner. Proper formulation is dependent in part upon the route of administration selected. Routes of administration include, but are not limited to parenteral (e.g., intravenous, intra-arterial, subcutaneous, rectal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intraperitoneal, or intrasternal), topical (nasal, transdermal, intraocular), intravesical, intrathecal, enteral, pulmonary, intralymphatic, intracavitary, vaginal, transurethral, intradermal, aural, intramammary, buccal, orthotopic, intratracheal, intralesional, percutaneous, endoscopic, transmucosal, sublingual and intestinal administration. Preferably, the composition is administered parenterally or is inhaled (e.g., intranasal).

[0181] Pharmaceutically acceptable excipients for use in the compositions of the present invention are selected based upon a number of factors including the particular compound used, and its concentration, stability and intended bioavailability; the subject, its age, size and general

condition; and the route of administration.

[0182] The pharmaceutical compositions can also be formulated for parenteral administration, e.g., formulated for injection via intravenous, intra-arterial, subcutaneous, rectal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intraperitoneal, or intrasternal routes. Dosage forms suitable for parenteral administration include solutions, suspensions, dispersions, emulsions or any other dosage form that can be administered parenterally.

[0183] Pharmaceutically acceptable excipients are identified, for example, in The Handbook of Pharmaceutical Excipients, (American Pharmaceutical Association, Washington, D.C., and The Pharmaceutical Society of Great Britain, London, England, 1968). Additional excipients can be included in the pharmaceutical compositions of the invention for a variety of purposes. These excipients can impart properties which enhance retention of the compound at the site of administration, protect the stability of the composition, control the pH, facilitate processing of the compound into pharmaceutical compositions, and so on. Other excipients include, for example, fillers or diluents, surface active, wetting or emulsifying agents, preservatives, agents for adjusting pH or buffering agents, thickeners, colorants, dyes, flow aids, non-volatile silicones, adhesives, bulking agents, flavorings, sweeteners, adsorbents, binders, disintegrating agents, lubricants, coating agents, and antioxidants.

[0184] In various embodiments, the pharmaceutical composition according to the invention can comprise at least one additional antibody or antigen binding fragment targeting the influenza virus. In these embodiments, the pharmaceutical composition comprises a cocktail or a mixture of antibodies. The additional antibodies may be selective for a hemagglutinin (HA) protein or different immunogenic structure present on the influenza virus (such as M2). The additional antibodies may selectively bind the head or the stalk of the hemagglutinin protein.

[0185] In some embodiments, the composition may further comprise at least one other therapeutic, prophylactic and/or diagnostic agent. Preferably, said therapeutic and/or prophylactic 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. The therapeutic and/or prophylactic agent can comprise an M2 inhibitor (e.g., amantadine, rimantadine) and/or a neuraminidase inhibitor (e.g., zanamivir, oseltamivir). In various embodiments, the anti-viral agent can comprise baloxavir, oseltamivir, zanamivir, peramivir or

any combination thereof.

[0186] The additional antibodies or therapeutic/prophylactic and/or diagnostic agents may be used in combination with the antibodies and antigen-binding fragments of the present invention. "In combination" herein, means simultaneously, as separate formulations, or as one single combined formulation or according to a sequential administration regiment 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.

[0187] The pharmaceutical composition can be formulated without blood, plasma or a major component of blood or plasma (e.g., blood cells, fibrin, hemoglobin, albumin, etc.). The pharmaceutical composition can also be free of or substantially free (e.g., less than 1 wt.% or even less than 0.1 wt.%) of blood, plasma or a major component of blood or plasma.

Universal Influenza Vaccine

[0188] In various embodiments a vaccine is provided for preventing an influenza infection. Advantageously the vaccine can provide protection from Influenza A and Influenza B viruses. In various embodiments, the vaccine comprises any of the antibodies or antigen-binding fragments disclosed herein that are selective for a neuraminidase. In various embodiments, the vaccine may comprise a universal neuraminidase epitope, such as, for example a polypeptide comprising the residues targeted by the antibodies or antigen-binding fragments described herein. For example, the epitope can comprise an amino acid sequence comprising at least about 70% sequence identity to SEQ ID NO: 46 and containing at least one of the residues selected from the group consisting of: R118, E119, L134, D151, R152, R156, W178, I222, R224, E276, E277, R371, and Y406. In some cases, the epitope comprises at least about 70% identity to SEQ ID NO: 46 and comprises the following residues: R118, E119, D151, R152, I222, R224, E276, E277, R371, and Y406. In some cases, the epitope comprises at least about 70% identity to SEQ ID NO: 46 and comprises the following residues: R118, E119, D151, R152, I222, R224, E277, R371, and Y406. In some cases, the epitope comprises at least about 70% identity to SEQ ID NO: 46 and comprises the following residues: R118, E119, L134, D151, R152, R156, W178, I222, R224, E276, E277, R371, and Y406. In all of the epitopes described herein, residues are numbered according to the amino acid numbering of SEQ ID NO: 46. In some embodiments, the

vaccine may comprise a nucleic acid (e.g., RNA or DNA) that encodes any of the epitopes (e.g., polypeptides) described above.

[0189] In various embodiments, the vaccine further comprises an adjuvant to stimulate an immune response. Suitable adjuvants are known in the art and can include, for example, alum, aluminum hydroxide, monophosphoryl lipid A (MPL) or combinations thereof. Further, the vaccine may be prepared using suitable carriers and excipients according to pharmaceutical compositions described herein above.

[0190] In various embodiments, the vaccine can elicit an immunological response to prevent an influenza infection. The influenza infection may be caused by an influenza A virus or an influenza B virus. In some embodiments, the influenza infection is caused by an influenza A virus selected from the group consisting of: HXN1, HXN2, H2N3, HXN4, HXN5, HXN6, HXN7, HXN8, HXN9 or any combination thereof, wherein X is any integer between 1 and 18.

Methods of treating

[0191] In various embodiments, a method of preventing or treating influenza in a subject in need thereof is provided. The method can comprise administering any antibody or antigen binding fragment (including any nucleic acid or expression vector that encodes the antibody or antigen-binding fragment), any vaccine, or any composition as described herein to the subject.

[0192] In various embodiments, the composition is administered parentally (e.g., systemically). In other embodiments, the composition is inhaled orally (e.g., intranasally). In both cases the composition is formulated (e.g., with excipients) according to its mode of administration as described above.

[0193] In various embodiments the composition is administered via intranasal, intramuscular, intravenous, and/or intradermal routes. In some embodiments, the composition is provided as an aerosol (e.g., for nasal administration).

[0194] Dosing regimens can be adjusted to provide the optimum desired response (e.g., a prophylactic or therapeutic response). Therefore, the dose used in the methods herein can vary depending on the intended use (e.g., for prophylactic vs. therapeutic use). Nevertheless, the compositions described herein may be administered at a dose of about 1 to about 100 mg/kg body weight, or from about 1 to about 70 mg/kg body weight. Furthermore, 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 of the therapeutic situation.

[0195] In various embodiments, the antibody or antigen binding fragment is delivered using a gene therapy technique. Such techniques are well known in the art and generally comprise administering a viral vector comprising a nucleic acid that codes for a gene product of interest to a subject in need thereof. Therefore, in certain embodiments, the antibody or antigen-binding fragment described herein is delivered to a subject in need thereof by administering a viral vector or vectors (e.g., an adenovirus) containing one or more of the necessary nucleic acids (such as, for example, the nucleic acids provided herein) for expressing the antibody or antibody binding fragment in vivo. Similar delivery methods have successfully lead to the expression of protective antibodies in other disease contexts. For example, see Sofer-Podesta C. et al., "Adenovirus-mediated delivery of an Anti-V Antigen Monoclonal Antibody Protects Mice against a Lethal Yersinia pestis Challenge" Infection and Immunity Mar 2009, 77 (4) 1561-1568, the entire disclosure of which is incorporated herein by reference.

[0196] In various embodiments, the influenza to be treated is an influenza A virus or an influenza B virus. In some embodiments, the influenza to be treated is selected from the group consisting of: HXN1, HXN2, H2N3, HXN4, HXN5, HXN6, HXN7, HXN8, HXN9 or any combination thereof, wherein X is any integer between 1 and 18.

[0197] Having described the invention in detail, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims.

EXAMPLES

[0198] The following non-limiting examples are provided to further illustrate the present invention.

Materials and Methods

[0199] The following materials and methods were used to perform the experiments described in Examples 1 to 4.

Patient

[0200] Human peripheral blood mononuclear cells (PBMC) were obtained from a single subject, 1718003, enrolled into the Barnes Jewish Hospital Emergency Department Influenza – EDFLU - prospective observational cohort study. The EDFLU study was reviewed and approved by the Washington University in Saint Louis Institutional Review Board (Approval #

2017-10-220). 1718003 was recruited during the 2017-2018 H3N2-predominant influenza season and PBMCs were obtained on the 5th day of symptomatic illness following presentation for medical attention to the Barnes Jewish Hospital Emergency Department. The subject received the 2017-2018 seasonal influenza vaccine prior to the onset of illness and other seasonal influenza vaccines in previous influenza seasons. The subject was briefly admitted to the hospital and discharged 1½ days after admission without complication.

PBMC isolation

[0201] Blood was collected in ethylenediaminetetraacetic acid (EDTA)-anticoagulated sample tubes using standard phlebotomy techniques. PBMCs were prepared within 8 hours of collection by layering over Ficoll and centrifuging at 400 g for 30 minutes. The PBMC layer at the Ficoll interface was collected, washed with 1x phosphate buffered saline (PBS) and re-suspended in Roswell Park Memorial Institute (RPMI)-1640 media. Cell counts were obtained, and cells were cryogenically preserved in RPMI-1640 media supplemented with 10% dimethyl sulfoxide (DMSO) and 40% fetal bovine serum (FBS).

Cell sorting

[0202] Staining for sorting was performed using cryo-preserved PBMCs resuspended in PBS supplemented with 2% FBS and 1mM EDTA. Cells were stained for 30 min at 4 °C with CD71-FITC (Biolegend, clone CY1G4), CD19-PE (Biolegend, clone HIB19), IgD-PerCP-Cy5.5 (Biolegend, clone IA6-2), CD38-BV605 (Biolegend, clone HIT2), and Zombie Aqua (Biolegend). Cells were then washed twice and single ASCs (live singlet CD19+ CD4- IgDlo CD38+ CD71+) were sorted using a FACS Aria II into 96-well plates containing 10 µL of 10 mM tris(hydroxymethyl)aminomethane (Tris) supplemented with 1 U/µL RNase inhibitor (Promega) and immediately frozen on dry ice.

Monoclonal antibody generation

[0203] Antibodies were cloned as previously described (1). Generally, three antibody secreting cells (ASCs) that express IgA antibodies were isolated from the subject as described above. The variable genes (V_H and V_κ) were cloned from the cells cDNA into IgG1 expression vectors to express non-naturally occurring IgG antibodies having the specificity of the IgA antibodies in vivo. In brief, V_H and V_κ genes were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) and nested PCR reactions from singly-sorted antibody

secreting cells (ASCs) using cocktails of primers specific for IgG and Igκ using primer sets detailed in (2) and then sequenced. To generate recombinant antibodies, restriction sites were incorporated by PCR with primers to the VH3-20, JH2, VK1-9, and JK3 genes. The amplified V_H and V_κ genes were cloned into IgG1 and Igκ expression vectors, respectively, as previously described (1-3). Nucleotide sequences used to express the antibodies, are shown in the following table.. Heavy and light chain plasmids were co-transfected into Expi293F cells (Gibco) for expression and antibody was purified with protein A agarose (Invitrogen).

Name (SEQ ID NO:)	Sequence
1G01_H <i>Homo sapiens</i> [clone=1G01H] Human heavy chain clone 1G01 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 47)	GAGGTGCAGCTGGTGGAGTCTGGGGG ACGTGCGCTACGGCCTGGGGGGTCCCT GAGACTCTCCTGTGCAGCCTCTGGATT CAAGTTTGATGATTATGCCATGAGCTG GGTCCGCCAGGTTCCAGGGAAGGGAC TGGAGTTTGTCTCTGGTCTTAATTGGA ATGGTGACATCACAGCTTATACAGACT CTGTGAAGGGCCGATTCACTGTCTCCA GAGACAACGCCAAGAAGTCCCTGTATC TGCACATCAACAGTCCGAAGCCCGAG GACACGGCCCTCTATTATTGTGCGAGA ACTAGCTCATGGGGCGATTATACAAGA GGGCCTGAGCCAAAGATTACCTGGTAC TTCGATCTCTGGGGCCGGGGCACTCTG GTCACCGTCTCCTCAG
1G04_H <i>H. sapiens</i> [clone=1G04H] Human heavy chain clone 1G04 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 48)	GAGGTGCAGCTGGTGGAGTCTGGGGG ACGTGTGGTACGGCCGGGGGGATCCCT GCGACTCTCCTGTGCAGCCTCTGGATT CACCTTTGATGACTATGGCATGAGCTG GGTCCGCCAAGCTCCAGGGAAGGGGC TGAATTTGTCTCTGGTCTTAAGTGA ATGGAGATATCACAGCTTTCACAGACT CTGTGAAGGGCCGATTACCATCTCCA GAGACAACGCCAAGAGCTCCCTGTATC TGCAAATGAACAGTCTGAGAGCCGAC GACACGGCCTTCTATTACTGTGCGAGA GTGCGGACGTGGGGCGAGTATACAAC AAGAGAGGAGCCAATTCACAGCTGGT ACTTCGATCTCTGGGGCCGTGGCACCC TGGTCACCGTCTCCTCAG
1E01_H <i>H. sapiens</i> [clone=1E01H] Human heavy chain clone 1E01 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 49)	GAGGTGCAGCTGGTGGAGTCTGGGGG ACGTGTGGTACGGCCGGGGGGTCCCT GAGACTCTCCTGTGCCGCCTCTGGATT CACGTTTGATGATTATGGCATGAGTTG GGTACGCCAACCTCCAGGGAAGGGGC TGGAGTTTGTCTCTGGTCTTAAGTGA

Name (SEQ ID NO:)	Sequence
	ATGGAGATATCACAGCTTTTACAGACT CTGTGAAGGGCCGATTACCATCTCCA GAGACAACGTCAAGAGTTCCCTGTATT TGCAAATGAACAGTCTGAGAGCCGAC GACACGGCCTTCTATTACTGTGCGAGA GTACGGACATGGGGCGACTATACAAC AGGAGAGGAGATAATCAACAGCTGGT ACTTCGATCTCTGGGGCCGTGGCACCC TGGTCACCGTCTCCTCAG
1E01_L <i>H. sapiens</i> [clone=1E01L] Human Light chain clone 1E01 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 50)	GACATCCAGTTGACCCAGTCTCCATCC TTCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGTCAG GACATTAGCAGCTATTTAGCCTGGTAT CAGCAAAAACCAGGGAATGCCCTAA GCTCCTGATCTATGCTGCATCCCTTTTG CAAAGTGGGGTCCCATCAAGGTTACAGC GCCTTTGGATCTGGGACAGAATTCAC CTCACAATCAGCAGCCTGCAGCCTGAA GATTTTGCAACTTATTACTGTCAACAC CTTAAGAGTTACCCCCTGTTCACTTTC GGCCCTGGGACCAAAGTGGATATCAA AC
1G01_L <i>H. sapiens</i> [clone=1G01L] Human Light chain clone 1G01 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 51)	GACATCCAGTTGACCCAGTCTCCATCC TTCCTGTCTGCATCTGTTGGAGACAGA ATAACCATCACTTGCCGGGCCAGTCAG GGCATTGATGGTTATTTAGCCTGGTAT CAGCAAAGACCAGGGAAGCCCTAA CCTCCTGATCTATGCTGCATCCCTTTTG CAAAGTGGGGTCCCATCAAGGTTACAGC GGCAGTGGATATGGGACAGAATTCAC TCTCACAATCAGCAGCCTGCAGCCTGA GGACTTTGCAACTTATTACTGTCAACA TCTTGACAGTTACCCCCTGTTCACTTTC GGCCCTGGGACCAAAGTGGATATCAA AC
1G04_L <i>H. sapiens</i> [clone=1G04L] Human Light chain clone 1G04 immunoglobulin variable region, mRNA, partial CDS (SEQ ID NO: 52)	GACATCCAGTTGACCCAGTCTCCATCC TTCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGTCAG GACATTAGCAGTTTTTTAGCCTGGTAT CAGCAAAAACCAGGGAATGCCCTAA GGTCCTGATCTATGCTGCGTCCCTTTTG CAGAGTGGGGTCCCATCAAGGTTACAGC GGCAGTGGATCTGGGACAGATTTCACT CTCACAATCAGCAGCCTGCAACCTGAA GATTTGCAACTTATTACTGTCAACAG CTTAATAGTTACCCCCTGTTCACTTTCG

Name (SEQ ID NO:)	Sequence
	GCCCTGGGACCAAAGTGGATATCAAA C

Cells, viruses and proteins

[0204] Human embryonic kidney cells (293T) were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco) containing penicillin–streptomycin antibiotics mix (100 U/mL of penicillin, 100 µg/mL streptomycin; Gibco) and FBS (10%; Corning) resulting in complete DMEM (cDMEM). Madin Darby canine kidney (MDCK) cells were grown and maintained in cDMEM. BTI-TN-5B1-4 (*Trichoplusia ni*) cells were grown in serum-free SFX medium (HyClone) supplemented with antibiotics (100 U/mL of penicillin, 100 µg/mL streptomycin; Gibco). Sf9 (*Spodoptera frugiperda*) cells were maintained in TNM-FH medium (Gemini Bio-Products) in the presence of 10% FBS and pen-strep antibiotics mix. Single-use aliquots of ADCC bioeffector FcγRIIIa cells (Promega) were thawed before usage. Influenza viruses were grown in 8-10 day old embryonated chicken eggs (Charles River Laboratories) at 33°C (influenza B viruses) for 3 days or at 37°C (influenza A viruses) for 2 days, respectively. Virus reassortants were rescued by plasmid-based reverse genetic techniques as previously described (4). A full list of viruses used in this study can be found in Table 6. Recombinant proteins were expressed in the baculovirus expression system as previously described in detail (5). All NAs were expressed as ectodomains with an N-terminal vasodilator-stimulated phosphoprotein tetramerization domain and a hexahistidine tag for purification. A full list of recombinant neuraminidases can be found in Table 4, below.

Table 4: Recombinant NA proteins used in this study.

Subtype	Strain name	From subtype
N2	A/chicken/Hong Kong/G9/1997	H9N2
N2	A/Hong Kong/4801/2014	H3N2
N2	A/Texas/50/2012	H3N2
N3	A/swine/Missouri/4296424/2006	H6N3
N7	A/mallard/IA/10BM01929/2010	H10N7
N6	A/caspian seal/Russia/T1/2012	H4N6
N9	A/Anhui/1/2013	H7N9
N9	A/Hong Kong/4801/2014	H7N9
N1	A/Michigan/45/2015	pH1N1
N1	A/Vietnam/1204/2004 (N1)	H5N1
N4	A/mallard/Sweden/24/2002 (N4)	H8N4
N5	A/mallard/Sweden/86/2003 (N5)	H12N5

Subtype	Strain name	From subtype
N8	A/chicken/Netherlands/14015531/2014	H5N8
B NA	B/Malaysia/2506/2004	B (V)
B NA	B/Florida/04/2006	B (Y)

Enzyme-linked immunosorbent assay (ELISA).

[0205] Ninety six-well microtiter plates (Thermo Fisher) were coated with 50 μ L recombinant NA at a concentration of 2 μ g/mL in 1X coating buffer (KPL coating solution; SeraCare) at 4°C overnight. Two hundred twenty μ L blocking solution (PBS (Gibco) supplemented with 0.1% Tween-20 (T-PBS; Fisher Scientific), 3% goat serum (Life Technologies) and 0.5% milk powder (American-Bio)) were added to all wells and the plates incubated for 1 h at room temperature. The mAbs were diluted to a starting concentration of 30 μ g/mL, serially diluted 1:3, and incubated for 2 h at room temperature. The plates were washed three times with T-PBS and 50 μ L anti-human IgG (Fab specific) horseradish peroxidase antibody (HRP, produced in goat; Sigma, #A0293) diluted 1:3000 in blocking solution was added to all wells and incubated for 1 h at room temperature. The microtiter plates were washed four times and 100 μ L SigmaFast o-phenylenediamine dihydrochloride (OPD; Sigma) was added to all wells. The reaction was stopped after 10 min with 50 μ L 3M hydrochloric acid (Thermo Fisher) and the plates were read at a wavelength of 490 nm with a microtiter plate reader (Bio-Tek). The data were analyzed in Microsoft Excel and GraphPad Prism 7. The cutoff value was defined as the average of all blank wells plus three times the standard deviation of the blank wells. The minimal binding concentration was defined as the last well that has a signal higher than the cutoff value.

Enzyme-linked lectin assay (ELLA)

[0206] Ninety six-well microtiter plates (Thermo Fisher) were coated with 100 μ L/well fetuin (Sigma) at a concentration of 25 μ g/mL in 1X coating buffer (KPL coating solution; SeraCare) at 4°C overnight. The next day, the plates were washed three times with T-PBS and blocked in 200 μ L blocking buffer (5% bovine serum albumin (BSA, Biomedicals) in PBS) at room temperature. On a separate 96-well plate virus was serially diluted 1:2 in PBS. Seventy-five μ L of PBS was added to all wells and the plates incubated for 1.5 h on a shaker at room temperature. After blocking, the plates were washed three times with T-PBS, 100 μ L of serially diluted virus was transferred well by well to the fetuin coated plates and the plates were incubated for 2 hours at 33°C or 37°C depending on the virus type. The plates were washed four

times and 100 μL peanut agglutinin (PNA) conjugated to HRP at a concentration of 5 $\mu\text{g/mL}$ was added to all wells. The plates were incubated in the dark for two hours, the plates washed four times and developed with 100 μL SigmaFast OPD. The reaction was stopped after 5 min with 50 μL 3M hydrochloric acid (Thermo Fisher) and the plates were read at a wavelength of 490 nm with a microtiter plate reader (Bio-Tek). The data were analyzed in Microsoft Excel and GraphPad Prism 7, and the 50% effective concentration (EC_{50}) was calculated for each virus.

[0207] To perform neuraminidase inhibition assays, the microtiter plates were coated and blocked as described above. During the blocking of the fetuin coated plates the mAbs were diluted to a starting concentration of 30 $\mu\text{g/mL}$ and serially diluted 1:2 in PBS in a separate 96-well plate. Seventy-five μL of virus diluted to 2 x effective concentration 50 (EC_{50}) was added to wells of the serially diluted mAbs and incubated for 1.5 h on a shaker. The fetuin coated plates were washed three times with T-PBS and the virus/mAb mixture was added to the plates and incubated for 2 hours at 33°C or 37°C. The remainder of the assay was performed as described above. The data were analyzed in Microsoft Excel and GraphPad Prism 7, and the inhibitory concentration 50 (IC_{50}) was calculated.

NA-Star assay

[0208] The NA-Star Influenza Neuraminidase Inhibitor Resistance Detection Kit (Applied Biosystems) was used to measure the inhibition of the viral NA to cleave a small, soluble, chemiluminescent substrate by the anti-NA mAbs. The assay was performed according to the manufacturer's protocol. In brief, the mAbs were diluted to a concentration of 30 $\mu\text{g/mL}$, serially diluted 1:2 and 25 μL transferred to a white, flat bottom 96-well cell culture plate. Twenty-five μL of virus at the determined $2\times\text{EC}_{50}$ concentration was added to each well and plates were shaken and incubated for 20 min at 37°C. The NA-Star substrate was prepared shortly before use and 10 $\mu\text{L/well}$ of substrate was added to all wells. The plates were incubated for 30 minutes at room temperature and 60 $\mu\text{L/well}$ of NA-Star accelerator solution was added to all wells right before reading the plates using a microtiter plate reader (Bio-Tek). The data were analyzed using Microsoft Excel and GraphPad Prism 7, and inhibition curves visualized.

Microneutralization assay

[0209] Hundred $\mu\text{L/well}$ MDCK cells at a concentration of 1.5×10^5 cells/mL were seeded in 96-well cell culture plates (Sigma) and incubated at 37 °C overnight. Next day, in a separate 96-well cell culture plate the mAbs were diluted to a starting concentration of 30

µg/mL, serially diluted 1:2 in UltraMDCK media (Lonza) supplemented with tosyl phenylalanyl chloromethyl ketone (TPCK)-treated trypsin (infection media; Sigma) at a concentration of 1 µg/mL. The viruses were diluted to a concentration of 100 x TCID₅₀/50 µL in infection medium and 60 µL of virus dilution was incubated with 60 µL of serially diluted mAbs and incubated on a shaker at room temperature for 1 h. Next, the MDCK cell plates were washed with PBS (Gibco) and 100 µL of the virus/mAb mixture was added. The plates were incubated at 33°C for one hour and after the incubation time the virus/mAb mixture was removed and replaced with diluted mAb at the previous concentration. The plates were incubated at 33°C for 48 h and the readout was performed by the means of classical hemagglutination assay. In brief, chicken red blood cells (RBCs; Lampire Biological Laboratories) were diluted to a concentration of 0.5% in PBS and added to 50 µL of cell supernatant in v-bottom 96-well plates (Corning). The plates were incubated at 4 °C for 30-45 min, scanned and the results analyzed in Microsoft Excel and visualized in GraphPad Prism 7.

ADCC reporter bioassay

[0210] Hundred µL/well MDCK cells at a concentration of 1.5 x 10⁵ cells/mL were seeded in white, flat bottom 96-well cell culture plates (Corning) and incubated at 37°C overnight. The next day, the cells were washed with PBS, 100 µL of virus diluted to 1.5 x 10⁶ plaque forming units (PFU)/mL (corresponding to a multiplicity of infection (MOI) of about 5) in serum-free UltraMDCK media was added to each well and the plates were incubated for 24 h at 37°C. In a separate 96-well cell culture plate the mAbs were diluted to a starting concentration of 60 µg/mL and serially diluted 1:2 in RPMI 1640 medium (Life Technologies). Human ADCC bioeffector FcγRIIIa cells (Promega) were thawed in a 37°C water bath. The media was taken off the MDCK cell plates and 25 µL of RPMI 1640 media, 25 µL of serially diluted mAbs and 25 µL of bioeffector cells (6.25 x 10⁴ cells/25 µL) were added. After incubation for 6 h at 37°C, 75 µL of Bio-Glo luciferase (Promega) was added to each well. The plates were incubated for 10 minutes in the dark and the luciferase-induced luminescence measured with a microplate reader (BioTek). The results were analyzed in Microsoft Excel and Graphpad Prism 7, and the area under the curve (AUC) values determined. The cutoff value was defined as the average of the values of the blank wells plus 3 times the standard deviation of the blank wells.

Cloning, baculovirus expression and purification of neuraminidases for crystal structure determination.

[0211] The ectodomains of Hunan N9, Japan57 N2 and CA04 N1 NAs were expressed in a baculovirus system essentially as previously described (6). Briefly, the ectodomain (residues 83-470, 82-468 in N2 numbering) of N9 NA from A/Hunan/02650/2016 (H7N9) (Hunan N9, GISAID accession number EPI961189), the ectodomain (residues 82-469) of N2 NA from A/Japan/305/1957 (H2N2) (Japan N2, GenBank accession number CY045806) and the ectodomain (residues 82-469, 82-470 in N2 numbering) of N1 NA from A/California/04/2009 (H1N1) (CA04 N1, GenBank accession number FJ969517) were expressed in a baculovirus system for structural analyses. The cDNAs corresponding to the NA ectodomains were incorporated into a baculovirus transfer vector, pFastbacHT-A (Invitrogen) with an N-terminal gp67 signal peptide, thrombin cleavage site, and hexahistidine-tag (6). The constructed plasmids were used to transform DH10bac competent bacterial cells by site-specific transposition (Tn-7 mediated) to form a recombinant bacmid with beta-galactosidase blue-white receptor selection. The purified recombinant bacmids were used to transfect Sf9 insect cells (Thermo Fisher Scientific) for overexpression. NA proteins were produced in suspension cultures of Sf9 cells with recombinant baculovirus at an MOI of 5-10 and incubated at 28°C shaking at 110 rounds per minute. After 72 hours, Sf9 cells were removed by centrifugation and supernatants containing secreted, soluble NA proteins were concentrated and buffer-exchanged into 20 mM Tris pH 8.0, 150 mM NaCl, and then further purified by metal affinity chromatography using Ni-nitrilotriacetic acid (NTA) resin (Qiagen). For crystallization, the NA ectodomains were digested with thrombin to remove the hexahistidine-tag and purified further by size exclusion chromatography on a Hiload 16/90 Superdex 200 column (GE healthcare) in 20 mM Tris pH 8.0, 150 mM NaCl and 0.02% NaN₃. The purified NAs were measured by optical absorbance at 280 nm, and purity and integrity were analyzed by reducing and non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Expression of recombinant antibody Fabs in mammalian cells for crystal structure determination.

[0212] The light and heavy chain variable regions of human antibodies 1G04, 1E01 and 1G01 were cloned into the vector phCMV containing the corresponding lambda or kappa CL region, and the CH1 region of human IgG1 appended to hexahistidine-tag, respectively. The Fab was expressed by transient co-transfection of the expression vectors containing heavy chain and

light chain into ExpiCHO cells (Thermo Fisher Scientific). Recombinant Fab was purified from culture supernatant using metal affinity chromatography using NTA resin (Qiagen) followed by size exclusion chromatography using a Superdex 200 column (GE Healthcare). Purified Fabs in 20 mM Tris pH 8.0, 150 mM NaCl and 0.02% NaN₃ were measured by optical absorbance at 280 nm, and purity and integrity were analyzed by reducing and non-reducing SDS-PAGE.

Crystal structure determination

[0213] Crystallization experiments were set up using the sitting drop vapor diffusion method. Diffraction quality crystals for the complex of 1G04 Fab with Hunan N9 NA at 6.0 mg/ml were grown in 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 5% (w/v) polyethylene glycol 3000, 30% (w/v) polyethylene glycol 400 and 10% (v/v) glycerol at 20°C. The complex of 1E01 Fab with Japan57 N2 NA at 6.0 mg/ml was crystallized in 20% (v/v) glycerol, and 24% (w/v) polyethylene glycol 1500 at 20°C. Finally, the complex of 1G01 Fab with CA04 N1 NA at 3.0 mg/ml was crystallized in 0.1 M Tris, pH 8.5, 10% (v/v) glycerol, 20% (w/v) polyethylene glycol 300 and 5% (w/v) polyethylene glycol 8000 at 20°C. All crystals were flashed-cooled at 100 K without additional cryo-protectant.

[0214] Diffraction data were collected at synchrotron beamlines (Table 5). Data for all crystals were integrated and scaled with HKL2000 (7). Data collection statistics are outlined in Table 5.

Table 5: Data collection and refinement statistics of crystal structures.

Data set	1G04 Fab Hunan N9	1E01 Fab Japan57 N2	1G01 Fab CA04 N1
Data Collection			
X-ray source	APS 23ID-B	SSRL 12-2	APS 23ID-B
Space group	I4	P4 ₂ 12	P2 ₁
Unit cell (Å)	$a = b = 180.2,$ $c = 148.1$	$a = b = 160.5,$ $c = 87.2$	$a = 106.6, b =$ 235.0, $c = 106.3$
β angle (deg.)			$\beta = 106.4$
Resolution (Å) ^a	47.4-3.45 (3.51-3.45)	47.0-2.45 (2.49-2.45)	43.3-3.27 (3.38- 3.27)

Data set	1G04 Fab Hunan N9	1E01 Fab Japan57 N2	1G01 Fab CA04 N1
Unique reflections	30,994	40,758	70,214
Redundancy ^a	4.4 (4.1)	13.8 (10.1)	2.7 (2.2)
Average I/σ(I) ^a	14.6 (1.0)	22.5 (1.1)	4.5 (1.1)
Completeness ^a	99.1 (99.5)	99.5 (98.2)	91.9 (73.1)
$R_{\text{sym}}^{\text{a,b}}$	0.09 (0.87)	0.12 (0.99)	0.18 (0.57)
$R_{\text{pim}}^{\text{a,b}}$	0.05 (0.48)	0.03 (0.30)	0.12 (0.43)
$\text{CC}_{1/2}^{\text{a}}$	0.997 (0.844)	0.998 (0.942)	0.952 (0.876)
No. molecules per ASU ^c	2	1	4
Refinement			
Reflections in refinement	30,967	40,669	66,845
Refined residues	1,664	830	3,314
Refined waters	-	63	-
$R_{\text{cyst}}^{\text{d}}$	0.197	0.217	0.201
$R_{\text{free}}^{\text{e}}$	0.247	0.272	0.244
Twin operator/twin fraction	-	-	-l, -k, -h / 0.335
B-values (Å ²)			
Protein	136	37	62
Waters	-	57	-
Wilson B-values (Å ²)	126	50	53
Ramachandran values (%) ^f	93.7, 0.5	93.6, 0.5	94.8, 0.5
r.m.s.d. bond (Å)	0.010	0.003	0.009
r.m.s.d. angle (deg.)	1.27	0.67	1.35
PDB codes	AAAA	BBBB	CCCC
^a Parentheses denote outer-shell statistics. ^b $R_{\text{sym}} = \sum_{hkl} \sum_i I_{hkl,i} - \langle I_{hkl} \rangle / \sum_{hkl} \sum_i I_{hkl,i}$ and $R_{\text{pim}} = \sum_{hkl} [1/(N-1)]^{1/2} \sum_i I_{hkl,i} - \langle I_{hkl} \rangle / \sum_{hkl} \sum_i I_{hkl,i}$, where $I_{hkl,i}$ is the scaled intensity of the i th measurement of reflection h, k, l , $\langle I_{hkl} \rangle$ is the average intensity for that reflection, and N is the redundancy. $R_{\text{pim}} = \sum_{hkl} (1/(n-1))^{1/2} \sum_i I_{hkl,i} - \langle I_{hkl} \rangle / \sum_{hkl} \sum_i I_{hkl,i}$, where n is the redundancy ^c No. molecules for complexes refers to number of HA protomers plus antibody per			

Data set	1G04 Fab Hunan N9	1E01 Fab Japan57 N2	1G01 Fab CA04 N1
asymmetric unit (ASU) or, for antibodies, single chain constructs per ASU. ^d $R_{\text{cryst}} = \sum_{hkl} F_o - F_c / \sum_{hkl} F_o $, where F_o and F_c are the observed and calculated structure factors. ^e R_{free} was calculated as for R_{cryst} , but on 5% of data excluded before refinement. ^f The values are percentage of residues in the favored and outliers regions analyzed by MolProbity (14)			

[0215] All of the crystal structures were determined by molecular replacement using the program Phaser (8). The complex structure of 1E01 Fab with Japan57 N2 NA was determined using N2 NA from A/R1/5+/1957 (H2N2) (PDB 3TIA) and Fab light and heavy chains (PDB codes 5ITB and 4FQL, respectively) as input MR models. The complex structure of 1G04 Fab with Hunan N9 NA was determined using N9 NA from A/Shanghai/2/2013 (H7N9) (PDB 5L14) and the refined 1E01 Fab structure as input MR models. Finally, the complex structure of 1G01 Fab with CA04 N1 NA was determined using the apo CA04 N1 NA structure (PDB code 3NSS) and the refined 1E01 Fab structure as input MR models. For structural refinement of 1E01 Fab with Japan57 N2 NA and 1G04 Fab with Hunan N9 NA, initial rigid body refinement was performed in REFMAC5 (9), and simulated annealing and restrained refinement (including TLS refinement) were carried out in PHENIX (10). For structural refinement of 1G01 Fab in complex with CA04 N1 NA, pseudomerothedral twinning was detected with a twinned P21 space group where a is nearly equal to c (Table 5) and higher apparent symmetry corresponding to a C-centered orthorhombic space group. Initial rigid body refinement and restrained twin refinement with twin operator $-I, -k, -h$ and refined twin fraction 0.335 were all performed in REFMAC5 (9). Between rounds of refinements of these three structures, model building was carried out with the program Coot (11). Final statistics for these structures are summarized in Table 5. The quality of the structures was analyzed using the JCSG validation suite. All figures were generated with PyMol.

Passive transfer experiments in mice

[0216] Passive transfer experiments to test prophylactic and therapeutic efficacy of the mAbs were performed as described previously (12). In short, for the prophylactic setting, 6-8-week-old female BALB/c mice ($n=5$ mice/group) or 6-8-week-old female DBA/2J mice ($n=5$ mice/group) were given 100 μ L mAb 1G04, 1E01 or 1G01 at a concentration of 5 mg/kg

intraperitoneally. The negative control mice received 100 μ L of irrelevant human IgG control mAb at a concentration of 5 mg/kg. Two hours post transfer, the mice were anesthetized with a ketamine–xylazine–water mixture (0.15 mg ketamine/kg and 0.03 mg/kg xylazine of body weight; 100 μ L intraperitoneally) and challenged intranasally with $5 \times \text{LD}_{50}$ of challenge virus, except for the H7N2 A/feline/New York/16-040082/2016 and B/Malaysia/2506/2004 challenge experiments where $2 \times \text{LD}_{50}$ or $7.5 \times \text{LD}_{50}$ were given, respectively. BALB/c mice were used for all challenge experiments except for the H4N6 A/duck/Czechoslovakia/1956 virus challenge where DBA/2J mice were used because avian H4N6 virus does not induce weight loss in BALB/c mice. A full list of viruses used for challenge and the corresponding LD_{50} information can be found in Table 10 (Example 5). Weight loss was monitored daily for 14 days and the humane endpoint was defined as a loss of 25% of the initial day 0 weight.

[0217] For testing the therapeutic efficacy, mice were infected with $5 \times \text{LD}_{50}$ of H3N2 A/Philippines/2/1982 virus. The mAbs 1G04, 1E01, 1G01 and a human negative control mAb were administered 48 or 72 h post infection at a concentration of 5 mg/kg. Weight loss was measured daily for 14 days and mice that lost 25% or more of their day 0 weight were euthanized according to institutional guidelines.

[0218] To determine the reduction of lung viral titers, mice were given 5 mg/kg mAbs 1G04, 1E01, 1G01 and negative control human IgG via intraperitoneal injection, and after two hours were infected with $0.1 \times \text{LD}_{50}$ of H3N2 A/Philippines/2/1982 virus. At day 3 ($n=3$ mice/mAb) and day 6 ($n=3$ mice/mAb) post infection, lungs were harvested and homogenized using a BeadBlaster24 (Benchmark). Lung virus titers were assessed by standard plaque assay as described before (13). The results were analyzed in Microsoft Excel and GraphPad Prism 7.

NA sequences

[0219] The sequences for generation of the phylogenetic tree were downloaded from the Influenza Resource Database and the Global Initiative on Sharing Avian Influenza Data).

Example 1: Isolation and Identification of Human monoclonal antibodies following an infection of seasonal influenza.

[0220] Plasmablasts were single cell sorted from peripheral blood mononuclear cells (PBMCs) on day 5 after onset of symptomatic illness and the corresponding immunoglobulin heavy and light variable (IGHV and IGLV) chain genes were cloned and expressed as previously described (23). The mAbs were then screened for binding to recombinant H3 HA,

N2 NA, nucleoprotein and matrix protein 1. Three antibodies from this screening, mAbs 1G04, 1E01 and 1G01, bound to the N2 NA of the seasonal influenza virus strain A/Hong Kong/4801/2014 (H3N2), which is presumably closely related to the strain that caused the infection. 1G04, 1E01 and 1G01 form a three-member clonal family where IGHV3-20 and IGKV1-9 encode the IGHV and IGLV genes, respectively (FIG. 1A). The three mAbs most likely originate from memory B cells as evidenced by the accumulation of large number of somatic hypermutations, particularly in the IGHV genes. Alignment of the amino-acid sequences of each mAb to the inferred unmutated common ancestor (UCA) showed that the heavy chains of 1G04, 1E01 and 1G01 differ from the UCA at 12, 14 and 19 positions, respectively (FIG. 1B).

Example 2: Characterization of isolated mAb binding to recombinant N2 proteins using ELISA.

[0221] We first measured the binding affinity and cross-reactivity of the three mAbs to recombinant neuraminases (NA) from various seasonal and avian influenza viruses using an enzyme linked immunosorbent assay (ELISA). Upon further characterization, we found that the three antibodies displayed broad binding to recombinant N2 NA (group 2) from seasonal and avian influenza viruses (FIG. 1C, Table 4). Furthermore, 1G04 showed some cross-reactivity to N3 and N6 (group 2), and N1, N5 and N8 (group 1) as well as weak binding to influenza B NA (FIG. 1C). 1E01 showed an even broader binding pattern that included group 2 NAs N3, N6, N7 and N9, group 1 NAs N1, N5 and N8 as well as strong binding to influenza B NA from the B/Victoria/2/87 lineage and weak binding to the NA of the B/Yamagata/16/88 lineage. Finally, mAb 1G01 showed the broadest binding activity that covered all group 1 NAs (N1, N4, N5 and N8) and group 2 NAs (N2, N3, N6, N7 and N9), as well as NAs from both influenza B virus lineages (FIG. 1C).

Example 3: Functional capacity of mAbs to inhibit neuraminidase.

[0222] We next examined the functional capacity of the three mAbs in an enzyme linked lectin assay (ELLA) that measures neuraminidase inhibition (NI) (FIGS. 1D and 2A). The virus strains assessed are listed in Table 6, below. 1G04 inhibited N2, N3 and certain N1 NAs, while 1E01 inhibited group 2 NAs, N1 and B/Victoria/2/87 lineage NAs. 1G01 remarkably inhibited the activity of all A/group 2 and group 1 NAs and B/Victoria/2/87 lineage NA (FIGS. 1D and 2A). NA activity can be inhibited by antibodies binding directly to epitopes within the enzyme

active site or through steric hindrance when antibodies bind proximal to the active site. However, NI through steric hindrance is only observed in the ELLA when large natural substrates like fetuin are used. When small molecules are used as substrate, like in an NA-Star assay, antibodies that do not bind directly to the active site do not inhibit (9, 10). When tested in the NA-Star assay, all three mAbs potently inhibited NA activity, hinting at a binding footprint within the NA active site (FIG. 2B and FIGS. 6A-6D). For 1G01, this was further confirmed using the antigen-binding fragment (Fab) instead of the full antibody in ELLA that also removes a possible contribution of steric hindrance to inhibition. The 1G01 Fab still showed potent inhibition further suggesting that the antibody may be directly targeting the active site (FIG. 2C). Interestingly, a germline version of these three mAbs also potently inhibited NA activity in the ELLA assays (FIG. 2D).

[0223] Importantly, all three antibodies inhibited an oseltamivir resistant H3N2 virus at similar potency as a susceptible control virus (FIGS. 7A-7B). Typically, anti-NA antibodies do not show activity in in vitro neutralization assays (9), but all three mAbs here showed strong inhibitory activity in assays against various N2 viruses (FIG. 2E and FIGS. 8A-8C). While this effect might not be entirely due to inhibition of viral entry into cells, it might reflect the strong NI activity of the mAbs that efficiently blocks virus egress and subsequent virus replication necessary for a positive readout in that assay. In addition to Fab-based antiviral activity, Fc-FcR mediated effector functions like antibody dependent cellular cytotoxicity (ADCC) have been shown to be important for broadly protective anti-HA antibodies and have also been detected for anti-NA antibodies (9, 24). All three mAbs showed activity against a panel of different NAs in an ADCC bioreporter assay, even when they bound only at higher concentrations (e.g., 1G04 binding to influenza B NA) (FIG. 2F and FIGS. 9A-9D).

Table 6: Virus strains used in this study.

Subtype	HA	NA	Backbone
H1N1	A/Singapore/GP1908/2015	A/Singapore/GP1908/2015	A/Texas/1/1977
H1N1	A/New Caledonia/20/1999	A/New Caledonia/20/1999	A/New Caledonia/20/1999
H5N1	A/Indonesia/5/2005 ^S	A/Indonesia/5/2005	A/PR/8/1934
H5N1	A/Vietnam/1204/2004 ^S	A/Vietnam/1204/2004	A/PR/8/1934
cH6/1 N4*	head: A/mallard/Sweden/81/2002 stalk: A/PR/8/1934	A/mallard/Sweden/24/2002	A/PR/8/1934

Subtype	HA	NA	Backbone
H9N4	A/guinea fowl/HK/WF10/99	A/mallard/Sweden/24/2002	A/PR/8/1934
H10N8	A/Jiangxi-Donghu/346/2013	A/Jiangxi-Donghu/346/2013	A/PR/8/1934
H5N8	A/gyrfalcon/Washington/41088-6/2014	A/gyrfalcon/Washington/41088-6/2014	A/PR/8/1934
H4N8	A/duck/Czech/1956	A/mallard/Sweden/50/2002	A/PR/8/1934
H12N5	A/mallard/Interior Alaska/7MP0167/2007	A/mallard/Interior Alaska/7MP0167/2007	A/mallard/Interior Alaska/7MP0167/2007
H15N5	A/mallard duck/Sweden/139579/2012	A/PR/8/1934	A/PR/8/1934
H7N9	A/Shanghai/1/2013	A/Shanghai/1/2013	A/PR/8/1934
H5N6	A/Shenzhen/1/2016 [#]	A/Shenzhen/1/2016	A/PR/8/1934
H4N6	A/duck/Czechoslovakia/1956	A/duck/Czechoslovakia/1956	A/PR/8/1934
H10N7	A/mallard/IA/10BM01929/2010	A/mallard/IA/10BM01929/2010	A/mallard/IA/10BM01929/2010
H1N7	A/PR/8/1934	A/chicken/Netherlands/1/2003	A/PR/8/1934
H3N2	A/Philippines/2/1982	A/Philippines/2/1982	A/PR/8/1934
H3N2	A/Hong Kong/4801/2014	A/Hong Kong/4801/2014	A/PR/8/1934
H3N2 [#]	A/Washington/01/2007	A/Washington/01/2007	A/Washington/01/2007
H3N2 ^{&}	A/Texas/2/2007	A/Texas/2/2007	A/Texas/2/2007
H9N2	A/chicken/Hong Kong/G9/1997	A/chicken/Hong Kong/G9/1997	A/PR/8/1934
H7N2	A/feline/New York/16-040082-1/2016	A/feline/New York/16-040082-1/2016	A/PR/8/1934
H6N3	A/swine/Missouri/4296424/2006	A/swine/Missouri/4296424/2006	A/PR/8/1934
B	B/Malaysia/2506/2004	B/Malaysia/2506/2004	B/Malaysia/2506/2004
B	B/Florida/04/2006	B/Florida/04/2006	B/Florida/04/2006
B	B/Yamagata/16/1988	B/Yamagata/16/1988	B/Yamagata/16/1988
* This virus has a chimeric HA consisting of the head domain of the A/mallard/Sweden/81/2002 virus and the stalk domain of A/PR/8/1934. [#]Osetamivir susceptible E119 variant ^{&}Osetamivir resistant E119V variant ^SPolybasic cleavage site removed.			

Example 4: Structural determination of binding epitope for the monoclonal antibodies.

[0224] To elucidate the epitopes of the three mAbs and the structural basis of their broad protection, we determined crystal structures of 1G04 with the N9 NA from the recent H7N9

epidemic isolate A/Hunan/02650/2016 (Hunan N9) at 3.45 Å, 1E01 in complex with the N2 NA of the 1957 H2N2 pandemic isolate A/Japan/305/1957 (Japan57 N2) at 2.45 Å and 1G01 in complex with the N1 NA of the 2009 pandemic H1N1 isolate A/California/04/2009 (CA04 N1) at 3.27 Å resolution (FIG. 3, NA strains are listed in Table 4). In all three Fab-NA complex structures, one Fab bound to one NA protomer of the NA tetramer. Significantly, all three Fabs bound to the NAs such that they fully blocked the NA active site (FIG. 3). 1G04, 1E01 and 1G01 recognized their epitopes using both heavy and light chains; the heavy chains especially complementarity determining regions (CDR) H3 in three antibodies played the dominant role in their NA interactions. The total buried surface areas on the NA by 1G04, 1E01 and 1G01 was 1030 Å², 900 Å² and 800 Å², respectively, of which 87%, 84% and 77% arose from the heavy chain, and 67%, 66% and 77% from the extended CDR H3 loop.

[0225] Antibody 1G04 interacted with Hunan N9 NA using CDRs L1, L2, H1, H2 and H3 (FIG. 3A, FIG. 10, Table 7). The long 21-residue CDR H3 of 1G04 contributed most of the Fab- interactions to 20 N9 NA residues including 10 active site residues, which are conserved in group 1, group 2 and influenza B NAs: R118, E119, D151, R152, I222, R224, E276, E277, R371 and Y406. CDRs L1, L2, H1 and H2 contacted some non-conserved N9 NA residues. Similarly, 1E01 interacted with Japan57 N2 using the same five CDR loops as 1G04. The 21-residue CDR H3 of 1E01 dominated with interaction to 16 N2 NA residues including all of the conserved active site epitope residues recognized by 1G04 except E276 (FIG. 3B, FIG. 11, Table 8). Interestingly, 1G01 bound CA04 N1 NA using only CDRs L1, L2 and H3 as well as one residue, Y67, from the framework region (FR) L3. The 21-residue CDR H3 of 1G01 interacted with 19 CA04 N1 NA residues, which include 13 NA-conserved active site (10 of which are conserved with 1G04) or second-shell residues including L134, R156, W178 (FIG. 3C, FIG. 12, Table 9). The epitopes of these three antibodies are unique compared to the structurally characterized mouse NA antibodies, which bind to epitopes distant from the active site or on the rim of the active site (12, 25-27).

[0226] These reactivity profiles suggest that these antibodies were initially induced by an H3N2 infection and have acquired affinity to N1 and influenza B virus NA antigens through subsequent exposures of this individual to H1N1 and influenza B viruses. The recent H3N2 infection of this individual then recalled these clones. It is likely, that the actual serum antibody titer in the individual was not high enough to mediate protection, which would explain why the person got infected. It is therefore possible that the antibody clones are present in the memory B compartment of the individual but not in the long-lived plasma cell compartment in the bone

marrow.

Table 7: Sequence conservation of 1G04 epitope by NA subtype.

NA residue number	Hunan N9	Flu A Group 1				Flu A Group 2					Flu B
		N1 (9,744) ^a	N4 (149)	N5 (237)	N8 (1,290)	N2 (12,826)	N3 (772)	N6 (1,218)	N7 (473)	N9 (587)	B NA (3,469)
118	<u>R</u>	R ^b (99.9) ^c	R (99.3)	R (99.6)	R (99.8)	R (100)	R (100)	R (100)	R (100)	R (99.8)	R (100)
119	<u>E</u>	E (99.5)	E (99.3)	E (100)	E (99.9)	E (99.7)	E (100)	E (100)	E (100)	E (99.7)	E (99.9)
149	I	V (54.4)	V (98.7)	V (99.6)	V (99.5)	V (65.2)	I (90.3)	I (99.2)	I (97.3)	I (99.5)	R (99.9)
151	<u>D</u>	D (99.2)	D (100)	D (99.6)	D (99.9)	D (93.5)	D (99.1)	D (99.9)	D (100)	D (99.7)	D (99.4)
152	<u>R</u>	R (100)	R (100)	R (99.6)	R (99.8)	R (99.9)	R (99.7)	R (99.8)	R (99.8)	R (99.3)	R (99.9)
198	N	D (99.5)	D (100)	D (99.6)	D (99.9)	D (99.6)	D (99.4)	N (97.8)	N (100)	N (99.5)	D (99.4)
199	N	N (81.7)	A (56.4)	D (98.7)	A (66.0)	K (59.3)	N (97.8)	N (98.1)	D (88.4)	N (99.8)	S (52.7)
220	R	N (85.0)	G (100)	K (98.7)	G (99.7)	K (80.5)	K (74.9)	G (99.1)	G (56.4)	R (98.1)	N (51.9)
221	N	N (72.0)	N (100)	Q (98.7)	D (97.8)	N (50.6)	D (90.2)	N (99.6)	N (98.9)	N (99.8)	N (81.4)
222	<u>I</u>	I (99.2)	I (99.3)	I (99.6)	I (99.7)	I (99.3)	I (99.4)	I (99.3)	I (99.8)	I (100)	I (99.5)
224	<u>R</u>	R (99.9)	R (100)	R (99.2)	R (99.9)	R (99.9)	R (99.2)	R (99.5)	R (100)	R (100)	R (99.8)
245	P	P (99.5)	P (100)	P (99.6)	P (100)	S (89.0)	P (99.4)	P (99.4)	S (96.8)	S (92.7)	S (52.7)
246	A	S (99.2)	S (99.3)	A (99.6)	A (99.5)	A (99.8)	A (99.5)	A (99.5)	A (100)	A (99.5)	A (99.9)
247	T	N (59.7)	D (98.7)	N (92.4)	N (99.0)	S (89.7)	A (89.8)	N (99.0)	S (96.0)	T (99.7)	D (77.5)
276	<u>E</u>	E (99.9)	E (100)	E (99.6)	E (99.9)	E (99.9)	E (99.6)	E (99.6)	E (100)	E (100)	E (100)
277	<u>E</u>	E (100)	E (100)	E (99.6)	E (100)	E (100)	E (99.6)	E (99.6)	E (100)	E (100)	E (100)
295	W	W (99.9)	W (100)	W (99.6)	W (100)	W (99.9)	W (99.6)	W (99.8)	W (100)	W (100)	S (66.3)
296	Q	H (99.9)	R (98.7)	N (99.6)	T (99.7)	K (97.6)	K (99.0)	K (97.9)	Q (99.8)	Q (99.3)	Y (99.9)
345	N	G (100)	G (100)	N (95.8)	G (98.9)	G (99.9)	G (99.6)	P (99.7)	G (98.7)	N (98.1)	S (95.8)
347	N	N (66.2)	Y (100)	Y (100)	Y (100)	H (73.1)	P (89.5)	P (99.8)	P (99.6)	N (99.3)	G (99.7)
370	S	S (89.7)	S (99.3)	S (99.6)	S (99.9)	S (70.9)	G (89.8)	S (99.6)	S (98.1)	S (100)	K (45.5)
371	<u>R</u>	R	R	R	R	R	R	R	R	R	R

NA residue number	Hunan N9	Flu A Group 1				Flu A Group 2					Flu B
		N1 (9,744) ^a	N4 (149)	N5 (237)	N8 (1,290)	N2 (12,826)	N3 (772)	N6 (1,218)	N7 (473)	N9 (587)	B NA (3,469)
		(99.9)	(100)	(100)	(100)	(99.9)	(100)	(100)	(100)	(100)	(99.9)
<u>406</u>	<u>Y</u>	Y (99.9)	Y (100)	Y (99.6)	Y (99.9)	Y (99.9)	Y (100)	Y (100)	Y (100)	Y (100)	Y (99.9)
<u>432</u>	<u>K</u>	K (76.1)	K (99.3)	E (97.0)	E (99.8)	E (58.1)	N (99.6)	K (98.9)	E (100)	K (99.3)	- ^d

NA residues conserved in influenza A N1 to N9 and Influenza B NA are underlined.

^a Number of non-redundant influenza NA sequences for subtype from the Influenza Virus Resource at the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/genomes/FLU/>) in Nov 28, 2018.

^b Most common residue at position for subtype.

^c Percent identity for most common residue.

^d Sequence deletion as aligned to N2 NA.

Table 8: Sequence conservation of 1E01 epitope by NA subtype.

NA residue number	Japan57 N2	Flu A Group 1				Flu A Group 2					Flu B
		N1 (9,744) ^a	N4 (149)	N5 (237)	N8 (1,290)	N2 (12,826)	N3 (772)	N6 (1,218)	N7 (473)	N9 (587)	B NA (3,469)
<u>118</u>	<u>R</u>	R ^b (99.9) ^c	R (99.3)	R (99.6)	R (99.8)	R (100)	R (100)	R (100)	R (100)	R (99.8)	R (100)
<u>119</u>	<u>E</u>	E (99.5)	E (99.3)	E (100)	E (99.9)	E (99.7)	E (100)	E (100)	E (100)	E (99.7)	E (99.9)
<u>151</u>	<u>D</u>	D (99.2)	D (100)	D (99.6)	D (99.9)	D (93.5)	D (99.1)	D (99.9)	D (100)	D (99.7)	D (99.4)
<u>152</u>	<u>R</u>	R (100)	R (100)	R (99.6)	R (99.8)	R (99.9)	R (99.7)	R (99.8)	R (99.8)	R (99.3)	R (99.9)
<u>198</u>	<u>D</u>	D (99.5)	D (100)	D (99.6)	D (99.9)	D (99.6)	D (99.4)	N (97.8)	N (100)	N (99.5)	D (99.4)
<u>199</u>	<u>R</u>	N (81.7)	A (56.4)	D (98.7)	A (66.0)	K (59.3)	N (97.8)	N (98.1)	D (88.4)	N (99.8)	S (52.7)
<u>220</u>	<u>Q</u>	N (85.0)	G (100)	K (98.7)	G (99.7)	K (80.5)	K (74.9)	G (99.1)	G (56.4)	R (98.1)	N (51.9)
<u>221</u>	<u>N</u>	N (72.0)	N (100)	Q (98.7)	D (97.8)	N (50.6)	D (90.2)	N (99.6)	N (98.9)	N (99.8)	N (81.4)
<u>222</u>	<u>I</u>	I (99.2)	I (99.3)	I (99.6)	I (99.7)	I (99.3)	I (99.4)	I (99.3)	I (99.8)	I (100)	I (99.5)
<u>224</u>	<u>R</u>	R (99.9)	R (100)	R (99.2)	R (99.9)	R (99.9)	R (99.2)	R (99.5)	R (100)	R (100)	R (99.8)
<u>245</u>	<u>S</u>	P (99.5)	P (100)	P (99.6)	P (100)	S (89.0)	P (99.4)	P (99.4)	S (96.8)	S (92.7)	S (52.7)
<u>246</u>	<u>A</u>	S (99.2)	S (99.3)	A (99.6)	A (99.5)	A (99.8)	A (99.5)	A (99.5)	A (100)	A (99.5)	A (99.9)
<u>249</u>	<u>R</u>	Q (89.5)	Q (100)	Q (99.6)	Q (99.9)	K (73.1)	S (88.2)	R (84.5)	Q (99.6)	P (100)	V (53.8)
<u>277</u>	<u>E</u>	E (100)	E (100)	E (99.6)	E (100)	E (100)	E (99.6)	E (99.6)	E (100)	E (100)	E (100)

NA residue number	Japan57 N2	Flu A Group 1				Flu A Group 2					Flu B
		N1 (9,744) ^a	N4 (149)	N5 (237)	N8 (1,290)	N2 (12,826)	N3 (772)	N6 (1,218)	N7 (473)	N9 (587)	B NA (3,469)
295	W	W (99.9)	W (100)	W (99.6)	W (100)	W (99.9)	W (99.6)	W (99.8)	W (100)	W (100)	S (66.3)
296	K	H (99.9)	R (98.7)	N (99.6)	T (99.7)	K (97.6)	K (99.0)	K (97.9)	Q (99.8)	Q (99.3)	Y (99.9)
342	N	S (68.8)	N (100)	G (100)	G (99.9)	N (97.9)	N (89.9)	T (97.5)	T (99.6)	P (99.8)	D (77.5)
<u>371</u>	<u>R</u>	R (99.9)	R (100)	R (100)	R (100)	R (99.9)	R (100)	R (100)	R (100)	R (100)	R (99.9)
<u>406</u>	<u>Y</u>	Y (99.9)	Y (100)	Y (99.6)	Y (99.9)	Y (99.9)	Y (100)	Y (100)	Y (100)	Y (100)	Y (99.9)
432	Q	K (76.1)	K (99.3)	E (97.0)	E (99.8)	E (58.1)	N (99.6)	K (98.9)	E (100)	K (99.3)	- ^d

NA residues conserved in influenza A N1 to N9 and Influenza B NA are underlined.

^a Number of non-redundant influenza NA sequences for subtype from the Influenza Virus Resource at the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/genomes/FLU/>) in Nov 28, 2018.

^b Most common residue at position for subtype.

^c Percent identity for most common residue.

^d Sequence deletion as aligned to N2 NA.

Table 9: Sequence conservation of 1G01 epitope by NA subtype.

NA residue number	CA04 N1	Flu A Group 1				Flu A Group 2					Flu B
		N1 (9,744) ^a	N4 (149)	N5 (237)	N8 (1,290)	N2 (12,826)	N3 (772)	N6 (1,218)	N7 (473)	N9 (587)	B NA (3,469)
<u>118</u>	<u>R</u>	R ^b (99.9) ^c	R (99.3)	R (99.6)	R (99.8)	R (100)	R (100)	R (100)	R (100)	R (99.8)	R (100)
<u>119</u>	<u>E</u>	E (99.5)	E (99.3)	E (100)	E (99.9)	E (99.7)	E (100)	E (100)	E (100)	E (99.7)	E (99.9)
<u>134</u>	<u>L</u>	L (99.9)	L (100)	L (99.6)	L (99.8)	L (99.9)	L (99.9)	L (99.7)	L (100)	L (99.8)	L (99.8)
150	K	K (99.5)	K (100)	K (99.2)	K (99.5)	H (65.9)	K (98.4)	H (99.3)	H (98.7)	H (100)	E (53.3)
<u>151</u>	<u>D</u>	D (99.2)	D (100)	D (99.6)	D (99.9)	D (93.5)	D (99.1)	D (99.9)	D (100)	D (99.7)	D (99.4)
<u>152</u>	<u>R</u>	R (100)	R (100)	R (99.6)	R (99.8)	R (99.9)	R (99.7)	R (99.8)	R (99.8)	R (99.3)	R (99.9)
<u>156</u>	<u>R</u>	R (99.8)	R (100)	R (99.6)	R (99.8)	R (99.9)	R (99.9)	R (99.9)	R (100)	R (99.1)	R (99.9)
<u>178</u>	<u>W</u>	W (99.9)	W (100)	W (99.6)	W (99.8)	W (99.0)	W (99.9)	W (100)	W (100)	W (100)	W (100)
197	P	P (99.8)	P (100)	A (99.2)	P (98.6)	D (80.9)	N (97.0)	P (99.5)	N (97.5)	P (100)	P (99.5)
198	D	D (99.5)	D (100)	D (99.6)	D (99.9)	D (99.6)	D (99.4)	N (97.8)	N (100)	N (99.5)	D (99.4)
199	N	N (81.7)	A (56.4)	D (98.7)	A (66.0)	K (59.3)	N (97.8)	N (98.1)	D (88.4)	N (99.8)	S (52.7)

NA residue number	CA04 N1	Flu A Group 1				Flu A Group 2					Flu B
		N1 (9,744) ^a	N4 (149)	N5 (237)	N8 (1,290)	N2 (12,826)	N3 (772)	N6 (1,218)	N7 (473)	N9 (587)	B NA (3,469)
220	N	N (85.0)	G (100)	K (98.7)	G (99.7)	K (80.5)	K (74.9)	G (99.1)	G (56.4)	R (98.1)	N (51.9)
221	N	N (72.0)	N (100)	Q (98.7)	D (97.8)	N (50.6)	D (90.2)	N (99.6)	N (98.9)	N (99.8)	N (81.4)
<u>222</u>	<u>I</u>	I (99.2)	I (99.3)	I (99.6)	I (99.7)	I (99.3)	I (99.4)	I (99.3)	I (99.8)	I (100)	I (99.5)
<u>224</u>	<u>R</u>	R (99.9)	R (100)	R (99.2)	R (99.9)	R (99.9)	R (99.2)	R (99.5)	R (100)	R (100)	R (99.8)
245	P	P (99.5)	P (100)	P (99.6)	P (100)	S (89.0)	P (99.4)	P (99.4)	S (96.8)	S (92.7)	S (52.7)
246	S	S (99.2)	S (99.3)	A (99.6)	A (99.5)	A (99.8)	A (99.5)	A (99.5)	A (100)	A (99.5)	A (99.9)
247	N	N (59.7)	D (98.7)	N (92.4)	N (99.0)	S (89.7)	A (89.8)	N (99.0)	S (96.0)	T (99.7)	D (77.5)
<u>276</u>	<u>E</u>	E (99.9)	E (100)	E (99.6)	E (99.9)	E (99.9)	E (99.6)	E (99.6)	E (100)	E (100)	E (100)
<u>277</u>	<u>E</u>	E (100)	E (100)	E (99.6)	E (100)	E (100)	E (99.6)	E (99.6)	E (100)	E (100)	E (100)
347	N	N (66.2)	Y (100)	Y (100)	Y (100)	H (73.1)	P (89.5)	P (99.8)	P (99.6)	N (99.3)	G (99.7)
<u>371</u>	<u>R</u>	R (99.9)	R (100)	R (100)	R (100)	R (99.9)	R (100)	R (100)	R (100)	R (100)	R (99.9)
<u>406</u>	<u>Y</u>	Y (99.9)	Y (100)	Y (99.6)	Y (99.9)	Y (99.9)	Y (100)	Y (100)	Y (100)	Y (100)	Y (99.9)
431	P	P (99.3)	T (99.3)	P (99.6)	P (100)	K (69.3)	P (99.7)	P (100)	P (100)	P (100)	G (99.8)
456	W	W (100)	W (100)	W (100)	W (99.9)	G (99.9)	G (100)	W (99.9)	G (100)	W (99.8)	W (99.9)

NA residues conserved in influenza A N1 to N9 and Influenza B NA are underlined.

^a Number of non-redundant influenza NA sequences for subtype from the Influenza Virus Resource at the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/genomes/FLU/>) in Nov 28, 2018.

^b Most common residue at position for subtype.

^c Percent identity for most common residue.

Example 5: Protective capacity of monoclonal antibodies in mouse models of influenza.

[0227] Next, we assessed the protective capacity of the three mAbs in vivo in the mouse model. We tested the protective effect against viruses expressing a human N2, an avian N2, a swine N3, and avian N6, N7 and N9 NAs (all group 2), a human N1, an avian N1, as well as avian N4, N5 and N8 NAs (all group 1) and an influenza B virus from the B/Victoria/2/88 lineage (FIGS. 4A, 4C-4M, Table 10). All were lethal challenge models except for avian N2 and N6 where only severe weight loss was observed in the animals that received the control

antibody, but no mortality. Animals received mAbs at 5 mg/kg 2 hours prior to infection, morbidity (weight loss) and mortality were then monitored for 14 days. The mAbs protected from both weight loss and mortality in a manner that was consistent with their reactivity pattern, meaning if binding was observed they were also protective. Notably, 1G01 provided full protection from lethality against every single challenge virus. With the exception of the N4 challenge (FIG. 4J), where some transient weight loss was observed, 1G01 provided solid protection against weight loss. For the H3N2 challenge, we also monitored virus replication in the lungs of challenged animals on day 3 and day 6 post infection and could not detect any replicating virus suggesting that the strong in vitro inhibiting and neutralizing activity of the three mAbs translates to sterilizing immunity in vivo, at least on the sampled days (FIG. 4B). To determine if the mAbs could be useful if given as a therapeutic, we infected mice with a lethal dose of H3N2 virus and treated them with respective antibody 48- or 72-hours post-infection (FIG. 4N and FIG. 4O). While transient weight loss was observed, all animals recovered when treated with a low dose of 5 mg/kg mAb suggesting that these anti-NA mAbs have therapeutic potential.

Table 10: LD₅₀s of challenge viruses used in this study.

Subtype	NA	LD ₅₀
pH1N1	A/Singapore/GP1908/2015	320 PFU
H5N1	A/Vietnam/1204/2004	32 PFU
cH6/1 N4	A/mallard/Sweden/24/2002	5.6x10 ⁴ PFU
H10N8	A/Jiangxi-Donghu/346/2013	56 PFU
H15N5	A/mallard duck/Sweden/139579/2012	3200 PFU
H7N9	A/Shanghai/1/2013	4000 PFU
H4N6	A/duck/Czechoslovakia/1956	1.4x10 ⁴ PFU
H1N7	A/chicken/Netherlands/1/2003	5.6 PFU
H3N2	A/Philippines/2/1982	6 PFU
H7N2	A/feline/New York/16-040082-1/2016	3.16x10 ⁴ TCID ₅₀
H6N3	A/swine/Missouri/4296424/2006	182 PFU
B	B/Malaysia/2506/2004	316 PFU

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[0228] When introducing elements of the present invention or the preferred embodiments(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

[0229] In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained.

[0230] As various changes could be made in the above methods, processes, and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

CLAIMS:

1. An antibody or antigen-binding fragment, wherein the antibody or antigen-binding fragment comprises:
 - a) an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 70% identity to SEQ ID NOs: 1, 2, 3, 7, 10, 13, 14, 40, 42 or 44;
 - b) an immunoglobulin light chain variable region comprising an amino acid sequence having at least about 70% identity to any one of SEQ ID NOs: 4, 5, 6, 8, 9, 11, 12, 15, 16, 41, 43, and 45; or
 - c) a combination thereof.
2. The antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment comprises:
 - (a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7, a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14, or a combination of any thereof;
 - (b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4, 8, 11 or 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9, a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16, or a combination of any thereof; or
 - (c) a combination thereof.
3. The antibody or antigen-binding fragment of claim 1 or 2, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7, or a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14.

4. The antibody or antigen-binding fragment of any one of claims 1 to 3 wherein the immunoglobulin heavy chain variable region comprises a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13.
5. The antibody or antigen-binding fragment of claim 4 wherein the immunoglobulin heavy chain variable region comprises a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1.
6. The antibody or antigen-binding fragment of claim 4 wherein the immunoglobulin heavy chain variable region comprises a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 13.
7. The antibody or antigen binding fragment of any one of claims 1 to 6, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7.
8. The antibody or antigen binding fragment of claim 7, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2.
9. The antibody or antigen-binding fragment of claim 7, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7.
10. The antibody or antigen-binding fragment of any one of claims 1 to 9, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14.
11. The antibody or antigen-binding fragment of claim 10, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3.
12. The antibody or antigen-binding fragment of claim 10, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 10.

13. The antibody or antigen-binding fragment of claim 10, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 14.

14. The antibody or antigen-binding fragment of any one of claims 1 to 13, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14.

15. The antibody or antigen-binding fragment of any one of claims 1 to 14, wherein the immunoglobulin heavy chain variable region comprises:

a) a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3;

b) a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence or SEQ ID NO: 10; or

c) a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 14.

16. The antibody or antigen-binding fragment of any one of claims 1 to 15, wherein the immunoglobulin light chain variable region comprises a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4, 8, 11 or 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9, or a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16.

17. The antibody or antigen-binding fragment of any one of claims 1 to 16 wherein the immunoglobulin light chain variable region comprises a CDR_{L1} having an amino acid sequence comprising any one of SEQ ID NOs: 4, 8, 11 or 15.

18. The antibody or antigen-binding fragment of claim 17, wherein the immunoglobulin light chain variable region comprises a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4.
19. The antibody or antigen-binding fragment of claim 17, wherein the immunoglobulin light chain variable region comprises a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 8.
20. The antibody or antigen-binding fragment of claim 17, wherein the immunoglobulin light chain variable region comprises a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 11.
21. The antibody or antigen-binding fragment of claim 17, wherein the immunoglobulin light chain variable region comprises a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 15.
22. The antibody or antigen-binding fragment of any one of claims 1 to 21, wherein the immunoglobulin light chain variable region comprises a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9.
23. The antibody or antigen-binding fragment of claim 22, wherein the immunoglobulin light chain variable region comprises a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5.
24. The antibody or antigen-binding fragment of claim 22, wherein the immunoglobulin light chain variable region comprises a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9.
25. The antibody or antigen-binding fragment of any one of claims 1 to 24 wherein the immunoglobulin light chain variable region comprises a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16.
26. The antibody or antigen-binding fragment of claim 25, wherein the immunoglobulin light chain variable region comprises a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6.

27. The antibody or antigen-binding fragment of claim 25, wherein the immunoglobulin light chain variable region comprises a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 12.

28. The antibody or antigen-binding fragment of claim 25, wherein the immunoglobulin light chain variable region comprises a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 16.

29. The antibody or antigen-binding fragment of any one of claims 1 to 28 wherein the immunoglobulin light chain variable region comprises a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4, 8, 11 or 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16.

30. The antibody or antigen-binding fragment of any one of claims 1 to 29, wherein the immunoglobulin light chain variable region comprises:

a) a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 8, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6; or

b) a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 11, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 12; or

c) a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 16.

31. The antibody or antigen-binding fragment of any one of claims 1 to 30, wherein the antibody or antigen-binding fragment comprises:

(a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1 or 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 2 or 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3, 10 or 14; and

(b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 4, 8, 11 or 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 5 or 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6, 12 or 16.

32. The antibody or antigen-binding fragment of claim 31, wherein the antibody or antigen-binding fragment comprises:

a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 3; and

b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 8, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 6.

33. The antibody or antigen-binding fragment of claim 31, wherein the antibody or antigen-binding fragment comprises:

a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 1, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 10; and

b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 11, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 12.

34. The antibody or antigen-binding fragment of claim 31, wherein the antibody or antigen-binding fragment comprises:

a) an immunoglobulin heavy chain variable region comprising a CDR_{H1} having an amino acid sequence comprising SEQ ID NO: 13, a CDR_{H2} having an amino acid sequence comprising SEQ ID NO: 7, and a CDR_{H3} having an amino acid sequence comprising SEQ ID NO: 14; and

b) an immunoglobulin light chain variable region comprising a CDR_{L1} having an amino acid sequence comprising SEQ ID NO: 15, a CDR_{L2} having an amino acid sequence comprising SEQ ID NO: 9, and a CDR_{L3} having an amino acid sequence comprising SEQ ID NO: 16.

35. The antibody or antigen-binding fragment of any one of claims 1 to 34, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 70% identity to any one of SEQ ID NOs: 40, 42 and 44.

36. The antibody or antigen-binding fragment of any one of claims 1 to 35, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 75% identity to any one of SEQ ID NOs: 40, 42, and 44.

37. The antibody or antigen-binding fragment of any one of claims 1 or 36, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 80% identity to any one of SEQ ID NOs: 40, 42, and 44.

38. The antibody or antigen-binding fragment of any one of claims 1 to 37, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 85% identity to any one of SEQ ID NOs: 40, 42, and 44.

39. The antibody or antigen-binding fragment of any one of claims 1 to 38, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 90% identity to any one of SEQ ID NOs: 40, 42, and 44.

40. The antibody or antigen-binding fragment of any one of claims 1 to 39, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 95% identity to any one of SEQ ID NOs: 40, 42, and 44.

41. The antibody or antigen-binding fragment of any one of claims 1 to 40, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region

comprising an amino acid sequence having at least about 96% identity to any one of SEQ ID NOs: 40, 42, and 44.

42. The antibody or antigen-binding fragment of any one of claims 1 to 41, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 97% identity to any one of SEQ ID NOs: 40, 42, and 44.

43. The antibody or antigen-binding fragment of any one of claims 1 to 42, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 98% identity to any one of SEQ ID NOs: 40, 42, and 44.

44. The antibody or antigen-binding fragment of any one of claims 1 to 43, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 99% identity to any one of SEQ ID NOs: 40, 42, and 44.

45. The antibody or antigen-binding fragment of any one of claims 1 to 44, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 99.5% identity to any one of SEQ ID NOs: 40, 42, and 44.

46. The antibody or antigen-binding fragment of any one of claims 1 to 45, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 99.9% identity to any one of SEQ ID NOs: 40, 42, and 44.

47. The antibody or antigen-binding fragment of any one of claims 1 to 46, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 70% identity to any one of SEQ ID NOs: 41, 43, and 45.

48. The antibody or antigen-binding fragment of any one of claims 1 to 47, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 75% identity to any one of SEQ ID NOs: 41, 43, and 45.

49. The antibody or antigen-binding fragment of any one of claims 1 to 48, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 80% identity to any one of SEQ ID NOs: 41, 43, and 45.
50. The antibody or antigen-binding fragment of any one of claims 1 to 49, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 85% identity to any one of SEQ ID NOs: 41, 43, and 45.
51. The antibody or antigen-binding fragment of any one of claims 1 to 50, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 90% identity to any one of SEQ ID NOs: 41, 43, and 45.
52. The antibody or antigen-binding fragment of any one of claims 1 to 51, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 95% identity to any one of SEQ ID NOs: 41, 43, and 45.
53. The antibody or antigen-binding fragment of any one of claims 1 to 52, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 96% identity to any one of SEQ ID NOs: 41, 43, and 45.
54. The antibody or antigen-binding fragment of any one of claims 1 to 53, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 97% identity to any one of SEQ ID NOs: 41, 43, and 45.
55. The antibody or antigen-binding fragment of any one of claims 1 to 54, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 98% identity to any one of SEQ ID NOs: 41, 43, and 45.
56. The antibody or antigen-binding fragment of any one of claims 1 to 55, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 99% identity to any one of SEQ ID NOs: 41, 43, and 45.
57. The antibody or antigen-binding fragment of any one of claims 1 to 56, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 99.5% identity to any one of SEQ ID NOs: 41, 43, and 45.

58. The antibody or antigen-binding fragment of any one of claims 1 to 57, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising at least about 99.9% identity to any one of SEQ ID NOs: 41, 43, and 45.

59. The antibody or antigen-binding fragment of any one of claims 1 to 58, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5% sequence, or at least about 99.9% identity to any one of SEQ ID NOs: 40, 42 and 44 and an immunoglobulin light chain variable region comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.9% identity to any one of SEQ ID NOs: 41, 43, and 45.

60. The antibody or antigen-binding fragment of claim 59, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising any one of SEQ ID NOs: 40, 42 and 44 and an immunoglobulin light chain variable region comprising any one of SEQ ID NOs: 41, 43 and 45.

61. The antibody or antigen-binding fragment of claim 60, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising SEQ ID NO: 40.

62. The antibody or antigen-binding fragment of claim 60, wherein antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising SEQ ID NO: 42.

63. The antibody or antigen-binding fragment of claim 60, wherein antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising SEQ ID NO: 44.

64. The antibody or antigen-binding fragment of claim 60, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising SEQ ID NO: 41.

65. The antibody or antigen-binding fragment of claim 60, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising SEQ ID NO: 43.
66. The antibody or antigen-binding fragment of claim 60, wherein the antibody or antigen-binding fragment comprises an immunoglobulin light chain variable region comprising SEQ ID NO: 45.
67. The antibody or antigen-binding fragment of claim 60, wherein the antibody or antigen-binding fragment comprises: an immunoglobulin heavy chain variable region comprising SEQ ID NO: 40 and an immunoglobulin light chain variable region comprising SEQ ID NO: 41.
68. The antibody or antigen-binding fragment of claim 60, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising SEQ ID NO: 42 and an immunoglobulin light chain variable region comprising SEQ ID NO: 43.
69. The antibody or antigen-binding fragment of claim 60, wherein the antibody or antigen-binding fragment comprises an immunoglobulin heavy chain variable region comprising SEQ ID NO: 44 and an immunoglobulin light chain variable region comprising SEQ ID NO: 45.
70. An antibody or antigen-binding fragment thereof that comprises a polypeptide that has a tertiary structure that is structurally similar to a polypeptide having an amino acid sequence of any one of SEQ ID NOs: 1 to 46.
71. The antibody or antigen-binding fragment of claim 70, wherein the polypeptide has a tertiary structure that is structurally similar to a single CDRH3 loop comprising any one of SEQ ID NOs: 3, 10, and 14.
72. The antibody or antigen-binding fragment of any one of claims 1 to 71, wherein the antibody or antigen-binding fragment binds an influenza virus.
73. The antibody or antigen-binding fragment of claim 72, wherein the antibody or antigen-binding fragment specifically binds to a neuraminidase.

74. The antibody or antigen-binding fragment of claim 73, wherein the antibody or antigen-binding fragment inhibits the neuraminidase with an IC_{50} of about 0.0001 $\mu\text{g/ml}$ to about 30 $\mu\text{g/ml}$.

75. The antibody or antigen-binding fragment of 73 or 74, wherein the CDR_{H3} region of the antibody or antigen-binding fragment interacts with at least one active site residue of the neuraminidase.

76. The antibody or antigen-binding fragment of claim 75, wherein the neuraminidase is an N1 neuraminidase, the CDR_{H3} region comprises SEQ ID NO: 3 and the active site residue of the N1 neuraminidase comprises at least one of: R118, E119, D151, R152, I222, R224, E276, E277, R371, and Y406, wherein the amino acid numbering is according to SEQ ID NO: 46.

77. The antibody or antigen-binding fragment of claim 75, wherein the neuraminidase is an N1 neuraminidase, CDR_{H3} comprises SEQ ID NO: 10 and the active site residue comprises at least one of R118, E119, D151, R152, I222, R224, E277, R371, and Y406, wherein the amino acid numbering is according to SEQ ID NO: 46.

78. The antibody or antigen-binding fragment of claim 75, wherein neuraminidase is an N1 neuraminidase, the CDR_{H3} comprises SEQ ID NO: 14 and the active site residue comprises at least one of R118, E119, L134, D151, R152, R156, W178, I222, R224, E276, E277, R371, and Y406, wherein the amino acid numbering is according to SEQ ID NO: 46.

79. The antibody or antigen-binding fragment of any one of claims 73 to 78, wherein the neuraminidase comprises an influenza Type A neuraminidase.

80. The antibody or antigen-binding fragment of claim 79, wherein the neuraminidase comprises any one of N1 to N9.

81. The antibody or antigen-binding fragment of any one of claims 73 to 78, wherein the neuraminidase comprises an influenza Type B neuraminidase.

82. The antibody or antigen-binding fragment of any one of claims 73 to 81, wherein the neuraminidase is expressed on a surface of the influenza virus.

83. The antibody or antigen-binding fragment of any one of claims 1 to 82, wherein the antibody or antigen-binding fragment is humanized.
84. The antibody or antigen binding fragment of any one of claims 1 to 83, wherein the antibody or antigen binding fragment is a monoclonal antibody and/or is an IgG antibody.
85. An antibody or antigen binding fragment having specific affinity for an N1 neuraminidase of an influenza virus, and wherein the antibody or antigen binding fragment binds to an active site residue of the N1 neuraminidase comprising at least about 70% of SEQ ID NO: 46 and containing at least one residue selected from R118, E119, L134, D151, R152, R156, W178, I222, R224, E276, E277, R371, and Y406 according to the amino acid numbering of SEQ ID NO: 46.
86. The antibody or antigen bind fragment of any one of claims 1 to 85, wherein the antibody or antigen bind fragment comprises a hinge region or Fc region that contains at least one amino acid substitution, deletion, or insertion relative to the sequence of a wild-type hinge region or a wild-type Fc region.
87. The antibody or antigen bind fragment of any one of claims 1 to 86, wherein the antibody or antigen bind fragment comprises an Fc region that contains at least one amino acid substitution, deletion, or insertion relative to the sequence of a wild-type Fc region.
88. The antibody or antigen bind fragment of claim 87, wherein the substitution, deletion, or insertion prevents or reduces recycling of the antibody or antigen-binding fragment.
89. The antibody or antigen-binding fragment of any one of claims 1 to 88 wherein the antibody or antigen-binding fragment comprises a heavy chain variable region and/or a light chain variable region having an amino acid sequence comprising at least one amino acid substitution, insertion, or deletion as compared to any of SEQ ID NOs: 40 – 46.
90. The antibody of any one of claims 1 to 89.
91. The antigen-binding fragment of any one of claims 1 to 89.

92. A nucleic acid comprising a nucleotide sequence encoding the immunoglobulin heavy chain variable region of the antibody or antigen-binding fragment of any one of claims 1 to 91.
93. The nucleic acid of claim 92, wherein the nucleic acid comprises any one of SEQ ID NOs: 47 – 49.
94. A nucleic acid comprising a nucleotide sequence encoding an immunoglobulin light chain variable region of the antibody or antigen-binding fragment of any one of claims 1 to 91.
95. The nucleic acid of claim 94, wherein the nucleic acid comprises SEQ ID NOs: 50 – 52.
96. An expression vector comprising the nucleic acid of claim 92 or 93 and/or the nucleic acid of any one of claims 94 or 95.
97. A host cell comprising the expression vector of claim 96.
98. A method of producing an antibody or antigen-binding fragment that binds a neuraminidase of an influenza virus, the method comprising growing the host cell of claim 97 under conditions so that the host cell expresses a polypeptide or polypeptides comprising the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region, thereby producing the antibody or antigen-binding fragment and purifying the antibody or antigen-binding fragment.
99. A vaccine comprising an antibody or antigen-binding fragment of any one of claims 1 to 91.
100. A vaccine comprising a polypeptide or a nucleic acid encoding the polypeptide, wherein the polypeptide comprises an amino acid sequence comprising at least about 70% identity to an epitope targeted by an antibody or antigen binding fragment of any one of claims 1 to 91.
101. The vaccine of claim 100, wherein the polypeptide comprises an amino acid residue selected from R118, E119, D151, R152, W178, R156, I222, R224, E276, E277, R371, and Y406 according to a reference sequence comprising SEQ ID NO: 46.
102. The vaccine of claim 100 or 101 wherein the nucleic acid comprises DNA or RNA.
103. The vaccine of any one of claims 99 to 102, wherein the vaccine provides an effective immunological response to an influenza virus when administered to a subject.

104. A pharmaceutical composition, the composition comprising an antibody or antigen-binding fragment of any one of claims 1 to 91 and a pharmaceutically acceptable carrier or excipient.
105. The pharmaceutical composition of claim 104, wherein the composition further comprises a monoclonal antibody selective for a hemagglutinin of the influenza virus.
106. The pharmaceutical composition of claim 104 or 105, wherein the composition further comprises an antiviral drug.
107. The pharmaceutical composition of claim 106, where the antiviral drug comprises baloxavir, oseltamivir, zanamivir, peramivir or any combination thereof.
108. The pharmaceutical composition of any one of claims 104 to 107, wherein the pharmaceutically acceptable carrier or excipient comprises at least one of a filler, a diluent, a surface active, a wetting or emulsifying agent, a preservative, an agent for adjusting pH, a buffering agent, a thickener, a colorant, a dye, a flow aid, a non-volatile silicone, an adhesive, a bulking agent, a flavoring, a sweetener, an adsorbent, a binder, a disintegrating agent, a lubricant, a coating agent, or an antioxidant.
109. The pharmaceutical composition of any one of claims 104 to 108, wherein the composition is formulated without blood, plasma or a major component of blood or plasma.
110. The pharmaceutical composition of any one of claims 104 to 109, wherein the composition is free of or substantially free of blood, plasma or a major component of blood or plasma.
111. A method of preventing or treating influenza in a subject in need thereof, the method comprising administering the antibody or antigen-binding fragment of any one of claims 1 to 91, the nucleic acid of any one of claims 92 to 95, the expression vector of claim 96, the vaccine of any one of claims 99 to 103, or the composition of any one of claims 104 to 110 to the subject.
112. The method of claim 111, wherein the influenza infection is caused by an influenza A virus or an influenza B virus.

113. The method of claim 112, wherein the serotype of the influenza A virus is selected from the group consisting of: HXN1, HXN2, H2N3, HXN4, HXN5, HXN6, HXN7, HXN8, HXN9 or any combination thereof, wherein X is an integer between 1 and 18.

114. The method of any one of claims 111 to 113, wherein the antibody or antigen-binding fragment, nucleic acid, expression vector, vaccine or composition is parenterally administered.

115. The method of claim 114, wherein the antibody or antigen-binding fragment, nucleic acid, expression vector, vaccine or composition is administered via intramuscular, intravenous, or intradermal delivery or is administered intranasally via an aerosol.

116. The method of any one of claims 111 to 115, wherein the antibody or antigen-binding fragment, nucleic acid, expression vector, vaccine or composition is administered therapeutically to treat an active influenza infection.

117. The method of any one of claims 111 to 116, wherein the antibody or antigen-binding fragment, nucleic acid, expression vector, vaccine or composition is administered prophylactically to prevent an influenza infection.

118. The method of any one of claims 111 to 117, wherein antibody or antigen-binding fragment, nucleic acid, expression vector, vaccine or composition is administered at a dose of about 1 to 70 mg/kg.