

NANOBODY-DRUG ADDUCTS AND USES THEREOF**RELATED APPLICATIONS**

This application claims the benefit of priority under 35 U.S.C. §119(e) to U.S. provisional application number 63/300,995, filed on January 19, 2022; and U.S. provisional
5 application number 63/423,667, filed on November 8, 2022; the entire contents of each of which are incorporated by reference herein.

FEDERALLY SPONSORED RESEARCH

This invention was made with government support under Grant Number AI150593,
10 awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND

The fragment crystallizable (Fc) receptor is an immunoglobulin receptor located on the
15 cell membrane of many immune cell types, including macrophages, dendritic cells, natural killer cells, neutrophils, basophils, eosinophils, and mast cells. These cells typically have only limited ability to target antigens directly, such as those located on the surface of viruses, microbial pathogens, and cancer cells. However, these immune cells can bind to polyclonal antibodies via Fc receptors on their surface. These polyclonal antibodies can in turn bind to antigens located on
20 the surface of cells (including bacteria, parasites, and fungi) or viruses, thereby enabling immune cells to act upon these targets.

SUMMARY

The immune system includes a variety of specialized cell types, many of which are
25 responsible for actively targeting and eliminating viruses, foreign cells, especially pathogenic microorganisms (e.g., bacteria, parasites, and fungi), and cells that are not foreign but have undergone certain deleterious phenotypic changes (e.g., damaged cells, virus-infected cells, and transformed cells, such as cancer cells). Although immune cells protect their host from this broad range of pathogenic or otherwise hazardous cell types, these immune cells are generally
30 unable to bind to their targets directly. Instead, many specialized immune cells bind to immunoglobulins produced by their host through Fc receptors on their surface, which are in turn specific for a particular antigen located on the surface of a target cell or pathogen. Although this system enables effective immunity against a broad range of possible targets, it is limited by the

quantity of immunoglobulins specific for a particular antigen that are present in a host at any given time. The levels of immunoglobulins specific for a given target antigen require prior exposure to these antigens, for example through a natural infection or via deliberate immunization with that particular antigen.

5 Molecules that enhance the interaction between immune cells and potential targets, irrespective of the specificity of host immunoglobulins, would be particularly useful for the treatment and prevention of various diseases. Described herein is one strategy for designing and producing such molecules, which involves conjugating a first agent that is specific for immunoglobulins produced by a host to a second agent that is specific for an antigen on the
10 surface of a cell or pathogen. By selecting a first agent that binds specifically to a structural feature shared by a broad range of host immunoglobulins, such as a kappa light chain or a lambda light chain, such conjugates can bind simultaneously to a viral or cellular surface antigen and to any one of a wide range of host immunoglobulins, which in turn can then bind to a Fc receptor-positive immune cell, such as a natural killer (NK) cell, a macrophage or other cells of
15 the myeloid lineage. In this way, these conjugates can be used to recruit Fc receptor-positive immune cells to a target cell or pathogen, without relying on the clonality or specificity of the immunoglobulin that then links the immune cell to the target. These conjugates may be tailored to target immune cells to any conceivable antigen and are useful for enhancing or eliciting an immune response toward a particular cell or a pathogen in a subject.

20 Some aspects of the present disclosure provide a conjugate comprising a first agent that binds to an immunoglobulin and a second agent that binds to a target on the surface of a cell or a pathogen, wherein the first agent and the second agent are covalently conjugated via a linker in a chemical reaction.

 In some embodiments, the first agent is an antibody fragment comprising a variable
25 region that is capable of binding to an antigen. In some embodiments, the antibody fragment comprises a heavy chain variable region. In some embodiments, the first agent is a single domain antibody fragment. In some embodiments, the immunoglobulin recruited by the conjugate comprises an immunoglobulin kappa light chain or an immunoglobulin lambda light chain. In some embodiments, the immunoglobulin kappa light chain is a human immunoglobulin
30 kappa light chain, and the immunoglobulin lambda light chain is a human immunoglobulin lambda light chain. In some embodiments, the first agent binds to the human immunoglobulin kappa light chain.

 In some embodiments, the second agent comprises a small molecule, a peptide, a protein, a carbohydrate, a lipid, a nucleotide, a nucleic acid, an oligonucleotide, an aptamer, or an
35 antibody. In some embodiments, the second agent is an antibody that is a single domain

antibody. In some embodiments, the second agent has a therapeutic effect when administered to a subject.

In some embodiments, the linker comprises a cleavable or a non-cleavable linker. In some embodiments, the linker comprises a cleavable linker. In some embodiments, the cleavable linker is a peptide, disulfide, or hydrazone linker.

In some embodiments, the cell is a cell infected by a pathogen, a cancer cell, a transformed cell, a healthy cell, a cell that is undergoing or has undergone a phenotypic change in response to cellular stress. In some embodiments, the pathogen is a virus, a bacterium, a parasite, or a fungus.

In some embodiments, the pathogen is a virus selected from an influenza virus, a coronavirus, an adenovirus, an enterovirus, a rotavirus, a norovirus, a herpesvirus, a lentivirus, a poxvirus, a paramyxovirus, a rhabdovirus, an arenavirus, a flavivirus, a togavirus, a hantavirus, a pneumovirus, or an ebolavirus.

In some embodiments, the influenza virus is an influenza A virus or an influenza B virus. In some embodiments, the second agent binds to an influenza virus neuraminidase or an influenza virus hemagglutinin. In some embodiments, the second agent comprises a small molecule that binds to an influenza virus neuraminidase. In some embodiments, the second agent comprises zanamivir or an analog thereof. In some embodiments, the linker is a triglycine dibenzylcyclooctyne (DBCO) linker. In some embodiments, the second agent comprises an antibody or antibody fragment that binds to an influenza virus neuraminidase.

In some embodiments, the coronavirus is a beta coronavirus. In some embodiments, the beta coronavirus is a Middle East Respiratory Syndrome coronavirus (MERS-CoV), a Severe Acute Respiratory Syndrome (SARS)-associated coronavirus (SARS-CoV)-1, or a SARS-CoV-2. In some embodiments, the second agent binds to a MERS-CoV spike protein, a SARS-CoV-1 spike protein, or a SARS-CoV-2 spike protein. In some embodiments, the second agent binds to a MERS-CoV spike protein receptor binding domain (RBD), a SARS-CoV-1 spike protein RBD, or a SARS-CoV-2 spike protein RBD.

In some embodiments, the lentivirus is a human immunodeficiency virus (HIV). In some embodiments, the second agent binds to a HIV envelope glycoprotein gp120.

In some embodiments, the pneumovirus is a human respiratory syncytial virus (RSV). In some embodiments, the second agent binds to a RSV fusion (F) protein.

In some embodiments, the pathogen is a bacterium selected from a *Pasteurella* species, a *Staphylococcus* species, a *Streptococcus* species, a *Bacillus* species, a *Corynebacterium* species, a *Diphtheroids* species, a *Listeria* species, an *Erysipelothrix* species, a *Clostridium* species, a *Neisseria* species, a *Branhamella* species, an *Escherichia* species, an *Enterobacter* species, a

Proteus species, a *Pseudomonas* species, a *Klebsiella* species, a *Salmonella* species, a *Shigella* species, a *Serratia* species, an *Acinetobacter* species, a *Haemophilus* species, a *Brucella* species, a *Yersinia* species, a *Francisella* species, a *Pasturella* species, a *Vibrio cholera* species, a *Flavobacterium* species, a *Pseudomonas* species, a *Campylobacter* species, a *Bacteroides* species, a *Fusobacterium* species, a *Calymmatobacterium* species, a *Streptobacillus* species, or a *Legionella* species.

In some embodiments, the pathogen is a parasite selected from a *Plasmodium* species, a *Trypanosoma* species, a *Toxoplasma* species, a *Leishmania* species, or a *Cryptosporidium* species. In some embodiments, the *Plasmodium* is *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Plasmodium ovale curtisi*, or *Plasmodium ovale wallikeri*. In some embodiments, the second agent binds a plasmodium surface protein. In some embodiments, the plasmodium surface protein is a merozoite surface protein 1 (MSP-1). In some embodiments, the second agent is an antibody that binds to MSP-1, optionally wherein the antibody is a nanobody. In some embodiments, the antibody comprises a CDR-H1, a CDR-H2, and a CDR-H3 of any one of the antibodies listed in Table 1. In some embodiments, the antibody comprises an amino acid sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%)) identical to of any one of SEQ ID NOs: 6-17. In some embodiments, the antibody comprises the amino acid sequence of any one of SEQ ID NOs: 6-17.

In some embodiments, the cancer cell is a hematological cancer cell, a lung cancer cell, a breast cancer cell, a brain cancer cell, a gastrointestinal cancer cell, a liver cancer cell, a kidney cancer cell, a bladder cancer cell, a pancreatic cancer cell, an ovarian cancer cell, a testicular cancer cell, a prostate cancer cell, an endometrial cancer cell, a muscle cancer cell, a bone cancer cell, a neuroendocrine cancer cell, a connective tissue cancer cell, a head or neck cancer cell, or a skin cancer cell. In some embodiments, the second agent binds to a tumor-associated antigen. In some embodiments, the tumor-associated antigen comprises a MHC class I polypeptide-related sequence A (MICA) protein, a MHC class I polypeptide-related sequence B (MICB) protein, a folate receptor, a fibronectin splice variant, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), hepatocyte growth factor receptor (HGFR), vascular endothelial growth factor receptor 2 (VEGFR-2), C-X-C chemokine receptor type 4 (CXCR4), urokinase plasminogen activator surface receptor (uPAR), follicle-stimulating hormone receptor (FSHR), epithelial cell adhesion molecule (EpCAM), epithelial cadherin (ECAD), carcinoembryonic antigen (CEA), or mesothelin (MSLN). In some embodiments, the second agent is an antibody that binds to MICA, optionally wherein the antibody is a nanobody. In some embodiments, the antibody comprises a CDR-H1, a CDR-H2, and a CDR-H3 of any

one of the antibodies listed in Table 2. In some embodiments, the antibody comprises the amino acid sequence of any one of SEQ ID NOs: 19-27.

In some embodiments, the cell is a cancerous or healthy bone marrow cell. In some
embodiments, the bone marrow-associated antigen is cluster of differentiation antigen 45
5 (CD45).

In some embodiments, the cell is a cancerous or healthy immune cell. In some
embodiments, the cell is a cancerous or healthy T cell or B cell. In some embodiments, the
second agent binds to an immune cell-associated antigen. In some embodiments, the immune
cell-associated antigen is cluster of differentiation antigen 4 (CD4), cluster of differentiation
10 antigen 8 (CD8), a T cell receptor (TCR), or a B cell receptor (BCR).

In some embodiments, the conjugate provides a therapeutic effect when administered to
a subject. In some embodiments, the conjugate enhances association between one or more
immune cells expressing a fragment crystallizable (Fc) receptor and the cell or pathogen when
administered to a subject. In some embodiments, the conjugate results in killing of the cell or
15 pathogen when administered to a subject. In some embodiments, the conjugate results in
inactivation of the cell or pathogen when administered to a subject. In some embodiments, the
subject is a mammal. In some embodiments, the subject is a human.

In another aspect, the present disclosure provides a composition comprising any one of
the conjugates described herein. In some embodiments, such a composition further comprises a
20 pharmacologically acceptable excipient.

In another aspect, the present disclosure provides a method for enhancing an immune
response to a cell or a pathogen in a subject, the method comprising administering to the subject
an effective amount of any one of the conjugates or compositions described herein. In some
embodiments, the cell is a cell infected by a pathogen, a cancer cell, a transformed cell, a healthy
25 cell, a cell that is undergoing or has undergone a phenotypic change in response to cellular
stress. In some embodiments, the pathogen is a virus, a bacterium, a parasite, or a fungus. In
some embodiments, the cell is a cell of the subject.

In some embodiments, the pathogen is a virus selected from an influenza virus, a
coronavirus, an adenovirus, an enterovirus, a rotavirus, a norovirus, a herpesvirus, a lentivirus, a
30 poxvirus, a paramyxovirus, a rhabdovirus, an arenavirus, a flavivirus, a togavirus, a hantavirus, a
pneumovirus, or an ebolavirus. In some embodiments, the virus is an influenza A virus or an
influenza B virus. In some embodiments, the virus is a Middle East Respiratory Syndrome
coronavirus (MERS-CoV), a Severe Acute Respiratory Syndrome (SARS)-associated
coronavirus (SARS-CoV)-1, or a SARS-CoV-2. In some embodiments, the virus is a human

immunodeficiency virus (HIV). In some embodiments, the virus is a human respiratory syncytial virus (RSV).

In some embodiments, the pathogen is a bacterium selected from a *Pasteurella* species, a *Staphylococcus* species, a *Streptococcus* species, a *Bacillus* species, a *Corynebacterium* species, a *Diphtheroids* species, a *Listeria* species, an *Erysipelothrix* species, a *Clostridium* species, a *Neisseria* species, a *Branhamella* species, an *Escherichia* species, an *Enterobacter* species, a *Proteus* species, a *Pseudomonas* species, a *Klebsiella* species, a *Salmonella* species, a *Shigella* species, a *Serratia* species, an *Acinetobacter* species, a *Haemophilus* species, a *Brucella* species, a *Yersinia* species, a *Francisella* species, a *Pasturella* species, a *Vibrio cholera* species, a *Flavobacterium* species, a *Pseudomonas* species, a *Campylobacter* species, a *Bacteroides* species, a *Fusobacterium* species, a *Calymmatobacterium* species, a *Streptobacillus* species, or a *Legionella* species.

In some embodiments, the pathogen is a parasite selected from a *Plasmodium* species, a *Trypanosoma* species, a *Toxoplasma* species, a *Leishmania* species, or a *Cryptosporidium* species. In some embodiments, the parasite is *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Plasmodium ovale curtisi*, or *Plasmodium ovale wallikeri*.

In some embodiments, the cancer cell is a hematological cancer cell, a lung cancer cell, a breast cancer cell, a brain cancer cell, a gastrointestinal cancer cell, a liver cancer cell, a kidney cancer cell, a bladder cancer cell, a pancreatic cancer cell, an ovarian cancer cell, a testicular cancer cell, a prostate cancer cell, an endometrial cancer cell, a muscle cancer cell, a bone cancer cell, a neuroendocrine cancer cell, a connective tissue cancer cell, a head or neck cancer cell, or a skin cancer cell.

In some embodiments, the cell is a cancerous or healthy bone marrow cell.

In some embodiments, the cell is a cancerous or healthy immune cell. In some embodiments, the cell is a cancerous or healthy T cell or B cell.

In some embodiments, the immune response comprises an innate immune response. In some embodiments, the conjugate binds to the cell or pathogen and to an immunoglobulin of the subject, wherein the immunoglobulin further binds to an immune cell of the subject which expresses a fragment crystallizable (Fc) receptor on its surface. In some embodiments, the immunoglobulin of the subject comprises an immunoglobulin kappa light chain or an immunoglobulin lambda light chain. In some embodiments, the immunoglobulin of the subject comprises an immunoglobulin kappa light chain. In some embodiments, the immune cell is a macrophage, a dendritic cell, a natural killer cell, a neutrophil, a basophil, an eosinophil, or a mast cell. In some embodiments, the administration induces the production of one or more

cytokines or chemokines by the immune cell. In some embodiments, the one or more cytokines or chemokines are proinflammatory cytokines or chemokines. In some embodiments, the administration induces phagocytosis of the cell or pathogen by the immune cell. In some embodiments, the administration results in killing of the cell or pathogen. In some embodiments, the administration results in inactivation of the cell or pathogen.

In some embodiments, the subject is a subject that has or is at risk of developing a viral infection. In some embodiments, the subject is a subject that has or is at risk of developing cancer. In some embodiments, the cancer is metastatic cancer. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. In some embodiments the subject is a human neonate, a human infant, a human adult, or an elderly human. In some embodiments the subject is a companion animal, a research animal, or a domesticated animal.

In some embodiments, the administration is intravenous, intramuscular, intradermal, subcutaneous, or inhaled. In some embodiments, the administration occurs more than once. In some embodiments, the administration is prophylactic.

In another aspect, the present disclosure provides a method for treating a disease or reducing the risk of a disease in a subject in need thereof, the method comprising administering to the subject an effective amount of any one of the conjugates or compositions described herein. In some embodiments, the disease is a disease caused by a virus, a bacterium, a parasite, a fungus, or a cancer.

In some embodiments, the virus is an influenza virus, a coronavirus, an adenovirus, an enterovirus, a rotavirus, a norovirus, a herpesvirus, a lentivirus, a poxvirus, a paramyxovirus, a rhabdovirus, an arenavirus, a flavivirus, a togavirus, a hantavirus, a pneumovirus, or an ebolavirus. In some embodiments, the virus is an influenza A virus or an influenza B virus. In some embodiments, the virus is a Middle East Respiratory Syndrome coronavirus (MERS-CoV), a Severe Acute Respiratory Syndrome (SARS)-associated coronavirus (SARS-CoV)-1, or a SARS-CoV-2. In some embodiments, the virus is a human immunodeficiency virus (HIV). In some embodiments, the virus is a human respiratory syncytial virus (RSV).

In some embodiments, the bacterium is a *Pasteurella* species, a *Staphylococcus* species, a *Streptococcus* species, a *Bacillus* species, a *Corynebacterium* species, a *Diphtheroids* species, a *Listeria* species, an *Erysipelothrix* species, a *Clostridium* species, a *Neisseria* species, a *Branhamella* species, an *Escherichia* species, an *Enterobacter* species, a *Proteus* species, a *Pseudomonas* species, a *Klebsiella* species, a *Salmonella* species, a *Shigella* species, a *Serratia* species, an *Acinetobacter* species, a *Haemophilus* species, a *Brucella* species, a *Yersinia* species, a *Francisella* species, a *Pasturella* species, a *Vibrio cholera* species, a *Flavobacterium* species, a *Pseudomonas* species, a *Campylobacter* species, a *Bacteroides* species, a

Fusobacterium species, a *Calymmatobacterium* species, a *Streptobacillus* species, or a *Legionella* species.

In some embodiments, the parasite is a *Plasmodium* species, a *Trypanosoma* species, a *Toxoplasma* species, a *Leishmania* species, or a *Cryptosporidium* species. In some
5 embodiments, the parasite is *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*,
Plasmodium knowlesi, *Plasmodium ovale curtisi*, or *Plasmodium ovale wallikeri*.

In some embodiments, the cancer is a hematological cancer, a lung cancer, a breast
cancer, a brain cancer, a gastrointestinal cancer, a liver cancer, a kidney cancer, a bladder
cancer, a pancreatic cancer, an ovarian cancer, a testicular cancer, a prostate cancer, an
10 endometrial cancer, a muscle cancer, a bone cancer, a neuroendocrine cancer, a connective
tissue cancer, a head or neck cancer, or a skin cancer. In some embodiments, the cancer is
metastatic cancer.

In some embodiments, the subject is a mammal. In some embodiments, the subject is a
human. In some embodiments, the subject is a human neonate, a human infant, a human adult, or
15 an elderly human. In some embodiments, the subject is a companion animal, a research animal,
or a domesticated animal.

In some embodiments, the administration is intravenous, intramuscular, intradermal,
subcutaneous, or inhaled. In some embodiments, the administration occurs more than once. In
some embodiments, the administration is prophylactic.

20 Other aspects of the present disclosure provide antibodies comprising a CDR-H1, a
CDR-H2, and a CDR-H3 of any one of the antibodies listed in Table 1. In some embodiments,
the antibody comprises an amino acid sequence at least 80% (e.g., at least 80%, at least 85%, at
least 90%, at least 95%, or at least 99%)) identical to of any one of SEQ ID NOs: 6-17. In
some embodiments, the antibody comprises the amino acid sequence of any one of SEQ ID
25 NOs: 6-17.

Other aspects of the present disclosure provide antibodies comprising a CDR-H1, a
CDR-H2, and a CDR-H3 of any one of the antibodies listed in Table 2. In some embodiments,
the antibody comprises an amino acid sequence at least 80% (e.g., at least 80%, at least 85%, at
least 90%, at least 95%, or at least 99%)) identical to of any one of SEQ ID NOs: 19-27. In
30 some embodiments, the antibody comprises the amino acid sequence of any one of SEQ ID
NOs: 19-27.

The summary above is meant to illustrate, in a non-limiting manner, some of the
embodiments, advantages, features, and uses of the technology disclosed herein. Other
embodiments, advantages, features, and uses of the technology disclosed herein will be apparent
35 from the Detailed Description, the Drawings, the Examples, and the Claims.

BRIEF DESCRIPTION OF DRAWINGS

The accompanying drawings are not intended to be drawn to scale. In the drawings, each identical or nearly identical component that is illustrated in various figures is represented by a like numeral. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

FIG. 1A: A schematic depicting the mechanism by which nanobody-drug adducts enhance immunity toward cells and/or pathogens in a subject. Nanobody-drug adducts comprise an antibody fragment (e.g., VHH_{kappa}) that has sufficient affinity to bind kappa light chains of host immunoglobulins, which in turn interact with Fc rQVQeceptors on host immune cells. Simultaneously, nanobody-drug adducts comprise an agent (e.g., zanamivir) that is sufficient for binding to the surface of a target cell and/or pathogen (e.g., a cell infected by a pathogen, e.g., a cell infected by influenza virus), leading to inactivation and/or killing of the cell and/or pathogen by host immune cells.

FIG. 1B: A schematic overview of the mode of action of the viral neuraminidase-targeted VHH_{kappa}-zanamivir adduct and the viral hemagglutinin-targeted VHH_{kappa}-SD36 adduct. Conjugation with VHH_{kappa} extends the circulatory half-life of zanamivir and SD36 and enables them to kill virus-infected cells by attracting immune effectors.

FIG. 1C: Structures of VHH_{kappa}-zanamivir and VHH_{kappa}-SD36. VHH_{kappa}-zanamivir is prepared by a sortase-mediated conjugation of triglycine modified zanamivir to VHH_{kappa}. VHH_{kappa}-SD36 is expressed as a genetically fused hetero-bivalent nanobody with a C-terminal sortase recognition motif (LPETG).

FIG. 2: VHH_{kappa} binds to mouse IgG with nanomolar affinity. The affinity of VHH_{kappa}-biotin constructs were assessed with mouse IgG2b coated ELISA plates. HRP-conjugated streptavidin was used as a secondary reagent for detection. SD36 is a nanobody that recognizes influenza hemagglutinin (HA). Affinities are reported as dissociation constants (K_d). Binding for each construct is depicted according to the color code.

FIGs. 3A-3C: Schematics depicting steps in the synthesis nanobody-drug adducts comprising Gly-Gly-Gly-zanamivir. **FIG. 3A:** Schematics depicting the synthesis of a zanamivir targeting ligand from zanamivir. **FIG. 3B:** Schematics depicting the synthesis of Gly-Gly-Gly-zanamivir from Gly-Gly-Gly-DBCO and the zanamivir targeting ligand depicted in **FIG. 3A**. **FIG. 3C:** Schematics depicting tethering of VHH_{kappa} to Gly-Gly-Gly-zanamivir through a sortase reaction (e.g., SrtA) to produce a VHH_{kappa}-zanamivir nanobody-drug adduct.

FIGs. 4A and 4B: Assessment of VHH_{kappa}-zanamivir nanobody-drug adduct synthesized as depicted in **FIGs. 3A-3C**. **FIG. 4A:** 15% reducing SDS-PAGE indicates that VHH_{kappa}-zanamivir is primarily synthesized as a single species with a mass of approximately 14 kDa. **FIG. 4B:** Mass spectra of VHH_{kappa}-zanamivir indicates purity of VHH_{kappa}-zanamivir synthesized as depicted in **FIGs. 3A-3C**.

FIGs. 5A-5D: VHH_{kappa}-zanamivir binds to influenza neuraminidase with nanomolar affinity. **FIG. 5A:** Madin-Darby Canine Kidney (MDCK) cells were infected with influenza A virus A/Wisconsin/629-D00015/2009 (H1N1) and expressed influenza A neuraminidase within 24 hours post-infection, at which point infected cells were treated with VHH_{kappa}-zanamivir nanobody-drug adducts. Affinity of VHH_{kappa}-zanamivir for cell surface-localized influenza A neuraminidase was assessed by a saturation binding assay. Mouse IgG-phycoerythrin (PE) was used as a secondary antibody for assessing VHH_{kappa}-zanamivir affinity. Nanomolar affinity of VHH_{kappa}-zanamivir for influenza A neuraminidase was determined from a logarithmic regression of observed binding and is reported as a dissociation constant (K_d). **FIG. 5B:** Affinity of VHH_{kappa}-zanamivir for influenza A neuraminidase was assessed as in **FIG. 5A**, however MDCK cells were instead infected with influenza A virus A/Hong Kong/8/1968 (H3N2). Nanomolar affinity of VHH_{kappa}-zanamivir for influenza A neuraminidase was determined from a logarithmic regression of observed binding and is reported as a dissociation constant (K_d).

FIG. 5C: Madin-Darby Canine Kidney (MDCK) cells were infected with influenza B virus -- B/Florida/4/2006 and expressed influenza B neuraminidase within 24 hours post-infection, at which point infected cells were treated with VHH_{kappa}-zanamivir nanobody-drug adducts. Affinity of VHH_{kappa}-zanamivir for cell surface-localized influenza B neuraminidase was assessed by a saturation binding assay. Mouse IgG-phycoerythrin (PE) was used as a secondary antibody for assessing VHH_{kappa}-zanamivir affinity. Nanomolar affinity of VHH_{kappa}-zanamivir for influenza B neuraminidase was determined from a logarithmic regression of observed binding and is reported as a dissociation constant (K_d). **FIG. 5D:** Affinity of VHH_{kappa}-zanamivir for influenza B neuraminidase was assessed as in **FIG. 5A**, however MDCK cells were instead infected with influenza B virus -- B/Brisbane/60/2008. Nanomolar affinity of VHH_{kappa}-zanamivir for influenza B neuraminidase was determined from a logarithmic regression of observed binding and is reported as a dissociation constant (K_d).

FIGs. 6A-6E: A single intraperitoneal injection of VHH_{kappa}-zanamivir protects mice against lethal flu infection. **FIG. 6A:** Mice were infected with 50 mL influenza virus A/Puerto Rico/8/1934 (H1N1) (10 LD₅₀) at day 0. Mice received either intraperitoneal PBS control, a single dose of VHH_{kappa}-zanamivir or its components intraperitoneally at day 0 post-infection, or a dose of VHH_{kappa}-zanamivir intraperitoneally at days 0, 2, and 4 post-infection. Percent change

in body weight of infected mice was monitored daily for up to 14 days post-infection. Mice were treated with VHH_{kappa}-zanamivir or its components as depicted according to the color code.

FIG. 6B: Infected mice were treated as in **FIG. 6A** and survival rate was monitored daily for up to 14 days post-infection. Mice treated with 1 mg/kg VHH_{kappa}-zanamivir or greater on day 0

5 post-infection did not exhibit influenza lethality within 14 days post-infection. Mice were treated with VHH_{kappa}-zanamivir or its components as depicted according to the color code. **FIG. 6C:**

Infected mice were treated as in **FIG. 6A** and survival rate was monitored daily for up to 14 days post-infection. Efficacy of VHH_{kappa}-zanamivir against the different strains of influenza

virus indicated. **FIG. 6D:** Delayed addition of VHH_{kappa}-zanamivir on day 1, 2 or 3 post-

10 infection. **FIG. 6E:** Infection of mice with influenza A/Puerto Rico /8/1934 (H1N1) 7 days after a single dose of VHH_{kappa}-zanamivir.

FIGs. 7A-7E: Synthesis of SD36-VHH_{kappa} adduct through copper-free click reaction.

FIG. 7A: Synthesis of SD36-azide, sortase A catalyzes the addition of a triglycine azido-lysine peptide to the C-terminal of SD36. **FIG. 7B:** Synthesis of VHH_{kappa}-DBCO, sortase A catalyzes

15 the addition of a triglycine DBCO-functionalized cysteine peptide to the C-terminal of anti-

mouse VHH_{kappa}. **FIG. 7C:** SD36-azide is conjugated to VHH_{kappa}-DBCO through a copper-free click reaction. **FIG. 7D:** Schematic of genetically fused anti-mouse VHH_{kappa}-SD36. **FIG. 7E:**

15% reducing SDS-PAGE indicates that VHH_{kappa}-SD36 is primarily synthesized as a single species with a mass of approximately 30 kDa. Mass spectra of VHH_{kappa}-SD36 indicates purity.

20 **FIGs. 8A-8C:** Synthesis of VHH_{kappa}-biotin, VHH_{kappa}-SD36-biotin and SD36-biotin.

FIG. 8A: Sortase A catalyzes the addition of a triglycine biotin-functionalized cysteine peptide to the C-terminal of anti-mouse VHH_{kappa}. **FIG. 8B:** Sortase A catalyzes the addition of a

triglycine biotin-functionalized cysteine peptide to the C-terminal of a genetically fused anti-mouse VHH_{kappa}-SD36 conjugate. **FIG. 8C:** Sortase A catalyzes the addition of a triglycine

25 biotin-functionalized cysteine peptide to the C-terminal of SD36.

FIGs. 9A-9C: Binding affinity of SD36 and VHH_{kappa}-SD36 for various influenza virus hemagglutinins. **FIG. 9A:** Saturation binding curves of SD36-biotin to hemagglutinins (HA)

expressed on influenza virus-infected MDCK cells. Streptavidin-Phycoerythrin (PE) was used to quantify the amount of SD36-biotin bound to HAs. **FIG. 9B:** Saturation binding curves of anti-

30 mouse VHH_{kappa}-SD36-biotin (genetic fusion) to hemagglutinins (HAs) expressed on influenza virus-infected MDCK cells. Streptavidin-Phycoerythrin (PE) was used to quantify the amount of

anti-mouse VHH_{kappa}-SD36-biotin bound to HAs. **FIG. 9C:** Saturation binding curves of anti-mouse VHH_{kappa}-SD36 (C to C conjugation by click reaction) to hemagglutinins (HAs)

expressed on influenza virus-infected MDCK cells. A mouse IgG-Phycoerythrin (PE) was used

to quantify the amount of anti-mouse VHH_{kappa}-SD36 bound to HAs. Data are presented as mean values $\pm$ SD (n=3).

FIGs. 10A-10C: A single intraperitoneal injection of VHH_{kappa}-SD36 protects against a lethal influenza virus infection. Mice were infected intranasally with 50 μ L of influenza virus A/Puerto Rico/8/1934 (H1N1) (=10 LD₅₀). Mice received a single dose of anti-mouse VHH_{kappa}-SD36 genetic fusion, **FIG. 10A**, or anti-mouse VHH_{kappa}-SD36 (C to C conjugation by click reaction, **FIG. 10B**, intraperitoneally on the day of infection. **FIG. 10C**, delayed addition of VHH_{kappa}-SD36 at 1, 2 or 3 post-infection. Mice that lost more than 25% of their initial body weight or became moribund were considered as dead for the survival curve. Body weight change curve (left) and survival curve (right) are shown for each treatment. Body weight change values (%) are presented as mean values $\pm$ SD.

FIGs. 11A and 11B: Preparation of SD36-DFO (⁸⁹Zr chelated) and VHH_{kappa}-SD36-DFO (⁸⁹Zr chelated). **FIG. 11A:** Sortase A catalyzes the addition of a triglycine desferrioxamine (DFO) peptide to the C-terminal of SD36. Zirconium-89 (⁸⁹Zr) chelates with DFO under pH 7 at room temperature. **FIG. 11B:** Sortase A catalyzes the addition of a triglycine desferrioxamine (DFO) peptide to the C-terminal of anti-mouse VHH_{kappa}-SD36 conjugate. Zirconium-89 (⁸⁹Zr) chelates with DFO under pH 7 at room temperature.

FIG. 12: Immuno-PET imaging of influenza virus infection using VHH_{kappa}-SD36-DFO (⁸⁹Zr chelated) and SD36-DFO (⁸⁹Zr chelated). Mice were infected intranasally with 50 μ L of influenza virus A/Hong Kong/8/1968 (H3N2) (=10 LD₅₀). Mice were retro-orbitally injected with a single dose of 60 μ Ci of SD36-DFO (⁸⁹Zr chelated) or anti-mouse VHH_{kappa}-SD36-DFO (⁸⁹Zr chelated) at day 4 post-infection. Mice were scanned for 10 minutes by a PET scanner. Images were processed through Vivoquant using same intensity settings. Images were taken at different time points after the injection of imaging agent. Images taken at 48h post-injection of imaging agent were expanded to check the detailed virus infection in the chest of mice.

FIGs. 13A-13D: VHH nanobodies bind specifically to *Plasmodium falciparum* merozoite surface protein 1 (MSP-1). **FIG. 13A:** Schematic of the PfMSP-1 pro-peptide and the four inclusive subunits: p83, p30, p38, and p42. **FIG. 13B:** Purified anti-p84 B4, anti-p38 B8, anti-p42 A6, and anti-p42 G11 VHHs were biotinylated and these VHHs were incubated with plate bound subunits proteins as indicated. Binding ELISA was detected by using streptavidin-HRP and tetramethylbenzidine (TMB). Data are represented as optical density (OD). Error bars show SEM. **FIG. 13C:** Western blot of VHHs against the three PfMSP-1 subunits, the full length p190 pro-peptide, and some 3D7 lysates from ~38-44 hour 3D7 schizonts. **FIG. 13D:** Flow cytometry on synchronized ~38-44 hour 3D7 schizonts using fluorescently (Cy5) labeled VHHs.

FIGs. 14A-14C: Synthesis of VHH_{kappa}-VHH7 adduct through copper-free click reaction. **FIG. 14A:** Synthesis of VHH7-azide, sortase A catalyzes the addition of a triglycine azido-lysine peptide to the C-terminal of VHH7. **FIG. 14B:** Synthesis of VHH_{kappa}-DBCO, sortase A catalyzes the addition of a triglycine DBCO-functionalized cystine peptide to the C-terminal of anti-mouse VHH_{kappa}. **FIG. 14C:** VHH7-azide is conjugated to VHH_{kappa}-DBCO through copper-free click reaction.

FIGs. 14D: 6-9 week old female BALB/c mice were infected with 10 LD₅₀ of influenza virus. Mice were treated with the indicated doses of VHH_{kappa}-SD36, a mixture of VHH_{kappa} and SD36, or with an equal volume of PBS by intraperitoneal injection. Mice were euthanized when they lost 25% of their body weight or became moribund. Weight loss curves (left) and survival curves (right) are shown. For weight loss curves, body weight change (%) values represent mean $\pm$ standard deviation. The mean of the % body weight change over the 14 days between any two groups were compared using one-way ANOVA analysis with Tukey's multiple comparisons test, and the statistical differences between the indicated group and PBS-treated group are shown (*P < 0.05, **P < 0.01, ***P < 0.001, see all the comparisons and P values from Table. S1). For survival curves, statistical differences between the indicated group and PBS-treated group were calculated by Log-rank (Mantel-Cox) test (*P < 0.05, **P < 0.01).

FIGs. 15A and 15B: Complement-dependent cytotoxicity (CDC) of A20 cells induced by VHH_{kappa}-VHH7 adduct. **FIG. 15A:** Schematic showing the experimental procedure of the complement-dependent cytotoxicity assay. **FIG. 15B:** Bar graph showing the percent cell cytotoxicity induced by the VHHs. Error bars represent standard deviation (n=4).

FIGs. 16A and 16B: Antibody-dependent cellular cytotoxicity (ADCC) of A20 cells induced by VHH_{kappa}-VHH7 adduct. **FIG. 16A:** Schematic showing the experimental procedure of the antibody-dependent cellular cytotoxicity assay. **FIG. 16B:** Bar graph showing the percent total cell lysis induced by the VHHs. Error bars represent standard deviation (n=3).

FIGs. 17A-17E: Development of MHC class I polypeptide-related sequence A (MICA)-specific nanobodies. **FIG. 17A:** Comparison of amino acid sequences of 9 MICA-specific nanobodies that were identified and cloned into a pHen6 expression vector. **FIG. 17B:** Immunoblots showing specific binding of biotinylated A1 and H3 anti-MICA nanobody clones for purified MICA*009 antigen in whole cell lysate. **FIG. 17C:** Quantification of ELISA cross-competition assays to determine binding epitopes for anti-MICA nanobodies. A decrease in intensity measured at 450 nm as compared to a single nanobody alone is indicative of binding to the same epitope. Cross-competition is shown for A1 (left) and H3 (right) anti-MICA nanobody clones. **FIG. 17D:** Quantification of anti-MICA nanobody binding to MICA allelic products as determined by ELISA. A significant increase in intensity measured at 450 nm as compared to

uncoated ELISA control is indicative of binding. **FIG. 17E:** Flow cytometry of B16F10 cells transfected with empty vector, MICA, or MHC class I polypeptide-related sequence B (MICB) using A1 and H3 anti-MICA nanobody clones.

FIGs. 18A-18C: Affinity of VHH_{kappa}-biotin and VHH_{kappa}-SD36-biotin for mouse immunoglobulins. **FIG. 18A:** Saturation binding curves of VHH_{kappa}-biotin and VHH_{kappa}-SD36-biotin to mouse polyclonal IgG. **FIG. 18B:** Saturation binding curves of VHH_{kappa}-biotin and VHH_{kappa}-SD36-biotin to a monoclonal mouse IgM. **FIG. 18C:** Saturation binding curves of VHH_{kappa}-biotin and VHH_{kappa}-SD36-biotin to mouse polyclonal IgA coated on ELISA plates. Streptavidin-Phycoerythrin (PE) was used to quantify the amount of VHHs bound to mouse immunoglobulins.

FIGs. 19A-19B: Neuraminidase inhibition assay. The neuraminidase inhibition activities of VHH_{kappa}-zanamivir, ALB1-zanamivir, zanamivir, and VHH_{kappa} were measured by the NA-Star™ Influenza Neuraminidase Inhibitor Resistance Detection Kit. Various of influenza strains were used as the neuraminidase source. **FIG. 19A:** Summary of the half maximal inhibitory concentration (IC₅₀) values for the tested molecules. **FIG. 19B:** Dose-Inhibition curves for the tested molecules.

FIGs. 20A-20B: Saturation binding curves of VHH_{kappa}-SD36 to hemagglutinins expressed on influenza virus-infected MDCK cells. Mouse IgG-Phycoerythrin (PE) was used to quantify the amount of VHH_{kappa}-SD36 bound to hemagglutinins. Data represent mean ± standard deviation (n=3).

FIG. 21: Comparison of the therapeutic efficacy among VHH_{kappa}-zanamivir, MEDI8852, and VHH_{kappa}-E11. 6-9 week old female BALB/c mice were infected with 10 LD₅₀ of influenza virus. Mice were treated with the indicated dose of VHH_{kappa}-zanamivir, MEDI8852, or VHH_{kappa}-E11 (SARS CoV-2 spike-specific nanobody) by intraperitoneal injection. Mice were euthanized when they lost 25% of their body weight or became moribund. Weight loss curves (left) and survival curves (right) are shown. For weight loss curves, % body weight change represents the mean ± standard deviation. The mean of the % body weight change over 14 days between any two groups were compared using one-way ANOVA analysis with Tukey's multiple comparisons test. Statistical differences between the indicated group and the PBS-treated group are shown (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). For survival curves, statistical differences between the indicated group and the PBS-treated group were calculated by Log-rank (Mantel-Cox) test (*P < 0.05, **P < 0.01).

FIGs. 22A-22B: Preparation of SD36-DFO and VHH_{kappa}-SD36-DFO for PET imaging. **FIG. 22A:** The nanobody-DFO adduct was prepared by a sortase-mediated conjugation of

triglycine modified DFO to a nanobody. **FIG. 22B:** The final product of SD36-DFO (left) and VHH_{kappa}-SD36-DFO (right) were analyzed by SDS-PAGE.

FIGs. 23A-23E: VHH_{kappa}-zanamivir induces CDC and ADCC. **FIG. 23A:** Influenza virus-infected MDCK cells were killed by VHH_{kappa}-zanamivir in the presence of rabbit complement and mouse polyclonal mouse IgG. Differences in the % cytotoxicity for the VHH_{kappa}-zanamivir-treated groups and the group treated with the individual components as a mixture were analyzed by t-test (Data represent mean $\pm$ standard deviation, n = 5, *P < 0.05, **P < 0.01, ***P < 0.001). **FIG. 23B:** Virus-infected MDCK cells induced expression of luciferase in reporter cells that express luciferase upon engagement of mouse Fc γ RIV receptor in the presence of VHH_{kappa}-zanamivir and mouse polyclonal mouse IgG. Induction of ADCC was calculated by dividing the luminescence intensity of the indicated samples by the mean of control samples containing virus-infected cells and reporter cells with no VHHs. Differences in -fold induction between the VHH_{kappa}-zanamivir-treated groups and individual component mixture-treated groups were analyzed by t test (Data represent mean $\pm$ standard deviation, n = 5, *P < 0.05, **P < 0.01, ***P < 0.001). The results represent at least two independent experiments for the CDC and ADCC assays. **FIG. 23C:** Weight loss curve (left) and survival curve (right) for the comparison of efficacy between VHH_{kappa}-zanamivir and ALB1-zanamivir. **FIG. 23D:** Measure of the clearance rate of nanobodies after retro-orbital injection of their ⁸⁹Zr-labelled constructs. Each individual measurement of the ⁸⁹Zr disintegration rate as count per minute (CPM) from 10 μ L of whole blood (y-axis) is shown as a blue square (VHH_{kappa}-DFO-⁸⁹Zr, n = 3), red circle (ALB1-DFO-⁸⁹Zr, n = 4) or black triangle (SD36-DFO-⁸⁹Zr, n = 3) for each blood draw timepoint after initial injection of the construct (x-axis: 10 min, 1h, 24h, 48h, 72h, 96h, and 144h). All mice received an initial dose of 250 μ Ci of ⁸⁹Zr-labelled VHH (equals to 1 mg/kg VHH). Each data point represent mean $\pm$ standard deviation. The half-life (fast phase and slow phase) for each VHH was estimated using a two-phase decay model. Total VHH exposure across the first 144h post-injection was calculated by integrating the concentration of VHH in blood over time. It is expressed as 'area under the curve' (AUC). **FIG. 23E:** Weight loss curve (left) and survival curve (right) for the virus-infected Rag1 knockout mice having received anti-mouse VHH_{kappa}-zanamivir plus mouse polyclonal IgG. For panel (C) and (E), % body weight change represents mean $\pm$ standard deviation in panel (C), while the body weight change curves for individual mice are shown in panel (E). The mean of the % body weight change over 14 days between any two groups were compared using one-way ANOVA analysis with Tukey's multiple comparisons test. The statistical differences between the indicated group and PBS-treated group are shown (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). For survival curves, statistical differences between the indicated group and PBS-treated group were calculated by Log-rank (Mantel-Cox) test (*P < 0.05, **P < 0.01).

FIGs. 24A and 24B: VHH_{kappa}-SD36 induces ADCC but not CDC. **FIG. 24A:** Virus-infected MDCK cells induced expression of luciferase in reporter cells that express luciferase upon engagement of mouse FcγRIV receptor in the presence of VHH_{kappa}-SD36 and mouse polyclonal mouse IgG. Fold induction was calculated by dividing the luminescent intensity of the indicated samples by the mean of control samples containing virus-infected cells and reporter cells with no VHHs. Differences in fold induction between the VHH_{kappa}-SD36-treated groups and individual component mixture-treated groups were analyzed by t test (Data represent mean ± standard deviation, n = 5, *P < 0.05, **P < 0.01, ***P < 0.001). **FIG. 24B:** Influenza virus-infected MDCK cells were not significantly killed by VHH_{kappa}-SD36 in the presence of rabbit complement and polyclonal mouse IgG. Differences in the % cytotoxicity for the VHH_{kappa}-SD36-treated groups and the group treated with the individual components as a mixture were analyzed by t-test (Data represent mean ± standard deviation, n = 5, *P < 0.05, **P < 0.01, ***P < 0.001). The results represent at least two independent experiments for the ADCC and CDC assays.

FIG. 25A and 25B: Preparation of ALB1-zanamivir. **FIG. 25A:** Amino acid sequence of the anti-serum albumin nanobody (ALB1). A sortase recognition motif (LPETG) is attached to the C terminus of the nanobody. **FIG. 25B:** ALB1-zanamivir was prepared by sortase-mediated conjugation of triglycine-modified zanamivir to ALB1. The identity of the final product, ALB1-zanamivir, was confirmed by SDS-PAGE (left) and mass spectrometry (right).

FIG. 26: Preparation of VHH_{kappa}-DFO, ALB1-DFO, and SD36-DFO for PET imaging. The nanobody-DFO adduct was prepared by sortase-mediated conjugation of triglycine-modified DFO to a nanobody. The nanobody-DFO adducts were analyzed by SDS-PAGE (For each gel, in order from left to right: 1: sortase, 2: unconjugated nanobody, 3: reaction mixture, 4-9: different fractions obtained after PD-10 column elution, nanobody-DFO adducts shown as #6 on the gels were used for PET imaging).

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

Some aspects of the present disclosure are based, at least in part, on the finding that conjugate molecules which bind specifically to both an antigen located on the surface of a cell or pathogen and to a structural feature shared by many distinct immunoglobulins produced in a host, such as a kappa light chain or lambda light chain sequence, may be used to recruit immune cells with cell surface Fc receptors to the cell or pathogen expressing the antigen, irrespective of the clonality or specificity of the host immunoglobulins. As described herein, these conjugates may be used to enhance or elicit an immune response toward specific viruses or cells in a subject. This approach is useful for treating or reducing the risk for a disease associated with the

targeted viruses or cells in a subject. In some embodiments, the conjugates described herein are useful for treating (both prophylactically and therapeutically) one or more diseases in a subject, including infections caused by pathogens (e.g., viruses, bacteria, parasites, fungi) and cancers.

As further described, the disclosed conjugates are also useful for ablating a specific type of cell in a subject, whether or not the cell is associated with a disease.

Conjugates

Without wishing to be bound by theory, during an innate (cell-mediated) immune response, a variety of immune cell types directly target potential pathogens or otherwise harmful cells, causing these cells to be killed or inactivated (i.e., prevented from reproducing). In this way, immune cells such as macrophages, dendritic cells, natural killer cells, neutrophils, basophils, eosinophils, and mast cells contain and destroy pathogenic infections and cancers that occur in their host. However, these immune cells are generally incapable of interacting directly with their targets, as they lack receptors on their surface for doing so. Instead, many immune cells express a receptor protein on their surface that is referred to as the fragment crystallizable (Fc) receptor. Fc receptors are able to bind to host immunoglobulins of the subject, each of which is specific for a particular antigen. When an Fc receptor-bound immunoglobulin binds to its target antigen, such as an antigen occurring on the surface of a pathogen or other target cell, it brings the target into sufficiently close proximity of the immune cell, leading to the killing or inactivation of the pathogen or other target cell.

Although this system allows for protection against a broad range of possible targets, the efficacy of many immune cells is largely based upon the quantity of immunoglobulins in a subject that are specific for a particular antigen. In other words, if there is an insufficient quantity of immunoglobulins that are specific for an antigen on the surface of a pathogen or cancer cell, certain immune cell types are limited in their ability to mount an effective response. Fortunately, an immune response may be enhanced or elicited in a subject by providing a molecule that is capable of enhancing the proximity between Fc receptor-positive immune cells and their targets. Provided herein are such molecules, which are conjugates between a first agent that is specific for immunoglobulins produced by a host and a second agent that is specific for an antigen on the surface of a cell or pathogen. By selecting a first agent that binds specifically to a structural feature shared by many host immunoglobulins, such as a kappa light chain or a lambda light chain, these conjugates can bind simultaneously to a target and to an immunoglobulin bound by a Fc receptor-positive immune cell. By effectively bridging immune cells and their targets, these conjugates enable enhanced immunity toward virtually any potential

target without relying on the specificity of host immunoglobulins, simply by customizing the target specificity of the second agent.

Accordingly, some aspects of the present disclosure describe a conjugate comprising a first agent that binds to an immunoglobulin and a second agent that binds to a target on the surface of a cell or a pathogen, wherein the first agent and the second agent are covalently conjugated via a linker. In some embodiments, the first agent binds specifically to an immunoglobulin. In some embodiments, the second agent binds specifically to a target on the surface of a cell or pathogen.

In some embodiments, the first agent is an antibody or an antibody fragment thereof (e.g., a recombinant antibody fragment, such as, but not limited to, a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a single domain antibody (sdAb)). In some embodiments, the first agent is a single domain antibody, which is alternately referred to in the art as a “nanobody.” In some embodiments, the first agent is a single domain antibody that is a heavy-chain antibody, i.e., a single domain antibody fragment derived from an immunoglobulin that only comprises heavy chains, as typically found in mammalian species that belong to the Camelid family. Such fragments are referred to in the art as a “VHH,” expressed recombinantly as a “nanobody.” In some embodiments, the first agent is a recombinant single domain antibody, such as a recombinant VHH or nanobody.

The first agent of a conjugate described herein binds to an immunoglobulin. An “immunoglobulin” refers to an antibody protein complex that is produced and secreted by lymphocytes or plasma cells. An immunoglobulin typically comprises one or more heavy chains and one or more light chains, which are covalently linked to one another by disulfide bonds. The heavy chain(s) and light chain(s) of an immunoglobulin may each comprise a variable region, which comprises an amino acid sequence that varies between immunoglobulin clones and determines which antigen is bound by an immunoglobulin (e.g., a heavy chain variable region and a light chain variable region), and a constant region, which is constant across antibody clones (e.g., a heavy chain constant region and a light chain constant region). An immunoglobulin may belong to any one of several structural classes (isotypes), according to its overall mass and number of antigen binding sites. For example, an immunoglobulin may be an immunoglobulin A (IgA), an immunoglobulin D (IgD), an immunoglobulin E (IgE), an immunoglobulin G (IgG), or an immunoglobulin M (IgM). An immunoglobulin may further belong to a particular structural subclass, such as, for example, IgG subclass 1 (IgG1), IgG subclass 2 (IgG2), IgG subclass 3 (IgG3), and IgG subclass 4 (IgG4). The class and subclass of an immunoglobulin determine many functional characteristics of the immunoglobulin, such as, but not limited to, its biodistribution. In some embodiments, an immunoglobulin described

herein comprises a heavy chain that comprises a fragment crystallizable (Fc) region. The Fc region is comprised by the heavy chain constant region and is required for binding to an Fc receptor, such as an Fc receptor on the surface of an immune cell (e.g., a macrophage, a dendritic cell, a natural killer cell, a neutrophil, a basophil, an eosinophil, a mast cell). All circulating immunoglobulins comprise an Fc region.

The present disclosure particularly relates to immunoglobulins comprising a light chain that is a kappa (κ) light chain or a lambda (λ) light chain. Kappa light chains and lambda light chains are expressed from genes within different genetic loci, referred to as *IGK* (NCBI Gene ID: 50802) and *IGL* (NCBI Gene ID: 3535), respectively, which are located on different chromosomes (e.g., in humans, chromosome 2 for *IGK* and chromosome 22 for *IGL*). In some embodiments, an immunoglobulin of the present disclosure comprises a kappa light chain expressed from the *IGK* locus. In some embodiments, an immunoglobulin of the present disclosure comprises a lambda light chain expressed from the *IGL* locus.

In some embodiments, the first agent binds to an immunoglobulin kappa light chain or an immunoglobulin lambda light chain. In some embodiments, the first agent is a nanobody or single domain antibody (e.g., a VHH) that binds to an immunoglobulin kappa light chain or an immunoglobulin lambda light chain. In some embodiments, the first agent is a nanobody or single domain antibody (e.g., a VHH) that binds to an immunoglobulin kappa light chain or an immunoglobulin lambda light chain of a particular species, such as a human. In some embodiments, the first agent is a nanobody or single domain antibody (e.g., a VHH) that binds specifically to an immunoglobulin kappa light chain, such as a human immunoglobulin kappa light chain. As used herein, a nanobody or single domain antibody that is a VHH and is specific for a kappa light chain is referred to as “VHH_{kappa}.” Example VHH_{kappa} amino acid sequences are as follows:

Anti-mouse IgG kappa light chain VHH:

QVQLVESGGGWVQPGGSLRLSCAASGFTFSDTAMMWVRQAPGKGREWVAAIDTGGG
YTTYADSVKGRFTISRDNKNTLYLQMNSLKPEDTARYYCAKTYSGNYYSNYTVANY
GTTGRGTLVTVSSGG (SEQ ID NO: 1)

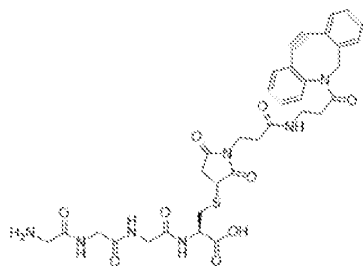
Anti-human IgG kappa light chain VHH:

QVQLQESGGGLVQPGGSLRLSCAASGRITISRYAMSWFRQAPGKEREFVAVARRSGDGA
FYADSVQGRFTVSRDDAKNTVYLQMNSLKPENTAVYYCAIDSDTFYSGSYDYWGQGT
QVTVSSGG (SEQ ID NO: 2)

In some embodiments, the second agent comprises a small molecule, a peptide, a protein, a carbohydrate, a lipid, a nucleotide, a nucleic acid, an oligonucleotide, an aptamer, or an antibody or antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a nanobody or a single domain antibody (sdAb)). In some
5 embodiments, the second agent is a small molecule, a peptide, a protein, a carbohydrate, a lipid, a nucleotide, a nucleic acid, an oligonucleotide, an aptamer, or an antibody or antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a nanobody or a single domain antibody (sdAb)). In some embodiments, the second agent is a small molecule (e.g., a small molecular inhibitor). In some embodiments, the second
10 agent is an antibody or an antibody fragment thereof. In some embodiments, the second agent is a single domain antibody. In some embodiments, the second agent has a therapeutic effect when administered to a subject (e.g., when the second agent is administered to a subject alone). In some embodiments, the second agent does not have a therapeutic effect when administered to a subject (e.g., when the second agent is administered to a subject alone). In some embodiments,
15 the second agent binds specifically to a target on the surface of a particular species of cell or pathogen (e.g., a target on the surface of a particular virus, bacterium, parasite, fungus, or human cell, such as a cancer cell), or of a range of related species (e.g., where the species express substantially similar versions of the target on their surface).

In some embodiments, the first agent and the second agent are covalently linked through
20 a linker. In some embodiments, the linker comprises a cleavable or a non-cleavable linker. A “cleavable linker” refers to a linker within which one or more covalent bonds are cleaved (broken) under certain conditions, such as those occurring in a cell or subject. A “non-cleavable” linker refers to a linker which for all intents and purposes cannot be efficiently or reliably cleaved under certain conditions, such as those occurring in a cell or subject. Examples of
25 cleavable linkers familiar to those of skill in the relevant art include peptide linkers (e.g., Val-Cit), disulfide linkers, and hydrazone linkers, which are cleaved by proteolysis, reduction, and low pH, respectively. Examples of non-cleavable linkers include, for example, N-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) and polyethylene glycol (PEG). Additional examples of linkers known in the art include, for example, those of Lu, et al.
30 “Linkers Having a Crucial Role in Antibody-Drug Conjugates” *Int J Mol Sci*, 17(4), 561., the contents of which are incorporated herein by reference.

In some embodiments, the linker is a triglycine dibenzylcyclooctyne (DBCO) linker, which is as follows:



In some embodiments, the second agent is a protein or peptide (e.g., an antibody or antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a nanobody or a single domain antibody (sdAb)) and the linker is a peptide bond, an isopeptide bond, or a disulfide bond. In some embodiments, the linker comprises one or more amino acids linking the first agent and the second agent, such as, for example, a repeating linker comprising glycine and/or serine, or another amino acid linker that is generally known in the art. In some embodiments, the first agent and the second agent are translated separately (e.g., in an *in vitro* translation system or recombinantly expressed in a cell) and post-translationally linked. In some embodiments, the first agent and second agent are translated as a fusion protein (e.g., in an *in vitro* translation system or recombinantly expressed in a cell), wherein the first agent and the second agent are linked by one or more peptide bonds. In some embodiments, the conjugate is produced recombinantly, by inserting one or more nucleic acids (e.g., DNA or RNA) encoding the first agent and the second agent to a prokaryotic (e.g., bacterial) or eukaryotic (e.g., fungal or mammalian) cell for expression, and subsequently isolating the conjugate using one or more techniques that are generally known in the art, such as, for example, affinity chromatography or size and/or size exclusion chromatography. In some embodiments, the first agent and the second agent are encoded by different nucleic acids (e.g., DNA or RNA). In some embodiments, the first agent and the second agent are encoded by the same nucleic acid (e.g., DNA or RNA). In some embodiments, the first agent and second agent are encoded by one or more plasmids or mRNAs and inserted (transfected) into cells by any means known in the art (e.g., electroporation). In some embodiments, nucleic acids encoding the first agent and second agent are inserted (transfected) into the cell using a viral vector (e.g., an adenoviral vector, a lentiviral vector), by any means known in the art. In some embodiments, nucleic acids encoding the first agent and second agent are chromosomally inserted into cell by any means known in the art.

In some embodiments, the second agent is linked to the first agent by means of a sortase enzyme (e.g., sortase A). Sortases are a class of enzyme that specifically targets the amino acid motif LPXTG, where X is any amino acid, by cleaving C-terminal to the threonine residue, generating a peptide bond between the threonine and sortase that is subsequently transferred to

the N-terminus of another protein. In some embodiments, the C-terminus of the first agent (e.g., VHH_{kappa}) is labeled with a sortase recognition sequence, such as LPETGGHHHHHH (SEQ ID NO: 3), prior to being linked to the second agent. In some embodiments, the second agent is a protein or peptide (e.g., an antibody or antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a nanobody or a single domain antibody (sdAb)) that is labeled at its C-terminus with a sortase recognition sequence, prior to being linked to the first agent.

In some embodiments, the second agent binds specifically to a target present on the surface of a particular cell, such as a cell that is infected by a pathogen (e.g., a virus, a bacterium, a parasite, a fungus, a cancer cell, a transformed cell, a healthy cell, or a cell that is undergoing or has undergone a phenotypic change in response to cellular stress. A cell that is undergoing or has undergone a phenotypic change in response to cellular stress may be a cell that is undergoing or has undergone a phenotypic change in response to cellular stress caused by, but not limited to, oxidative stress, nutritional stress, hypoxia, heat shock, ionizing radiation, exposure to heavy metals, or exposure to mutagens, or physical damage.

In some embodiments, the second agent binds specifically to a target present on the surface of a pathogen, such as a pathogenic virus, bacterium, parasite, or fungus. In some embodiments, the pathogen is a virus. In some embodiments, the virus is an influenza virus, a coronavirus, an adenovirus, an enterovirus, a rotavirus, a norovirus, a herpesvirus, a lentivirus, a poxvirus, a paramyxovirus, a rhabdovirus, an arenavirus, a flavivirus, a togavirus, a hantavirus, a pneumovirus, or an ebolavirus.

In some embodiments, the virus is an influenza virus. In some embodiments, the virus is an influenza A virus or an influenza B virus. In some embodiments, the target to which the second agent binds is an influenza virus neuraminidase or an influenza virus hemagglutinin, such as an influenza virus neuraminidase or an influenza virus hemagglutinin expressed on the surface of an influenza A virus or an influenza B virus. In some embodiments, the second agent is a small molecule inhibitor, such as a small molecule inhibitor that binds to an influenza virus neuraminidase. In some embodiments, the second agent comprises zanamivir, oseltamivir, peramivir, or an analog thereof. In some embodiments, the second agent is an antibody or an antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a single domain antibody (sdAb)) that binds to an influenza virus neuraminidase or an influenza virus hemagglutinin. In some embodiments, the second agent is a VHH that binds to an influenza virus hemagglutinin. An example of a VHH that binds to influenza virus hemagglutinin is as follows:

Anti-HA VHH (SD36):

EVQLVESGGGLVQAGGSLKLSCAASGRITYAMGWFRQAPGKEREFVAHINALGTRTTY
SDSVKGRFTISRDNANKNTEYLEMNNLKPEDTAVYYCTAQGQWRAAPVAVAAEYEFWG
QGTQVTVSSGG (SEQ ID NO: 4).

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In some embodiments the first agent and the second agent are linked by a triglycine dibenzylcyclooctyne (DBCO) linker. In some embodiments the first agent and the second agent are linked by a triglycine dibenzylcyclooctyne (DBCO) linker and the second agent is zanamivir.

In some embodiments, the virus is a beta coronavirus. In some embodiments, the beta coronavirus is a Middle East Respiratory Syndrome coronavirus (MERS-CoV), a Severe Acute Respiratory Syndrome (SARS)-associated coronavirus (SARS-CoV)-1, or a SARS-CoV-2. In some embodiments, the target to which the second agent binds is a MERS-CoV spike protein, a SARS-CoV-1 spike protein, or a SARS-CoV-2 spike protein. In some embodiments, the target to which the second agent binds is a MERS-CoV spike protein receptor binding domain (RBD), a SARS-CoV-1 spike protein RBD, or a SARS-CoV-2 spike protein RBD. In some
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embodiments, the second agent is an antibody or an antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a single domain antibody (sdAb)) that binds to a target on the surface of MERS-CoV, SARS-CoV-1, or SARS-CoV-2, such as a MERS-CoV spike protein, a SARS-CoV-1 spike protein, or a SARS-CoV-2 spike protein, or a MERS-CoV spike protein RBD, a SARS-CoV-1 spike protein RBD, or a SARS-CoV-2 spike protein RBD. In some embodiments, the second agent specifically binds to a target expressed on the surface of SARS-CoV-2. In some embodiments, the second agent specifically binds to a target expressed on the surface of the originally discovered SARS-CoV-2. In some embodiments, the second agent specifically binds to a target expressed on the surface of an identified SARS-CoV-2 variant, such as a variant of concern (VOC) as identified by the United States Centers for Disease Control and Prevention (CDC), such as, but not limited to, the extant variants B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma), B.1.617.2 (delta), B.1.427 and B.1.429 (epsilon), B.1.525 (eta), B.1.526 (iota), B.1.617.1 (kappa), B.1.1.529 (omicron), B.1.621 (mu), and P.2 (zeta) variant and those yet to emerge for SARS-CoV-2.

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In some embodiments, the virus is a lentivirus. In some embodiments, the lentivirus is a human immunodeficiency virus (HIV). In some embodiments, the target to which the second agent binds is a HIV envelope glycoprotein gp120. In some embodiments, the second agent specifically binds to a target expressed on the surface of HIV. In some embodiments, the second agent is an antibody or an antibody fragment thereof (e.g., a fragment antigen binding (Fab)

fragment, a single-chain variable fragment (ScFv), or a single domain antibody (sdAb)) that binds to a target on the surface of HIV, such as a HIV envelope glycoprotein gp120.

In some embodiments, the virus is a pneumovirus. In some embodiments, the pneumovirus is a human respiratory syncytial virus (RSV). In some embodiments, the target to which the second agent binds is a RSV fusion (F) protein. In some embodiments, the second agent specifically binds to a target expressed on the surface of RSV. In some embodiments, the second agent is an antibody or an antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a single domain antibody (sdAb)) that binds to a target on the surface of RSV, such as a RSV F protein.

In some embodiments, the pathogen is a bacterium. In some embodiments, the bacterium is a *Pasteurella* species, a *Staphylococcus* species, a *Streptococcus* species, a *Bacillus* species, a *Corynebacterium* species, a *Diphtheroids* species, a *Listeria* species, an *Erysipelothrix* species, a *Clostridium* species, a *Neisseria* species, a *Branhamella* species, an *Escherichia* species, an *Enterobacter* species, a *Proteus* species, a *Pseudomonas* species, a *Klebsiella* species, a *Salmonella* species, a *Shigella* species, a *Serratia* species, an *Acinetobacter* species, a *Haemophilus* species, a *Brucella* species, a *Yersinia* species, a *Francisella* species, a *Pasturella* species, a *Vibrio* species, a *Flavobacterium* species, a *Pseudomonas* species, a *Campylobacter* species, a *Bacteroides* species, a *Fusobacterium* species, a *Calymmatobacterium* species, a *Streptobacillus* species, or a *Legionella* species. In some embodiments, the second agent specifically binds to a target expressed on the surface of the bacterium. In some embodiments, the second agent is an antibody or an antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (scFv), or a single domain antibody (sdAb)) that binds to a target on the surface of the bacterium.

In some embodiments, the pathogen is a parasite. In some embodiments, the parasite is a *Plasmodium* species, a *Trypanosoma* species, a *Toxoplasma* species, a *Leishmania* species, or a *Cryptosporidium* species. In some embodiments, the *Plasmodium* species is *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Plasmodium ovale curtisi*, or *Plasmodium ovale wallikeri*. In some embodiments, the target to which the second agent binds is a *Plasmodium* surface protein, such as, for example, a merozoite surface protein 1 (MSP-1). In some embodiments, the second agent specifically binds to a target expressed on the surface of the parasite. In some embodiments, the second agent is an antibody or an antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a nanobody or a single domain antibody (sdAb)) that binds to a target on the surface of the parasite, such as, for example, a target on the surface of a *Plasmodium* species. In some embodiments, the target on the surface of a *Plasmodium* species is a plasmodium surface

protein, e.g., merozoite surface protein 1 (MSP-1). In some embodiments, the second agent is an antibody that binds to MSP-1, optionally wherein the antibody is a nanobody. In some embodiments, the antibody comprises a CDR-H1, a CDR-H2, and a CDR-H3 of any one of the antibodies listed in Table 1. In some embodiments, the antibody comprises an amino acid
5 sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%)) identical to of any one of SEQ ID NOs: 6-17. In some embodiments, the antibody comprises the amino acid sequence of any one of SEQ ID NOs: 6-17.

In some embodiments, the second agent binds specifically to a target present on the surface of a cancer cell. In some embodiments, the cancer cell is a hematological cancer cell, a
10 lung cancer cell, a breast cancer cell, a brain cancer cell, a gastrointestinal cancer cell, a liver cancer cell, a kidney cancer cell, a bladder cancer cell, a pancreatic cancer cell, an ovarian cancer cell, a testicular cancer cell, a prostate cancer cell, an endometrial cancer cell, a muscle cancer cell, a bone cancer cell, a neuroendocrine cancer cell, a connective tissue cancer cell, a head or neck cancer cell, or a skin cancer cell. In some embodiments, the cancer cell is a human
15 cancer cell. In some embodiments, the target to which a second agent binds is a tumor-associated antigen, such as, but not limited to, a MHC class I polypeptide-related sequence A (MICA) protein, a MHC class I polypeptide-related sequence B (MICB) protein, a folate receptor, a fibronectin splice variant, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), hepatocyte growth factor receptor (HGFR), vascular
20 endothelial growth factor receptor 2 (VEGFR-2), C-X-C chemokine receptor type 4 (CXCR4), urokinase plasminogen activator surface receptor (uPAR), follicle-stimulating hormone receptor (FSHR), epithelial cell adhesion molecule (EpCAM), epithelial cadherin (ECAD), carcinoembryonic antigen (CEA), or mesothelin (MSLN). In some embodiments, the second agent is a small molecule (e.g., a small molecular inhibitor) that binds to a target on the surface
25 of the cancer cell, such as a tumor-associated antigen. In some embodiments, the second agent is an antibody or an antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a single domain antibody (sdAb)) that binds to a target on the surface of the cancer cell, such as a tumor-associated antigen. In some embodiments, the second agent is an antibody that binds to MICA, optionally wherein the antibody is a nanobody.
30 In some embodiments, the antibody comprises a CDR-H1, a CDR-H2, and a CDR-H3 of any one of the antibodies listed in Table 2. In some embodiments, the antibody comprises an amino acid sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%)) identical to of any one of SEQ ID NOs: 19-27. In some embodiments, the antibody comprises the amino acid sequence of any one of SEQ ID NOs: 19-27.

In some embodiments, the cell is a cancerous or healthy bone marrow cell. In some embodiments, the target to which a second agent binds is a bone marrow-associated antigen, such as, but not limited to, a cluster of differentiation antigen 45 (CD45). In some embodiments, the second agent is an antibody or an antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a single domain antibody (sdAb)) that binds to a target on the surface of the bone marrow cell, such as a bone marrow-associated antigen.

In some embodiments, the cell is a cancerous or healthy immune cell, such as, but not limited to, a cancerous or healthy T cell or B cell. In some embodiments, the target to which a second agent binds is an immune cell-associated antigen, such as, but not limited to, a cluster of differentiation antigen 4 (CD4), a cluster of differentiation antigen 8 (CD8), a T cell receptor (TCR), or a B cell receptor (BCR). In some embodiments, the second agent is an antibody or an antibody fragment thereof (e.g., a fragment antigen binding (Fab) fragment, a single-chain variable fragment (ScFv), or a single domain antibody (sdAb)) that binds to a target on the surface of the immune cell, such as an immune cell-associated antigen.

In some embodiments, a conjugate provided herein provides a therapeutic effect when administered to a subject. In some embodiments, a conjugate provided herein enhances the association (proximity) between one or more immune cells (e.g., one or more immune cell types) expressing a fragment crystallizable (Fc) receptor and a cell or pathogen when the conjugate is administered to a subject. In some embodiments, administration of a conjugate provided herein to a subject results in the killing of a cell or pathogen in the subject. In some embodiments, administration of a conjugate provided herein to a subject results in the inactivation of a cell or pathogen in the subject. In some embodiments, the subject to which a conjugate provided herein provides a therapeutic effect is a mammal. In some embodiments, the subject to which a conjugate provided herein provides a therapeutic effect is a human.

Compositions

In some embodiments, compositions (e.g., pharmaceutical compositions) of the present disclosure comprise a conjugate described herein. In some embodiments, compositions (e.g., pharmaceutical compositions) of the present disclosure comprise two or more conjugates described herein. In some embodiments, a composition comprising two or more conjugates comprises two or more conjugates specific for the same antigen. In some embodiments, a composition comprising two or more conjugates comprises only one conjugate specific for each antigen. As contemplated herein, the terms “composition” and “formulation” may be used interchangeably.

In some embodiments, a composition may comprise one or more conjugates described herein and one or more pharmacologically acceptable excipients. A pharmacologically acceptable excipient may enhance stability of a conjugate described herein, enhance delivery of the conjugate to cells (*e.g.*, immune cells) of a subject to which the composition is administered, permit sustained or delayed release of the conjugate upon administration, alter the biodistribution of the conjugate (*e.g.*, target the conjugate to specific tissues or cell types), or reduce host immunity against the conjugate. Examples of pharmacologically acceptable excipients includes any and all solvents, dispersion media, diluents or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, and preservatives, as are known in the art. In some embodiments, a pharmacologically acceptable excipient comprises an aqueous solution or buffer. In some embodiments, the composition is isotonic, relative to a biological fluid of a subject (*i.e.*, blood) to which the composition is to be administered. In some embodiments, the composition has a pH between 7 and 8, or optimally a pH of about 7.4.

Kits

Also encompassed by the present disclosure are kits (*e.g.*, pharmaceutical packs). The kits provided may comprise a pharmaceutical composition or conjugate described herein and a container (*e.g.*, a vial, ampule, bottle, syringe, and/or dispenser package, or other container suitable for storage and/or administration). In some embodiments, provided kits may optionally further include a second container comprising a pharmaceutical excipient for dilution or suspension of a pharmaceutical composition or conjugate described herein. In some embodiments, the pharmaceutical composition or conjugate described herein is provided in the first container and is combined with the second container to form one dosage unit.

Thus, in one aspect, provided herein are kits including a first container comprising a conjugate or pharmaceutical composition described herein. In certain embodiments, the kits are useful for enhancing or eliciting an immune response toward a particular cell or pathogen in a subject (*e.g.*, a pathogenic cell or a cell of the subject). In certain embodiments, the kits are useful for treating a disease (*e.g.*, a disease caused by a virus, a disease caused by a bacterium, a disease caused by a parasite, a disease caused by a fungus, a cancer) in a subject in need thereof. In certain embodiments, the kits are useful for preventing a disease (*e.g.*, a disease caused by a virus, a disease caused by a bacterium, a disease caused by a parasite, a disease caused by a fungus, a cancer) in a subject in need thereof.

In certain embodiments, a kit described herein further includes instructions for using the pharmaceutical composition or conjugate included in the kit. A kit described herein may also

include information as required by a regulatory agency such as the U.S. Food and Drug Administration (FDA). In certain embodiments, the information included in the kits is prescribing information. In certain embodiments, the kits and instructions provide for enhancing or eliciting an immune response toward a cell or pathogen in a subject (*e.g.*, a pathogenic cell or a cell of the subject). In certain embodiments, the kits and instructions provide for treating a disease (*e.g.*, a disease caused by a virus, a disease caused by a bacterium, a disease caused by a parasite, a disease caused by a fungus, a cancer) in a subject in need thereof. In certain embodiments, the kits and instructions provide for preventing a disease (*e.g.*, a disease caused by a virus, a disease caused by a bacterium, a disease caused by a parasite, a disease caused by a fungus, a cancer) in a subject in need thereof. A kit described herein may include one or more additional pharmaceutical agents described herein as a separate composition.

Administration of conjugates

The terms “treatment,” “treat,” and “treating” refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease (*e.g.*, a disease caused by a virus, a disease caused by a bacterium, a disease caused by a parasite, a disease caused by a fungus, a cancer) described herein. In some embodiments, treatment may be administered after one or more signs or symptoms of the disease have developed or have been observed in a subject. In other embodiments, treatment may be administered in the absence of signs or symptoms of the disease. For example, treatment may be administered to a susceptible subject prior to the onset of symptoms (*e.g.*, in light of a history of symptoms for the disease, in light of a risk of relapse or reoccurrence of the disease, and/or in light of exposure to a pathogen that is causative for the disease or the likelihood for future exposure to a pathogen that is causative for the disease). Treatment may also be continued after symptoms have resolved, for example, to delay or prevent relapse or recurrence. Prophylactic treatment refers to the treatment of a subject who is not and was not with a disease but is at risk of developing the disease or who was with a disease and is at risk of relapse or regression of the disease. In some embodiments, the subject is at a higher risk of developing the disease or at a higher risk of relapse or regression of the disease than an average healthy member of a population.

An “effective amount” of a composition described herein refers to an amount sufficient to elicit the desired biological response. An effective amount of a composition described herein may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of a conjugate described herein, the condition being treated, the mode of administration, and the age and health of the subject. In some embodiments, an effective amount is a therapeutically effective amount. In some embodiments, an effective amount is an amount sufficient for

prophylactic treatment. In some embodiments, an effective amount is the amount of a conjugate described herein administered in a single dose. In some embodiments, an effective amount is the combined amount (sum) of a conjugate described herein administered in multiple doses. Where an effective amount of a composition is referred to herein, the amount that is therapeutically and/or prophylactically effective is signified, depending on the subject and/or the disease to be treated. Determining the effective amount and/or dosage is within the abilities of one skilled in the art.

The terms “administer,” “administering,” or “administration” refers to implanting, absorbing, ingesting, injecting, inhaling, or otherwise introducing a composition or conjugate described herein in or on a subject. A composition or conjugate described herein may be administered systemically (*e.g.*, via intravenous injection) or locally (*e.g.*, via local injection). In some embodiments, the composition or conjugate described herein is administered orally, intravenously, topically, intranasally, or sublingually. Parenteral administering is also contemplated. The term “parenteral” as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, intradermally, and intracranial injection or infusion techniques. In some embodiments, the administering is done intramuscularly, intradermally, orally, intravenously, topically, intranasally, intravaginally, or sublingually. In some embodiments, the composition or conjugate described herein is administered prophylactically.

In some embodiments, a composition or conjugate described herein is administered once or is administered repeatedly (*e.g.*, 2, 3, 4, 5, or more times). For multiple administrations, the administrations may be done over a period of time (*e.g.*, 1 day, 1 week, 1 month, 6 months, 1 year, 2 years, 5 years, 10 years, or longer). For repeating administrations, the administrations may be done over a fixed period of time (*e.g.*, 1 day, 1 week, 1 month, 6 months, 1 year, 2 years, 5 years, 10 years, or longer), or a variable period of time. In some embodiments, the composition or conjugate described herein is administered twice (*e.g.*, Day 0 and Day 7, Day 0 and Day 14, Day 0 and Day 21, Day 0 and Day 28, Day 0 and Day 60, Day 0 and Day 90, Day 0 and Day 120, Day 0 and Day 150, Day 0 and Day 180, Day 0 and 3 months later, Day 0 and 6 months later, Day 0 and 9 months later, Day 0 and 12 months later, Day 0 and 18 months later, Day 0 and 2 years later, Day 0 and 5 years later, or Day 0 and 10 years later). In some embodiments, the composition or conjugate described herein is administered more than twice, is administered until a subject is free of symptoms of a disease (*e.g.*, a disease caused by a virus, a disease caused by a bacterium, a disease caused by a parasite, a disease caused by a fungus, a cancer), or is administered until the risk of developing the disease subsides.

In some embodiments, a composition or conjugate described herein is administered to a subject for the purpose of enhancing or eliciting an immune response toward a cell or pathogen in a subject (*e.g.*, a pathogenic cell or a cell of the subject). In some embodiments, a composition or conjugate described herein is administered to a subject for the purpose of treating or
5 preventing an infection by a pathogen. In some embodiments, a composition or conjugate described herein is administered to a subject for the purpose of treating or preventing a viral infection. In some embodiments, a composition or conjugate described herein is administered to a subject for the purpose of treating or preventing a bacterial infection. In some embodiments, a composition or conjugate described herein is administered to a subject for the purpose of
10 treating or preventing a parasitic infection. In some embodiments, a composition or conjugate described herein is administered to a subject for the purpose of treating or preventing a fungal infection. In some embodiments, a composition or conjugate described herein is administered to a subject for the purpose of treating or preventing a cancer.

In some embodiments, administration of a composition or conjugate described herein to
15 a subject enhances or elicits an innate (cell-mediated) immune response in the subject. In some embodiments, following administration of a composition or conjugate described herein to a subject, the conjugate binds to a cell or pathogen in the subject and to an immunoglobulin of the subject, wherein the immunoglobulin further binds to a subject's immune cell that expresses fragment crystallizable (Fc) receptors on its surface. In some embodiments, the subject's
20 immunoglobulin comprises an immunoglobulin kappa light chain or an immunoglobulin lambda light chain. In some embodiments, the subject's immunoglobulin comprises an immunoglobulin kappa light chain. In some embodiments, the immune cell is a macrophage, a dendritic cell, a natural killer cell, a neutrophil, a basophil, an eosinophil, or a mast cell. In some embodiments, administration of a composition or conjugate described herein induces the production of one or
25 more cytokines or chemokines by the immune cell. In some embodiments, administration of a composition or conjugate described herein induces the production of one or more proinflammatory cytokines or proinflammatory chemokines by the immune cell. In some embodiments, administration of a composition or conjugate described herein induces phagocytosis of a cell or pathogen in the subject by the immune cell of the subject. In some
30 embodiments, administration of a composition or conjugate described herein results in killing of a cell or pathogen in the subject. In some embodiments, administration of a composition or conjugate described herein results in inactivation of a cell or pathogen in the subject (*i.e.*, the cell or pathogen is no longer able to replicate or reproduce).

In some embodiments, the subject is a subject that has or is at risk for developing an
35 infection by a pathogen (*e.g.*, a viral infection, a bacterial infection, a parasitic infection, a

fungal infection). In some embodiments, the subject has or is at risk for developing a cancer, such as, but not limited to, a hematological cancer, a lung cancer, a breast cancer, a brain cancer, a gastrointestinal cancer, a liver cancer, a kidney cancer, a bladder cancer, a pancreatic cancer, an ovarian cancer, a testicular cancer, a prostate cancer, an endometrial cancer, a muscle cancer, a bone cancer, a neuroendocrine cancer, a connective tissue cancer, a head or neck cancer, or a skin cancer. In some embodiments, the cancer is a metastatic cancer.

As defined herein, a “subject” refers to a living organism to which administration is contemplated. In some embodiments, a subject is a mammal. In some embodiments, the subject is a non-human animal (*e.g.*, primate (*e.g.*, cynomolgus monkey or rhesus monkey), a commercially relevant mammal (*e.g.*, cattle, pig, horse, sheep, goat, cat, or dog), or a bird (*e.g.*, commercially relevant bird, such as chicken, duck, goose, or turkey)). In some embodiments the subject is a domesticated animal (*e.g.*, cattle, pig, horse, sheep, goat) or a companion animal (*i.e.*, a pet or service animal, *e.g.*, cat or dog). In some embodiments, the subject is a fish, reptile, or amphibian. The non-human animal may be a male or female at any stage of development. The non-human animal may be a transgenic animal or genetically engineered animal.

In some embodiments, the subject is a human. In some embodiments, the subject is a human infant. In some embodiments, the human infant is a neonate that is less than 28 days of age. In some embodiments, the human infant is less than 1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 days of age at the time of administration.

In some embodiments, the human subject is more than 28 days of age (*e.g.*, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 2 years, 3 years, 4 years, 5 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years of age). In some embodiments, the human subject is an adult (*e.g.*, more than 18 years of age). In some embodiments, the human subject is an elderly subject (*e.g.*, more than 60 years of age). In some embodiments, the human subject is 60 years, 65 years, 70 years, 75 years, 80 years, 85 years, 90 years, 95 years, 100 years, or more than 100 years of age.

In some embodiments, the human subject is part of one or more immunologically vulnerable populations. In some embodiments, the human subject is frail (*e.g.*, a subject having frailty syndrome, a malnourished subject, or a subject with a chronic disease causing frailty). In some embodiments, the human subject has a weak immune system, such as an undeveloped (*e.g.*, an infant or a neonate subject), immunosenescent (*e.g.*, an elderly subject), or compromised immune system. Immunosenescent subjects include, without limitation, subjects exhibiting a decline in immune function associated with advanced age. Immunocompromised

subjects include, without limitation, subjects with primary immunodeficiency or acquired immunodeficiency such as those suffering from sepsis, HIV infection, and cancers, including those undergoing chemotherapy and/or radiotherapy, as well as subjects to which immunosuppressants are administered, as for organ or tissue transplantation. In some
5 embodiments, the human subject has or is suspected of having one or more disorders or diseases that reduce immune system function and/or increase the risk of infection in the subject by one or more pathogens (*e.g.*, a virus, a bacterium, a parasite, a fungus). In some embodiments, the human subject is, for example, a subject that has or is suspected of having chronic lung disease, asthma, cardiovascular disease, cancer, a metabolic disorder (*e.g.*, obesity or diabetes mellitus),
10 chronic kidney disease, or liver disease.

Some of the embodiments, advantages, features, and uses of the technology disclosed herein will be more fully understood from the Examples below. The Examples are intended to illustrate some of the benefits of the present disclosure and to describe particular embodiments,
15 but are not intended to exemplify the full scope of the disclosure and, accordingly, do not limit the scope of the disclosure.

EXAMPLES

Example 1 – Nanobody-drug adducts for treatment of influenza

20 Influenza is an acute and potentially life-threatening respiratory infection that is caused by influenza viruses. Influenza in humans can be caused by influenza A viruses and influenza B viruses, which typically spread during seasonal influenza epidemics. The human cost of annual influenza epidemics is high, resulting in an estimated three to five million cases of severe influenza each year and 250,000 to 500,000 annual fatalities, including approximately 12,000 to
25 79,000 annual fatalities in the United States. Influenza viruses also pose a significant health risk to human societies due to their high transmissibility and the possibility for new influenza variants to be transmitted to humans from animal reservoirs (*e.g.*, a non-human mammal or avian species), including many migratory bird species. Due to these factors, influenza viruses are especially likely to cause pandemics. Indeed, at least four separate influenza pandemics have
30 occurred during the last century (*e.g.*, from 1918–1920, 1957–1958, 1968–1969, and 2009–2010) and caused many tens of millions of deaths collectively.

Fortunately, several therapeutics have been clinically approved for use in treating influenza infections. These include several antivirals that specifically target molecular features on the surface of influenza virions and infected cells, and include, for example, zanamivir,

oseltamivir, and peramivir, which specifically bind to and inhibit the neuraminidase enzyme encoded by influenza A and influenza B viruses, thereby preventing the release of new virions from infected cells. Although timely administration of these therapeutics may reduce the duration of influenza infection by about 1-2 days, it is unclear whether administration actually
5 reduces one's risk of developing severe illness (e.g., illness requiring hospitalization). Moreover, it is unclear whether these therapeutics effectively prevent infection when administered prophylactically, therefore prophylactic use of these therapeutics is not recommended due to the risk for adverse drug reactions.

One approach for improving the efficacy of treatments for influenza is to fuse a
10 therapeutic agent that is specific for influenza to a separate agent that is capable of binding to polyclonal immunoglobulins produced by the host. Bound immunoglobulins may then recruit immune cells that express Fc receptors on their surface, which then kill or inactivate the virions or infected cells they are recruited to. This strategy may be achieved, for example, by fusing an existing therapeutic specific for influenza, such as zanamivir, to an antibody or antibody
15 fragment that binds broadly to host immunoglobulins, such as the variable region of a heavy chain of a camelid antibody that is specific for kappa light chains (VHH_{kappa}) (**FIG. 1**). This strategy could potentially be used to stimulate a stronger immune response toward influenza than could otherwise be achieved, as any host immunoglobulin comprising kappa light chains and a Fc region can be used to recruit immune cells to influenza virions or infected cells,
20 regardless of specificity of the host immunoglobulin. Although zanamivir fused to VHH_{kappa} may retain its inhibitory activity, in principle any agent that is specific for one or more targets on the surface of influenza virions and/or influenza-infected cells may be used, including, for example an antibody or antibody fragment that is specific for viral neuraminidase or hemagglutinin. Additionally, fusion to VHH_{kappa} may also enhance the circulatory half life of
25 zanamivir or another therapeutic specific for influenza, as compared to the free drug alone, thus further enhancing the efficacy of the therapeutic.

To initially evaluate the efficacy of this strategy, the specificity of VHH_{kappa} for immunoglobulins was tested *in vitro*. Varying concentrations of VHH_{kappa} fused to biotin (VHH_{kappa}-biotin) were exposed to mouse IgG2b-coated enzyme-linked immunosorbent assay
30 (ELISA) plates. For this assay, a commercially available anti-mouse kappa chain VHH was used (nanobody clone TP1170). A horseradish peroxidase fused to streptavidin (HRP-streptavidin) was subsequently used as a secondary agent to detect binding between VHH_{kappa}-biotin and IgG2b of the plate. As a control, a separate nanobody specific for influenza hemagglutinin, rather than kappa light chains, SD36, was also fused to biotin and tested (SD36-biotin), as well
35 as fusions between SD36-biotin and VHH_{kappa} (VHH_{kappa}-SD36-biotin-1 and VHH_{kappa}-SD36-

biotin-2). After incubation at ambient temperature for 2 hours, ELISA plates were washed and then incubated with streptavidin-HRP, then subsequently washed, treated with TMB substrate solution (Biolegend, cat. no. 421101), quenched with H₂SO₄, and measured. The dissociation constant (K_d) was calculated from a plot of average absorbance values at 450 nm versus the concentration of VHHs. VHH_{kappa}-biotin, VHH_{kappa}-SD36-biotin-1, and VHH_{kappa}-SD36-biotin-2 each bound to mouse IgG2b with low nanomolar affinity (dissociation constant, K_d, of 2.0–2.4 nM) (**FIG. 2**). In contrast, no binding between SD36-biotin and IgG2b was detected. These results confirm the high specificity of VHH_{kappa} for host immunoglobulins and suggest that fusing VHH_{kappa} to an agent specific for influenza could effectively recruit host immunoglobulins and immune cells to influenza virions and infected cells.

A method of covalently linking VHH_{kappa} to influenza-specific agents, such as zanamivir, was then devised. Although any cleavable or non-cleavable linker could be used in principle, a method of modifying zanamivir by attaching a triglycine dibenzylcyclooctyne (DBCO) linker to the 7-hydroxyl group of zanamivir was developed. First a zanamivir targeting ligand was prepared (**FIG. 3A**) which was then reacted with Gly-Gly-Gly-DBCO to produce Gly-Gly-Gly-zanamivir (**FIG. 3B**). Gly-Gly-Gly-zanamivir was then fused to the C-terminal amino acid residues LPETGGH₆ of VHH_{kappa} using a sortase (sortase A; SrtA) reaction, in order to produce a VHH_{kappa}-zanamivir adduct (**FIG. 3C**). Briefly, pentamutant sortase A was used to catalyze the addition of sortase-ready nucleophiles to the C-terminal LPETG motif of VHH_{kappa}. Sortase reactions were conducted in PBS containing 20 μM sortase A, and 10 μM CaCl₂. After incubation overnight at 4°C, Ni-NTA beads were added to the reaction mixture and incubated for 30 min. at 4°C to remove the unreacted VHHs and His tagged sortase A. The reaction mixture was then loaded onto a PD-10 desalting column to remove excess nucleophile. VHH_{kappa}-zanamivir adducts synthesized by this method were subsequently determined by SDS-PAGE and mass spectrometry to be approximately 14 kDa in size and substantially pure (**FIGs. 4A and 4B**).

The specificity of VHH_{kappa}-zanamivir adducts for influenza-infected cells was then assessed *in vitro* with an influenza-infected cell culture. Madin-Darby Canine Kidney (MDCK) cells were infected with influenza virus and express neuraminidase 24 hours post-infection, at which point infected MDCK cells were treated with varying concentrations of VHH_{kappa}-zanamivir adducts. Binding between VHH_{kappa}-zanamivir adducts and infected cells was measured by a saturation binding assay. Briefly, media was removed from the infected cells, and replaced with media containing various concentrations of adduct. After incubation for 1 hour, infected cells were washed and treated with mouse IgG-Phycoerythrin (R&D Systems #IC002P) in fresh serum free medium. After incubation for 30 min., the infected cells were washed and

dissolved in 1% aqueous sodium dodecyl sulfate (SDS) and cell-associated fluorescence was measured using an excitation wavelength at 560 nm and an emission wavelength at 620 nm. The dissociation constant (K_d) was calculated from a plot of the cell bound fluorescence intensity versus the concentration of VHHs. Similar to the specificity of VHH_{kappa} for IgG observed previously (**FIG. 2**), VHH_{kappa}-zanamivir adducts exhibited low nanomolar specificity for influenza A virus (**FIGs. 5A** and **5B**). Moreover, VHH_{kappa}-zanamivir adducts exhibited low nanomolar specificity for MDCK cells infected with two separate strains of influenza A, A/Wisconsin/629-D00015/2009, a H1N1 influenza subtype (**FIG. 5A**), and A/Hong Kong/8/1968, a H3N2 influenza subtype (**FIG. 5B**). Similarly, VHH_{kappa}-zanamivir adducts exhibited low nanomolar specificity for MDCK cells infected with two separate strains of influenza B, B/Florida/4/2006 (**FIG. 5C**), and B/Brisbane/60/2008 (**FIG. 5D**). These results confirm that VHH_{kappa}-zanamivir adducts are as specific for influenza neuraminidase as free zanamivir and should be effective at treating a range of influenza subtypes.

After establishing the efficacy of VHH_{kappa}-zanamivir adducts *in vitro*, VHH_{kappa}-zanamivir adducts were then evaluated in an animal model. Mice were infected on day 0 with 50 μ L influenza A virus A/Puerto Rico/8/1934, an H1N1 subtype. 50 μ L of A/Puerto Rico/8/1934 influenza A virus is the equivalent to 10 times the LD₅₀ of A/Puerto Rico/8/1934 influenza A. Mice were then dosed intraperitoneally with varying amounts of VHH_{kappa}-zanamivir adduct (0.1, 1 mg/kg, or 3 mg/kg), 1 mg/kg of VHH_{kappa} and zanamivir which were not covalently fused, or phosphate buffered saline (PBS) mock treatment. Mice receiving 1 mg/kg VHH_{kappa}-zanamivir adduct received VHH_{kappa}-zanamivir adduct either on day 0 post-infection only, or on days 0, 2, and 4. After administration of VHH_{kappa}-zanamivir adduct body weight (mass) and survival rate of infected mice was monitored once per day over 14 days (**FIGs. 6A** and **6B**). Mice treated with PBS mock treatment and 0.1 mg/kg VHH_{kappa}-zanamivir adduct exhibited substantial weight loss and complete lethality by days 8 and 10, respectively, post-infection. Importantly, mice treated with 1 mg/kg VHH_{kappa} and zanamivir (separately) also exhibited weight loss and lethality by day 9 post-infection. However, mice treated with either 1 or 3 mg/kg VHH_{kappa}-zanamivir adduct did not exhibit weight loss or lethality as a result of influenza infection. In fact, mice treated with 3 mg/kg VHH_{kappa}-zanamivir adduct or 1 mg/kg VHH_{kappa}-zanamivir adduct on days 0, 2, and 4 post-infection modestly gained weight during the 14 days post-infection.

To establish this efficacy across multiple viral species, mice were similarly infected with Influenza A – A/California/07/2009 (H1N1); Influenza A – A/Hong Kong/1/1968; or Influenza B – B/Florida/4/2006; and treated with a single dose of VHH_{kappa}-zanamivir (3 mg/kg) on the

same day (FIG. 6C). VHH_{kappa}-zanamivir was shown to lead to high survival rates for all infected mice for up to 14 days.

Additional experiments were performed to delay addition of VHH_{kappa}-zanamivir (10 mg/kg) until day 1, 2 or 3 post-infection with Influenza A – A/Puerto Rico/8/1934 (FIG. 6D).

5 The treated mice showed high survival rates for up to 14 days.

Finally, prophylactic experiments were performed. Mice were dosed with VHH_{kappa}-zanamivir (5 mg/kg) seven days *before* infection with Influenza A – A/Puerto Rico/8/1934 (FIG. 6E). These data showed that administration of VHH_{kappa}-zanamivir prior to infection caused high rates of survival.

10 These results show that administration of a fusion between VHH_{kappa} and a influenza-specific agent is highly effective for enhancing immunity toward influenza and reducing the risk of serious illness.

Although these results were obtained in a mouse model, using a mouse-specific VHH, a VHH_{kappa}-zanamivir adduct effective for treating influenza in humans can be obtained by simply exchanging the mouse-specific VHH_{kappa} with a VHH_{kappa} specific for human kappa light chains. As mentioned previously, similar results may be obtained with any adduct comprising an agent specific for influenza virions and/or influenza infected cells, such as a small molecule or peptide specific for hemagglutinin, or an antibody or antibody fragment (e.g., a nanobody) specific for neuraminidase, hemagglutinin, or another target (e.g., protein) on the surface of influenza virions and/or infected cells. Unless designed for specificity toward influenza A or influenza B, adducts comprising VHH_{kappa} are very likely to be effective for treating both types of influenza, particularly as zanamivir and other influenza therapeutics are known to be active toward both influenza A and influenza B.

25 ***Example 2 – Nanobody-nanobody adducts for treatment of influenza***

Alternatively, adducts useful for the treatment of influenza may be developed that comprise an immune cell-specific nanobody (e.g., VHH_{kappa}) and an influenza virus-specific nanobody, rather than an influenza-specific small molecule, such as zanamivir. To this end, a nanobody VHH_{kappa} was conjugated to a previously reported single domain antibody (VHH) that is specific for influenza virus hemagglutinin, SD36 (see, e.g., Laursen, et al. “Universal protection against influenza infection by a multidomain antibody to influenza hemagglutinin.” 2018. Science, 362(6414), 598–602). A VHH_{kappa}-SD36 adduct was synthesized by first preparing a version of SD36 comprising a terminal azide (**FIG. 7A**) and VHH_{kappa} linked via Gly-Gly-Gly-Cys-DBCO (SEQ ID NO: 28) (**FIG. 7B**), each via a sortase reaction as described in Example 1. Each VHH was then combined to produce the VHH_{kappa}-SD36 adduct (**FIG. 7C**).

The final product was purified by size exclusion chromatography using a Superdex 75 10/300 column. Additionally, a genetically fused version of the VHH_{kappa}-SD36 adduct was recombinantly expressed and isolated, in which VHH_{kappa} is N-terminally linked to SD36 by a flexible linker (GGGGS)₃ (SEQ ID NO: 29) (**FIG. 7D**). Biotinylated versions of VHH_{kappa}, SD36, and genetically conjugated VHH_{kappa}-SD36 were further produced (**FIGs. 8A-8C**). The amino acid sequence of the genetically fused conjugate is as follows:

VHH_{kappa}-SD36 nanobody conjugate:

QVQLVESGGGWVQPGGSLRLSCAASGFTFSDTAMMWVRQAPGKGREWVAIDTGGG
 YTTYADSVKGRFTISRDNALNTLYLQMNSLKPEDTARYYCAKTYSGNYYSNYTVANY
 GTTGRGTLVTVSSAAAGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLKLSCAASG
 RTYAMGWFRQAPGKEREFVAHINALGTRTYSDSVKGRFTISRDNALNTLEYLMNNL
 KPEDTAVYYCTAQGWRAAPVAVAAEYEFWGQGTQVTVSSGGLPETGGHHHHHHH
 (SEQ ID NO: 5).

Next, binding between biotinylated SD36, VHH_{kappa}-SD36, and genetically conjugated VHH_{kappa}-SD36 as assessed using the same saturation binding assay as described previously (**FIGs. 5A and 5B**), with the exception that 10 µg/mL Streptavidin-PE (Biolegend #405204) was used to treat cells incubated with SD36-biotin. As expected, each VHH and VHH conjugate bound to influenza A hemagglutinin with low nanomolar affinity (**FIGs. 9A-9C**). While both VHH_{kappa}-SD36, and genetically conjugated VHH_{kappa}-SD36 bound to influenza A virus-infected MDCK cells, VHH_{kappa}-SD36 synthesized via click chemistry bound with higher affinity (**FIG. 9C**).

After establishing the efficacy of VHH_{kappa}-SD36 conjugates *in vitro*, each conjugate was then evaluated in an animal model, as previously (**FIGs. 6A and 6B**). Mice treated with as little as 2 mg/kg of either genetically fused VHH_{kappa}-SD36 (**FIG. 6A**) or VHH_{kappa}-SD36 conjugated through click chemistry (**FIG. 6B**) were fully protected from a lethal dose of influenza A virus, although mice treated with 10 mg/kg of either conjugate further exhibited no weight loss as a result of the infection (**FIGs. 10A and 10B**).

Additional experiments were performed to delay addition of VHH_{kappa}-SD36 (10 mg/kg) until day 1, 2 or 3 post-infection with Influenza A – A/Hong Kong/1/1968 (**FIG. 10C**). The treated mice showed high survival rates for up to 14 days.

These results show that, similar to the VHH_{kappa}-zanamivir adduct of Example 1, administration of a fusion between VHH_{kappa} and a nanobody targeting influenza antigens is

highly effective for enhancing immunity toward influenza and reducing the risk of serious illness.

Finally, the *in vivo* half-life of VHH_{kappa}-SD36 conjugates was assessed. To this end, a Zirconium-89 (⁸⁹Zr) radiolabeled version of VHH_{kappa}-SD36 (produced via a click chemistry reaction) was synthesized by treating VHH_{kappa}-SD36-DFO, or SD36-DFO control, with ⁸⁹Zr⁴⁺ stock solution at pH 6.8-7.5 with 2.0 M Na₂CO₃ for 1 hour (**FIGs. 11A and 11B**). The location and half-life of radiolabeled conjugate in mice was then assessed via immuno-positron emission tomography (Immuno-PET). Briefly, mice infected with a lethal dose (10 LD₅₀) of influenza virus A/Hong Kong/8/1968 (H3N2) were injected with either radiolabeled SD36-DFO or VHH_{kappa}-SD36-DFO at 4 days post-infection and scanned every 24 hours to determine the location and total level of SD36-DFO or VHH_{kappa}-SD36-DFO (**FIG. 12**). Anti-mouse VHH_{kappa}-SD36-DFO was observed to have prolonged half-life *in vivo* compared to SD36 alone, and accumulated to a greater extent at sites of infection, particularly in the lung.

Together, these results demonstrate the flexibility of VHH_{kappa} conjugates, as conjugates comprising different modalities of binding to influenza antigens (e.g., a small molecule for binding neuraminidase, as compared to a nanobody for binding hemagglutinin) have been shown to be effective for treating influenza and for preventing severe disease.

Example 3 – Development of malaria-specific nanobodies

Next, adducts comprising VHH_{kappa} were developed for specificity toward another pathogen, *Plasmodium falciparum*. *P. falciparum* is a unicellular protozoan species that is one of five parasites known to cause malaria in humans when transmitted by an infected female *Anopheles* mosquito. The World Health Organization estimates that there were approximately 241 million cases of malaria worldwide in 2020, resulting in approximately 627,000 deaths. Cases caused by *P. falciparum* have the highest risk of mortality. Although malaria is both treatable and curable, particularly through the use of artemisinin-based combination therapy (ACT), drug resistant malaria has emerged as a growing global health threat. Moreover, though generally effective, ACT is somewhat toxic and can cause fatigue, headache, dizziness, nausea, vomiting, and abdominal pain.

Whereas Example 1 demonstrates the use of a small molecule, zanamivir, for influenza virions and influenza infected cells, here a set of VHH nanobodies were developed that are specific for different regions of the *P. falciparum* merozoite surface protein 1 (MSP-1). MSP-1 is a pro-peptide (referred to as p190) comprising four subunits, p83, p30, p38, and p42, that is expressed in *Plasmodium* parasites at the beginning of their asexual reproductive phase (**FIG. 13A**). Once cleaved, these subunits assemble to form mature MSP-1 complexes on the surface

of *Plasmodium* cells, where they are used to bind and infect red blood cells. Isolated VHH sequences that bind to MSP-1 are shown in Table 1 below.

Table 1: VHH amino acid sequences specific for *P. falciparum* MSP-1

Clone	SEQ ID NO	Sequence
A3	6	QLQLVESGGGLVQAGGSLRLSCVVSGRSLILYGMGWFRQAPGMEREFVAGIVWNG GWTTYADSVKGRFTISRDTAKNTVYLQMNSLKPEDTAVYSCAAGPGDGRRRVAPA DYNWYGQGTQVTVSSLPETGGHHHHHH
A6	7	QVQLVESGGGLVQAGGSLRLSCAASARTFSYYGAAWFRQAPGKEREFVAAVSWNG DDTTYADSVKGRFTISRDNKTNTVSLQMNALKPEDTAVYYCEAAAYGRDWRQSAN YVYWGQGTQVTVSSLPETGGHHHHHH
A8	8	QLQLVESGGGLVQPGGSLRLSCAASGETFSNYHMGWFRQAPGKEREFVAVIRRTGE STYADSVKDRFTISRDNKNTVYLQMNALKAEDTAVYYCAARRLLGIGAIDEYDYW GQGTQVTVSSLPETGGHHHHHH
G9	9	QVQLVESGGGVVQAGGSLRLSCVASGRTFSSYGMGWFRQAPGLEREFVAAISWRGS TTLYADSVKGRFTISRDNKNTVYLQMNSLKPEDTAVYYCGGSENFAGLRRAEYKF RGQGTQVTVSSLPETGGHHHHHH
H3	10	QLQLVESGGGLVQAGGSLRLSCAASARFVSYYGLAWFRQAPGKEREFVAAVSWNG DSADYADSVKGRFTISRDNPKSTVSLQMSNLKPEDTAVYYCAAAAYGRDWRESAN YVYWGQGTQVTVSSLPETGGHHHHHH
H10	11	QVQLVETGGGLVQPGGSLRLACTASIEDFIHYHMGWFRQAPGKERKFIALLIRSGETT YADSVKDRFTISRDNKNTVYLQMNSLKTEDTADYYCAARRLLGIGAFDEYDYWG QGTQVTVSSLPETGGHHHHHH
B4	12	QLQLVESGGGLAQAGGSLRLSCAASGRTSSTKPMGWFRQAPGLEREFVASISWLGG SLYSDSVKGRFTISRDNKNTVYLQMNSLKSEDTAVYYCAGGTSAYSGRDSSRYEY WGQGTQVTVSSLPETGGHHHHHH
E10	13	QVQLVETGGGLAQAGGSLRLSCAASGRITASIKPMGWFRQAPGKEREFVASISWLGS SLYSDSVKGRFTISRDNKNTVYLQMNSLESEDTAVYYCAGGSTAYAGRSTSEY WGQGTQVTVSSLPETGGHHHHHH
F1	14	QVQLVETGGGLVQAGGSLRLSCAASGRTSSTAKPMTWFRQGPGEREFVAAISWSGG STLYSDSVKGRFTISRDNKNTVSLQMNSLKSDDTAVYYCAGGTTAYSGRDSSKYE YWGQGTQVTVSSLPETGGHHHHHH
F2	15	QLQLVESGGGLVQPGGSLRLSCAASGFIPDYLLIGWFRQAPGKERDGVSLISNVDGST YYADSVKGRFTISADNAKNTVYLQMNSLKPEDTAVYYCAVRDIGRALLDEVGNTGR NSSGVMDYWGKGTQVTVSSLPETGGHHHHHH
G11	16	QVQLVETGGGLVQAGGSLRLSCVVSGRSLILYGMGWFRQAPGKEREFVAGIVWNG GWTTYADSVKGRFTISRDTAKNTVYLQMNSLKPEDTAVYSCAAGPGDGRRRVAPA EYNYWGQGTQVTVSSLPETGGHHHHHH

H11	17	QVQLVETGGDLVQAGGSLRLSCVVSIGISINAMRWYHQAPGKQREL VATISPSGLT DHADSVKGRFTISKDNSKNMVYLMNSLKPEDTGAYYCNAPFGDYWGQGTQVTVS SLPETGGHHHHHH
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Purified VHH nanobodies were biotinylated and tested for specificity toward isolated MSP-1 subunits. Anti-p83 B4, anti-p38 B8, anti-p42 A6, and anti-p42 G11 VHHs were each incubated with plate-bound p83, p38, p42, and p42, and binding to the plates was detected by an enzyme-linked immunoassay (ELISA) using streptavidin-horseradish peroxidase (HRP) and tetramethylbenzidine (TMB). Each VHH was observed to bind specifically to one subunit of the MSP-1 complex, while a control VHH did not bind (**FIG. 13B**). This binding was further confirmed by subsequent gel electrophoresis, in which each antibody was shown to bind to one subunit of MSP-1 and to the MSP-1 pro-peptide (p190), but not to any other subunit (**FIG. 13C**). VHH clones B4 and B8 were also tested against lysate from ~38-44 hour 3D7 schizonts and were observed to bind. Finally, purified VHHs were tested against live ~38-44 hour 3D7 schizonts in a flow cytometry assay. Cy5 fluorescently labeled VHHs were observed to specifically bind to live *Plasmodium* schizonts, binding with greater fluorescent intensity than that observed with a control VHH, anti-major histocompatibility complex II (MHC-II) (**FIG. 13D**).

Together, these data demonstrate the specificity of VHH nanobodies targeting *Plasmodium* parasites and provide a proof-of-concept that adducts comprising VHH_{kappa} and any one of these VHHs could be used effectively treat malaria in a similar fashion as demonstrated for influenza, as demonstrated in Example 1.

Example 4 – Nanobody-drug adducts for enhancing immunity against cancer cells

The efficacy of adducts targeting pathogens has been shown above (Examples 1 and 2), as well as the development of nanobodies for use in nanobody-nanobody adducts for treating diseases caused by pathogens (Examples 2 and 3). However, adducts comprising VHH_{kappa} could further be useful for enhancing or eliciting immune responses against cancer. To this end, an adduct comprising VHH_{kappa} and a previously reported VHH targeting murine major histocompatibility complex II (MHC-II), VHH7 (see Fang, et al., “Structurally Defined α MHC-II Nanobody-Drug Conjugates: A Therapeutic and Imaging System for B-Cell Lymphoma.” 2016. *Angewandte Chemie*, 55(7), 2416–2420), was developed. Although typically located on the surface of professional antigen presenting cells (e.g., dendritic cells), MHC-II is also expressed on the surface of lymphoid cells, including certain lymphomas. Therefore, an adduct comprising a nanobody targeting MHC-II could be used to recruit immune cells to lymphoma

cells. A VHH_{kappa}-VHH7 adduct was synthesized using a click chemistry reaction (**FIGs. 14A-14C**), similarly to that prepared previously to target influenza hemagglutinin (**FIGs. 7A-7C**).

The amino acid sequence of VHH7 prior to conjugation is as follows:

5 VHH7 nanobody:

QVQLQESGGGLVQAGDSLRLSCAASGRFTSRGVMGWFRRAPGKEREFVAIFSGSSWSG
RSTYYSDSVKGRFTISRDNKNTVYLQMNGLPEDTAVYYCAAGYPEAYSAYGRESTY
DYWGQGTQVTVSSGG (SEQ ID NO: 18).

10 The VHH_{kappa}-VHH7 adduct was subsequently tested *in vitro* against A20 cells, a mouse lymphoma cell line, in a complement-dependent cytotoxicity (CDC) assay. Briefly, A20 cells were plated in 96 well white-walled plates and treated with either anti-mouse VHH_{kappa}-VHH7 adduct or a mixture of anti-mouse VHH_{kappa} and VHH7 (final concentration: 10 nM and 100 nM). After incubation at ambient temperature for 30 min., fresh serum-free medium containing
15 normal mouse IgG isotype control (Invitrogen #10400C) and 40% (v/v) rabbit complement serum (Sigma-Aldrich #S7764) were added to the cells. After 4 hours further incubation at 37°C, cell viability was measured by CellTiter-Glo® Luminescent Cell Viability Assay (Promega #G7572). Maximum killing was measured by treating cells with 5% H₂O₂. The percent cytotoxicity induced by VHH_{kappa}-VHH7 adduct was calculated as:
20 % Cytotoxicity = (luminescence_{without VHH} – luminescence_{with VHH}) / (luminescence_{without VHH} – luminescence_{maximum killing}) × 100. The VHH_{kappa}-VHH7 adduct was found to be effective at eliciting cytotoxicity of A20 cells at both 10 nM and 100 nM, while treatment with each of the components separately was not (**FIGs. 15A and 15B**).

The efficacy of the VHH_{kappa}-VHH7 adduct was further demonstrated using an antibody-
25 dependent cellular cytotoxicity (ADCC) assay. Briefly, murine natural killer (NK) cells were harvested from the spleen of BALB/c mice by EasySep™ Mouse NK Cell Isolation Kit (STEMCELL, #19855RF) to be used as effector cells *in vitro*. A20 cells were plated in 96 well plates and treated with either anti-mouse VHH_{kappa}-VHH7 adduct or a mixture of anti-mouse VHH_{kappa} and VHH7 (final concentration: 10 nM and 100 nM), followed by a mouse IgG2a
30 kappa isotype control antibody (final concentration: 20 µg/mL). After incubation for 30 min., a suspension of murine NK cells were added at 1 × 10⁶ cells/well and incubated for a further 4 hours at 37°C. Cell viability was measured by CytoTox 96® Non-Radioactive Cytotoxicity Assay (LDH) (Promega, #G1780), in which absorbance at 490 nm is measured. The spontaneous signal produced by effector cells alone was also assessed. The percent total lysis
35 induced by VHH_{kappa}-VHH7 adduct was calculated as: % Total lysis = (A₄₉₀_{experimental} –

$A490_{\text{effector spontaneous}} / (A490_{\text{target maximum}}) \times 100$. As expected, treatment with the VHH_{kappa}-VHH7 adduct caused approximately 30%-40% of A20 cells to be lysed within this timeframe, at both 10 nM and at 100 nM (**FIGs. 16A and 16B**). Moreover, the efficacy of the VHH_{kappa}-VHH7 adduct was greater than that of VHH_{kappa} and VHH7 separately. These data demonstrate that in addition to their use for treating pathogenic infection, VHH_{kappa} adducts can also be used to effectively target and treat cancer cells by enhancing the immune cell responses.

VHH_{kappa}-SD36 adducts were further tested in 6-9 week old female BALB/c mice infected with 10 LD₅₀ of influenza virus. Mice were treated with the indicated doses of VHH_{kappa}-SD36, a mixture of VHH_{kappa} and SD36, or with an equal volume of PBS by intraperitoneal injection. Mice were euthanized when they lost 25% of their body weight or became moribund. Weight loss curves (left) and survival curves (right) are shown (**FIG. 14D**). These data demonstrate that VHH_{kappa}-SD36 conjugates are effective in treating infected mice, as shown by their high survival rates.

Example 5 – Nanobody-drug adducts for enhancing immunity against target pathogens or other cell types

The efficacy of adducts useful for the treatment of influenza infection and malaria infection has been demonstrated in Examples 1-3. Furthermore, the efficacy of adducts for treating cancer has been demonstrated in Example 4. However, the VHH_{kappa} adduct may be modified in several ways for specificity toward different pathogens and/or cell types. For example, VHH_{kappa} may instead be linked to an agent specific for another target, such as a target (e.g., protein) located on the surface of another pathogen, such as another virus, a bacterium, a parasite, or a fungus. For example, rather than zanamivir, VHH_{kappa} may be linked to a small molecule, peptide, protein, carbohydrate, lipid, nucleotide, nucleic acid, oligonucleotide, aptamer, or antibody (or antibody fragment) that specifically recognizes a target on the surface of a beta coronavirus, such as Middle East Respiratory Syndrome coronavirus (MERS-CoV), Severe Acute Respiratory Syndrome (SARS)-associated coronavirus (SARS-CoV)-1, or SARS-CoV-2. For example, VHH_{kappa} may be linked to an agent that specifically recognizes a MERS-CoV, SARS-CoV-1, or SARS-CoV-2 spike protein, or spike protein receptor binding domain (RBD) thereof, to generate a VHH_{kappa} adduct specific for and useful for the treatment of MERS-CoV, SARS-CoV-1, or SARS-CoV-2 infection. Similarly, VHH_{kappa} may be linked, for example, to an agent specific for human immunodeficiency virus (HIV) envelope glycoprotein gp120 to generate a VHH_{kappa} adduct specific for and useful for the treatment of HIV infection. VHH_{kappa} may be linked, for example, or to an agent specific for human respiratory syncytial virus (RSV) fusion (F) protein to generate a VHH_{kappa} adduct specific for and useful for the treatment of RSV

infection. VHH_{kappa} adducts useful for the treatment of parasites, such as plasmodium, may be generated for example by linking VHH_{kappa} to another agent specific for merozoite surface protein 1 (MSP-1), or to an agent specific to another surface protein of a parasite. Similar strategies may also be employed to generate adducts capable of recruiting host immunoglobulins and immune cells to infectious bacteria and fungi.

Alternately, a related strategy may be used to target host immunoglobulins and immune cells to a subject's own cells. For example, a VHH_{kappa} adduct specific for and useful for the treatment of cancer may be generated by linking VHH_{kappa} to an agent specific for a tumor-associated antigen, such as, but not limited to, a folate receptor, a fibronectin splice variant, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), hepatocyte growth factor receptor (HGFR), vascular endothelial growth factor receptor 2 (VEGFR-2), C-X-C chemokine receptor type 4 (CXCR4), urokinase plasminogen activator surface receptor (uPAR), follicle-stimulating hormone receptor (FSHR), epithelial cell adhesion molecule (EpCAM), epithelial cadherin (ECAD), carcinoembryonic antigen (CEA), or mesothelin (MSLN). When administered systemically, such an adduct is useful for targeting cancer cells throughout the body and is therefore particularly useful for the treatment of metastatic cancers. Adducts may also be designed for targeting host immunoglobulins and immune cells to other cell types, including cells that are undergoing or have undergone a phenotypic change, such as in response to cellular stress, by linking VHH_{kappa} to an agent specific for a target that is disproportionately expressed on the surface of the target cells, as compared to non-target cells. Additionally, adducts may be designed for targeting host immunoglobulins and immune cells to healthy cells in addition to diseased cells. For example, adducts specific for bone marrow may be generated by linking VHH_{kappa} to an agent specific for a bone marrow cells or their immediate precursors. Such an agent may be useful for ablating bone marrow in a subject without the need for potentially harmful radiation or chemotherapeutics, as typically administered prior to bone marrow transplantation. Similarly, adducts may be generated that are specific for healthy and/or diseased immune cells, by linking VHH_{kappa} to an agent specific for the target cell(s), such as, for example, a cluster of differentiation antigen 4 (CD4), a cluster of differentiation antigen 8 (CD8), a T cell receptor (TCR), or a B cell receptor (BCR). Such an adduct may be used, for example, to ablate immune cells specific for an autoantigen (i.e., an antigen associated with an autoimmunity) or immune cells specific for a particular allergen.

Alternately or in addition to any of the examples stated previously, adducts may also be modified to comprise an alternate antibody or antibody fragment known in the art, such as, for example, a whole antibody, a Fab fragment, or a single-chain fragment variable (ScFv). Adducts

specific for and useful for enhancing an immune response against a target pathogen and/or cell may also be generated by linking an agent specific for such a pathogen and/or cell to a heavy chain of a camelid antibody that is specific for lambda light chains (VHH_{lambda}), rather than VHH_{kappa}. VHH_{lambda} adducts differ from VHH_{kappa} adducts only in their specificity for host immunoglobulins comprising lambda light chains, rather than kappa light chains. VHH_{lambda} adducts are capable of binding to any host immunoglobulin comprising lambda light chains and a Fc region in order to recruit immune cells to target pathogens and/or cells, regardless of the specificity of the host immunoglobulin.

As previously stated, in principle any linker known in the art may be used to covalently link VHH_{kappa} or VHH_{lambda} to a target-specific agent. A linker may be a cleavable linker, such as a peptide, disulfide, or hydrazone linker, or a non-cleavable linker for linking the target-specific agent to amino acid residues of VHH_{kappa} or VHH_{lambda}, such as those occurring at the C-terminus. The formation of the conjugate may be brought about by a chemical reaction between the individual components of said conjugate or through the generation of nucleic acid constructs, RNA- or DNA-based, that when expressed in bacteria or eukaryotic cells specify the amino acid sequence of the desired conjugates and yield the desired conjugate (e.g., a target-specific antibody or antibody fragment (e.g., a target-specific nanobody or single domain antibody) conjugated to VHH_{kappa} or VHH_{lambda}).

Example 6 – Development of MICA-specific nanobodies

MHC class I polypeptide-related sequence A (MICA), a class I MHC-like molecule, is a cell stress-induced glycoprotein that is frequently found to be enriched on the surface of malignantly transformed cells. MICA is recognized by natural killer group 2D (NKG2D), also known as Killer Cell Lectin Like Receptor K1 (KLRK1), an activating receptor on the surface of natural killer (NK) cells that enables immunity towards MICA-positive targets, such as tumor cells. High levels of MICA expression are positively correlated with improved prognosis, for example in cholangiocarcinoma (see, e.g., Oliviero B, et al. *Oncoimmunology*. 2022;11(1):2035919). Downregulation of MICA can occur through shedding, catalyzed by ADAM-family matrix metalloproteases (see, e.g., Waldhauer I, et al. *Cancer Res.* 2008; 68(15): 6368-76). Loss of MICA surface expression renders tumor cells less sensitive to NKG2D-positive NK cells, while soluble MICA may itself occupy NKG2D receptors on NK cells and compromise interactions between NK cells with MICA-positive targets. VHH nanobodies that are specific for MICA could be used to selectively direct an immune response toward MICA-positive tumor cells or to deliver cytotoxic or cytostatic agents to such cells for therapy as part of a VHH nanobody adduct.

The generation of VHH nanobodies that specifically recognize MICA is described herein. These nanobodies, which are expected to have a short circulatory half-life and excellent tissue penetration as compared with conventional two-chain immunoglobulins, have properties that are desirable for both *in vivo* imaging agents and immunotherapeutics. Because MICA is expressed on the surface of stressed and cancerous cells, the ability to non-invasively detect such aberrations *in vivo* would be an important diagnostic tool to detect premalignant and malignant lesions. MICA-specific nanobodies may also be as part of therapeutic nanobody adducts.

To obtain MICA-specific VHH nanobodies, an alpaca was immunized with the purified extracellular domain of MICA and a phage display library was created from which MICA-specific nanobody sequences were isolated. Briefly, the alpaca was immunized with 250 µg of purified MICA*009 in alum adjuvant, followed by 3 booster injections separated at 2-week intervals. The immune response of the immunized alpaca was monitored by immunoblotting serum samples collected prior to each booster injection. Although the signal produced in immunoblots cannot be distinguished between conventional or heavy chain-only (nanobody) immunoglobulins, a positive signal denoted successful immunization and subsequent immune response. Having determined that the immunization was successful after the final booster injection, a phage display library was constructed and screened using established techniques.

DNA from positive clones was sequenced and a total of 9 clones were selected for further characterization. The relevant VHH sequences were subcloned into the pHEN6 expression vector with certain modifications, namely that each nanobody sequence would carry a C-terminal LPETG motif which recognized by sortase A, and a His₆ tag to facilitate recovery and purification (**FIG. 17A**). This arrangement enables the installation of fluorophores, biotin, or other moieties by a site-specific and efficient sortase-catalyzed transpeptidation reaction. Because the LPETG sequence is cleaved during transpeptidation, the His₆ tag immediately C-terminal of the LPETG motif is lost upon combining the nanobodies with another moiety. This enables enrichment of the desired modified nanobodies by depletion of His₆-tagged sortase and unreacted input nanobody on a nickel-nitriloacetic acid (NiNTA) matrix, followed by recovery of the unbound fraction which contains the modified nanobodies. The amino acid sequences of the 9 isolated anti-MICA nanobody clones are shown in Table 2 below.

Table 2: VHH amino acid sequences specific for MICA

Clone	SEQ ID	Sequence
	NO	

A1	19	MAQVQLVESGGGLVQPGGSLRLSCAASGFTLDYYDIGWFRQAPGKEREGVSCITSSD GSTYYADSVKGRFTISRDNNAKNTVYLQMNSLKPEDTAVYYCAADCTKPQWKSKE AWGQGTQVTVSSGGLPETGGHHHHHH
B11	20	MAQVQLVETGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKEREGVSCITSD GNTYYADSVKGRFTISRDNNAKNTVYLQMNSLKPEDTAVYYCAADCLSTWRTNAY LGQGTQVTVSSGGLPETGGHHHHHH
C12	21	MAQVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKEREGVSCISSD GSTYYADSVKDRFTISRDNNAKNTVYLQMNSLKPEDTAVYYCAKDCLSSSWRTSAYV GQGTQVTVSSGGLPETGGHHHHHH
D8	22	MAQVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKEREGVSCISSD GSTYYADSVKGRFTISRDNNAKNTVYLQMNSLKPEDTAVYYCAADCLSTWRTSAYL GQGTQVTVSSGGLPETGGHHHHHH
E9	23	MAQVQLVETGGGLVQPGGSLRLSCAASGFTLEHYAIGWFRQAPGKEQEGVSCITSD GDTYYADSVKGRFTISRDNNAKNTVYLQMNSLKPEDTAVYYCAADCLSSNWRTSAY WAQGTQVTVSSGGLPETGGHHHHHH
H3	24	MAQVQLVETGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAGISW SGGSTYYGDSVKGRFTISRDNNAKNTVYLLMNSLKPEDTAVYYCAADLVRSYGSSWP SLFQFRNPEDVQDGM DYWGKGLTVTVSSGGLPETGGHHHHHH
2A9	25	MAQVQLVETGGGLVQPGGSLRLSCAASGFTLDNYAIGWFRQAPGKEREGVSCITSD GSTYYSDSVKGRFTISRDNNAKNTVYLQMNSLKPEDTAVYYCAPNCLSSNWSSGYW GQGTQVTVSSGGLPETGGHHHHHH
2B5	26	MAQVQLVESGGGLVQPGGSLRLSCAASGFTLDYFAIGWYRQAPGKEREGVSCIVSS DGSTYYADSVKGRFTISSDIKNTVYLQMNSLKSED TAVYYCAADCRQGRKDYWG QGTQVTVSSGGLPETGGHHHHHH
2D5	27	MAQLQLVETGGGLVQPGGSLRLSCAASGFTLDDYAIGWFRQAPGKEREGVACITSD GSTYYADSVKGRFTISRDNNAKNTVYLQMNSLKPEDTAVYYCAADCLSTWRTGAYL GQGTQVTVSSGGLPETGGHHHHHH

Nanobodies are not always suitable for immunoblotting experiments, however, biotinylated versions of clones A1 and H3 yielded a surprisingly strong and specific luminescent signal on immunoblots when used at a dilution of 1 ug/mL (**FIG. 17B**). The immunoblots were prepared by resolving samples of whole cell lysate to which purified MICA*009 antigen was added by SDS-PAGE, immunoblotting with the isolated nanobodies, and treating immunoblots with streptavidin-horseradish peroxidase (HRP) as a secondary detection agent.

The specificity of anti-MICA VHH nanobody binding was further assessed by performing ELISA cross-competition experiments to determine whether the isolated nanobodies recognized similar or distinct epitopes on the MICA antigen. Competition of unlabeled nanobodies with a biotinylated nanobody for binding to MICA showed that the isolated nanobodies recognize two distinct epitopes, one exemplified by the A1 nanobody and the other

exemplified by the H3 nanobody (**FIG. 17C**). Interestingly, none of the isolated nanobodies competed for binding with the 7C6 anti-MICA monoclonal antibody that has been reported previously (see, *e.g.*, Ferrari de Andrade, et al. *Science*. 2018; 359(6383):1537-1542), indicating that these nanobodies bind to previously unrecognized epitopes of the MICA antigen.

5 Next it was assessed whether the isolated anti-MICA VHH nanobodies were selective for certain MICA allelic products. MICA is a highly polymorphic locus of the human genome, leading to the expression of a wide variety of allelic products in human populations, including several variants which are associated with disease states (see, *e.g.*, Shi C, et al. *Open Rheumatol J.* 2015; 9:60-64). It is therefore possible that the isolated anti-MICA nanobodies preferentially
10 bind to some MICA variants over others. To determine if this was the case, the A1, B11, E9, and H3 anti-MICA nanobody clones, which were determined to bind to either of two distinct epitopes, were assessed in an ELISA assay for binding to a set of MICA variants, as well as a similarly stress-induced glycoprotein, MHC class I chain-related protein B (MICB), and a ferritin control. Each of the isolated nanobodies were observed to bind to MICA*008 and
15 MICA*009 variants, but were not observed to bind to MICA*002 (**FIG. 17D**). Given that MICA*008 and MICA*009 were observed to occur in slightly more than half of participants in a study of 1.2 million donors of German descent (see, *e.g.*, Klussmeier A, et al. *Front Immunol.* 2020; 11:314.), occurring in approximately 42.3% and 8.8% of participants, respectively, the isolated nanobodies would have broad utility for either imaging or therapeutic applications in
20 subjects with MICA-expressing tumors.

 To further assess whether the isolated anti-MICA VHH nanobodies would bind to MICA on the surface of cells, A1 and H3 nanobody clones were generated against soluble, recombinant MICA*009 and cell binding was assessed by flow cytometry. Binding was assessed using B16F10 melanoma cells transfected to express either MICA or MICB, or an empty vector (EV)
25 as a negative control. Biotinylated A1 and H3 nanobodies were labeled with streptavidin-phycoerythrin (PE) and used for cytofluorimetry of transfected B16F10 cells. Both A1 and H3 nanobody clones demonstrated robust staining of MICA-transfected cells (**FIG. 17E**). Furthermore, neither nanobody clone stained MICB-transfected cells or EV-transfected cells, confirming that the isolated nanobodies are capable of selectively binding to MICA-positive
30 tumor cells. These results further indicate the utility of the isolated anti-MICA nanobodies, *e.g.*, a part of MICA-specific nanobody adducts.

 To further establish the utility of the isolated anti-MICA VHH nanobodies, additional assays may be performed *in vivo*. First, binding of labeled nanobodies may be assessed in a mouse xenograft model expressing MICA-positive tumors. For example, C57/B6 mice may be
35 inoculated with MICA-positive B16F10 cells and subsequently treated with biotinylated

nanobodies. Given that mice do not express MICA or cross-reacting species, the biotinylated nanobodies are predicted to only bind to MICA-positive B16F10 tumors. The mice are then additionally treated with a streptavidin-conjugated fluorescent or luminescent agent and imaged. Fluorescent/luminescent signal that localizes to xenograft sites is indicative that the labeled nanobodies are specific for MICA-positive cancer cells. Second, the isolated nanobodies may be further tested as imaging agents for positron emission tomography (Immuno-PET) due to their small size, efficient tissue penetration, and short circulatory half-life. To test the utility of isolated nanobodies for imaging by Immuno-PET, C57/B6 mice may be engrafted with B16F10 control cells or MICA-positive B16F10 cells. Once B16F10 tumors are established, the mice may be subsequently treated with ⁸⁹Zr-labeled A1 or H3 nanobody clones and imaged via Immuno-PET. Given the high specificity of the isolated nanobodies (**FIG. 17E**), these studies are expected to further indicate the diagnostic and clinical utilities of these novel anti-MICA nanobodies.

15 *Example 7 – Assessment of Conjugates*

VHH_{kappa}-biotin and VHH_{kappa}-SD36-biotin conjugates were tested for their binding affinity for mouse immunoglobulins (**FIGs. 18A-18C**). 96-well ELISA high binding plates were coated with 100 µl of 5 µg/ml a mouse IgG overnight at 4 °C (mouse IgG isotype control: Invitrogen, cat. no. 10400C; mouse IgA isotype control: Invitrogen, cat. no. 14-4762-81; mouse IgM isotype control: BioLegend, cat. no. 401601). Plates were washed 3x with the wash buffer (PBS supplemented with 0.1% (v/v) Tween-20) and incubated with the blocking buffer (1% (w/v) BSA in PBS) at rt for 1h. After washing 4x with the wash buffer, each well was treated with 100 µl of serial 4-fold dilutions of VHH_{kappa}-biotin, VHH_{kappa}-SD36-biotin, or SD36-biotin in blocking buffer. After incubation at room temperature for 2h, the plates were washed 4x with the wash buffer and incubated with Streptavidin-HRP (1:1000 dilution, Biolegend, cat. no. 405210) at room temperature for 1h. After washing 4x with the wash buffer, each well was incubated with 100 µl of TMB substrate solution (Biolegend, cat. no. 421101) at room temperature for 10 min before addition of 100 µl of 1N H₂SO₄ to terminate the enzymatic reaction. The optical density was then read at OD₄₅₀. The dissociation constant (K_d) was calculated from a plot of average absorbance values at 450 nm versus the concentration of VHHs, using the saturation binding equation in GraphPad Prism 7. (Saturation binding equations, One site -- Total). These data demonstrate that VHH_{kappa}-biotin and VHH_{kappa}-SD36-biotin conjugates have high binding affinity for mouse immunoglobins.

The neuraminidase inhibition activities of VHH_{kappa}-zanamivir, ALB1-zanamivir, zanamivir, and VHH_{kappa} towards neuraminidase of various influenza species were measured by

the NA-Star™ Influenza Neuraminidase Inhibitor Resistance Detection Kit. The neuraminidase inhibition activities of VHH_{kappa}-zanamivir, ALB1-zanamivir, zanamivir, and VHH_{kappa} were measured by the NA-Star™ Influenza Neuraminidase Inhibitor Resistance Detection Kit (Invitrogen, cat. no. 4374422). The influenza strains indicated in Figure. S12 were used as the neuraminidase source. All the viruses were diluted to a signal:noise ratio of 40:1 (luminescence intensity of the virus containing wells : NA-Star assay buffer containing wells). In brief, a series dilutions of the tested molecules (25 µL) were incubated with 25 µL of virus in the NA-Star™ detection microplates for 20 min at 37 ° C. 10 µL of NA-Star substrate was then added to each well and incubated for 30 min at rt. Finally, 60 µL of NA-Star accelerator solution was added to all wells and their luminescent intensity were immediately read by the plate reader (SpectraMax® iD5, Molecular Devices). The half maximal inhibitory concentration (IC₅₀) values was calculated by GraphPad Prism 7. VHH_{kappa}-zanamivir and ALB1-zanamivir demonstrated effective rates of half maximal inhibitory concentration (IC₅₀) (**FIGs. 19A-FIG. 19B**).

The amino acid sequence of ALB1 is:

AVQLVESGGGLVQPGNSLRSLCAASGFTFRSFGMSWVRQAPGKEPEWVSSISGSGSDTL
YADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLRSSQGTQVTVSSGGL
PETGGHHHHHH (SEQ ID NO: 38)

The ability of VHH_{kappa}-SD36 to bind to hemagglutinins expressed on influenza virus-infected MDCK cells was tested. MDCK cells were seeded into 24-well plates and allowed to grow to confluence overnight. Infection of MDCK cells with influenza viruses (at 10 TCID₅₀) was performed according to the Manual for the laboratory diagnosis and virological surveillance of influenza (World Health Organization – 2011).

The affinity of VHHs for viral hemagglutinins on the surface of infected MDCK cells was determined using a saturation binding assay. Briefly, spent medium was aspirated from 24-well plates containing virus-infected MDCK cells and then replaced with 0.5 mL of fresh serum-free medium containing various concentrations of VHH_{kappa}-SD36. After incubation for 1 h at 37 °C, virus-infected cells were rinsed with fresh medium (2 × 0.5 mL) to remove unbound VHHs. To quantify the amount of VHHs bound to HAs, a mouse IgG-Phycoerythrin (PE) (1:20 dilution, R&D Systems, cat. no. IC002P) in 0.25 mL fresh serum-free medium was added to the VHH_{kappa}-SD36 containing wells. After incubation for 30 min at 37°C, virus-infected cells were again rinsed with fresh medium (2 × 0.5 mL) and then dissolved in 0.5 mL of 1% (w/v) sodium dodecyl sulfate (SDS). Cell-associated fluorescence was measured using an excitation wavelength of 560 nm and emission at 620 nm. The dissociation constant (K_d) was calculated from a plot of the cell bound fluorescence intensity versus the concentration of VHHs using the saturation

binding equation in GraphPad Prism 7. (Saturation binding equations, One site -- Total). VHH_{kappa}-SD36 bound hemagglutinins expressed on Influenza A virus strains at high affinity (FIGs. 20A-20B).

SD36-DFO and VHH_{kappa}-SD36-DFO were prepared for PET imaging. The nanobody-DFO adduct was prepared by a sortase-mediated conjugation of triglycine modified DFO to a nanobody. The final product of SD36-DFO (left) and VHH_{kappa}-SD36-DFO (right) were analyzed by SDS-PAGE.

Example 8 – In vivo ability of conjugates

The therapeutic efficacy among VHH_{kappa}-zanamivir, MEDI8852, and VHH_{kappa}-E11 was tested for comparison. 6-9 week old female BALB/c mice were infected with 10 LD₅₀ of influenza virus. Mice were treated with the indicated dose of VHH_{kappa}-zanamivir, MEDI8852 (monoclonal antibody (mAb) that neutralizes both group I and group II influenza A viruses (IAVs) *in vitro*), or VHH_{kappa}-E11 (SARS CoV-2 spike-specific nanobody) by intraperitoneal injection. Mice were euthanized when they lost 25% of their body weight or became moribund. Weight loss curves (left) and survival curves (right) are shown. For weight loss curves, % body weight change represents the mean ± standard deviation. The mean of the % body weight change over 14 days between any two groups were compared using one-way ANOVA analysis with Tukey's multiple comparisons test. Statistical differences between the indicated group and the PBS-treated group are shown (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). For survival curves, statistical differences between the indicated group and the PBS-treated group were calculated by Log-rank (Mantel-Cox) test (*P < 0.05, **P < 0.01). These data demonstrate that VHH_{kappa}-zanamivir outperforms MEDI8852 and VHH_{kappa}-E11 with respect to body weight and/or survival rate (FIG. 21)

Example 9

The ability of VHH_{kappa}-zanamivir to induce Complement-dependent cytotoxicity (CDC) and Antibody-dependent cellular cytotoxicity (ADCC) was tested.

For the CDC assay, MDCK cells at 10000 cells/well were seeded in a 96-well plate and incubated with 100 TCID₅₀ of influenza virus A/NWS/33 (H1N1) for 24 hours. Spent medium was aspirated from 96-well plates containing virus-infected MDCK cells and then treated with 50 µl of VHH_{kappa}-zanamivir (or VHH_{kappa}-SD36) or a mixture of VHH_{kappa} and zanamivir (or SD36) (final concentration: 10 nM). After incubation at room temperature for 30 min, 50 µl of fresh serum-free medium containing 40 µg/mL normal mouse IgG isotype control (Invitrogen, cat. no. 10400C) and 40% (v/v) rabbit complement serum (Sigma-Aldrich, cat. no. S7764) was added to

the cells. The plate was then incubated for 2.5h at 37°C. Cell viability was measured by CellTiter-Glo® Luminescent Cell Viability Assay (Promega, cat. no. G7572). Maximal cell killing was achieved by treating cells with 5% H₂O₂. The percent cytotoxicity induced by VHH_{kappa}-zanamivir was calculated as:

$$5 \quad \% \text{ Cytotoxicity} = \frac{\text{luminescence}_{\text{no VHH}} - \text{luminescence}_{\text{expt}}}{\text{luminescence}_{\text{no VHH}} - \text{luminescence}_{\text{maskilling}}} \times 100$$

Influenza virus-infected MDCK cells were killed by VHH_{kappa}-zanamivir in the presence of rabbit complement and mouse polyclonal mouse IgG, as evidenced by the high cytotoxicity in infected cells (**FIG. 23A**).

For the ADCC assay, MDCK cells at 10000 cells/well were seeded in a 96-well plate and
 10 incubated with 100 TCID₅₀ of influenza virus A/NWS/33 (H1N1) for 24 hours. Spent medium was aspirated from 96-well plates containing virus-infected MDCK cells and then treated with 25 µl of VHH_{kappa}-zanamivir (or VHH_{kappa}-SD36) or a mixture of VHH_{kappa} and zanamivir (or SD36) (final concentration: 10 nM), followed by addition of 25 µl of 40 µg/mL normal mouse IgG isotype control (Invitrogen, cat. no. 10400C). After incubation at room temperature for 30
 15 min, 25 µl of ADCC reporter cells (Promega, cat. no. 10400C) were added at 75,000 cells/well and incubated for 6h at 37°C. To measure luciferase generated by the ADCC reporter cells, 75µl of Bio-Glo™ Reagent (Promega, cat. no. 10400C) were added to each well and the luminescence intensity was measured by the plate reader (SpectraMax® iD5, Molecular Devices).

20 Virus-infected MDCK cells induced expression of luciferase in reporter cells that express luciferase upon engagement of mouse FcγRIV receptor in the presence of VHH_{kappa}-zanamivir and mouse polyclonal mouse IgG. Induction of ADCC was calculated by dividing the luminescence intensity of the indicated samples by the mean of control samples containing virus-infected cells and reporter cells with no VHHs. The VHH_{kappa}-zanamivir conjugate provided significant
 25 induction relative to the mixture of VHH_{kappa} and zanamivir in infected cells (**FIG. 23B**).

It was found that VHH_{kappa}-zanamivir and ALB1-zanamivir have similar clearance rates (**FIG. 23D**). Each individual measurement of the ⁸⁹Zr disintegration rate as count per minute (CPM) from 10 µL of whole blood (y-axis) is shown as a blue square (VHH_{kappa}-DFO-⁸⁹Zr, n = 3), red circle (ALB1-DFO-⁸⁹Zr, n = 4) or black triangle (SD36-DFO-⁸⁹Zr, n = 3) for each blood
 30 draw timepoint after initial injection of the construct (x-axis: 10 min, 1h, 24h, 48h, 72h, 96h, and 144h). All mice received an initial dose of 250µ Ci of ⁸⁹Zr-labelled VHH (equals to 1 mg/kg VHH). Each data point represent mean ± standard deviation. The half-life (fast phase and slow phase) for each VHH was estimated using a two-phase decay model. Total VHH exposure across the first 144h post-injection was calculated by integrating the concentration of VHH in blood over
 35 time. It is expressed as ‘area under the curve’ (AUC).

Using experiments similar to those performed for VHH_{kappa}-zanamivir, it was found that VHH_{kappa}-SD36 induced ADCC but not CDC (**FIG. 24A-24B**).

Example 10

- 5 An ALB1-zanamivir conjugate was prepared. ALB1 is an anti-serum albumin nanobody (ALB1) having the amino acid sequence as shown in **FIG. 25A**. A sortase recognition motif (LPETG) was attached to the C terminus of the nanobody. ALB1-zanamivir was prepared by sortase-mediated conjugation of triglycine-modified zanamivir to ALB1. The identity of the final product, ALB1-zanamivir, was confirmed by SDS-PAGE and mass spectrometry (**FIG. 25B**).
- 10 VHH_{kappa}-DFO, ALB1-DFO, and SD36-DFO were prepared for PET imaging. The nanobody-DFO adduct was prepared by sortase-mediated conjugation of triglycine-modified DFO to a nanobody. The nanobody-DFO adducts were analyzed by SDS-PAGE (For each gel, in order from left to right: 1: sortase, 2: unconjugated nanobody, 3: reaction mixture, 4-9: different fractions obtained after PD-10 column elution, nanobody-DFO adducts shown as #6 on the gels
- 15 were used for PET imaging).

EQUIVALENTS AND SCOPE

- Those skilled in the art will recognize, or be able to ascertain using no more than routine
- 20 experimentation, many equivalents of the embodiments described herein. The scope of the present disclosure is not intended to be limited to the above description, but rather is as set forth in the appended claims.

- Articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or”
- 25 between two or more members of a group are considered satisfied if one, more than one, or all of the group members are present, unless indicated to the contrary or otherwise evident from the context. The disclosure of a group that includes “or” between two or more group members provides embodiments in which exactly one member of the group is present, embodiments in which more than one members of the group are present, and embodiments in which all of the
- 30 group members are present. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

It is to be understood that the disclosure encompasses all variations, combinations, and permutations in which one or more limitation, element, clause, or descriptive term, from one or

more of the claims or from one or more relevant portion of the description, is introduced into another claim. For example, a claim that is dependent on another claim can be modified to include one or more of the limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that
5 methods of making or using the composition according to any of the methods of making or using disclosed herein or according to methods known in the art, if any, are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

Where elements are presented as lists, *e.g.*, in Markush group format, it is to be
10 understood that every possible subgroup of the elements is also disclosed, and that any element or subgroup of elements can be removed from the group. It is also noted that the term “comprising” is intended to be open and permits the inclusion of additional elements or steps. It should be understood that, in general, where an embodiment, product, or method is referred to as comprising particular elements, features, or steps, embodiments, products, or methods that
15 consist, or consist essentially of, such elements, features, or steps, are provided as well. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

Where ranges are given, endpoints are included. Furthermore, it is to be understood that
20 unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value within the stated ranges in some embodiments, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. For purposes of brevity, the values in each range have not been individually spelled out herein, but it will be understood that each of these
25 values is provided herein and may be specifically claimed or disclaimed. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

30 Where websites are provided, URL addresses are provided as non-browser-executable codes, with periods of the respective web address in parentheses. The actual web addresses do not contain the parentheses.

In addition, it is to be understood that any particular embodiment of the present disclosure may be explicitly excluded from any one or more of the claims. Where ranges are
35 given, any value within the range may explicitly be excluded from any one or more of the

claims. Any embodiment, element, feature, application, or aspect of the compositions and/or methods of the disclosure, can be excluded from any one or more claims. For purposes of brevity, all of the embodiments in which one or more elements, features, purposes, or aspects is excluded are not set forth explicitly herein.

CLAIMS

1. A conjugate comprising a first agent that binds to an immunoglobulin and a second agent that binds to a target on the surface of a cell or a pathogen, wherein the first agent and the second agent are covalently conjugated via a linker.
5
2. The conjugate of claim 1, wherein the first agent is an antibody fragment comprising a variable region that is capable of binding to an antigen.
- 3 The conjugate of claim 2, where in the antibody fragment comprises a heavy chain
10 variable region.
4. The conjugate of claim 1 or claim 2, wherein the first agent is a single domain antibody fragment.
- 15 5. The conjugate of any one of claims 1-4, wherein the immunoglobulin bound by the first agent comprises an immunoglobulin kappa light chain or an immunoglobulin lambda light chain.
6. The conjugate of claim 5, wherein the immunoglobulin kappa light chain is a human
20 immunoglobulin kappa light chain, and the immunoglobulin lambda light chain is a human immunoglobulin lambda light chain.
7. The conjugate of claim 6, wherein the first agent binds to the human immunoglobulin kappa light chain.
25
8. The conjugate of any one of claims 1-7, wherein the second agent comprises a small molecule, a peptide, a protein, a carbohydrate, a lipid, a nucleotide, a nucleic acid, an oligonucleotide, an aptamer, or an antibody.
- 30 9. The conjugate of claim 8, wherein the antibody is a single domain antibody.
10. The conjugate of any one of claims 1-9, wherein the second agent has a therapeutic effect when administered to a subject.

11. The conjugate of any one of claims 1-10 wherein the linker comprises a cleavable or a non-cleavable linker.
12. The conjugate of claim 11, wherein the linker comprises a cleavable linker.
13. The conjugate of claim 12, wherein the cleavable linker is a peptide, disulfide, or hydrazone linker.
14. The conjugate of any one of claims 1-13, wherein the cell is a cell infected by a pathogen, a cancer cell, a transformed cell, a healthy cell, a cell that is undergoing or has undergone a phenotypic change in response to cellular stress.
15. The conjugate of any one of claims 1-14, wherein the pathogen is a virus, a bacterium, a parasite, or a fungus.
16. The conjugate of any one of claims 1-15, wherein the pathogen is a virus selected from an influenza virus, a coronavirus, an adenovirus, an enterovirus, a rotavirus, a norovirus, a herpesvirus, a lentivirus, a poxvirus, a paramyxovirus, a rhabdovirus, an arenavirus, a flavivirus, a togavirus, a hantavirus, a pneumovirus, or an ebolavirus.
17. The conjugate of claim 16, wherein the influenza virus is an influenza A virus or an influenza B virus.
18. The conjugate of any one of claims 1-17, wherein the second agent binds to an influenza virus neuraminidase or an influenza virus hemagglutinin.
19. The conjugate of claim 18, wherein the second agent comprises a small molecule that binds to an influenza virus neuraminidase.
20. The conjugate of claim 19, wherein the second agent comprises zanamivir or an analog thereof.
21. The conjugate of claim 20, wherein the linker is a triglycine dibenzylcyclooctyne (DBCO) linker.

22. The conjugate of claim 18, wherein the second agent comprises an antibody or antibody fragment that binds to an influenza virus neuraminidase.
23. The conjugate of claim 16, wherein the coronavirus is a beta coronavirus.
24. The conjugate of claim 23, wherein the beta coronavirus is a Middle East Respiratory Syndrome coronavirus (MERS-CoV), a Severe Acute Respiratory Syndrome (SARS)-associated coronavirus (SARS-CoV)-1, or a SARS-CoV-2.
25. The conjugate of claim 24, wherein the second agent binds to a MERS-CoV spike protein, a SARS-CoV-1 spike protein, or a SARS-CoV-2 spike protein.
26. The conjugate of claim 24, wherein the second agent binds to a MERS-CoV spike protein receptor binding domain (RBD), a SARS-CoV-1 spike protein RBD, or a SARS-CoV-2 spike protein RBD.
27. The conjugate of claim 16, wherein the lentivirus is a human immunodeficiency virus (HIV).
28. The conjugate of claim 27, wherein the second agent binds to a HIV envelope glycoprotein gp120.
29. The conjugate of claim 16, wherein the pneumovirus is a human respiratory syncytial virus (RSV).
30. The conjugate of claim 29, wherein the second agent binds to a RSV fusion (F) protein.
31. The conjugate of any one of claims 1-15, wherein the pathogen is a bacterium selected from a *Pasteurella* species, a *Staphylococcus* species, a *Streptococcus* species, a *Bacillus* species, a *Corynebacterium* species, a *Diphtheroids* species, a *Listeria* species, an *Erysipelothrix* species, a *Clostridium* species, a *Neisseria* species, a *Branhamella* species, an *Escherichia* species, an *Enterobacter* species, a *Proteus* species, a *Pseudomonas* species, a *Klebsiella* species, a *Salmonella* species, a *Shigella* species, a *Serratia* species, an *Acinetobacter* species, a *Haemophilus* species, a *Brucella* species, a *Yersinia* species, a *Francisella* species, a *Pasturella* species, a *Vibrio* species, a *Flavobacterium* species, a *Pseudomonas* species, a

Campylobacter species, a *Bacteroides* species, a *Fusobacterium* species, a *Calymmatobacterium* species, a *Streptobacillus* species, or a *Legionella* species.

32. The conjugate of any one of claims 1-15, wherein the pathogen is a parasite selected
5 from a *Plasmodium* species, a *Trypanosoma* species, a *Toxoplasma* species, a *Leishmania* species, or a *Cryptosporidium* species.

33. The conjugate of claim 32, wherein the *Plasmodium* is *Plasmodium falciparum*,
Plasmodium malariae, *Plasmodium vivax*, *Plasmodium knowlesi*, *Plasmodium ovale curtisi*, or
10 *Plasmodium ovale wallikeri*.

34. The conjugate of claim 33, wherein the second agent binds a *Plasmodium* surface protein.

15 35. The conjugate of claim 34, wherein the plasmodium surface protein is a merozoite surface protein 1 (MSP-1).

36. The conjugate of claim 35, wherein the second agent is an antibody that binds to MSP-1, optionally wherein the antibody is a nanobody.
20

37. The conjugate of claim 36, wherein the antibody comprises a CDR-H1, a CDR-H2, and a CDR-H3 of any one of the antibodies listed in Table 1.

38. The conjugate of claim 37, wherein the antibody comprises the amino acid sequence of
25 any one of SEQ ID NOs: 6-17.

39. The conjugate of claim 14, wherein the cancer cell is a hematological cancer cell, a lung cancer cell, a breast cancer cell, a brain cancer cell, a gastrointestinal cancer cell, a liver cancer cell, a kidney cancer cell, a bladder cancer cell, a pancreatic cancer cell, an ovarian cancer cell, a
30 testicular cancer cell, a prostate cancer cell, an endometrial cancer cell, a muscle cancer cell, a bone cancer cell, a neuroendocrine cancer cell, a connective tissue cancer cell, a head or neck cancer cell, or a skin cancer cell.

40. The conjugate of claim 14 or claim 39, wherein the second agent binds to a tumor-associated antigen.
35

41. The conjugate of claim 40, wherein the tumor-associated antigen comprises a MHC class I polypeptide-related sequence A (MICA) protein, a MHC class I polypeptide-related sequence B (MICB) protein, a folate receptor, a fibronectin splice variant, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), hepatocyte growth factor receptor (HGFR), vascular endothelial growth factor receptor 2 (VEGFR-2), C-X-C chemokine receptor type 4 (CXCR4), urokinase plasminogen activator surface receptor (uPAR), follicle-stimulating hormone receptor (FSHR), epithelial cell adhesion molecule (EpCAM), epithelial cadherin (ECAD), carcinoembryonic antigen (CEA), or mesothelin (MSLN).
42. The conjugate of claim 41, wherein the second agent is an antibody that binds to MICA, optionally wherein the antibody is a nanobody.
43. The conjugate of claim 42, wherein the antibody comprises a CDR-H1, a CDR-H2, and a CDR-H3 of any one of the antibodies listed in Table 2.
44. The conjugate of claim 43, wherein the antibody comprises the amino acid sequence of any one of SEQ ID NOs: 19-27.
45. The conjugate of claim 14, wherein the cell is a cancerous or healthy bone marrow cell.
46. The conjugate of claim 45, wherein the second agent binds to a bone marrow-associated antigen.
47. The conjugate of claim 46, wherein the bone marrow-associated antigen is cluster of differentiation antigen 45 (CD45).
48. The conjugate of claim 14, wherein the cell is a cancerous or healthy immune cell.
49. The conjugate of claim 48, wherein the cell is a cancerous or healthy T cell or B cell.
50. The conjugate of claim 48 or claim 49, wherein the second agent binds to an immune cell-associated antigen.

51. The conjugate of claim 50, wherein the immune cell-associated antigen is a cluster of differentiation antigen 4 (CD4), a cluster of differentiation antigen 8 (CD8), a T cell receptor (TCR), or a B cell receptor (BCR).
- 5 52. The conjugate of any one of claims 1-51, wherein the conjugate provides a therapeutic effect when administered to a subject.
53. The conjugate of any one of claims 1-51, wherein the conjugate enhances association
between one or more immune cells expressing a fragment crystallizable (Fc) receptor and the
10 cell or pathogen when administered to a subject.
54. The conjugate of any one of claims 1-52, wherein the conjugate results in killing of the cell or pathogen when administered to a subject.
- 15 55. The conjugate of any one of claims 1-54, wherein the conjugate results in inactivation of the cell or pathogen when administered to a subject.
56. The conjugate of any one of claims 52-55, wherein the subject is a mammal.
- 20 57. The conjugate of any one of claims 52-56, wherein the subject is a human.
58. A composition comprising the conjugate of any one of claims 1-57.
59. The composition of claim 58, wherein the composition further comprises a
25 pharmacologically acceptable excipient.
60. A method for enhancing an immune response to a cell or a pathogen in a subject, the method comprising administering to the subject an effective amount of the conjugate of any one of claims 1-57 or the composition of claim 58 or claim 59.
30
61. The method of claim 60, wherein the cell is a cell infected by a pathogen, a cancer cell, a transformed cell, a healthy cell, a cell that is undergoing or has undergone a phenotypic change in response to cellular stress.

62. The method of claim 60 or claim 61, wherein the pathogen is a virus, a bacterium, a parasite, or a fungus.

63. The method of any one of claims 60-62, wherein the cell is a cell of the subject.

5

64. The method of any one of claims 60-63, wherein the pathogen is a virus selected from an influenza virus, a coronavirus, an adenovirus, an enterovirus, a rotavirus, a norovirus, a herpesvirus, a lentivirus, a poxvirus, a paramyxovirus, a rhabdovirus, an arenavirus, a flavivirus, a togavirus, a hantavirus, a pneumovirus, or an ebolavirus.

10

65. The method of claim 64, wherein the virus is an influenza A virus or an influenza B virus.

15

66. The method of claim 64, wherein the virus is a Middle East Respiratory Syndrome coronavirus (MERS-CoV), a Severe Acute Respiratory Syndrome (SARS)-associated coronavirus (SARS-CoV)-1, or a SARS-CoV-2.

67. The method of claim 64, wherein the virus is a human immunodeficiency virus (HIV).

20

68. The method of claim 64, wherein the virus is a human respiratory syncytial virus (RSV).

25

69. The method of any of claims 60-63, wherein the pathogen is a bacterium selected from a *Pasteurella* species, a *Staphylococcus* species, a *Streptococcus* species, a *Bacillus* species, a *Corynebacterium* species, a *Diphtheroids* species, a *Listeria* species, an *Erysipelothrix* species, a *Clostridium* species, a *Neisseria* species, a *Branhamella* species, an *Escherichia* species, an *Enterobacter* species, a *Proteus* species, a *Pseudomonas* species, a *Klebsiella* species, a *Salmonella* species, a *Shigella* species, a *Serratia* species, an *Acinetobacter* species, a *Haemophilus* species, a *Brucella* species, a *Yersinia* species, a *Francisella* species, a *Pasturella* species, a *Vibrio cholera* species, a *Flavobacterium* species, a *Pseudomonas* species, a *Campylobacter* species, a *Bacteroides* species, a *Fusobacterium* species, a *Calymmatobacterium* species, a *Streptobacillus* species, or a *Legionella* species.

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70. The method of any one of claims 60-63, wherein the pathogen is a parasite selected from a *Plasmodium* species, a *Trypanosoma* species, a *Toxoplasma* species, a *Leishmania* species, or a *Cryptosporidium* species.

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71. The method of claim 70, wherein the parasite is *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Plasmodium ovale curtisi*, or *Plasmodium ovale wallikeri*.

5

72. The method of any one of claims 61-63, wherein the cancer cell is a hematological cancer cell, a lung cancer cell, a breast cancer cell, a brain cancer cell, a gastrointestinal cancer cell, a liver cancer cell, a kidney cancer cell, a bladder cancer cell, a pancreatic cancer cell, an ovarian cancer cell, a testicular cancer cell, a prostate cancer cell, an endometrial cancer cell, a muscle cancer cell, a bone cancer cell, a neuroendocrine cancer cell, a connective tissue cancer cell, a head or neck cancer cell, or a skin cancer cell.

10

73. The method of any one of claims 60-63, wherein the cell is a cancerous or healthy bone marrow cell.

15

74. The method of any one of claims 60-63, wherein the cell is a cancerous or healthy immune cell.

75. The method of claim 74, wherein the cell is a cancerous or healthy T cell or B cell.

20

76. The method of any one of claims 60-75, wherein the immune response comprises an innate immune response.

77. The method of any one of claims 60-70, wherein the conjugate binds to the cell or pathogen and to an immunoglobulin of the subject, wherein the immunoglobulin further binds to an immune cell of the subject which expresses a fragment crystallizable (Fc) receptor on its surface.

25

78. The method of claim 77, wherein the immunoglobulin of the subject comprises an immunoglobulin kappa light chain or an immunoglobulin lambda light chain.

30

79. The method of claim 78, wherein the immunoglobulin of the subject comprises an immunoglobulin kappa light chain.

80. The method of any one of claims 77-79 wherein the immune cell is a macrophage, a dendritic cell, a natural killer cell, a neutrophil, a basophil, an eosinophil, or a mast cell.
81. The method of any one of claims 77-80, wherein the administration induces the
5 production of one or more cytokines or chemokines by the immune cell.
82. The method of claim 81, wherein the one or more cytokines or chemokines are proinflammatory cytokines or chemokines.
- 10 83. The method of any one of claims 77-82, wherein the administration induces phagocytosis of the cell or pathogen by the immune cell.
84. The method of any one of claims 77-82, wherein the administration results in killing of the cell or pathogen.
- 15 85. The method of any one of claims 60-82, wherein the administration results in inactivation of the cell or pathogen.
86. The method of any one of claims 60-85, wherein the subject is a subject that has or is at
20 risk of developing a viral infection.
87. The method of any one of claims 60-85, wherein the subject is a subject that has or is at risk of developing cancer.
- 25 88. The method of claim 87, wherein the cancer is metastatic cancer.
89. The method of any one of claims 60-88, wherein the subject is a mammal.
90. The method of any one of claims 60-89, wherein the subject is a human.
- 30 91. The method of claim 90, wherein the subject is a human neonate, a human infant, a human adult, or an elderly human.
92. The method of any one of claims 60-89, wherein the subject is a companion animal, a
35 research animal, or a domesticated animal.

93. The method of any one of claims 60-92, wherein the administration is intravenous, intramuscular, intradermal, subcutaneous, or inhaled.
- 5 94. The method of any one of claims 60-93, wherein the administration occurs more than once.
95. The method of any one of claims 60-94, wherein the administration is prophylactic.
- 10 96. A method for treating a disease or reducing the risk of a disease in a subject in need thereof, the method comprising administering to the subject an effective amount of the conjugate of any one of claims 1-57 or the composition of claim 58 or claim 59.
97. The method of claim 96, wherein the disease is a disease caused by a virus, a bacterium,
15 a parasite, a fungus, or a cancer.
98. The method of claim 97, wherein the virus is an influenza virus, a coronavirus, an adenovirus, an enterovirus, a rotavirus, a norovirus, a herpesvirus, a lentivirus, a poxvirus, a paramyxovirus, a rhabdovirus, an arenavirus, a flavivirus, a togavirus, a hantavirus, a
20 pneumovirus, or an ebolavirus.
99. The method of claim 97 or claim 98, wherein the virus is an influenza A virus or an influenza B virus.
- 25 100. The method of claim 97 or claim 98, wherein the virus is a Middle East Respiratory Syndrome coronavirus (MERS-CoV), a Severe Acute Respiratory Syndrome (SARS)-associated coronavirus (SARS-CoV)-1, or a SARS-CoV-2.
101. The method of claim 97 or claim 98, wherein the virus is a human immunodeficiency
30 virus (HIV).
102. The method of claim 97 or claim 98, wherein the virus is a human respiratory syncytial virus (RSV).

103. The method of claim 97, wherein the bacterium is a *Pasteurella* species, a *Staphylococcus* species, a *Streptococcus* species, a *Bacillus* species, a *Corynebacterium* species, a *Diphtheroids* species, a *Listeria* species, an *Erysipelothrix* species, a *Clostridium* species, a *Neisseria* species, a *Branhamella* species, an *Escherichia* species, an *Enterobacter* species, a
5 *Proteus* species, a *Pseudomonas* species, a *Klebsiella* species, a *Salmonella* species, a *Shigella* species, a *Serratia* species, an *Acinetobacter* species, a *Haemophilus* species, a *Brucella* species, a *Yersinia* species, a *Francisella* species, a *Pasturella* species, a *Vibrio cholera* species, a *Flavobacterium* species, a *Pseudomonas* species, a *Campylobacter* species, a *Bacteroides* species, a *Fusobacterium* species, a *Calymmatobacterium* species, a *Streptobacillus* species, or a
10 *Legionella* species.

104. The method of claim 97, wherein the parasite is a *Plasmodium* species, a *Trypanosoma* species, a *Toxoplasma* species, a *Leishmania* species, or a *Cryptosporidium* species.

15 105. The method of claim 104, wherein the parasite is *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Plasmodium ovale curtisi*, or *Plasmodium ovale wallikeri*.

106. The method of claim 97, wherein the cancer is a hematological cancer, a lung cancer, a
20 breast cancer, a brain cancer, a gastrointestinal cancer, a liver cancer, a kidney cancer, a bladder cancer, a pancreatic cancer, an ovarian cancer, a testicular cancer, a prostate cancer, an endometrial cancer, a muscle cancer, a bone cancer, a neuroendocrine cancer, a connective tissue cancer, a head or neck cancer, or a skin cancer.

25 107. The method of claim 97 or claim 106, wherein the cancer is metastatic cancer.

108. The method of any one of claims 96-107, wherein the subject is a mammal.

109. The method of any one of claims 96-108, wherein the subject is a human.
30

110. The method of claim 109, wherein the subject is a human neonate, a human infant, a human adult, or an elderly human.

111. The method of any one of claims 96-108, wherein the subject is a companion animal, a
35 research animal, or a domesticated animal.

112. The method of any one of claims 96-111, wherein the administration is intravenous, intramuscular, intradermal, subcutaneous, or inhaled.
- 5 113. The method of any one of claims 96-112, wherein the administration occurs more than once.
114. The method of any one of claims 96-113, wherein the administration is prophylactic.
- 10 115. An antibody comprising a CDR-H1, a CDR-H2, and a CDR-H3 of any one of the antibodies listed in Table 1.
116. The antibody of claim 115, wherein the antibody comprises the amino acid sequence of any one of SEQ ID NOs: 6-17.
- 15 117. An antibody comprising a CDR-H1, a CDR-H2, and a CDR-H3 of any one of the antibodies listed in Table 2.
118. The antibody of claim 117, wherein the antibody comprises the amino acid sequence of any one of SEQ ID NOs: 19-27.
- 20 119. An antibody comprising the amino acid sequence of SEQ ID NO: 38.