SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

In one example, the present disclosure provides targeted complement-activating molecules comprising a targeting or target-binding domain and a complement-activating serine protease effector domain. In one aspect, the present disclosure refers to a targeted complement-activating molecule comprising: (a) a target-binding domain; and (b) a complement-activating serine protease effector domain; wherein the complement-activating serine protease effector domain comprises C1r or a functional fragment thereof or C1s or a functional fragment thereof, and wherein the target-binding domain comprises an antibody that binds to an antigen present on a microbial pathogen, or an antigen-binding fragment thereof. In some examples, the target-binding domain is derived from an antibody. In some examples, the target-binding domain comprises an antigen-binding fragment of an antibody. In some examples, the complement-activating serine protease effector domain is catalytically active, while in other examples the complement-activating serine protease effector domain is in a zymogen form. In some examples, the target-binding domain binds to an antigen present on a cell, such as CD20, CD38, or CD52. In other examples, the target-binding domain binds to an antigen present on a microbial pathogen, such as a bacterial pathogen, a viral pathogen, a fungal pathogen, or a parasitic pathogen.

In some examples, the targeted complement-activating molecules comprise a fusion protein comprising the N-terminus of the serine protease effector domain fused to the C-terminus of the antibody heavy chain or fragment thereof or to the C-terminus of the antibody light chain or fragment thereof. In other examples, the fusion protein comprises the C-terminus of the serine protease effector domain fused to the N-terminus of the antibody heavy chain or fragment thereof or to the N-terminus of the antibody light chain or fragment thereof. In some examples, the targeted complement-activating molecules comprise such a fusion protein and a second antibody chain, which is a light chain or fragment thereof if the fusion protein comprises a heavy chain or fragment thereof and which is a heavy chain or fragment thereof if the fusion protein comprises a light chain or fragment thereof. In some examples, the targeted complement-activating molecules comprise a fusion protein comprising the N-terminus of the serine protease effector domain fused to the C-terminus of a single-chain antibody or fragment thereof, or a fusion protein comprising the C-terminus of the serine protease effector domain fused to the N-terminus of a single-chain antibody or fragment thereof.

In some examples, the serine protease effector domain comprises complement factor D or a fragment thereof, C1r or a fragment thereof, C1s or a fragment thereof, MASP-2 or a fragment thereof, MASP-3 or a fragment thereof, MASP-1 or a fragment thereof, C2a or a fragment thereof, or Bb or a fragment thereof.

Also provided herein are polynucleotides encoding targeted complement-activating molecules or portions thereof, and cloning vectors or expression cassettes comprising such polynucleotides. In one aspect, the present disclosure refers to a polynucleotide encoding the molecule as disclosed herein. In another aspect, the present disclosure refers to a cloning vector or expression cassette comprising the polynucleotide as disclosed herein.

Further provided herein are host cells expressing targeted complement-activating molecules and methods of producing targeted complement-activating molecules comprising culturing the host cells under conditions allowing for expression of the molecules and isolating the molecules. In one aspect, the present disclosure refers to a host cell expressing the molecule as disclosed herein, or comprising the cloning vector or expression cassette as disclosed herein. In one aspect, the present disclosure refers to a method of producing a molecule comprising: (a) a target-binding domain; and (b) a complement-activating serine protease effector domain; the method comprising culturing the host cell as disclosed herein under conditions allowing for expression of the molecule and isolating the molecule.

In one aspect, the present disclosure refers to a composition comprising the molecule as disclosed herein and one or more excipients.

Also provided herein are methods of activating at least one complement pathway in a mammalian subject using the targeted complement-activating molecules. In some examples, the targeted complement-activating molecules may be used to induce complement dependent cytotoxicity (CDC), complement-dependent cellular cytotoxicity (CDCC), or complement-dependent cellular phagocytosis (CDCP) in a target cell. In some examples, the targeted complement-activating molecules may be used to treat cancer, autoimmune disease, or a microbial infection, such as a bacterial infection, a viral infection, a fungal infection, or a parasitic infection. In one aspect, the present disclosure refers to use of the molecule as disclosed herein or the composition as disclosed herein in the manufacture of a medicament for treating a microbial infection.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 is a diagram illustrating the classical, lectin, and alternative complement pathways.

FIGURE 2 graphically illustrates the surface levels of CD20, CD55, and CD59 on CD20+ cancer cell lines Ramos, Kasumi-2, and SU-DHL-8. Cells were stained with primary

CLAIMS

What is claimed is:

1. A targeted complement-activating molecule comprising:

(a) a target-binding domain; and

(b) a complement-activating serine protease effector domain;

wherein the complement-activating serine protease effector domain comprises C1r or a functional fragment thereof or C1s or a functional fragment thereof, and wherein the target-binding domain comprises an antibody that binds to an antigen present on a microbial pathogen, or an antigen-binding fragment thereof.

2. The molecule of claim 1, wherein the target-binding domain binds to an antigen present on a bacterial pathogen, a viral pathogen, a fungal pathogen, or a parasitic pathogen.

3. The molecule of claim 2, wherein the pathogen is Neisseria meningitidis, Staphylococcus aureus, Borrelia burgdorferi, Escherichia coli, Klebsiella pneumoniae, Streptococcus pneumoniae, Serratia marcenscens, Haemophilus influenzae, Mycobacterium tuberculosis, Treponema pallidum, Neisseria gonorrhea, Clostridium difficile, a Salmonella species, a Helicobacter species, a Shigella species, a Campylobacter species, a Listeria species, an Epstein-Barr virus, a Human Immunodeficiency Virus 1 (HIV-1), a Herpesvirus, an Influenza virus, a West Nile virus, a Cytomegalovirus, a Coronavirus, Schistosoma mansoni, Plasmodium falciparum, or Trypanosoma cruzei.

4. The molecule any of claims 1-3, wherein the target-binding domain comprises an antibody or an antigen-binding fragment thereof.

5. The molecule of claim 4, wherein the molecule comprises:

a fusion protein comprising:

the N-terminus of the complement-activating serine protease effector domain fused to the C-terminus of the antibody heavy chain or fragment thereof; and

an antibody light chain or fragment thereof.

6. The molecule of claim 5, wherein the molecule comprises a fusion protein comprising:

the N-terminus of the complement-activating serine protease effector domain fused to the C-terminus of a single-chain antibody or fragment thereof or single-domain antibody or fragment thereof.

7. The molecule of any one of claims 1-6, wherein the target-binding domain and the serine protease effector domain are connected by a linker.

8. The molecule of any one of claims 1-7, wherein the target-binding domain comprises anti-fHbP antibody clone 19, anti-PspA antibody RX1MI005, anti-Fnbp antibody clone G, anti-Candida antibody 1A2, anti-PfRH5 antibody R5.004, anti-PfRH5 antibody R5.016, anti-GP120 antibody PGT121, bebtelovimab, anti-SARS-CoV-2 M protein antibody RB572, anti-SARS-CoV-2 M protein antibody RB574, anti-Aspergillus antibody hJF5, or an antigen-binding fragment of any of the foregoing.

9. The molecule of any one of claims 1-8, wherein:

a) the target-binding domain comprises a heavy chain as set forth in any one of SEQ ID NOs:103, 108, and 111 and a light chain as set forth in SEQ ID NO:104;

b) the target-binding domain comprises a heavy chain as set forth in SEQ ID NO: 122 or 123 and a light chain as set forth in SEQ ID NO:121;

c) the target-binding domain comprises a heavy chain as set forth in SEQ ID NO:126 or 127 and a light chain as set forth in SEQ ID NO:125;

d) the target-binding domain comprises a heavy chain as set forth in SEQ ID NO:130 or 131 and a light chain as set forth in SEQ ID NO:129;

e) the target-binding domain comprises a heavy chain as set forth in SEQ ID NO:138 or 139 and a light chain as set forth in SEQ ID NO:137;

f) the target-binding domain comprises a heavy chain as set forth in SEQ ID NO:142 or 143 and a light chain as set forth in SEQ ID NO:141;

g) the target-binding domain comprises a heavy chain as set forth in SEQ ID NO:146 or 147 and a light chain as set forth in SEQ ID NO:145;

h) the target-binding domain comprises a heavy chain as set forth in SEQ ID NO:150 or 151 and a light chain as set forth in SEQ ID NO:149; or

i) the target-binding domain comprises a heavy chain as set forth in SEQ ID NO:134 or 135 and a light chain as set forth in SEQ ID NO:133.

10. The molecule of any one of claims 1-9, wherein the serine protease effector domain comprises an amino acid sequence set forth in any one of SEQ ID NOs:61-74, 76, and 78-87.

11. The molecule of claim 5 or claim 6, comprising:

a) a fusion protein as set forth in any one of SEQ ID NOs:108, and a light chain as set forth in SEQ ID NO:104;

b) a fusion protein as set forth in SEQ ID NO:122 or 123 and a light chain as set forth in SEQ ID NO:121

c) a fusion protein as set forth in SEQ ID NO:126 or 127 and a light chain as set forth in SEQ ID NO:125

d) a fusion protein as set forth in SEQ ID NO:130 or 131 and a light chain as set forth in SEQ ID NO:129;

e) a fusion protein as set forth in SEQ ID NO:138 or 139 and a light chain as set forth in SEQ ID NO:137;

f) a fusion protein as set forth in SEQ ID NO:142 or 143 and a light chain as set forth in SEQ ID NO:141;

g) a fusion protein as set forth in SEQ ID NO:146 or 147 and a light chain as set forth in SEQ ID NO:145;

h) a fusion protein as set forth in SEQ ID NO:150 or 151 and a light chain as set forth in SEQ ID NO:149; or

i) a fusion protein as set forth in SEQ ID NO:134 or 135 and a light chain as set forth in SEQ ID NO:133.

12. The molecule of any one of claims 1-11, wherein the molecule binds to a target on a cell surface and activates a complement pathway when the molecule is to be administered to a mammalian subject.

13. A polynucleotide encoding the molecule of any one of claims 1-12.

14. A cloning vector or expression cassette comprising the polynucleotide of claim 13.

15. A host cell expressing the molecule of any one of claims 1-11, or comprising the cloning vector or expression cassette of claim 14.

16. A method of producing a molecule comprising:

(a) a target-binding domain; and

(b) a complement-activating serine protease effector domain; the method comprising culturing the host cell of claim 15 under conditions allowing for expression of the molecule and isolating the molecule.

17. A composition comprising the molecule of any one of claims 1-11 and one or more excipients.

18. Use of the molecule of any one of claims 1-11 or the composition of claim 17 in the manufacture of a medicament for treating a microbial infection.

19. The use of claim 18, wherein the infection is a bacterial infection, a viral infection, a fungal infection, or a parasitic infection.

20. The use of claim 19, wherein the infection is a Neisseria meningitidis, Staphylococcus aureus, Borrelia burgdorferi, Escherichia coli, Klebsiella pneumoniae, Streptococcus pneumoniae, Serratia marcenscens, Haemophilus influenzae, Mycobacterium tuberculosis, Treponema pallidum, Neisseria gonorrhea, Clostridium difficile, Salmonella species, Helicobacter species, Shigella species, Campylobacter species, Listeria species, Epstein-Barr virus, Human Immunodeficiency Virus 1 (HIV-1), Herpesvirus, Influenza virus, West Nile virus, Cytomegalovirus, Coronavirus, Schistosoma mansoni, Plasmodium falciparum, or Trypanosoma cruzei infection.