Abstract:
The invention features proteins including an antibody, or functional derivatives thereof, that bind hCD59 and have the activity of domain 4 of the Streptococcus intermedins intermedilysin (ILY) protein. In order to prevent the independent induction of CDC and ADCC, the antibodies of the invention can bind the same hCD59 epitope as ILYd4 and/or contain modifications that disrupt the interaction between the antibody and complement.
METHODS AND COMPOSITIONS FOR THE TREATMENT OF PROLIFERATIVE AND PATHOGENIC DISEASES

Background of the Invention

This invention relates to the treatment of proliferative and pathogenic diseases.


Cancer is a disease marked by the uncontrolled growth of abnormal cells. Cancer cells have overcome the barriers imposed in normal cells, which have a finite lifespan, to grow indefinitely. As the growth of cancer cells continues, genetic alterations may persist until the cancerous cell has manifested itself to pursue a more aggressive growth phenotype. If left untreated, metastasis, the
spread of cancer cells to distant areas of the body by way of the lymph system or bloodstream, may ensue, destroying healthy tissue.

CD59 is over-expressed in many cancer cells. Complement is a main mediator for antibody mediated cancer cytolysis. Up-regulation and high expression of CD59 can drive resistance to any antibody-mediated cancer therapy that activates complement as a component of its mechanism of activity. An example of resistance mediated by CD59 overexpression is resistance to the anti-CD20 chimeric MAb rituximab used for the treatment of B-cell non-Hodgkin lymphoma (B-NHL).

Accordingly, there exists a need for compounds and methods that sensitize pathogens and cancer cells to complement-mediated cell death.

**Summary of the Invention**

In one aspect, the invention features a protein including an antibody, or a functional derivative thereof (e.g., a single chain antibody (scFv), a Fv, a Fab, a Fab', or a F(ab')₂), that binds hCD59, inhibits binding of hCD59 to complement proteins C8 and/or C9, and does not independently induce antibody-dependent cellular toxicity (ADCC), complement dependent cytolysis (CDC), or apoptosis. These proteins can bind the same epitope of hCD59 as ILYd4 and/or contain modifications that disrupt the independent induction of ADCC, CDC, and apoptosis.

In any of the foregoing aspects, the light chain variable domain of the antibody, or functional derivative thereof, includes at least one (e.g., two or three) of the following complementary determining regions (CDRs): a CDRL1 including the sequence of GASQSVSSYLA (SEQ ID NO:11), a CDRL2 including the sequence of GASSRATGIPD (SEQ ID NO:12), and a CDRL3 including the sequence of YGSSPPVT (SEQ ID NO:13); and the heavy chain variable domain of the antibody, or functional derivative thereof, includes at least one (e.g., two or three) of the following CDRs: a CDRH1 including the sequence of SYDIN (SEQ ID NO:14), a CDRH2 including the sequence of WMNPNSSGNTGYAQQFQG (SEQ ID NO:15), and a CDRH3 including the
sequence of GKGSGYYNY (CDRH3; SEQ ID NO: 16). In one embodiment, the antibody, or functional derivative thereof, has a light chain variable domain sequence as set forth in SEQ ID NO:4 (e.g., a sequence with 80%, 90%, 95%, 99%, or 100%, sequence identity of SEQ ID NO:4) and a heavy chain variable domain sequence as set forth in SEQ ID NO:6 (e.g., a sequence with 80%, 90%, 95%, 99%, or 100%, sequence identity of SEQ ID NO:6).

In another aspect, the light chain variable domain of the antibody, or functional derivative thereof, includes at least one (e.g., two or three) of the following complementary determining regions (CDRs): a CDR1 including the sequence of TGTSSDVGYYNYVS (SEQ ID NO: 17), a CDR2 including the sequence of DVSNRPSGVSN (SEQ ID NO: 18), and a CDR3 including the sequence of YAGSSTLV (SEQ ID NO: 19) and the heavy chain variable domain of the antibody, or functional derivative thereof, includes at least one (e.g., two or three) of the following CDRs: a CDR1 including the sequence of SYDIN (SEQ ID NO: 14), a CDR2 including the sequence of WMNPNSSGNTGYAQKFQG (SEQ ID NO: 15), and a CDR3 including the sequence of GRGFDWLKNFDY (SEQ ID NO: 20). In one embodiment, the antibody, or functional derivative thereof, has a light chain variable domain sequence as set forth in SEQ ID NO:8 (e.g., a sequence with 80%, 90%, 95%, 99%, or 100%, sequence identity of SEQ ID NO:8) and a heavy chain variable domain sequence as set forth in SEQ ID NO: 10 (e.g., a sequence with 80%, 90%, 95%, 99%, or 100%, sequence identity of SEQ ID NO: 10).

In another aspect, the invention features a method for treating a proliferative disease (e.g., one in which neoplastic cells express hCD59) in a patient in need thereof, by administering to the patient any of the foregoing proteins and a therapeutic antibody (e.g., rituximab, MT201, 17-1A, herceptin, alemtuzumab, lym-1, bevacizumab, cetuximab, or IL-2 receptor alpha-directed monoclonal antibodies), wherein the protein of the invention and the therapeutic antibody are administered simultaneously (e.g., in the same formulation), or within 30 (e.g., 14) days of each other, in amounts that together are sufficient to treat the proliferative disease.
In another aspect, the invention features a method for treating a pathogenic disease (e.g., a disease associated with a pathogen expressing CD59 or a CD59-like molecule) in a patient in need thereof, by administering to the patient any of the foregoing proteins. This method may further include administering a therapeutic antibody (e.g., a therapeutic antibody specific for a virus selected from human cytomegalovirus, HCMV, human T-cell leukemia virus type 1, HIV-I, simian immunodeficiency virus, Ebola virus, Herpesvirus saimiri virus, influenza virus, and vaccinia virus or a microbial parasite such as Naegleria fowleri and Schistosoma mansoni), wherein the protein of the invention and the therapeutic antibody are administered simultaneously (e.g., in the same formulation), or within 30 (e.g., 14) days of each other, in amounts that together are sufficient to treat the pathogenic disease.

In yet another aspect, the invention features pharmaceutical compositions including any of the foregoing proteins and a pharmaceutically acceptable excipient.

In yet another aspect, the invention features kits including any of the foregoing proteins and a therapeutic antibody.

In certain embodiments, the protein is a substantially pure antibody.

By "patient" is meant any mammal, e.g., a human, mouse, pig, horse, dog, cat, or rat.

By "proliferative disease" is meant a disease featuring cell populations characterized by inappropriate accumulation in a tissue. This inappropriate accumulation may be the result of a genetic or epigenetic variation that occurs in one or more cells of the cell population. This genetic or epigenetic variation causes the cells of the cell population to grow faster, die slower, or differentiate slower than the surrounding, normal tissue. Examples of proliferative diseases are set forth herein.

By the "antibodies of the invention" is meant antibodies, or functional derivatives thereof, that possess ILYd4 activity.

By "CD59-like molecule" is meant a molecule expressed by a pathogen that binds domain 4 of the ILY polypeptide. Cells expressing CD59-like
molecules are resistant to the lytic effect of complement by inhibiting complete formation of the membrane attack complex of complement.

By a "pathogen expressing CD59 or a CD59-like molecule" is meant a microbe (e.g., a virus, bacteria, or microbial parasite) that contains CD59 or a CD59-like molecule on its outer membrane. The term is meant to include viruses which capture CD59 molecules from host cells during the process of maturation by budding, as well as pathogens that contain genes encoding for CD59 or CD59-like molecules.

By "intermedilysin" or "ILY" is meant a polypeptide having the activity of a *Streptococcus intermedins* intermedilysin polypeptide. ILY can be purified from *Streptococcus intermedius* or can be produced recombinantly. An exemplary Genbank Accession number corresponding to the nucleic acid sequence of ILY is AB029317, and an exemplary Genbank Accession number corresponding to the polypeptide sequence of ILY is BAE16324.

By "domain 4 of ILY polypeptide," "ILY domain 4 polypeptide," or "ILYd4" is meant a protein containing a peptide sequence

```
GALTLNHDGAFVARFYVYWEELGHADGYETIRSRWSNGYNRGAGHYSTTLRFKGNVRNIRVKV
GLATGLAWEPWRLIYSKNDLPLVPQRNIS
TWGTTLPQFEDKVVKDNTD (SEQ ID NO: 1) or
```

```
RNIRVKVLGATGLAWEWPRLIYSKNDLPLVPQRNISTWGTTLPQFEDKVVKDNTD (SEQ ID NO:2). This term explicitly excludes full length ILY.
```

By "fragment" is meant a portion of a polypeptide that contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more of the entire length of the polypeptide. A fragment may contain at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 114 amino acids or more.

By "ILY domain 4 activity" is meant the activity of a peptide that antagonizes human CD59 but does not directly cause substantial lysis of human red blood cells (RBCs) in the lysis assay described herein, does not independently cause complement dependent cytolysis (CDC), and does not independently cause antibody-dependent cellular cytotoxicity (ADCC) and apoptosis.
By "antagonism of hCD59" is meant a decrease of hCD59 binding to complement proteins C8 and/or C9, resulting in increased formation of the membrane attack complex of complement (MAC).

By "substantially pure antibody" is meant an antibody that has been separated from the components that naturally accompany it. Typically, the antibody is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably the antibody is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, pure.

An antibody is substantially free of naturally associated components when it is separated from those contaminants that accompany it in its natural state. Thus, an antibody that is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure antibodies include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes.

By "therapeutic antibody" is meant an antibody, or functional derivatives thereof, for the treatment of a proliferative or pathogenic disease.


Methods to determine identity are available in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program
package (Devereux et al., Nucleic Acids Research 12:387, 1984), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol. 215:403, 1990). The well known Smith Waterman algorithm may also be used to determine identity. The BLAST program is publicly available from NCBI and other sources (BLAST Manual, Altschul, et al., NCBI NLM NIH Bethesda, Md. 20894). Searches can be performed in URLs such as the following: http://www.ncbi.nlm.nih.gov/BLAST/unfinishedgenome.html; or http://www.tigr.org/cgi-bin/BlastSearch/blast.cgi. These software programs match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "complementarity determining region (CDR)" is meant the three hypervariable sequences in the variable regions within each of the immunoglobulin light and heavy chains. By "framework region (FR)" is meant the sequences of amino acids located on either side of the three hypervariable sequences (CDR) of the immunoglobulin light and heavy chains.

Common structural features among the variable regions of antibodies, or functional fragments thereof, are well known in the art. The DNA sequence encoding a particular antibody can generally be found following well known methods such as those described in Kabat, *et al.* 2987 *Sequence of Proteins of Immunological Interest* U.S. Department of Health and Human Services, Bethesda MD, which is incorporated herein as a reference. In addition, a general method for cloning functional variable regions from antibodies can be found in Chaudhary, V.K., *et al.*, 1990 Proc. Natl. Acad. Sci. USA 87:1066, which is incorporated herein as a reference.
**Detailed Description**

In general, the invention features proteins including an antibody, or functional derivatives thereof, that bind hCD59 and have the activity of domain 4 of the *Streptococcus intermedius* intermedilysin (ILY) protein. The following description focuses on antibodies and functional derivatives but is also generally applicable to recombinant proteins (e.g., fusion proteins) including the antibodies and functional derivatives, unless otherwise noted. In order to prevent the independent induction of CDC and ADCC, the antibodies of the invention can bind the same hCD59 epitope as ILYd4 and/or contain modifications that disrupt the interaction between the antibody and complement.

The antibodies of the invention antagonize hCD59 by decreasing the human CD59 binding to complement proteins C8 and/or C9, resulting in increased formation of MAC. Furthermore, the antibodies of the invention do not cause apoptosis or cell death of cells absent the binding of a second antibody to the cells. The second antibody can be a therapeutic antibody, or it can be a natively produced antibody.

1. **Antibodies of the invention**

   The invention features proteins, such as antibodies, and functional derivatives thereof, that bind hCD59 with ILYd4 activity. The development of antibodies is well understood in the art. In particular, the antibodies of the invention antagonize hCD59 without independently inducing apoptosis or ADCC. Such antibodies can be modified so that they do not directly interact with complement. For example, the antibodies might lack the Fc region (e.g., Fabs), or the Fc region can be modified (e.g., through mutation) not to interact with complement.

   Two Fabs were identified that compete with ILYd4 for binding to hCD59, each of which is set forth below.
Fab-2 Clone3

VL-CL(Nucleic Acid)

5'

gagctcgtgttgaagcagtctccagccacctgctctctcgcagtgctgctgctgtgtctgctgctgctgcagtgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctg
SGGTALGCLVKDYFPEPVTVSWSGALTSGVHTFPALQLSSGLYSLSVVTYPSGSLGTQTYICNVNHKPSNTKVSDKVEPKSCDKTS (SEQ ID NO:6)

5  Fab-3 Clone 7
VL-CL(Nucleic Acid)
5'-
cagctgcccctgacgcctccctcctctgtgtctgggtctcctggacagtcgatcaccatctcctgcactggaacca
gcaagtgacgttggtggtttataactatgtctctctgtagcaacacaccagagccacacattatga
tgtacgaatccgccteaggggtttaaatagetctctctcttccagaagctgctcaatctggctgcatctctctctctctctctcagtcgatcaccatctcctgcactggaacca
gcaagtgacgttggtggtttataactatgtctctctgtagcaacacaccagagccacacattatga
tgtacgaatccgccteaggggtttaaatagetctctctcttccagaagctgctcaatctggctgcatctctctctctctctctcagtcgatcaccatctcctgcactggaacca
gcaagtgacgttggtggtttataactatgtctctctgtagcaacacaccagagccacacattatga
tgtacgaatccgccteaggggtttaaatagetctctctcttccagaagctgctcaatctggctgcatctctctctctctctctcagtcgatcaccatctcctgcactggaacca
gcaagtgacgttggtggtttataactatgtctctctgtagcaacacaccagagccacacattatga
tgtacgaatccgccteaggggtttaaatagetctctctcttccagaagctgctcaatctggctgcatctctctctctctctctcagtcgatcaccatctcctgcactggaacca
gcaagtgacgttggtggtttataactatgtctctctgtagcaacacaccagagccacacattatga
tgtacgaatccgccteaggggtttaaatagetctctctcttccagaagctgctcaatctggctgcatctctctctctctctctcagtcgatcaccatctcctgcactggaacca
gcaagtgacgttggtggtttataactatgtctctctgtagcaacacaccagagccacacattatga
tgtacgaatccgccteaggggtttaaatagetctctctcttccagaagctgctcaatctggctgcatctctctctctctctctcagtcgatcaccatctcctgcactggaacca
5

VL-CL(Amino Acid)
(three CDRs underlined; variable region in boldface)

20 OLALTOPPSVSGPOSITISCTGTSSDVGGYNYVSYOOPKGAP
KLMIYDVSNRPSGVSNRFSGSKGNTASLTISGLOADEDEADYYCCSY
AGSSTLVFGGTTLVNLGOKAPSVTLPSSCOANAKTLVCLISD
FYPGAVTVAKWADGSPVAGYVETTPSFIGSNNKYAASSYLSLTPEQW
KSHRSYSCQVTHEGSTVEKTPAPEC (SEQ ID NO:8)

25

VH-CHL (Nucleic Acid)

5'-
gaggtgcagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctgtgggtggtggtggaagaagctttggtgcacagctg
VH-CHl (Amino Acid)
(three CDRs underlined; variable region in boldface)

EVOLVESGAEVKKPGASVKVSCKASGYTFTSYDINWVRQATGQGL
EWMGWMNPNSGNTGYAOKFOGRVTMTTRNTSISTAYMELSSLRSE
DTAVYYCARGRGFDWLKNNFDYWQQGTLYTVSPASTKGPVFPLAPSS
KSTSGGTAAALGCLVKDYFPEPVTVSWSGALTSGVHTPAVLQSSGLY
SLSSWTVPSLGTQTYICNVNHKPSNTKVDKKEPKSCDKT

The invention, in addition to featuring the above Fabs, also features methods of producing these Fabs using, e.g., plasmids and host cells containing, e.g., nucleic acids with the sequences of SEQ ID NOs: 3, 5, 7, and 9). Additionally, the invention features antibodies, or functional fragments thereof that contain at least one (e.g., 2, 3, 4, 5, or preferably 6) of the CDRs of the Fab-2 and Fab-3 proteins set forth above. These methods are discussed in more detail below.

Murine myeloma cell lines useful for the production of monoclonal antibodies can be obtained, for example, from the American Type Culture Collection (ATCC; Manassas, VA). Human myeloma and mouse-human heteromyeloma cell lines have also been described (Kozbor et al., *J. Immunol*, 133:3001-3005, 1984; Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, pp. 51-63, 1987).

The antibody may be prepared in any mammal, including mice, rats, rabbits, goats, camels, and humans. The antibody may be a member of one of the following immunoglobulin classes: IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof, and preferably is an IgG antibody. While the preferred animal for producing monoclonal antibodies is mouse, the invention is not so limited; in fact, human antibodies may be used and may prove to be preferable. Such antibodies can be obtained by using human hybridomas (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss Inc., p. 77-96, 1985). In the present invention, techniques developed for the production of chimeric antibodies by splicing the genes from a mouse antibody molecule of

The invention also includes antibody functional derivatives that have ILYd4 activity. Functional derivatives include polypeptides with amino acid sequences substantially identical to the amino acid sequence of the variable or hypervariable regions of an antibody of the invention. Functional derivatives have antigen binding characteristics comparable to those of the antibodies, and include, for example, chimeric, humanized, fully human, and single chain antibodies or antibody fragments, antigen-binding antibody fragments, and antibodies fused to a second protein, or otherwise derivatized as is known in the art. Methods of producing such functional derivatives are disclosed, for example, in PCT Publication No. WO93/21319; European Patent No. 0 239 400 Bl; PCT Publication No. WO89/09622; European Patent Application No. 0338,745; European Patent Application No. 0332424; U.S. Patent No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855, 1984; Boulianne et al., *Nature*, 312:643-646, 1984; Neuberger et al., *Nature*, 314:268-270, 1985, Smith et al., *FASEBJ*. 19:331-341 (2005); and U.S Patent Application Publication Nos. 20050208043 and 20050276802, each of which is hereby incorporated by reference.

Chimeric antibodies preferably have constant regions derived substantially or exclusively from human antibody constant regions and variable regions derived substantially or exclusively from the sequence of the variable region from a mammal other than a human. Methods for humanizing non-human antibodies are well known in the art (for reviews see Vaswani and Hamilton, *Ann. Allergy Asthma Immunol*, 81:105-119, 1998 and Carter, *Nature Reviews Cancer*, 1:118-129, 2001). Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain.
Humanization can be essentially performed following the methods known in the art (Jones et al., *Nature*, 321:522-525, 1986; Riechmann et al., *Nature*, 332:323-329, 1988; and Verhoeyen et al., *Science*, 239:1534-1536, 1988), by substituting rodent CDRs or other CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species (see for example, U.S. Patent No. 4,816,567). In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies (Presta, *Curr. Op. Struct. Biol*, 2:593-596, 1992).

Additional methods for the preparation of humanized antibodies can be found in U.S. Patent Nos. 5,821,337, and 6,054,297, and Carter, (supra) which are all incorporated herein by reference. The humanized antibody is selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG\(_1\), IgG\(_2\), IgG\(_3\), and IgG\(_4\). Where cytotoxic activity is not needed, such as in the present invention, the constant domain is preferably of the IgG\(_2\) class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.


Suitable mammals other than a human include any mammal from which monoclonal antibodies may be made. Examples of mammals other than a human include, for example a rabbit, rat, mouse, horse, goat, or primate; a mouse is preferred.
"Functional derivatives" of antibodies include single-chain antibody fragments, also known as single-chain antibodies (scFvs). Single-chain antibody fragments are recombinant polypeptides which typically bind antigens or receptors; these fragments contain at least one fragment of an antibody variable heavy-chain amino acid sequence (V_H) tethered to at least one fragment of an antibody variable light-chain sequence (V_L) with or without one or more interconnecting linkers. Such a linker may be a short, flexible peptide selected to assure that the proper three-dimensional folding of the V_L and V_H domains occurs once they are linked so as to maintain the target molecule binding-specificity of the whole antibody from which the single-chain antibody fragment is derived. Generally, the carboxyl terminus of the V_L or V_H sequence is covalently linked by such a peptide linker to the amino acid terminus of a complementary V_L and V_H sequence. Single-chain antibody fragments can be generated by molecular cloning, antibody phage display library or similar techniques. These proteins can be produced either in eukaryotic cells or prokaryotic cells, including bacteria.

Single-chain antibody fragments contain amino acid sequences having at least one of the variable regions or CDRs of the whole antibodies described in this specification but are lacking some or all of the constant domains of those antibodies. These constant domains are not necessary for antigen binding but constitute a major portion of the structure of whole antibodies. Single-chain antibody fragments may therefore overcome some of the problems associated with the use of antibodies containing part or all of a constant domain. For example, single-chain antibody fragments tend to be free of undesired interactions between biological molecules and the heavy-chain constant region, or other unwanted biological activity. Additionally, single-chain antibody fragments are considerably smaller than whole antibodies and may therefore have greater capillary permeability than whole antibodies, allowing single-chain antibody fragments to localize and bind to target antigen-binding sites more efficiently. Also, antibody fragments can be produced on a relatively large scale in prokaryotic cells, thus facilitating their production. Furthermore,
the relatively small size of single-chain antibody fragments makes them less likely than whole antibodies to provoke an immune response in a recipient.

"Functional derivatives" further include fragments of antibodies that have the same or comparable binding characteristics to those of the whole antibody. Such fragments may contain one or both Fab fragments or the F(\(\text{ab}'\))\(_2\) fragment (e.g., Fv, Fab, Fab', F(\(\text{ab}'\))\(_2\) or other antigen-binding subsequences of antibodies). Preferably the antibody fragments contain all six CDRs of the whole antibody, although fragments containing fewer than all of such regions, such as three, four or five CDRs, may also be functional.

Further, the functional derivatives may be or may combine members of any one of the following immunoglobulin classes: IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof.

Derivatives of antibodies are prepared by methods known in the art. For example, fragments of antibodies may be prepared enzymatically from whole antibodies. Preferably, equivalents of antibodies are prepared from DNA encoding such equivalents. DNA encoding fragments of antibodies may be prepared by deleting all but the desired portion of the DNA that encodes the full-length antibody.

DNA encoding chimeric antibodies may be prepared by recombining DNA substantially or exclusively encoding human constant regions and DNA encoding variable regions derived substantially or exclusively from the sequence of the variable region of a mammal other than a human. DNA encoding humanized antibodies may be prepared by recombining DNA encoding constant regions and variable regions other than the CDRs derived substantially or exclusively from the corresponding human antibody regions and DNA encoding CDRs derived substantially or exclusively from a mammal other than a human.

Suitable sources of DNA molecules that encode fragments of antibodies include cells, such as hybridomas, that express the full-length antibody. The fragments may be used by themselves as antibody derivatives, or may be recombined into derivatives, as described above.
The following methods are used to identify antibodies, and functional
derivatives thereof, of the invention.

Antibodies of the invention can bind the same epitope as ILYd4.

Erythrocytes from humans or hCD59RBC transgenic mice that
specifically express only human CD59 are pre-incubated with a candidate
antibody (for 10 minutes at room temperature) then incubated with ILYd4, or
mouse anti-hCD59 monoclonal antibody (0.2 µg/ml) (BRIC 229, Bristol, Great
Britain) at room temperature for 30 minutes followed by washing. The cells
are further incubated with FITC-conjugate anti-His antibody or a FITC-
conjugated corresponding secondary antibody, respectively. The cells are
washed with PBS three times followed by measuring the fluorescence intensity
using a FACScan (Becton Dickinson, Franklin Lakes, NJ). Cells pre-incubated
with antibodies of the invention that bind to the same epitope as ILYd4 do not
stain with either ILYd4 plus FITC anti-HIS or BRIC 229 plus FITC secondary
antibody. Cells not pre-incubated with an antibody of the invention only stain
with BRIC 229 plus FITC secondary antibody.

Antibodies of the invention can functionally interact with the same epitope
of hCD59 as ILYd4.

Erythrocytes from humans or hCD59RBC transgenic mice are pre-
incubated with a candidate antibody and ILYd4 (in different concentrations) at
room temperature for 10 minutes. Full length ILY is added to induce
hemolysis. Antibodies of the invention block full length ILY-mediated
hemolysis, and therefore, functionally block ILY access to human CD59 in the
cells.

Antibodies of the invention induce complement-mediated lysis in human
erythrocytes.

The sensitivity of human erythrocytes to human complement-mediated
lysis in the presence or absence of a candidate antibody compared to ILYd4 is
assessed by two methods: (1) cobra venom factor (CVF, 5 mg/L) lysis assay; and (2) anti-human erythrocyte antibody (Ab)-sensitized erythrocyte method, as described in Hu et al. Nat Med 14:98-103 (2008), which is hereby incorporated by reference in its entirety. Human serum (HS, 50% v/v) is used as a source of complement, and heat-inactivated human serum (HIS, 50%, v/v) is used as a control. Combined with the anti-human erythrocyte antibody, antibodies of the invention enhance complement-mediated lysis to a similar degree as ILYd4. Furthermore, when tested alone, antibodies of the invention do not induce hemolysis.

Antibodies of the invention do not alone cause apoptosis.

Candidate antibodies are incubated with hCD59 expressing FL lymphoma cells for 48 hours, and apoptosis is assessed (e.g., using terminal nucleotidyl transferase-mediated nick end labeling (TUNEL) assays (Roche) according to the manufacturer's instructions). Similarly to ILYd4, the antibodies of the invention do not induce apoptosis in this assay.

Antibodies of the invention do not alone cause ADCC.

FL lymphoma cells are stained with the green fluorescence cytoplasmic dye 5- and (6)-carboxyfluorescein diacetate, succinimidylester (CSFE; Molecular Probes, Inc.). After washing, labeled-FL cells are incubated with either a candidate antibody or ILYd4. Peripheral blood mononuclear effector (E) cells (PBMCs) are mixed with target (T) cells at 50:1 E/T cell ratio and incubated for 4 hours at 37°C in 5% CO2. The cells are centrifuged and assayed for dye release with Infinite F200 (Tecan). The percentage of specific lysis is determined as: [(test release - spontaneous release) / (total release - spontaneous release)] X 100. Dead cells will be stained with propidium iodide (50 Ag/mL; Sigma-Aldrich) and analyzed on a FACScalibur instrument to control the data obtained by measuring dye release. Similarly to ILYd4, the antibodies of the invention will not alone induce ADCC.
Additional experimental methods for measuring ILYd4 activity are described, e.g., in International Application Nos. PCT/US2008/004191 and PCT/US2008/004193, each of which is incorporated by reference in their entirety.

II. Methods of Administration

Therapy according to the invention may be performed alone or in conjunction with another therapy and may be provided at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment optionally begins at a hospital so that the doctor can observe the therapy's effects closely and make any adjustments that are needed, or it may begin on an outpatient basis. The duration of the therapy depends on the type of disease or disorder being treated, the age and condition of the patient, the stage and type of the patient's disease, and the patient response to the treatment. Additionally, a person having a greater risk of developing a proliferative or pathogenic disease may receive treatment to inhibit or delay the onset of symptoms.

Routes of administration for the various embodiments include, but are not limited to, topical, transdermal, transcranial, nasal, and systemic administration (such as, intravenous, intramuscular, subcutaneous, inhalation, rectal, buccal, vaginal, intraperitoneal, intraarticular, ophthalmic, otic, or oral administration). As used herein, "systemic administration" refers to all nondermal routes of administration, and specifically excludes topical and transdermal routes of administration.

The antibodies of the invention may be administered orally in the form of tablets, capsules, elixirs, or syrups, or rectally in the form of suppositories. The antibodies may also be administered topically in the form of foams, lotions, drops, creams, ointments, emollients, or gels. Parenteral administration of a compound is suitably performed, for example, in the form of saline solutions or with the compound incorporated into liposomes.
Dosages

The dosage of the antibody of the invention depends on several factors, including: the administration method, the disease to be treated, the severity of the disease, whether the disease is to be treated or prevented, and the age, weight, and health of the person to be treated. Additionally, pharmacogenomic (the effect of genotype on the pharmacokinetic, pharmacodynamic, or efficacy profile of a therapeutic) information about a particular patient may affect the dosage used.

Continuous daily dosing with an antibody of the invention may not be required. A therapeutic regimen may require cycles, during which time an antibody is not administered, or therapy may be provided on an as needed basis during periods of acute disease. The appropriate dosage and treatment regimen can be determined by one skilled in the art.

III. Indications

The antibodies of the invention are useful for treating any disease characterized by undesired hCD59 activity.

The antibodies of the invention are useful for the treatment of cancers and other disorders characterized by hyperproliferative cells (proliferative diseases). In these embodiments, the antibodies of the invention can be administered directly to a CD59-expressing neoplasia, or systemically to a subject having a neoplasia. Preferably, the antibodies of the invention will be administered with an anti-cancer therapeutic antibody.

In a separate embodiment, an antibody of the invention can be administered to a patient diagnosed with a proliferative disorder that is not characterized by the cell surface expression of CD59. Here, the compounds are administered in conjunction with an anti-cancer therapeutic antibody to prevent resistance to the therapeutic antibody based treatment.

Antibody therapy may be performed alone or in conjunction with another therapy (e.g., surgery, radiation therapy, chemotherapy, immunotherapy, anti-angiogenesis therapy, or gene therapy). Therapy may be
given in on-and-off cycles that include rest periods so that the patient's body has a chance to recovery from any as yet unforeseen side-effects.

Cancers include, without limitation, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblasts leukemia, acute promyelocyte leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, and multiple myeloma), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovia, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodenroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma).

The antibodies of the invention are also useful for the treatment of pathogens characterized by expression of CD59 or CD59-like molecules. For example, the antibodies of the invention are useful to treat viruses containing CD59 in their envelope, where the CD59 is captured during maturation by budding from a host cell expressing CD59 (e.g., human cytomegalovirus, HCMV, human T-cell leukemia virus type 1, HIV-I, simian immunodeficiency virus, Ebola virus, influenza virus, and vaccinia virus (a poxvirus) (Stoiber et al.)
et al. J Infect Dis 176:339-347 (1997)). The antibodies of the invention are
also useful for the treatment of patients infected with parasites or viruses that
directly express CD59 or a CD59-like molecule, such as Herpesvirus saimiri
virus, Schistosoma mansoni, and Naegleria fowleri (Parizade et al. J Exp Med
In these embodiments, an antibody of the invention can be administered
directly to a tissue infected with a pathogen or systemically to a subject
infected with a pathogen. Preferably, the antibody of the invention will be
administered with an antibody specific for the CD59- or CD59-like expressing
pathogen. Therapy may be performed alone or in conjunction with other anti-
microbial therapies.

IV. Therapeutic antibodies

The invention features the treatment of proliferative or pathogenic
diseases through the administration of the antibodies of the invention in
combination with a therapeutic antibody. Administration of the antibodies of
the invention may sensitize the cells targeted by antibody therapy to
complement-mediated cell lysis. Examples of therapeutic antibodies for use in
the methods of the invention for treating proliferative disorders are set forth in
Table 1.
Table 1: Relevant antibodies that are applied for cancer therapy

<table>
<thead>
<tr>
<th>Antibody name</th>
<th>Target antigen</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>B-cell lymphomas</td>
</tr>
<tr>
<td>MT201 and 17-IA</td>
<td>Ep-CAM</td>
<td>Colorectal and breast cancer</td>
</tr>
<tr>
<td>Herceptin</td>
<td>HER2</td>
<td>Breast cancer and lymphomas</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>CD52</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Lym-1</td>
<td>HLA-DR</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>VEGF</td>
<td>Cancer of the colon or rectum</td>
</tr>
<tr>
<td>IL-2Rα-directed monoclonal antibodies</td>
<td></td>
<td>T-cell leukemia</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>CD52</td>
<td>CLL</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>EGFR</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>EGFR</td>
<td>Colorectal cancer, head and neck cancer</td>
</tr>
</tbody>
</table>

Examples of therapeutic antibodies for use in the methods of the invention for treating HIV are the humanized antibody hNM-01 (Nakamura et al., Hybridoma, 19:427 (2000)), and the humanized KD-247 antibody (Matsushita et al., Hum Antibodies 14:81-88 (2005)). An example for treating RSV is Palivizumab. Other antibodies (preferably humanized antibodies) can be developed using any epitope of HIV or other CD59-expressing pathogen using standard methods.

In addition to the antibodies and indications listed above, the invention features co-administration of the antibodies of the invention in combination with any treatment that would be enhanced by inhibition of human CD59 activity.
V. Experimental Results

A human naive Fab library was screened against immobilized CD59. The binding of selected Fab to CD59 was tested for inhibition by D408Y (ILY domain 4 protein). Initially, plates were coated D408Y, CD59 was added, and the interaction D408Y and CD59 was detected using a specific anti-CD59 antibody. Subsequently, the first three rounds of screening were performed by directly coating CD59 and the 4th round of screening was performed by coating lysed CD59-expressing-red blood cells. From the 4th eluate, 40 clones were identified and phage ELISA was performed using plates coated with CD59. Subsequently, 32 positive clones were identified. Three unique Fabs (Fab-1, 2, and 3) were revealed by DNA sequencing from 32 positive clones. Meanwhile, another three Fabs (Fab 4, 5, and 6) were also identified from negative clones. Since there are unexpected internal 'TAG' amber stop codons within the genes of Fab-1 and Fab-6, 4 Fabs (Fabs 2, 3, 4, and 5) were subjected to soluble expression and soluble ELISA. The binding signals of two of the positive clones (clone 3 and 7; identified as Fab-2 and Fab-3, respectively) in the presence of CD59 were studied. The amino acid sequences for the variable domain heavy and light chains for Fab-2 and Fab-3 are set forth above.

Materials and Reagents

Target protein: CD59
Ligand: D408Y (ILY domain 4 protein)
Anti-CD59 antibody
Two tubes of red blood cells
Pre-made human naive Fab library (HuFabL)
M13K07 helper phage
Anti-HA mAb-HRP conjugate
Sequencing primers:
(VL-primer): 5'-AAGACAGCTATCGCGATTGCAG^' (SEQ ID NO:21)
(VH-primer): 5'-ACCTATTGCCTACGGCAGCCG-S' (SEQ ID NO:22)
LB Medium: Per liter: 10 g Bacto-Tryptone, 5 g yeast extract, 5 g NaCl.
LB Plates: LB medium + 15 g/L agarose.

2TY medium: 16 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl, made up to 1 L in deionized H₂O, and adjusted to pH 7.0 with 1 M NaOH. Autoclave.

2TY-G: 2TY containing 1% (w/v) glucose

2TY-AG: 2TY containing 100 µg/mL ampicillin (AMP) and 1% (w/v) glucose

2TY-A: 2TY containing 100 µg/mL ampicillin (AMP)

2TY-AK: 2TY containing 100 µg/mL ampicillin, 50 µg/mL kanamycin

TYE agar plates: Add 15 g agar to 1 L 2TY medium, autoclave, when cool, add glucose to 1% (w/v) and AMP.

PBS: Per liter: 8 g NaCl, 0.2 g KCl, 1.7 g Na₂HPO₄, 0.163 g KH₂PO₄, pH to 7.4 with HCl.

Blocking buffer: 0.1 M NaHCO₃ (pH 8.6), 5 mg/mL BSA, 0.02% NaN₃, 0.1 µg /mL streptavidin. Filter sterilize, store at 4°C.

Coating Buffer: 0.1 MNaHCO₃ (pH 8.6).

Acidic eluting buffer: 0.2 M Glycine-HCl (pH2.2), 1 mg/mL BSA.

Phage precipitant (PEG/NaCl): 20% (w/v) polyethylene glycol-8000, 2.5 M NaCl. Autoclave, store at room temperature.

TE buffer: 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

50% glycerol/PBS: Equal mix of PBS and Glycerol.

HRP-conjugated anti-M13 antibody.

Substrate of HRP: OPD from Sigma.

Substrate buffer: 2.6 g Citric acid and 6.9 g Na₂HPO₄ up to 500 mL. Adjust pH to 5.0 if needed.

Experimental procedure:

1. Prepared M13KO7 helper phage

Prepared helper phage by infecting log-phase TGl bacterial cells with M13K07 phage at different dilutions for 30 min at 37°C and plated in top agar onto 2TY plates.

Inoculated a small plaque in 3 mL liquid 2TY medium. Added 30 µL overnight culture of TGl and grow for 2 hours at 37°C.
Diluted the culture in 1 L 2TY medium and grow for 1 hour. Added kanamycin to 50 µg/mL and grow for 16 hours at 37°C.

Removed cells by centrifugation (10 min at 5000 g) and precipitated phage from the supernatant by addition of 0.25 vols of phage precipitant. After 30 min incubation on ice, collected the phage particles by centrifugation during 10 min at 5000 g. Resuspended the pellet in 5 mL PBS and sterilized through a 0.22 µm filter.

Titrated the helper phage by determining the number of plaque-forming units (pfu) on 2TY plates with top-agar layers containing 100 µL TGl (saturated culture) and dilutions of phage. Diluted the phage stock solution to 1 × 10^13 pfu/mL and stored in small aliquots at -20°C.

2. Prepare Library Phages
Inoculated 500 mL 2TY-G with the library glycerol stock and incubate at 37°C, shaking, at 250 rpm until the optical density at 600 nm reached 0.8-0.9.

Added M13KO7 helper phage to a final concentration of 5 × 10^9 pfu/mL, and incubated for 30 min at 37°C without shaking, followed by 30 min with gentle shaking (200 rpm), to allow phage infection.

Recovered the cells by centrifugation at 2200 g for 15 min and re-suspended the pellet in the same volume of 2TY-AK. Incubated overnight at 30°C with rapid shaking (300 rpm).

Pelleted the cells by centrifugation at 7000 g for 15 min at 4°C and recover the supernatant containing the phage into pre-chilled 1-L bottles.

Added 0.3 vols of phage precipitant. Mixed gently and allowed the phage to precipitate for 1 hour on ice.

Pelleted the phage by centrifugation twice at 7000 g for 15 min in the same bottle at 4°C. Removed as much of the supernatant as possible and re-suspended the pellet in 8 mL PBS.
Re-centrifuged the phage in smaller tubes at 12,000 g for 10 rain and recovered the phage via the supernatant. Ensured that any bacterial pellet that appeared was left undisturbed.

Titered phage stocks by infecting TGl cells with dilutions of phage stock, plated to 2TY-AG, incubated, and enumerated the ampicillin resistant colonies that appeared. The phage was then be stored in aliquots at 4°C for screening.

3. Panning

Coated the target proteins directly by incubating at 37°C for 2 hours.

Blocked panning plates (Nunc) with the blocking buffer at 4°C overnight.

Washed blocked wells 6 times with 0.1% PBST (PBS with 0.1% Tween 20(V/V)).

Mixed equal volumes of the phage library and 4% PBSM (PBS containing 4% milk) and added into panning wells. Incubated at room temperature for 60 min.

Washed 10-20 times with PBSMT (PBS containing 2% milk, a certain percent of Tween-20).

Added 200 µL acidic eluting buffer and incubated 5 min at room temperature. Transferred the supernatant containing the phages to a new tube and neutralize with Tris-HCl buffer.

Infected a fresh exponentially growing culture of *Escherichia coli* TGl with the eluted phages and amplified half of them for further rounds of selection. Stored the remaining eluate at 4°C.

4. Phage ELISA

1) Prepared single clones of antibody-displaying phages

Inoculated single clones of the eluate from the 4th round into 5 mL of 2YT-AG medium and incubated at 37°C overnight.
Prepared the glycerol stock for each clone with the overnight culture. Inoculated 100 µL of overnight culture into 20 mL of 2YT-AG medium. Grew for a few hours at 37°C until the optical density at OD600 reached 0.4-0.5.

Added VCSM13 helper phage at a multiplicity of infection of 20 (i.e., the number of phage particles/host cell). Infected the cells by incubating 30 min at 37°C without shaking and another 30 min with shaking.

Collected infected cells by centrifugation (10 min at 5000 g). Resuspended in 2YT-AK and grew the culture for 16 hours at 30°C.

Precipitated phage particles from the supernatant as described above.

Re-suspended the phage pellet in 1mL PBS and removed cellular debris by centrifugation (10 min at 5000 g).

To remove Ab fragments not associated to phage particles, carried out a second precipitation. Re-suspended the phage pellet in 250 µL PBS, clarified again by centrifugation.

2) Phage ELISA by coating the target proteins (CD59 or red cell membrane) directly.

Coated 100 µL target protein (10µg/mL) in coating buffer by incubating at 4°C overnight.

Shook out the coating solution and washed once with the washing buffer. Blocked all wells with 250 µL of blocking buffer. In order to test the binding of each selected sequence to BSA-coated plastic surface, enough uncoated wells were also blocked. Incubated the blocked plates 1-2 hours at 4°C.

Shook out the blocking buffer and washed the plate six times with the washing buffer.

Added 100 µL of phage solution in washing buffer per well. Incubated at room temperature for 1-2 hours.

Washed 6 times with the washing buffer. Diluted HRP-conjugated anti-M13 antibody (GE healthcare) 1:5,000 in blocking buffer. Added 100 µL of diluted conjugate per well and incubated at room temperature for 1 hour.
Washed 6 times with the washing buffer. Prepared the HRP substrate solution as follows: a stock solution of OPD can be prepared in advance by dissolving 22 mg OPD (Sigma) in 100 mL of 50 mM sodium citrate, pH 4.0. Filter-sterilize and store at 4°C. Immediately prior to the detection step, added 36 µL 30% H₂O₂ to 21 mL of OPD stock solution.

Added 100 µL of substrate solution per well and incubated at room temperature for 30 minutes.

Read plates using a microplate reader set at 490nm.

5. Soluble ELISA

Soluble expression in periplasm of HB2151 cells

Picked a single colony of HB2151 bacteria harboring the Fab expression vector from a LB plate and grew overnight at 37°C in 2TY.

Added 100 µL HB2151 culture to 50 mL 2TY and grew at 37°C (with shaking) to an OD600 of 0.6-0.8.

Infected HB2151 with phages of positive clones identified by phage ELISA. Inoculated 1 µL of phages into 200 µL aliquots of the log-phase HB2151 culture. Incubated for 30 min at 37°C (no shaking). Plated onto LB-AG plates and incubated overnight at 37°C.

Inoculated a single colony into 5mL aliquot of 2TY growth medium and grew overnight at 37°C (with gentle shaking).

Took a 250 µL aliquot of overnight culture from each well and transfer to 25 mL 2TY growth medium. Grew the cultures at 37°C until OD600 is approx 0.6.

Added 250 µL 2TY induction medium and grow overnight at 30°C. Collected cells via centrifugation at 3500 g for 10 min. Released soluble Fab by ultra-sonication of cell suspensions in 1/10 volume of PBS.

Centrifuged at 9000 g for 10 min and collected the supernatant containing soluble Fab.
2) Soluble Fab ELISA
Repeated steps outlined in 4-2.
Discarded the block solution and added 100 µL of supernatant containing soluble Fab. Incubated for 2 hours at room temperature.

Washed the wells 3 x with 200 µL PBST.
Added 100 µL of PBSM diluted HRP conjugate of rabbit anti Human Fab polyclonal antibody to each well and incubate for 1 hour at room temperature.
Washed the wells 3 x with 200 µL PBST.
Developed the HRP reaction using OPD and read optical density at 490 nM.

6. DNA Sequencing
Inoculated 2 µL glycerol stock TG I bacterial cells harboring the plasmid for each positive clone (determined by phage ELISA and/or soluble ELISA) into 5 mL LB-A medium (LB medium added with 100 µg/mL ampicillin). Grew overnight at 37°C with shaking.
Isolated plasmids for each positive clone from bacterial cells using a Plasmid Isolation Kit (e.g. Qiagen Miniprep kit).
Conducted DNA sequencing using "Vl-primer" and "VH-primer" as the primers.

7. Bioinformatics analysis
Translated returned sequences with professional software (Vector NTI®, Version 10). Aligned the protein sequences. Grouped the clones that encoded the same protein sequences.

Results
Validation of interaction between CD59 and D408Y
40 µg/mL D408Y was coated and 2.5-40 µg/mL CD59 added in a validation ELISA. As shown in Table 1, with 40 µg/mL CD59 a positive
binding signal was observed. Therefore, 40 µg/mL CD59 was coated in the first round of screening.

Table 1
The result of validation ELISA

<table>
<thead>
<tr>
<th>CD59 (µg/mL)</th>
<th>OD490</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.203</td>
</tr>
<tr>
<td>20</td>
<td>0.075</td>
</tr>
<tr>
<td>10</td>
<td>0.054</td>
</tr>
<tr>
<td>5</td>
<td>0.05</td>
</tr>
<tr>
<td>2.5</td>
<td>0.048</td>
</tr>
<tr>
<td>0</td>
<td>0.048</td>
</tr>
</tbody>
</table>

Direct coat 10 µg/mL CD59 | 0.644

The Panning:
As shown in the Table 2, only about 4,000 phages were eluted in the first round of screening. After 4 rounds of screening, the enriching effect was observed by reducing the enriching factor.

Table 2
Summarization of the panning process

<table>
<thead>
<tr>
<th>Round</th>
<th>Conditions</th>
<th>Input</th>
<th>Output</th>
<th>Enriching factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Target protein: 40 µg/mL CD59 Washing: 10 times with 0.1% Tween-20 PBST Elution: acidic elution buffer</td>
<td>$1.7 \times 10^{12}$</td>
<td>$4.8 \times 10^{4}$</td>
<td>$3.5 \times 10^{8}$</td>
</tr>
<tr>
<td>2nd</td>
<td>Target protein: 40 µg/mL CD59 Washing: 10 times with 0.3% Tween-20 PBST Elution: acidic elution buffer</td>
<td>$7.1 \times 10^{12}$</td>
<td>$6.9 \times 10^{4}$</td>
<td>$1.0 \times 10^{8}$</td>
</tr>
<tr>
<td>3rd</td>
<td>Target protein: 40 µg/mL CD59 Washing: 10 times with 0.3% Tween-20 PBST Elution: acidic elution buffer</td>
<td>$1.2 \times 10^{12}$</td>
<td>$4.3 \times 10^{4}$</td>
<td>$2.8 \times 10^{8}$</td>
</tr>
</tbody>
</table>
4th Target: 50µg/mL lysed CD59-expressing red-blood-cells
Control: 50µg/mL lysed non-CD59-expressing red-blood-cells
Washing: 10 times with 0.3% Tween-20 PBST
Elution: acidic elution buffer

Notes: Enriching factor=input/output

Phage ELISA

40 clones were randomly picked up from the 4th eluate and subjected to phage ELISA. As shown in Table 3, 32 positive clones were identified. When phage ELISA was conducted using membrane extracts of CD59 positive and negative red cells as antigens, the lower readings were observed for them in CD59 (-) wells than in CD59 (+) wells, as shown in Table 4.

Table 3

The result of the first phage ELISA

<table>
<thead>
<tr>
<th>Clone</th>
<th>Ag+ phage+</th>
<th>Ag-phage+</th>
<th>Clone</th>
<th>Ag+ phage+</th>
<th>Ag-phage+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.206</td>
<td>0.071</td>
<td>21</td>
<td>0.356</td>
<td>0.056</td>
</tr>
<tr>
<td>2</td>
<td>0.236</td>
<td>0.063</td>
<td>22</td>
<td>0.118</td>
<td>0.065</td>
</tr>
<tr>
<td>3</td>
<td>0.169</td>
<td>0.058</td>
<td>23</td>
<td>0.065</td>
<td>0.056</td>
</tr>
<tr>
<td>4</td>
<td>0.305</td>
<td>0.061</td>
<td>24</td>
<td>0.400</td>
<td>0.069</td>
</tr>
<tr>
<td>5</td>
<td>0.281</td>
<td>0.065</td>
<td>25</td>
<td>0.312</td>
<td>0.074</td>
</tr>
<tr>
<td>6</td>
<td>0.098</td>
<td>0.048</td>
<td>26</td>
<td>0.222</td>
<td>0.096</td>
</tr>
<tr>
<td>7</td>
<td>0.211</td>
<td>0.086</td>
<td>27</td>
<td>0.078</td>
<td>0.079</td>
</tr>
<tr>
<td>8</td>
<td>0.157</td>
<td>0.061</td>
<td>28</td>
<td>0.117</td>
<td>0.115</td>
</tr>
<tr>
<td>9</td>
<td>0.336</td>
<td>0.352</td>
<td>29</td>
<td>0.252</td>
<td>0.055</td>
</tr>
<tr>
<td>10</td>
<td>0.14</td>
<td>0.064</td>
<td>30</td>
<td>0.274</td>
<td>0.098</td>
</tr>
<tr>
<td>11</td>
<td>0.226</td>
<td>0.066</td>
<td>31</td>
<td>0.298</td>
<td>0.068</td>
</tr>
<tr>
<td>12</td>
<td>0.228</td>
<td>0.078</td>
<td>32</td>
<td>0.291</td>
<td>0.061</td>
</tr>
<tr>
<td>13</td>
<td>0.212</td>
<td>0.055</td>
<td>33</td>
<td>0.215</td>
<td>0.064</td>
</tr>
<tr>
<td>14</td>
<td>0.254</td>
<td>0.056</td>
<td>34</td>
<td>0.345</td>
<td>0.084</td>
</tr>
<tr>
<td>15</td>
<td>0.069</td>
<td>0.056</td>
<td>35</td>
<td>0.317</td>
<td>0.065</td>
</tr>
<tr>
<td>16</td>
<td>0.177</td>
<td>0.064</td>
<td>36</td>
<td>0.399</td>
<td>0.074</td>
</tr>
<tr>
<td>17</td>
<td>0.169</td>
<td>0.074</td>
<td>37</td>
<td>0.282</td>
<td>0.085</td>
</tr>
<tr>
<td>18</td>
<td>0.286</td>
<td>0.098</td>
<td>38</td>
<td>0.211</td>
<td>0.068</td>
</tr>
<tr>
<td>19</td>
<td>0.192</td>
<td>0.061</td>
<td>39</td>
<td>0.208</td>
<td>0.064</td>
</tr>
<tr>
<td>20</td>
<td>0.312</td>
<td>0.074</td>
<td>40</td>
<td>0.265</td>
<td>0.054</td>
</tr>
<tr>
<td>M13KO7</td>
<td>0.046</td>
<td>0.046</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 4
The result of the 2nd phage ELISA

<table>
<thead>
<tr>
<th>Clone</th>
<th>Ag⁺/phage⁺</th>
<th>Ag- phage⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD59(+)red cell</td>
<td>CD59(-)red cell</td>
</tr>
<tr>
<td>1</td>
<td>0.149</td>
<td>0.105</td>
</tr>
<tr>
<td>2</td>
<td>0.132</td>
<td>0.087</td>
</tr>
<tr>
<td>3</td>
<td>0.322</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>0.115</td>
<td>0.096</td>
</tr>
<tr>
<td>5</td>
<td>0.163</td>
<td>0.125</td>
</tr>
<tr>
<td>7</td>
<td>0.34</td>
<td>0.237</td>
</tr>
<tr>
<td>8</td>
<td>0.101</td>
<td>0.076</td>
</tr>
<tr>
<td>11</td>
<td>0.121</td>
<td>0.110</td>
</tr>
<tr>
<td>12</td>
<td>0.141</td>
<td>0.134</td>
</tr>
<tr>
<td>13</td>
<td>0.125</td>
<td>0.112</td>
</tr>
<tr>
<td>14</td>
<td>0.132</td>
<td>0.105</td>
</tr>
<tr>
<td>16</td>
<td>0.122</td>
<td>0.106</td>
</tr>
<tr>
<td>17</td>
<td>0.133</td>
<td>0.121</td>
</tr>
<tr>
<td>18</td>
<td>0.111</td>
<td>0.095</td>
</tr>
<tr>
<td>19</td>
<td>0.106</td>
<td>0.098</td>
</tr>
<tr>
<td>20</td>
<td>0.125</td>
<td>0.114</td>
</tr>
<tr>
<td>21</td>
<td>0.152</td>
<td>0.117</td>
</tr>
<tr>
<td>24</td>
<td>0.100</td>
<td>0.085</td>
</tr>
<tr>
<td>25</td>
<td>0.171</td>
<td>0.154</td>
</tr>
<tr>
<td>26</td>
<td>0.112</td>
<td>0.105</td>
</tr>
<tr>
<td>29</td>
<td>0.114</td>
<td>0.095</td>
</tr>
<tr>
<td>30</td>
<td>0.151</td>
<td>0.122</td>
</tr>
<tr>
<td>31</td>
<td>0.166</td>
<td>0.118</td>
</tr>
<tr>
<td>32</td>
<td>0.132</td>
<td>0.101</td>
</tr>
<tr>
<td>33</td>
<td>0.154</td>
<td>0.099</td>
</tr>
<tr>
<td>34</td>
<td>0.113</td>
<td>0.099</td>
</tr>
<tr>
<td>35</td>
<td>0.210</td>
<td>0.141</td>
</tr>
<tr>
<td>36</td>
<td>0.222</td>
<td>0.184</td>
</tr>
<tr>
<td>37</td>
<td>0.211</td>
<td>0.165</td>
</tr>
<tr>
<td>38</td>
<td>0.109</td>
<td>0.143</td>
</tr>
<tr>
<td>39</td>
<td>0.124</td>
<td>0.117</td>
</tr>
<tr>
<td>40</td>
<td>0.141</td>
<td>0.115</td>
</tr>
<tr>
<td>M13KO7</td>
<td>0.046</td>
<td>0.046</td>
</tr>
</tbody>
</table>

**DNA sequencing**

32 positive clones were DNA sequenced and three unique Fab sequences (Fab-1, 2, and 3) were identified. Three negative clones were also sequenced (clone 6, 9, and 10) and three additional Fab sequences were also identified (Fab-4, 5, and 6). Fab 1 and Fab 6 contained internal "TAG" amber
stop codons, which lead to no Fab expression in HB2151 host cells. Therefore, only four Fabs (Fabs 2, 3, 4 and 5) were subjected to soluble expression and soluble ELISA.

Table 5
Summary of DNA Sequencing

<table>
<thead>
<tr>
<th>Fab</th>
<th>Clones</th>
<th>Positions of Internal “TAG” stop codons</th>
<th>Positive clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>In VL and VH</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>In VL</td>
<td></td>
</tr>
</tbody>
</table>

**Other Embodiments**

Various modifications and variations of the described methods and compositions of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific desired embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in art are intended to be within the scope of the invention.

All publications, patent applications, and patents mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication was specifically and individually incorporated by reference.

What is claimed is:
CLAIMS

1. A protein comprising an antibody, or functional derivative thereof, that binds hCD59, inhibits binding of hCD59 to complement proteins C8 and/or C9, and does not independently induce antibody-dependent cellular toxicity.

2. The protein of claim 1, wherein said antibody, or functional derivative thereof, binds the same epitope of hCD59 as ILYd4.

3. The protein of claim 3, wherein said protein comprises said functional derivative of the antibody.

4. The protein of claim 3, wherein said functional derivative of the antibody is selected from a single chain antibody (scFv), a Fv, a Fab, a Fab', and a F(ab')₂.

5. The protein of claim 3, wherein said functional derivative of the antibody does not contain an Fc domain.

6. The protein of claim 1, wherein the antibody, or functional derivative thereof, comprises a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain comprises a complementarity determining region (CDRIl) comprising the sequence GASQSVSSSYLA (SEQ ID NO: 11), a CDRL2 comprising the sequence of ASSRATGIPD (SEQ ID NO: 12), and a CDRL3 comprising the sequence of YGSSPPVT (SEQ ID NO: 13); and wherein heavy chain variable domain comprises a CDRHI comprising the sequence of SYDIN (SEQ ID NO: 14), a CDRH2 comprising the sequence of WMNPNSSGNTGYAQKFQG (SEQ ID NO: 15), and a CDRH3 comprising the sequence of GKGSGGYNY (SEQ ID NO: 16).
7. The protein of claim 6, wherein light chain variable domain of said antibody, or functional derivative thereof, comprises the sequence of SEQ ID NO:4 and the heavy chain variable domain comprises the sequence of SEQ ID NO:6.

8. The protein of claim 1, wherein the antibody, or functional derivative thereof, comprises a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain comprises a CDRL1 comprising the sequence TGTSSDVGGYNYVS (SEQ ID NO: 17), a CDRL2 comprising the sequence of DVSNRPSGVSN (SEQ ID NO: 18), and a CDRL3 comprising the sequence of YAGSSTLV (SEQ ID NO: 19); and wherein heavy chain variable domain comprises a CDRH1 comprising the sequence of SYDIN (SEQ ID NO: 14), a CDRH2 comprising the sequence of WMNPNSSGNTGYA QKFQG (SEQ ID NO: 15), and a CDRH3 comprising the sequence of GRGFDWLKNFDY (SEQ ID NO:20).

9. The protein of claim 6, light chain variable domain of said antibody, or functional derivative thereof, comprises the sequence of SEQ ID NO:8 and the heavy chain variable domain comprises the sequence of SEQ ID NO:10.

10. A method for treating a proliferative disease in a patient in need thereof, said method comprising administering to said patient a protein of any one of claims 1-9 and a therapeutic antibody, wherein said protein and said therapeutic antibody are administered simultaneously, or within 14 days of each other, in amounts that together are sufficient to treat said proliferative disease.
11. The method of claim 10, wherein said therapeutic antibody is selected from a group consisting of rituximab, MT201, 17-IA, herceptin, alemtuzumab, lym-1, bevacizumab, cetuximab, and IL-2 receptor alpha-directed monoclonal antibodies.

12. The method of claim 11, wherein said protein and said therapeutic antibody are administered simultaneously.

13. The method of claim 12, wherein said protein is formulated together with said therapeutic antibody.

14. The method of claim 10, wherein said proliferative disease is characterized by neoplastic cells expressing CD59.

15. A method for treating a disease caused by a CD59- or CD59-like molecule expressing pathogen in a patient in need thereof, said method comprising administering to said patient the protein of any one of claims 1-9.

16. The method of claim 15, further comprising administering a therapeutic antibody against said pathogen, wherein said protein and said therapeutic antibody are administered simultaneously, or within 14 days of each other, in amounts that together are sufficient to treat said pathogenic disease.

17. The method of claim 16, wherein said therapeutic antibody is specific for a virus selected from the group consisting of human cytomegalovirus, HCMV, human T-cell leukemia virus type 1, HIV-I, simian immunodeficiency virus, Ebola virus, Herpesvirus saimiri virus, influenza virus, and vaccinia virus.
18. The method of claim 16, wherein said therapeutic antibody is specific for a microbial parasite selected from the group consisting of *Naegleria fowleri* and *Schistosoma mansoni*.

19. The method of claim 16, wherein said composition and said therapeutic antibody are administered simultaneously.

20. The method of claim 19, wherein said composition is formulated together with said therapeutic antibody.

21. A pharmaceutical composition comprising a protein of any one of claims 1-9 and pharmaceutically acceptable excipient.

22. A kit comprising a protein of any one of claims 1-9 and a therapeutic antibody.