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(54) **ANTI-C5 ANTIBODIES AND USES THEREOF**

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(57) **ABSTRACT**

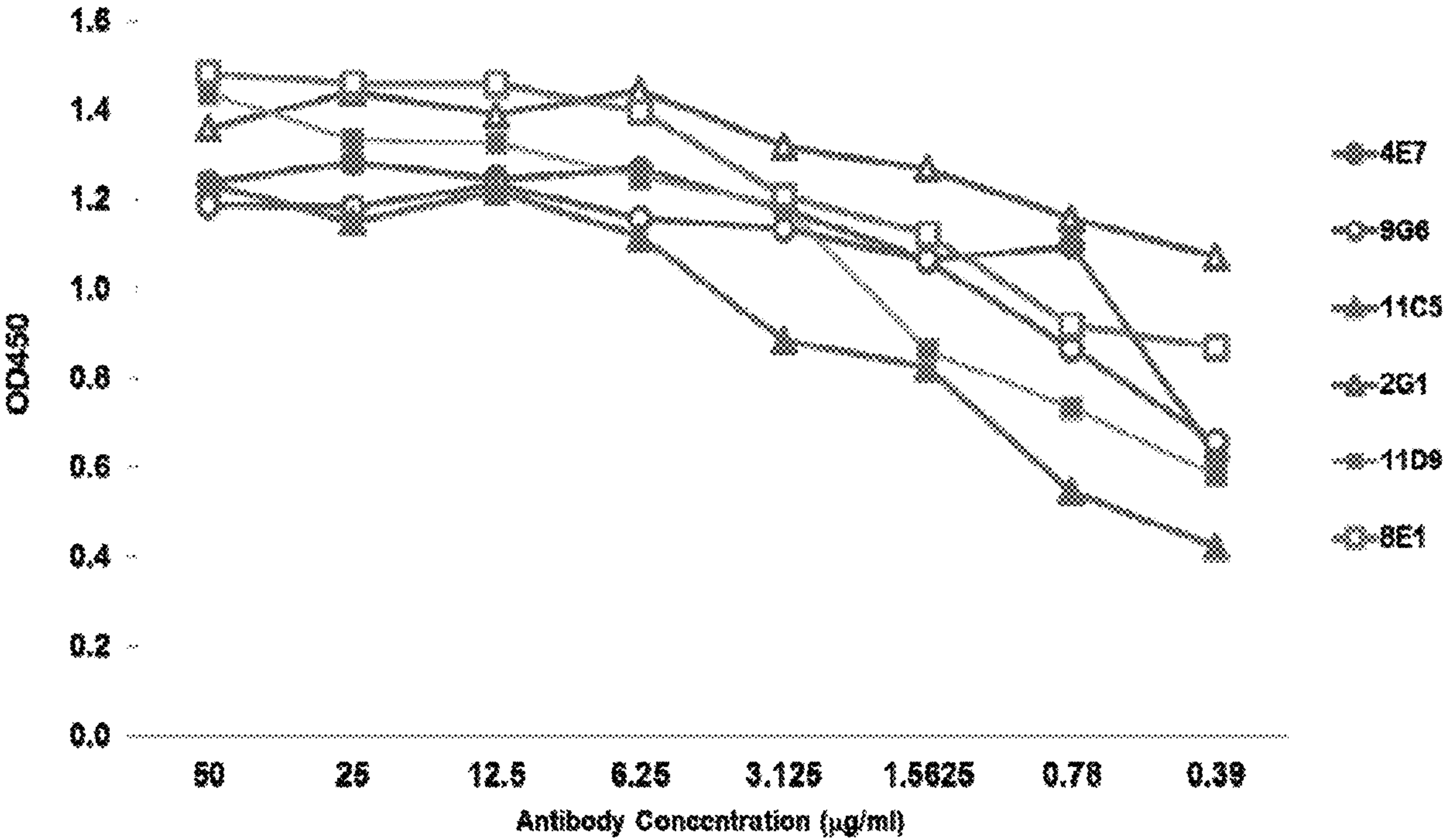
Related U.S. Application Data

(63) Continuation of application No. 16/491,675, filed on Sep. 6, 2019, now Pat. No. 11,578,137, filed as application No. PCT/US2018/021001 on Mar. 6, 2018.

ABSTRACT

This invention relates to inhibition of the complement signaling using an anti-C5 antibody. Specifically, the invention relates to methods of treating a complement-mediated disease or complement-mediated disorder in an individual by contacting the individual with an anti-C5 antibody.

Specification includes a Sequence Listing.



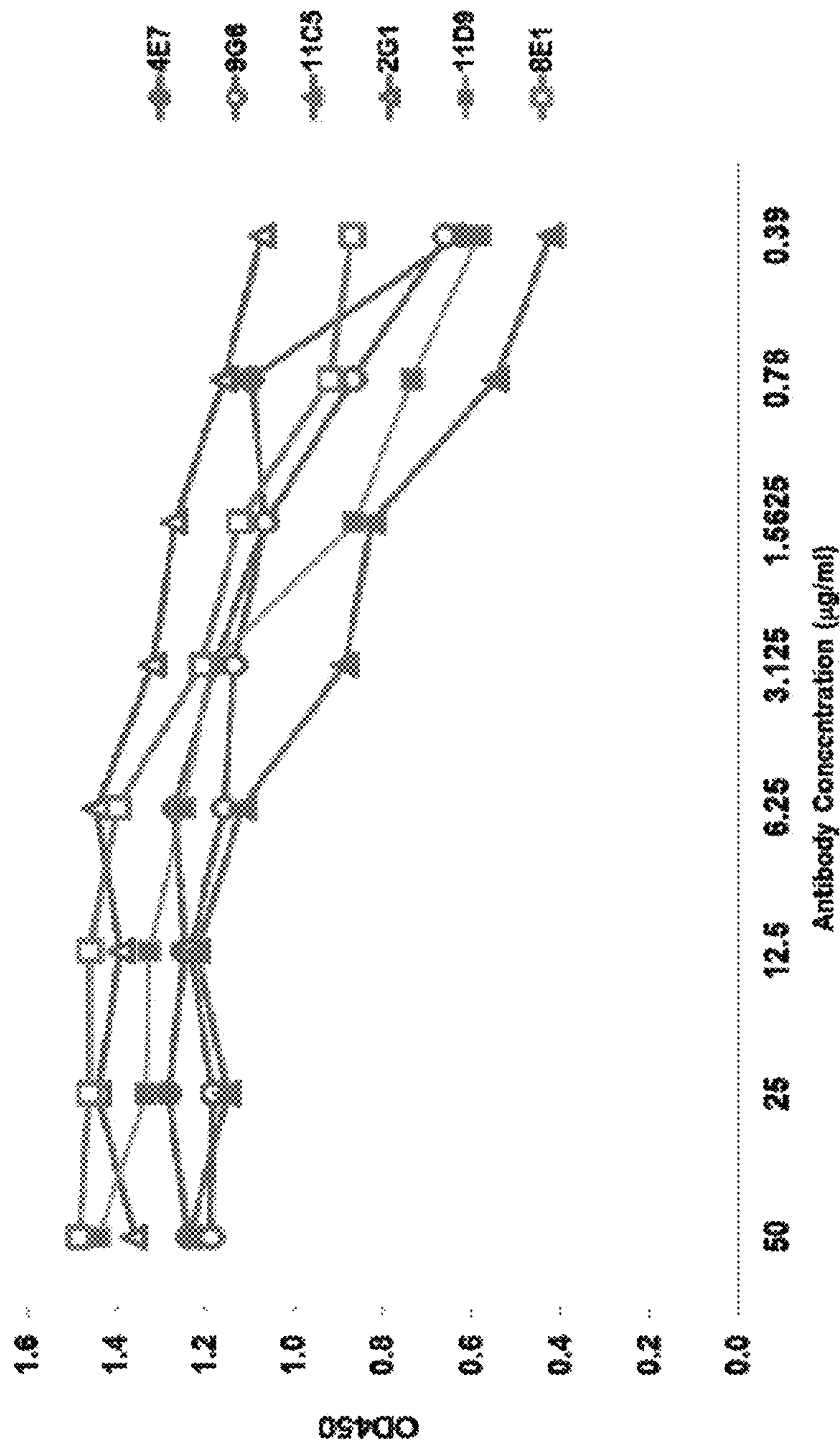


Figure 1

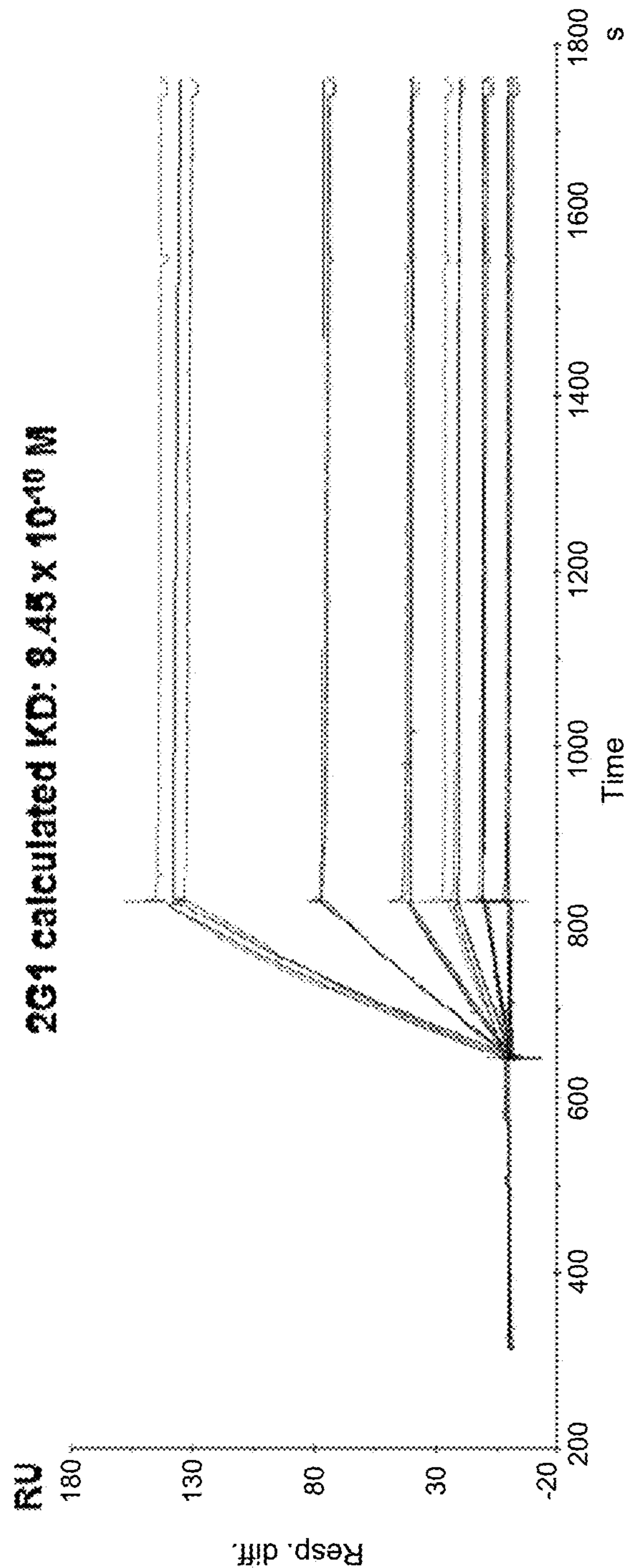


Figure 2

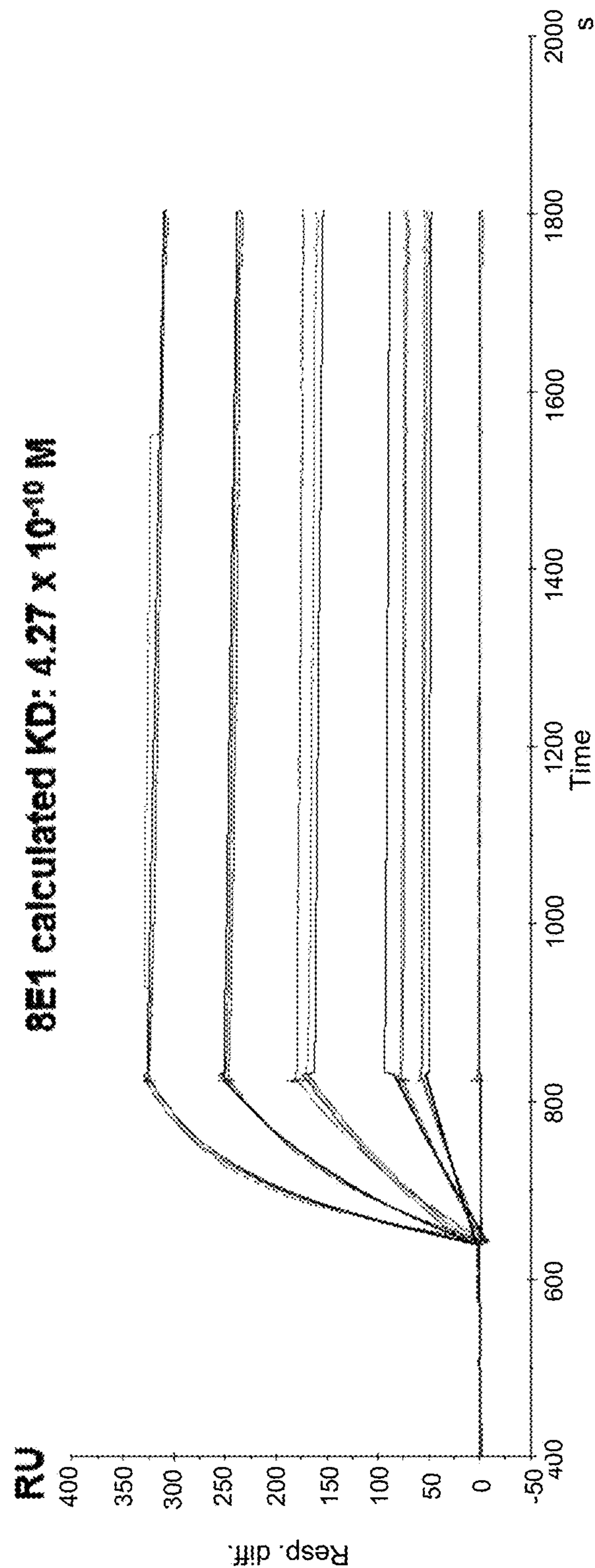


Figure 3

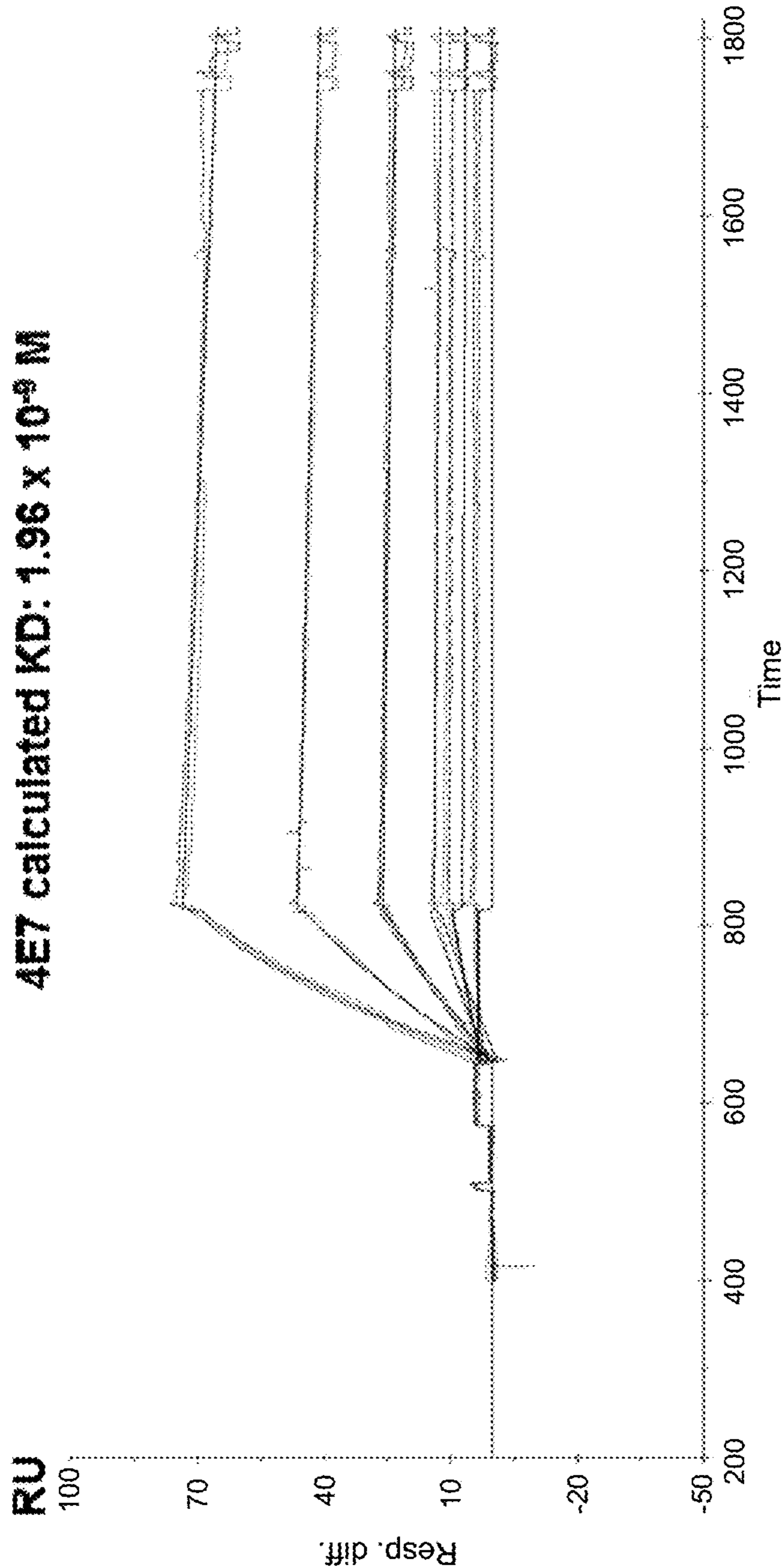


Figure 4

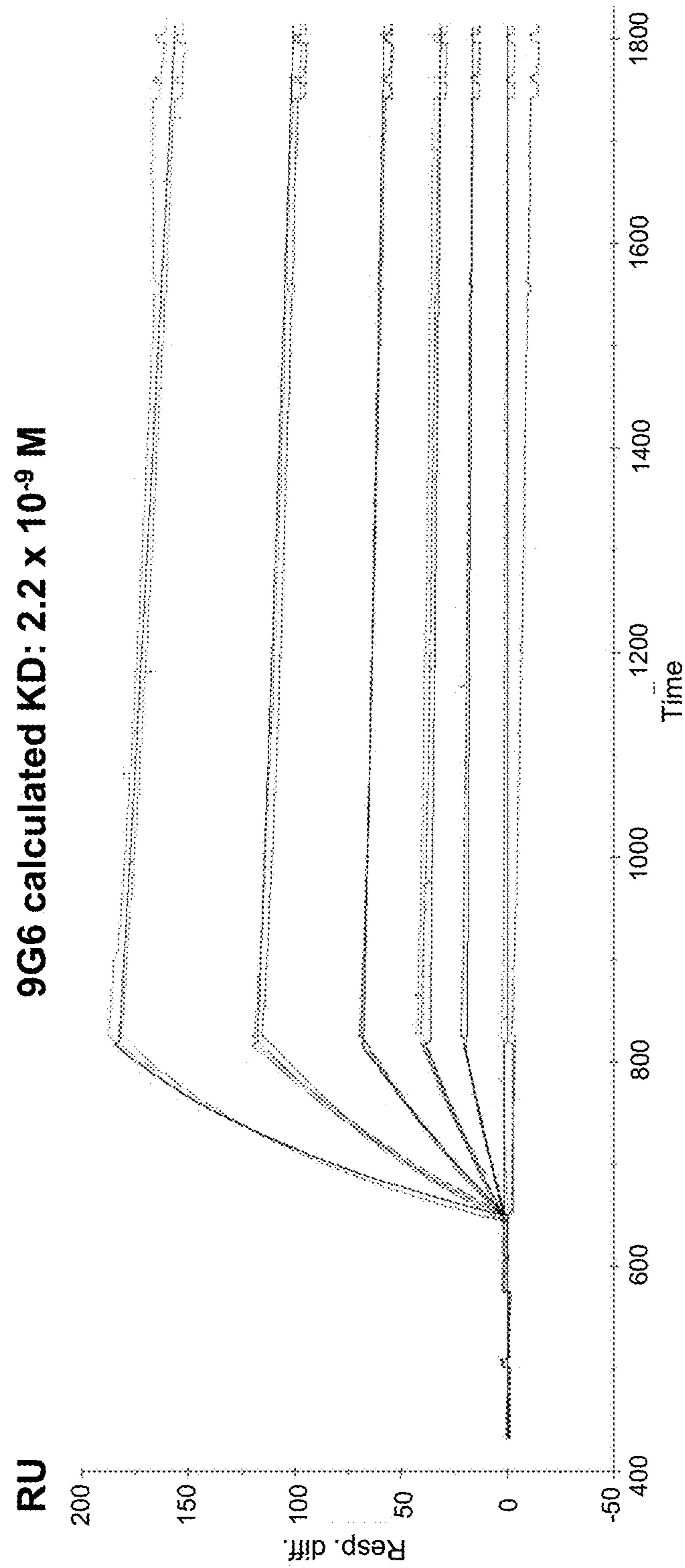


Figure 5

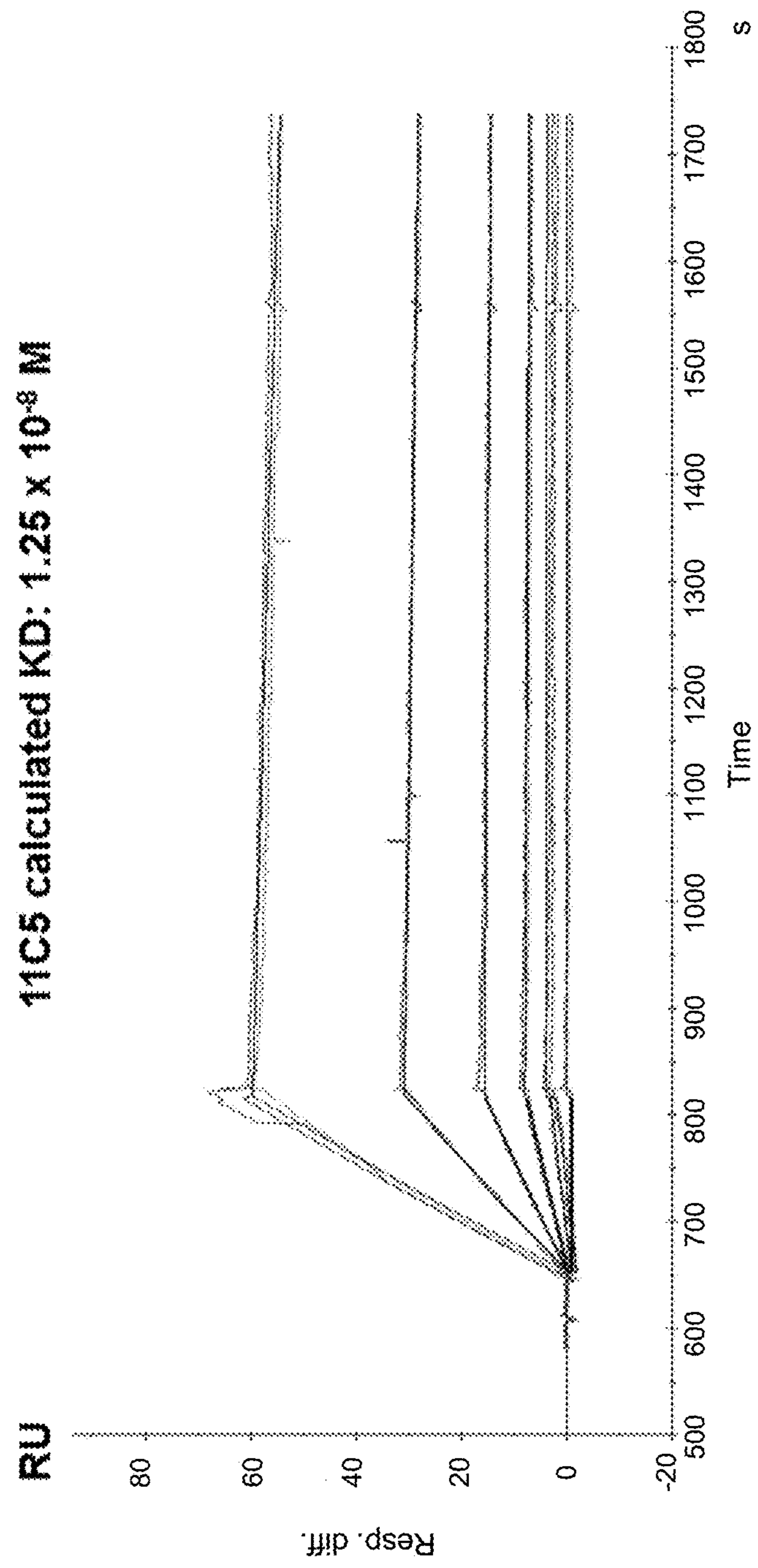


Figure 6

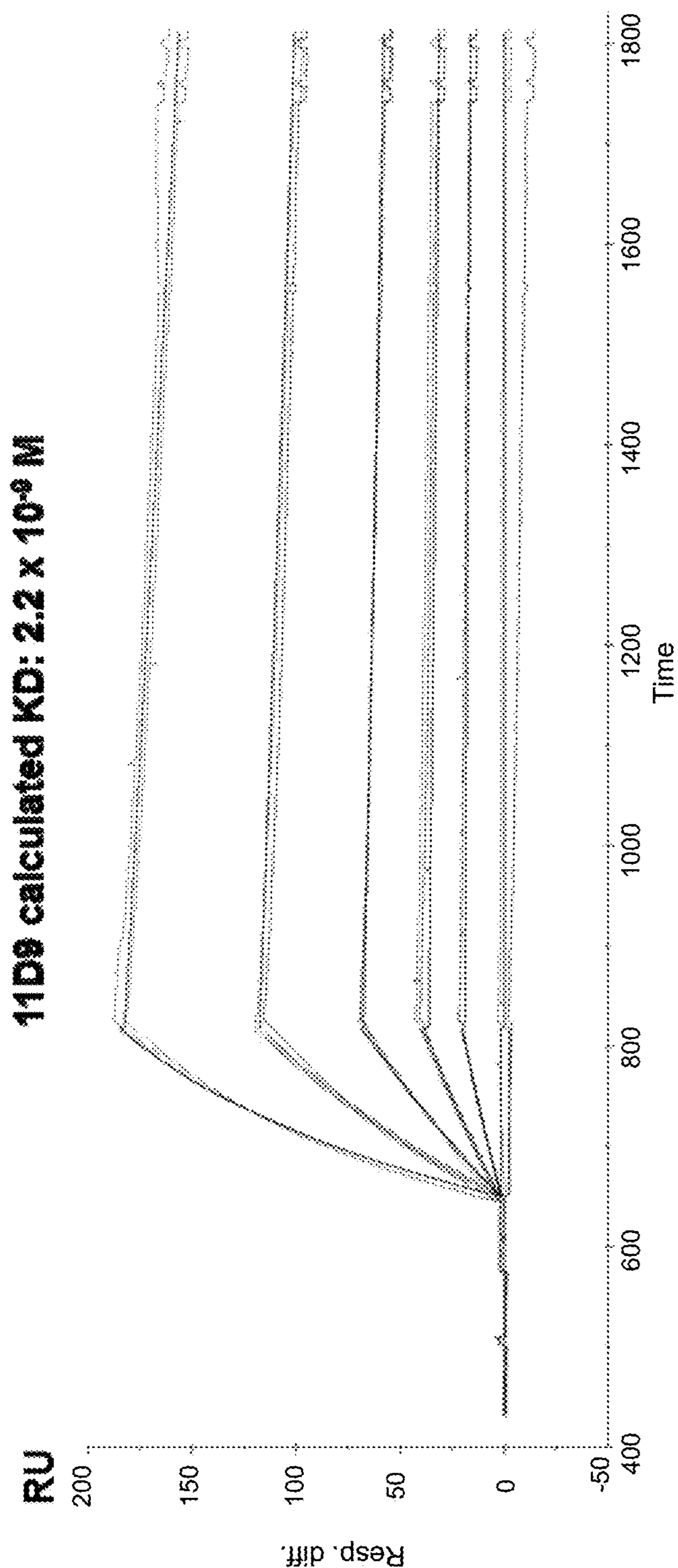


Figure 7

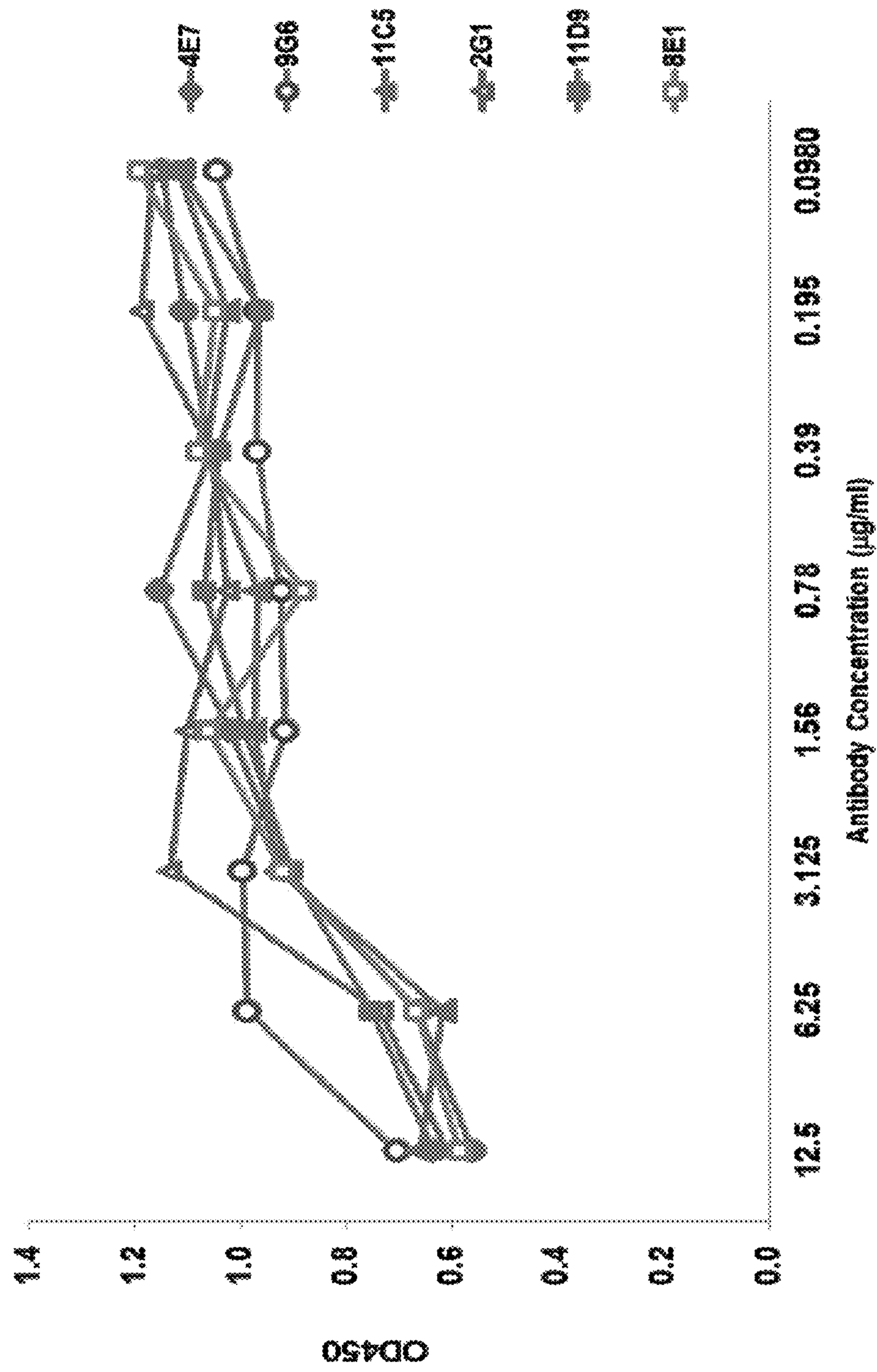


Figure 8

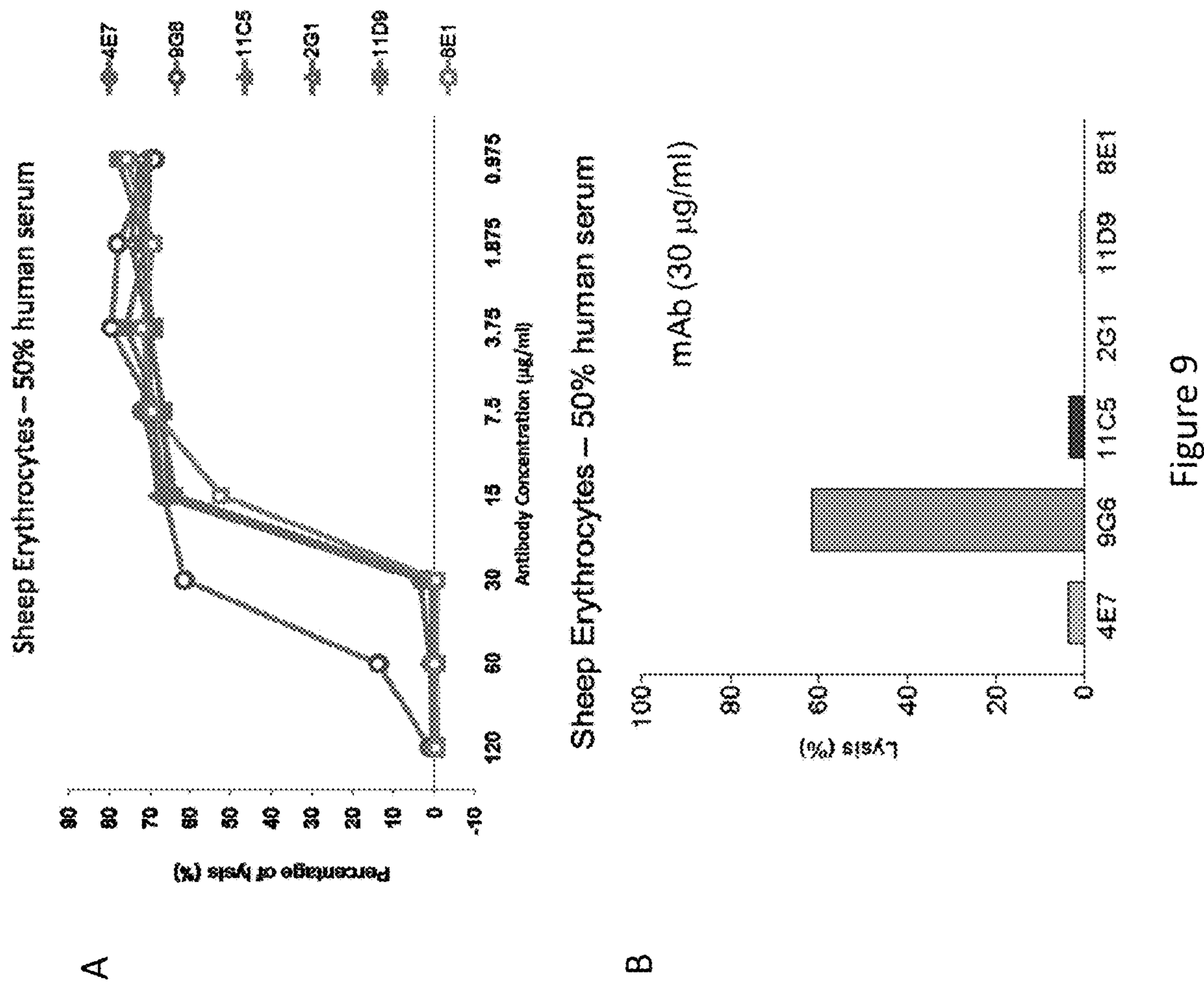


Figure 9

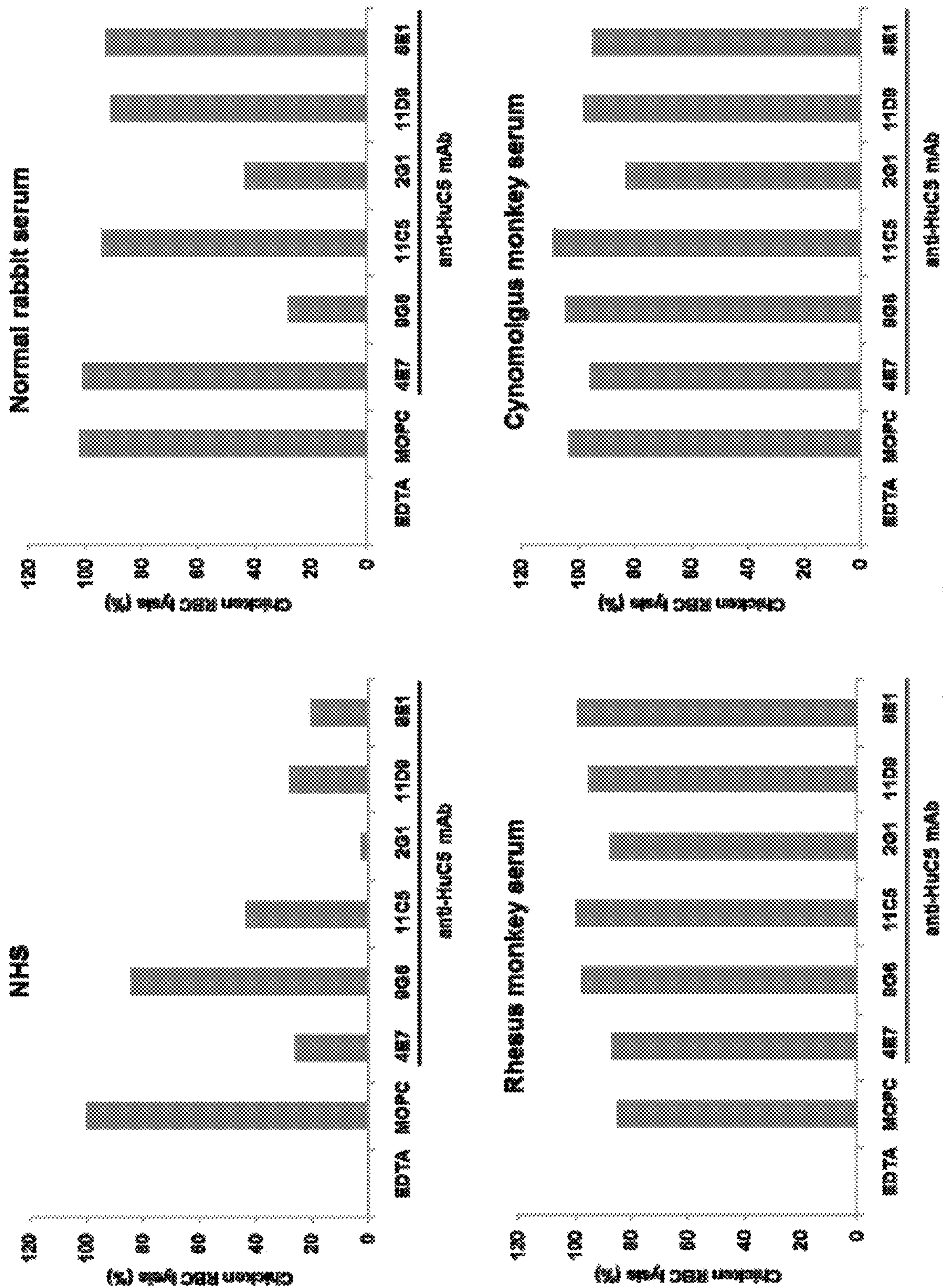


Figure 10

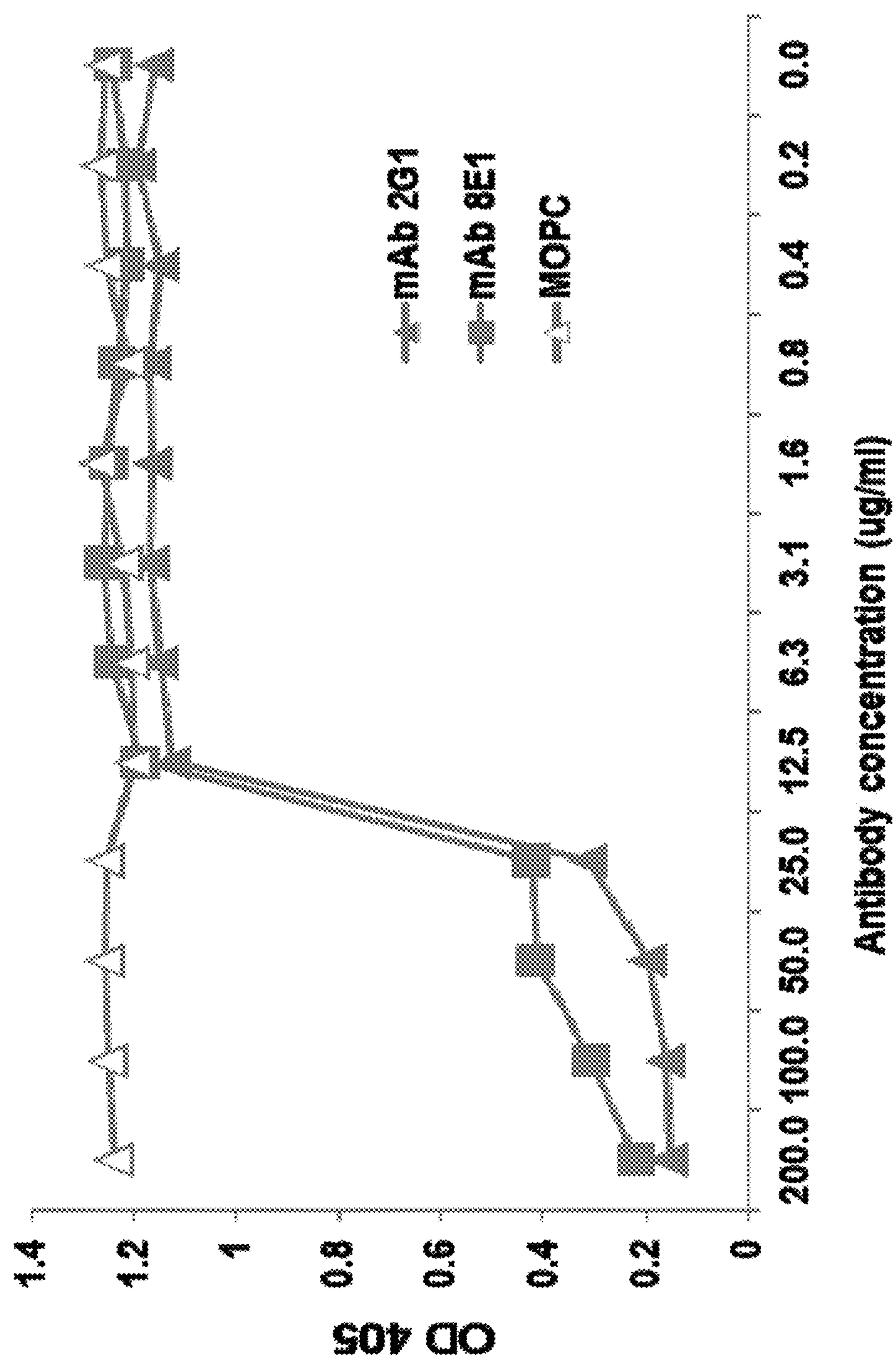


Figure 11

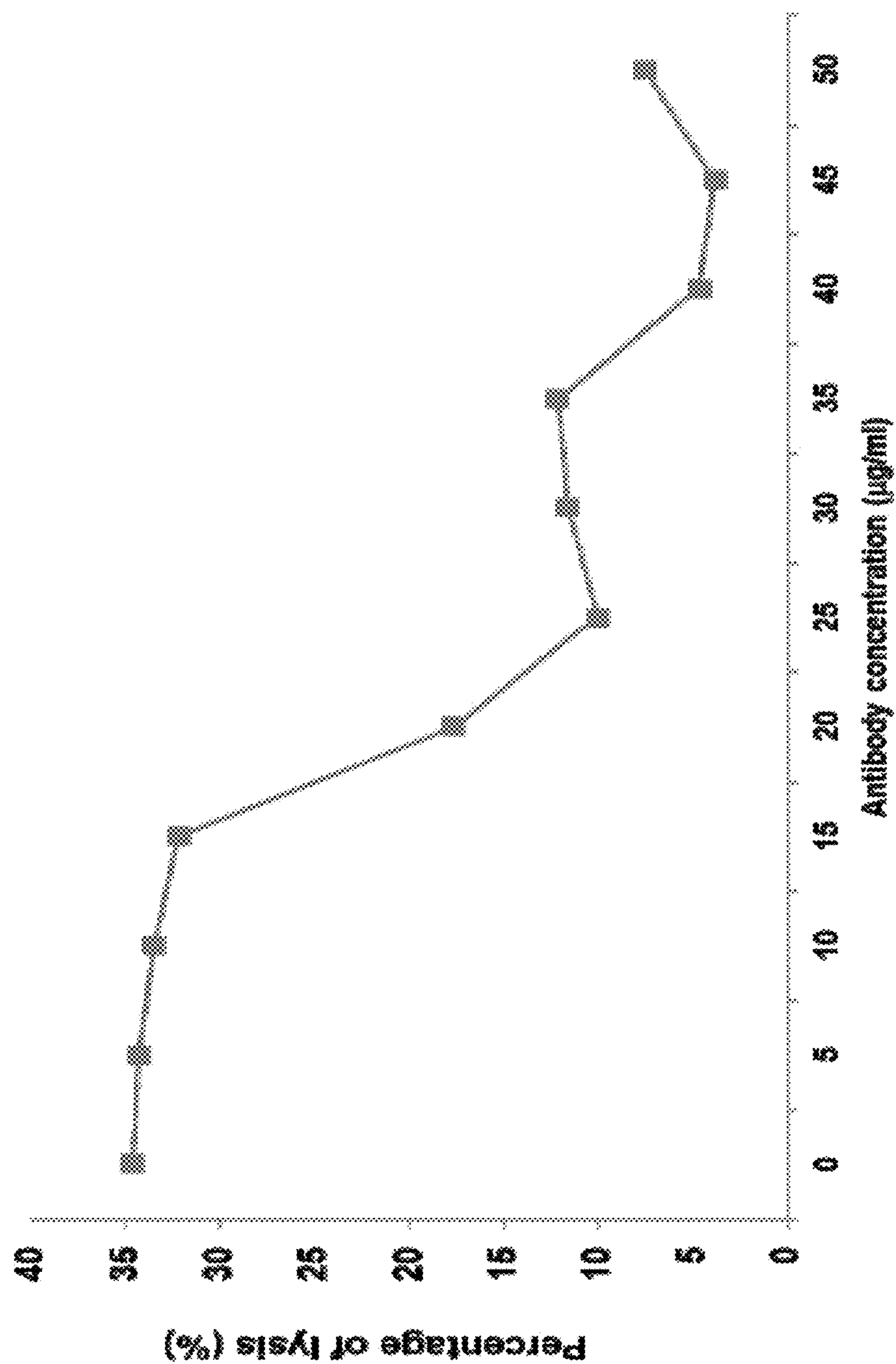


Figure 12

Nucleic acid and amino acid sequence of VH of mAb 2G1. Signal peptide is underlined and CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

VH

AACAACATCTGTCCAATGTCCTCTCTCCACAGGCACTGAACACACTGACTCTAACC	
atgggatggagctggatcttctctctctctctctctctcaggaactgcaggtgtctctctctgag	
M G W S W I F L L F L L S G T A G V L S E	
gtccagctgcaacagctctggaccagagctggtgaagcctgggcttcagtgaaagatatcc	
V Q L Q Q S G P E L V K P G A S V K I S	
tgcaaggcttctggatacacacaatcacagactacaatttggactgggtgaagcagagccat	
C K A S G Y T I T D Y N L D W V K Q S H	CDR1 (SEQ ID NO: 3)
ggaagagccttgagtggtggattggagatatattagtcctaactatggttatactatctacaac	
G K S L E W I G D I S P N Y G Y T I Y N	CDR2 (SEQ ID NO: 4)
cagaattcaaggacaaggccacattgactgtagacaggctcctccagcacagcctacatg	
Q K F K D K A T L T V D R S S S T A Y M	CDR3 (SEQ ID NO: 5)
gagctccgcagcctgacatctgaggacactgcagtttattactgtgcaagaaggacatt	
E L R S L T S E D T A V Y Y C A R R D I	
cgttactccggttaattcctacaaatggtactctgatgtctgtgggcacagggaccacggtc	
R Y S G N S Y K W Y F D V W G T G T T V	
accgtctctcagcccaaaacaacagcccccatcggtctatccactggcc (SEQ ID NO: 1)	
T V S S (SEQ ID NO: 2)	

Nucleic acid and amino acid sequence of VL of mAb 2G1. Signal peptide is underlined and CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

tcagttcctgcaggacacagtttagatatagaggttcagggttcagggttctgtgggtcctt	
M R F Q V Q V L G L L	
ctgctctggatatcaggtgcccagtggtgatgtccagataaaccagtcctccatcttatctt	
L L W I S G A Q C D V Q I T Q S P S Y L	
gctgcattcctctggagaaaccattactattactaattgcaggacacaagtaagagcataagcaaa	
A A S P G E T I T I N C R T S K S I S K	CDR1 (SEQ ID NO: 8)
tatttagccttggtatcaagagaaaacctgggaaaactaataagcttcttactactcttggga	
Y L A W Y Q E K P G K T N K L L I Y S G	
tccaccttgcaatctggaattccatcaagggttcaggggcagtggtgatctggtacagatttc	
S T L Q S G I P S R F R G S G S G T D F	CDR2 (SEQ ID NO: 9)
actctcaccatcagtagcctggagcctgaagatttttgcaatgtattactgtcaacaacat	
T L T I S S L E P E D F A M Y Y C Q R	CDR3 (SEQ ID NO: 10)
aatgaatacccgtaacagttcggaggggggacccaagctggaaataaaacgggctgatgct	
N E Y P Y F F G G G T K L E I K (SEQ ID NO: 7)	
gcacca (SEQ ID NO: 6)	

Figure 13

Nucleic acid and amino acid sequence of VH of mAb 8E1. Signal peptide is underlined and CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

AGCCTCCATCAGAGCatggtgtcctggggtgcttctctgtcctggtgactttcccaagc
M A V L G L L C L V T F P S
tgtgtcctgtcccaggtgcagctgaaggagtcaggacctggcctgggtggcgccctcacag
C V L S Q V Q L K E S G P G L V A P S Q
agcctgtccatcacatgcaccgtctcaggggttccctttaaacaaactatgggaatacactgg
S L S I T C T V S **G F P L N N Y G I H W**
gttcgccagcctccaggaagggtctggagtggtggcagtgatatggagtgatgggaaga
V R Q P P G K G L E W L A **V I W S D G R**
acaacctataattcagctctcaactccagactgagcatcagcaaggacaactccaagagc
T T Y N S A L N S R L S I S K D N S K S
caagtttctttaaaatgaacagttctccaaactgatgacacagccatgtactattgtgcc
Q V F F K M N S L Q T D D T A M Y Y C A
agacatgatggtcgggggactatagtaggactactgggtcaaggaaacctcagtcacc
R **H D G R G D Y S M D Y** W G Q G T S V T
Gtctcctcagccaaaacaacaccccccatca (SEQ ID NO: 11)
V S S (SEQ ID NO: 12)

CDR1 (SEQ ID NO: 13)

CDR2 (SEQ ID NO: 14)

CDR3 (SEQ ID NO: 15)

Nucleic acid and amino acid sequence of VL of mAb 8E1.

atggggaaagcatcctctcttccagctctcagagatggagacagacacactcctgttatgg
M E T D T L L L W
gtactgtgtctgtggcccttagttccgagctegtgtgatgacacagttcctgttccctta
V L L L W A L S S E L V M T Q S P A S L
gttgatatctgtgggcagaggggccaccatctcctcagggccagcaagggtgcagtaca
V V S L G Q R A T I S C **R A S K G V S T**
tctgtctacagttatatgcactgggtaccaacagaaaccaggacagccacccaaactcctc
S V Y S Y M H W Y Q Q K P G Q P K L L
atctatctgtgcataccaaacctagaatctbggggtccctgccaggttcagtgggcagtggtct
I Y **L A S N L E S** G V P A R F S G S
gggacagacttctccctcaacatccatcctgtgtggaggaggaggtgctgcaacctattac
G T D F S L N I H P V E E D A A T Y Y
tgtcagcaaaagtggggagcttccgctcacgttcggtgctgggaccacagctggagctgaaa
C **Q Q S G E L P L T** F G A G T K L E L K
cgggctgatgtgcaccaacaagggc (SEQ ID NO: 16)
R (SEQ ID NO: 17)

CDR1 (SEQ ID NO: 18)

CDR2 (SEQ ID NO: 19)

CDR3 (SEQ ID NO: 20)

Figure 14

Nucleic acid and amino acid sequence of VH of mAb 4E7. CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

```
atggggaaacaacatatgtccaatgtcctctcctcagacactgaacacactgactotaacc
      M S S P Q T L N T L T L T
atgggatggagctggtcttctcttctcctgtcagaaactgcaggagtcctctctgag
M G W S W I F L F L L S E T A G V L S E
gtccagctgaacaggggcttcaagtgaagatgtcctgttaagacttctcgatactcattc
V Q L Q Q G A S V K M S C K T S R Y S F CDR1 (SEQ ID NO: 23)
actgactacatcatgcactgggtgaagctgagccatggaaagagccttgagtgattgga
T D Y I M H W V K L S H G K S L E W I G
tatattaaccctaacaatggtgtactatatctacaaccagaagtccaaggacaaggccaca
Y I N P N N G G T I Y N Q K F K D CDR2 (SEQ ID NO: 24) K A T
ttgactgtaaacaagtcctccagcacagcctacatggaattccgcagcctgacatcggag
L T V N K S S S T A Y M E F R S L T S E
gattctgcagctctatttcttcaagaggggggtttattaccgggggtttgcttaactgg
D S A V Y F C S R G G V Y Y R G F A Y W CDR3 (SEQ ID NO: 25)
ggccaagggcacactgggtcactgtctctgcagccaaacgacacccccatct (SEQ ID NO: 21)
G Q G T L V T V S A (SEQ ID NO: 22)
```

Nucleic acid and amino acid sequence of VL of mAb 4E7.

```
atgggggaccaatattgaaaaagaatagacctgggttgtgaattatggcctggatttcactt
      M A W I S L
atactctctctcctggctctcagctcagggggccatttcccaggctgttgtgactcaggaa
I L S L L A L S S G A I S Q A V T Q E
tctgcactcaccacatcacctggtgaaacagtcacattcacttgctgcgtcaagtactggg
S A L T T S P G E T V T F T C R S S T G CDR1 (SEQ ID NO: 28)
gctgttacaaaacagtaactatgccaaactgggtccaagaaaaaacagatcatttttcaact
A V T N S N Y A N W V Q E K P D H F F T
ggctataatagggtgttaccaacaaccggaggtccagggtgttctcctgcccgatctctcaggctcc
G L I G V T N N R G P G V P A R F S G S CDR2 (SEQ ID NO: 29)
ctgattggagacaaggctgccctcaccatcacaaggggcacagactgaggatgagggaata
L I G D K A A L T I T G A Q T E D E A I
tatttctgtgctctatggtacagcaaccacttgggtgttcggtggagggaacaaactgac
Y F C A L W Y S N H L G V R W R N Q T D
tgtcctaggccagcccaagtcttcgccatcagt (SEQ ID NO: 26)
C P R P A Q V F A I S (SEQ ID NO: 27)
```

Figure 15

Nucleic acid and amino acid sequence of VH of mAb 9G6. CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

atggggaaggagtgaccagtttagttttaaggcaccactgagcccaagtttagacatcatg
M
gattgggtgtggaccttggtattctctgatatgacagctgccccaaagtgcccaagcacagatc
D W V W T L V F L I A A A Q S A Q A Q I
cagttggtgcagtcctggacctgagctgaagaagccctggagagacagtcagaatctcctgc
Q L V Q S G P E L K K P G E T V K I S C
aaggcttctgggtataccttcacagaatatccaatgcactgggtgaagcaggctccagga
K A S **G Y T F T E Y P M H W V K Q A P G** CDR1 (SEQ ID NO: 32)
aagagtttcaagtggatgggcattcctatacacogacactggagagcccaacatatgtgtgaa
K S F K W M G **T L Y T D T G E P T Y A E** CDR2 (SEQ ID NO: 33)
gagttcaggggacggtttgcccttctcttttggagacctctgccagcactgcctatttgcag
E P R G R F A F S L E T S A S T A Y L Q CDR3 (SEQ ID NO: 34)
atcaacaacctcaaaaatgaggacacgggtacatatattctgtgttcaactcagggtacgta
I N N L K N E D T A T Y F C V H **S G Y Y** (SEQ ID NO: 30)
ggctaactggggccaaggcaccactctcacagttctcatcagccaaacacaccccccatca
G Y W G Q G T T L T V S S (SEQ ID NO: 31)

Nucleic acid and amino acid sequence of VL of mAb 9G6. CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

atggggggtgtaatcagcatcacactgaaacacacacagacatgagtgtgcccactcagggtc
M S V P T Q V
ctgggggttgctgctgtgtggcttacagggtgccagatgtgacatccagatgactcagttct
L G L L L L W L T G A R C D I Q M T Q S
ccagcctccctatcagcatctgtgtgggagaacactgtcaccttcacatgtcgggcaagtga
P A S L S A S V G E T V T F T C **R A S E** CDR1 (SEQ ID NO: 37)
attatttacagttatttagtttgggtatcagcagaaacacagggaaaaatctcctcagctcctg
T I Y S Y L Y W Y Q Q K Q G K S P Q L L
gtctctaatagcacaaaaccttagcagaggggtgtgtgccatcaaggttcagttggcagttggatca
V S **N A K R L A E** G V P S R F S G S CDR2 (SEQ ID NO: 38)
ggcacacagttttctctgaagatcaatagcctgcagcctgaagattttgggagttattac
G T Q F S L K I N S L Q P E D F G S Y Y
tgtcaacattattatgtgtaatctctccacgttctggaggggggacccaagctggaaataaaa
C **Q H Y Y G N P P P** F G G G T K L E I K CDR3 (SEQ ID NO: 39)
cgg (SEQ ID NO: 35)
R (SEQ ID NO: 36)

Figure 16

Nucleic acid and amino acid sequence of VH of mAb 11C5. CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

acatgggrgctctgacagaggagccggtcctggattcgagttcctcacattcagtgatg	
agcactgaacaacggacccctcaccatgaacttcggggtcagcttgattttccttgctctt	M
S T E H G P L T M N F G L S L I F L V L	
gttttaaagggtgtccagtgtaagtgcagctagtggaagtctggggagggttagtgaag	
V L K G V Q C E V Q L V E S G G L V K	
cctggagggtccctgaaactctcctgtgcagcctctgtgattcactttcactacctatgcc	
P G G S L K L S C A A S G F T T Y A	CDR1 (SEQ ID NO: 42)
atgtcttgggttcgcagactccggaaaagaggtggagtggtcgcaaccatttagtgct	
M S W V R Q T P E K R L E W V A T I S A	
ggtggtacttacacctactattcagacaaatgtaaagggccgattcaccatctccagagac	
G G T Y T Y Y S D N Y K G R F T I S R D	CDR2 (SEQ ID NO: 43)
aatgccaagaacaacctgtacctgc aaatgagccatctgaagtctgaggacacagccatg	
N A K N N L Y L Q M S H L K S E D T A M	
ttttactgtgcaagagatcccgattactaactagcgtagaagcccgtttgcttactggggccaa	
F Y C A R P P D Y Y G R S P F A Y W G Q	CDR3 (SEQ ID NO: 44)
gggactctgtgtcaactgtctctgtgcagccaaaacacacccccatca (SEQ ID NO: 40)	
G T L V T V S A (SEQ ID NO: 41)	

Nucleic acid and amino acid sequence of VL of mAb 11C5. CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

atgggggtgagccacacaaaactcagggaagctcgaaagatggttttcacacctcagatactt	
ggacttatgcttttttggatttccagcctccagagggtgaaattgtgtcactcagttctcca	M V F T P Q I L
G L M L F W I S A S R G E I V L T Q S P	
gccacccctgtctgtgactccaggagagagcgtcagttctttcctgtaggggccagccaaagt	
A T L S V T P G E S V S L S C R A S Q S	CDR1 (SEQ ID NO: 47)
attagcaacaacctacagtggtatcaacaaaaatcacatgagttctccaagacttctcatc	
T S N N L Q W Y Q Q K S H E S P R L L I	
aaatatgcttccagtcctctctgtggatccccctccaggttcagtggtggcagtggtcaggg	
K Y A S Q S I S G I P S R F S G S G	CDR2 (SEQ ID NO: 48)
acagatttcaactctcagtatcaacagtggtggagactgaagattttggaatgtattctgt	
T D F T L S I N S V E T E D F G M Y F C	
caacagagtaacagctggccctctcaactgttcgggtgggacccaaaggtggagctgaaacgg (SEQ ID NO: 45)	
Q Q S N S W P L T F G G G T K V E L K R (SEQ ID NO: 46)	CDR3 (SEQ ID NO: 49)

Figure 17

Nucleic acid and amino acid sequence of VH of mAb 11D9. CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

atggggacatatgtacaatgtcctcaccacagacacactgaacacactgactctaaccatggga
M S S P Q T L N T L T L T M G
tggagctggatcttctctctctgtcaggaaactgcaggtgtcctctctctgaggtccag
W S W I F L L S G T A G V L S E V Q
ctgcaacaatctggacctgaggtggtgaagcctggggttcagtgaagatatcctgtaag
L Q Q S G P E V V K P G A S V K I S C K
gcttctggatacacgcttcaactgacttctacatgaactgggtgaaacagagccatggaaag
A S **G Y T F T D F Y N N** W V K Q S H G K CDR1 (SEQ ID NO: 52)
agccttgagtggtgatatattaatccttaacaatgggtgatactagttacaaccagaag
S L E W I G **Y I N P N N G D T S Y N Q K** CDR2 (SEQ ID NO: 53)
ttcaagggcaaggccacatcgactgtagacaggtcctccaacacagcctacatggagctc
F K G K A T S T V D R S S N T A Y M E L
cgcagcctgacatctgaggactctgcagctctattactgtgcaagactcattctctatggt
R S L T S E D S A V Y Y C A R **L I F Y G** CDR3 (SEQ ID NO: 54)
aactggtaacttctgtgtgtgggcaaggacacgggtcaccgtctcctcagccaaaaa
N W Y F D V W G T G T T V T V S S A K T (SEQ ID NO: 51)
acagccccatcg (SEQ ID NO: 50)

Nucleic acid and amino acid sequence of VL of mAb 11D9. CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

atggggaataattagccagagactgagatggaaacaaaatggatttttcagatgcagatt
M D F Q M Q I
atcagcttgctgtaataatcagtggtcacagtcatagtgtctaattggagaaattgtgtcacc
I S L L L I S V T V I V S N G E I V L T
cagttctccacccaccatggctgcattctccgggggagaagatcactatcacctgcagtgcc
Q S P P T M A A S P G E K I T I T C **S A** CDR1 (SEQ ID NO: 57)
agctcaagtataagttccaattacgtgcatgttggtatcagcagaagccaggattctccct
S S S I S S N Y V H W Y Q Q K P G F S P
aaactcttgatttataggacatccaatctggctctctggagtcaccagttcgttcaagtggc
K L L I Y **R T S N L A S** G V P V R F S G CDR2 (SEQ ID NO: 58)
agtgggtctgggacctcttactctctcacaattggcaccatggaggctgaagatgttgcc
S G S G T S Y S L T I G T M E A E D V A
acttactactgccagcaggtactagtataccgtggacgttcggtggagggcacccaaggtg
T Y Y C **Q Q G T S I P W T** F G G G T K V CDR3 (SEQ ID NO: 59)
gaaattaatcgg (SEQ ID NO: 55)
E I N R (SEQ ID NO: 56)

Figure 18

These sequences were used to construct chimeric (mouse variable region + human constant region) and humanized (humanized mouse variable region + human constant region) anti-human C5 antibody (2G1).

Amino acid sequence of human IgG4 Constant Heavy chain region with S228P mutation. Proline residue at position 228 is highlighted in gray.

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDPKPSNTKVDKRVESKYGPPCP**PCP**
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK
AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFFLYSRLTVDKSRWQEGNVFSCSVMHENHHTQKSLSLSPG
K (SEQ ID NO: 60)

Amino acid sequence of human Kappa Constant Light region

TVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQES
VTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 61)

Figure 19

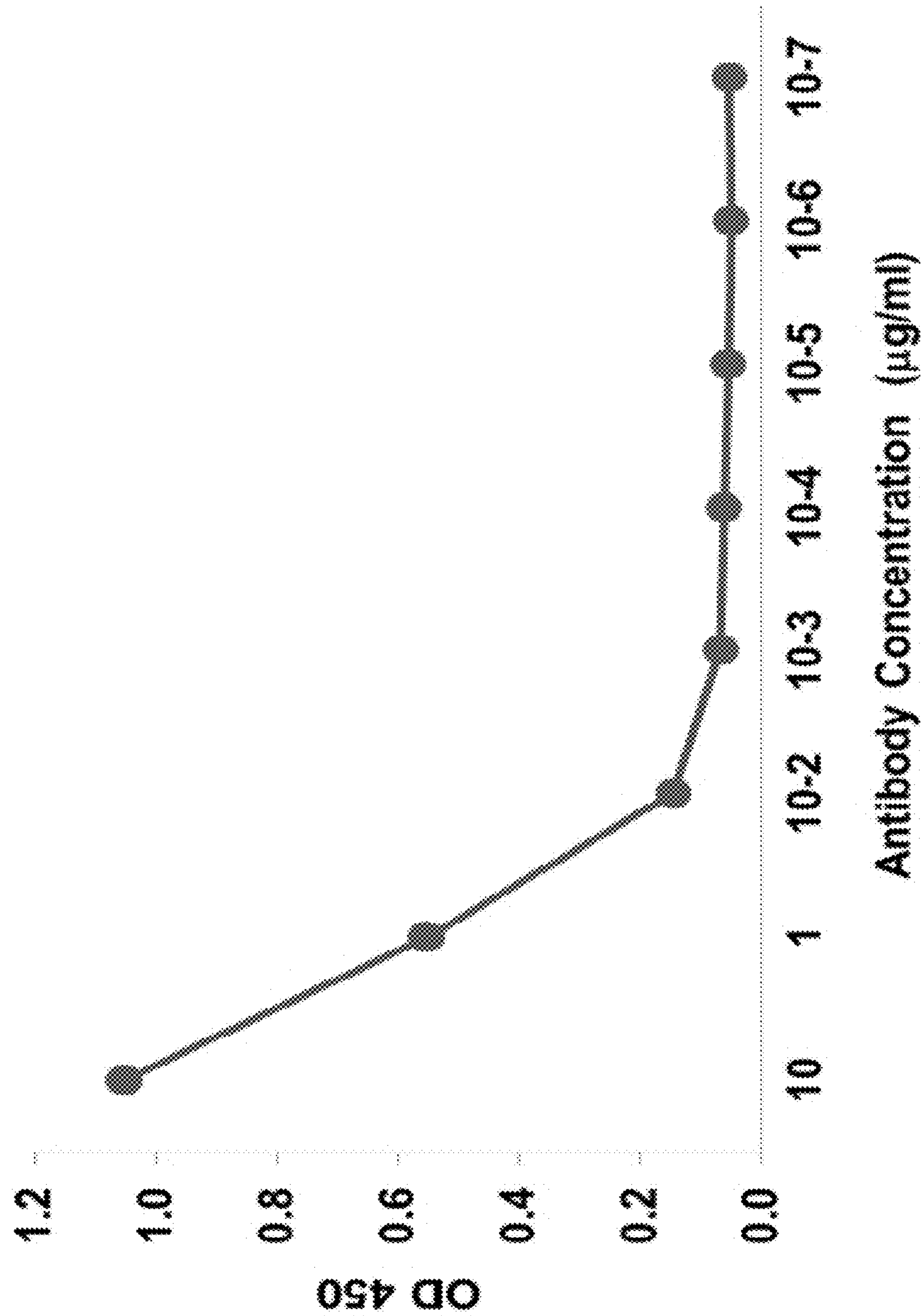


Figure 20

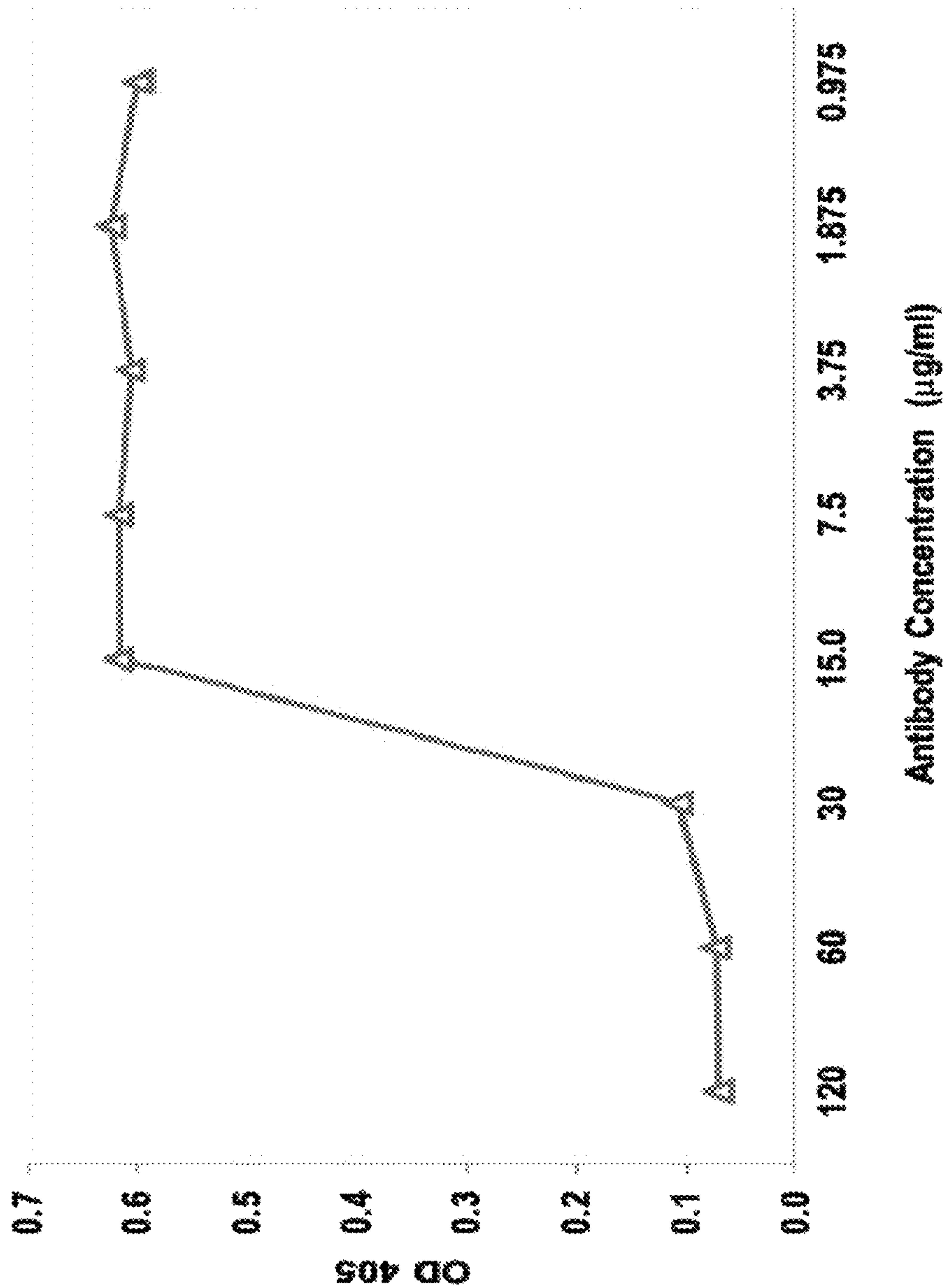


Figure 21

humanized 2G1 VH-11801

cagcatatgatcagtgctctctccaaagtccttgaacatagactctaaaccatggactggacc	
tgggtctttctctcctcctgtcagtaactgcaggtgtccactcccagggttcagctggtg	
W V F L F L L S V T A G V H S Q V Q L V	M D W T
cagctggagctgaggtgaagaagcctgggacctcagtgaaaggtctcctgcaaggcttct	
Q S G A E V K K P G A S V K V S C K A S	
ggatacacaatcacagactacaatttggactgggtgcgacagggccctggacaagggtt	
G Y T I T D Y N L D W V R Q A P G Q G L	CDR1 (SEQ ID NO: 64)
gagtggtgggagatattagtcctaactatggttatactatctacaaccagaaattcaag	
E W M G D I S P N Y G Y T I Y N Q K F K	CDR2 (SEQ ID NO: 65)
gacagagtcaccatgaccacagacacatccacgagcacagcctacatggagctgaggagc	
D R V T M T T D T S T S T A Y M E L R S	
ctgagatctgacgacacggccgtgtattactgtgcgagaagggacattcgttactccggt	
L R S D D T A V Y Y C A R R D I R Y S G	CDR3 (SEQ ID NO: 66)
aattccataaatgggtacttcgatgtctggggccaagggacaatgggtcacccgtctcttca	(SEQ ID NO: 62)
N S Y K W Y F D V W G Q G T M V T V S S	(SEQ ID NO: 63)

Figure 22

humanized 2G1 VH-16901

cagcatatgatcagtgctcctctccaaagtccttgaacatagactctaaccatggactggacc	
tgggtcttctctcctcctgtcagtaactgcaggtgtccactcccaggtgcagctggtg	M D W T
W V F L F L L S V T A G V H S Q V Q L V	
cagtcctgggctgaggtgaagaagcctgggtcctcgggtgaaggtctcctgcaaggcttct	
Q S G A E V K K P G S S V K V S C K A S	
ggatacaaatcacagactacaatttggactgggtgcgacagggccctggacaaggcctt	
G Y T I T D Y N L D W V R Q A P G Q G L	CDR1 (SEQ ID NO: 69)
gagtggtgggagatattagtccttaactataggttatctactatctacaaccagaaattcaag	
E W M G D I S P N Y G Y T I Y N Q K F K	CDR2 (SEQ ID NO: 70)
gacagagtcacgattaccggcggaatccacgagcacagcctacatggagctgagcagc	
D R V T I T A D E S T S T A Y M E L S S	
ctgagatctgaggacacggccgtgtattactgtgcgagaaggacattcgttactccggt	
L R S E D T A V Y Y C A R R D I R Y S G	CDR3 (SEQ ID NO: 71)
aattcctacaaatgggtacttctgtctggggccaaagggacaatgggtcaccgtctcttca	(SEQ ID NO: 67)
N S Y K W Y F D V W G Q G T M V T V S S	(SEQ ID NO: 68)

Nucleic acid and amino acid sequence of VL of the humanized 2G1. CDR1, CDR2 and CDR3 sequences are bolded and highlighted in gray.

Figure 23

humanized 2G1 VL-1901

gtcagagccctggggaggaactgctcagttaggaaccagagggaaccatggaagcccagct	
cagcttctcttcctcctgctactctggctcccagataccaccggagacatccagttgacc	
Q L L F L L L L W L P D T T G D I Q L T	M E A P A
cagtctccatccttcctgtctgcattctgtaggagacagagtcaccatcacttgcaggaca	
Q S P S F L S A S V G D R V T I T C R T	CDR1 (SEQ ID NO: 74)
agtaagagcataagcaaatatttagcctgggtatcagcaaaaaccagggaagcccctaag	
S K S I S K Y L A W Y Q Q K P G K A P K	
ctcctgatctattctggatccaccttgcaatctgggtcccatcaaggttcagcggcagt	
L L I Y S G S T L Q S G V P S R F S G S	CDR2 (SEQ ID NO: 75)
ggatctgggacagaattcactctcacaaatcagcagcctgcagcctgaagattttgcaact	
G S G T E F T L T I S S L Q P E D F A T	
tattactgtcaacaacataatgaatacccggtacacgtttggccaggggaccaagctggag	
Y Y C Q Q H N E Y P Y T F G Q G T K L E	CDR3 (SEQ ID NO: 76)
atcaaa (SEQ ID NO: 72)	
I K (SEQ ID NO: 73)	

Figure 24

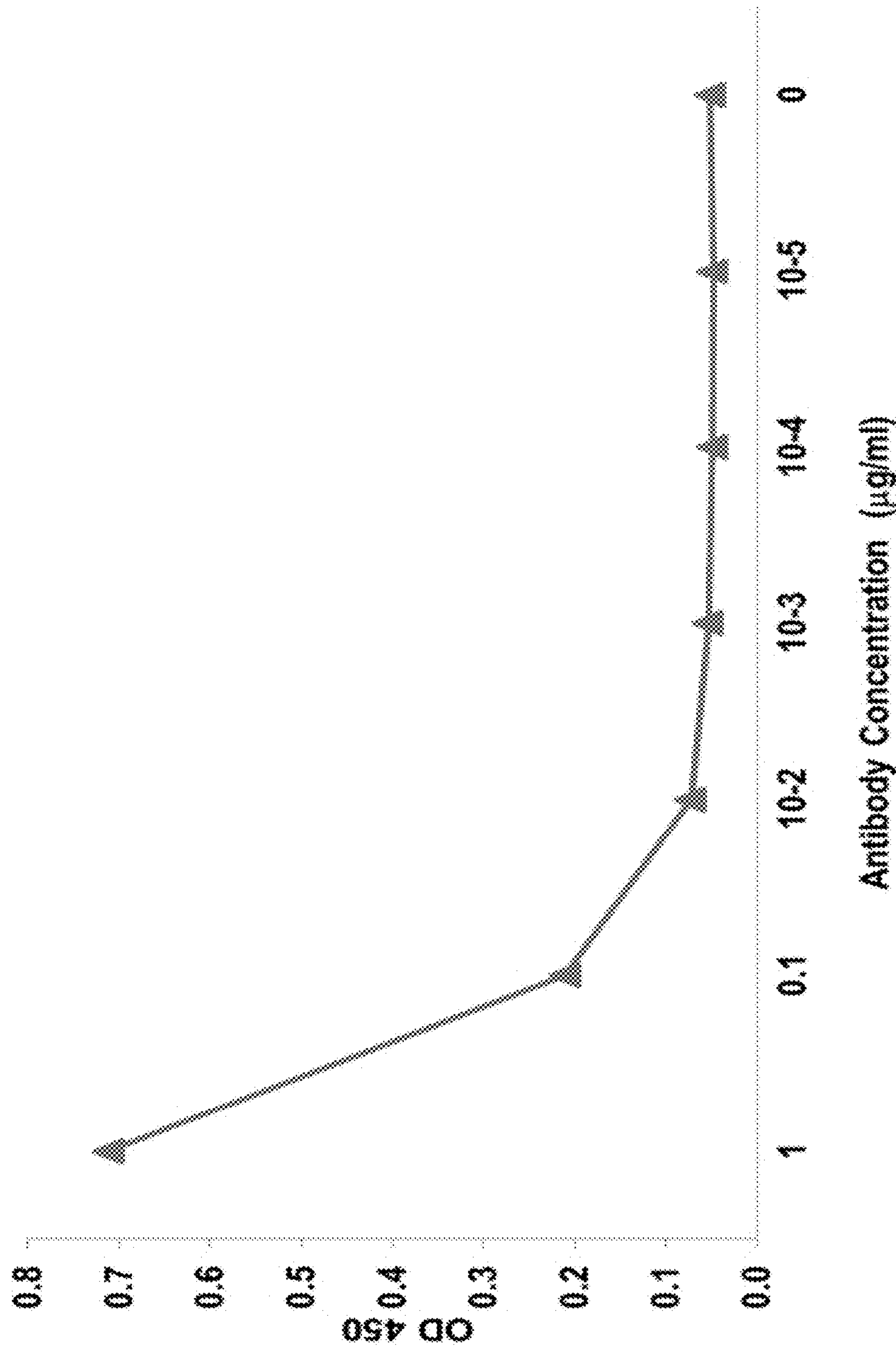


Figure 25

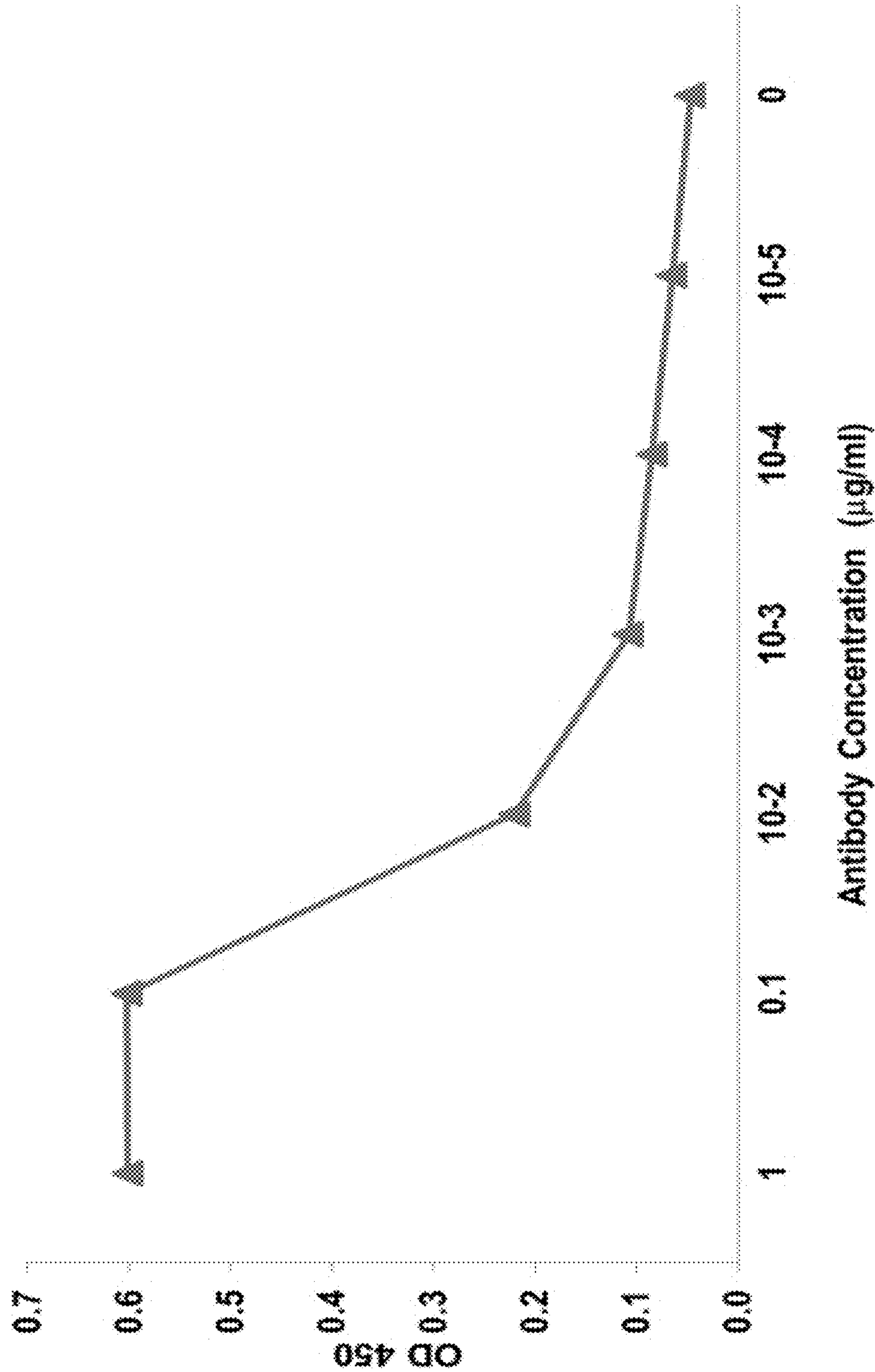


Figure 26

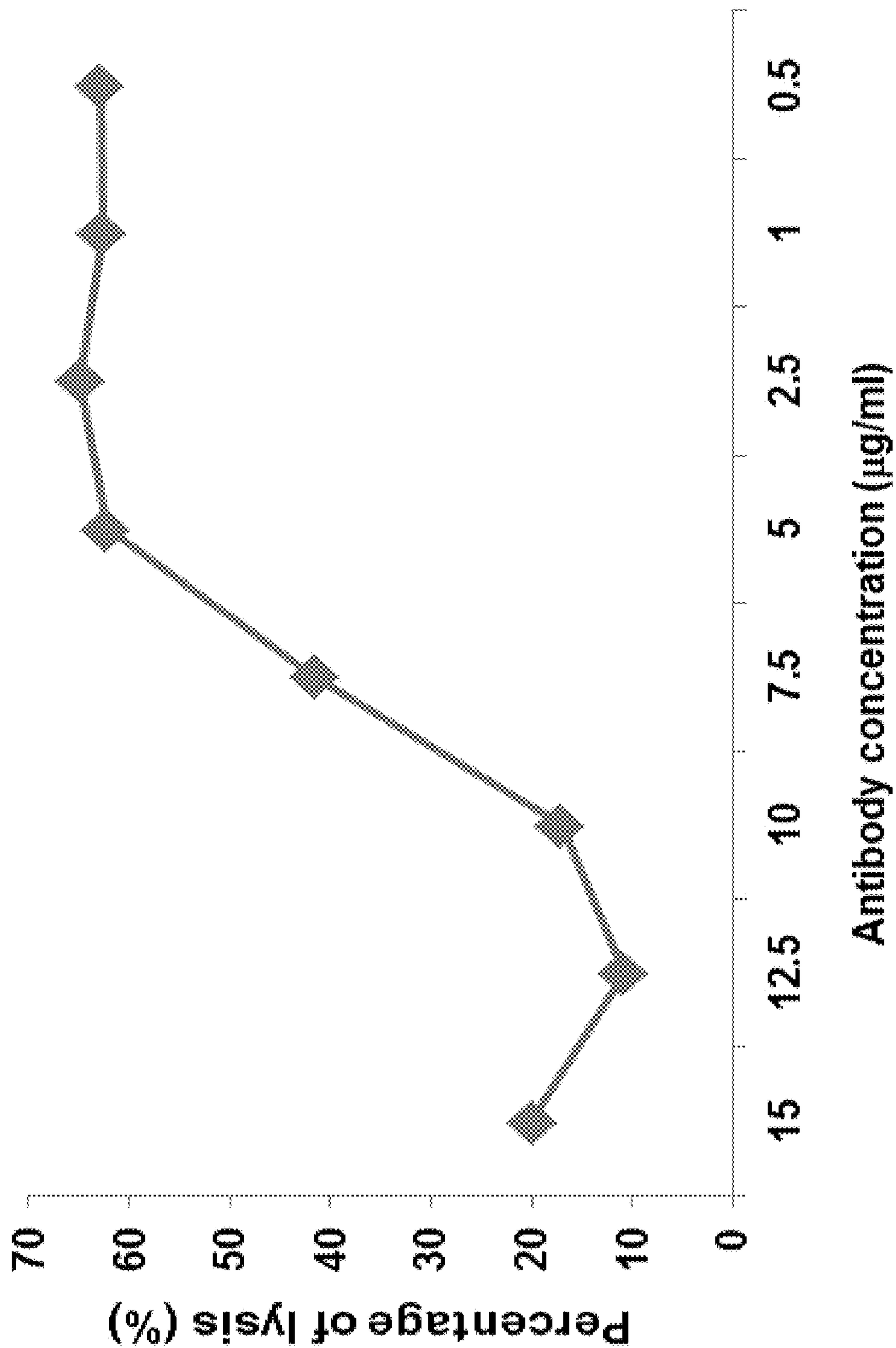


Figure 27

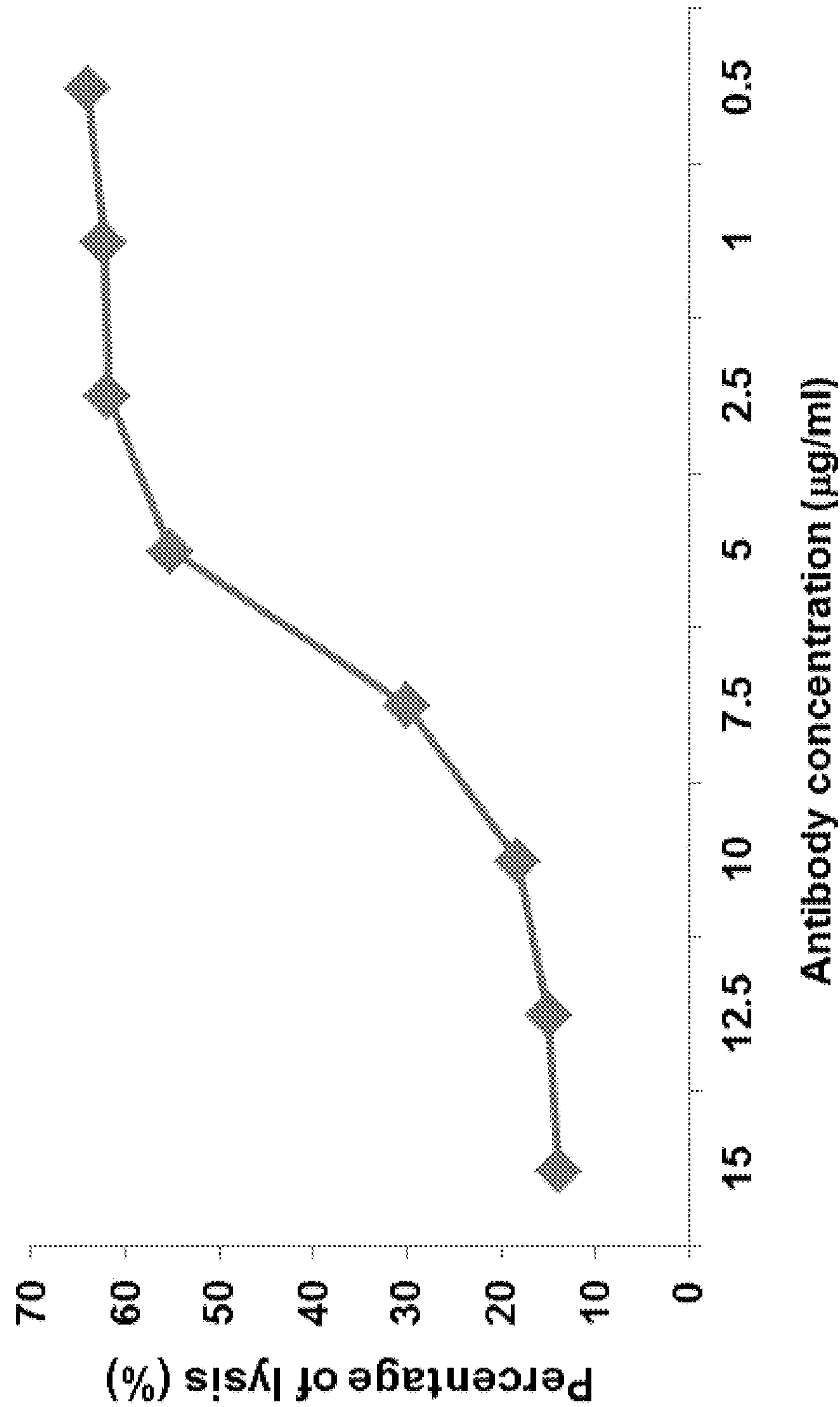


Figure 28

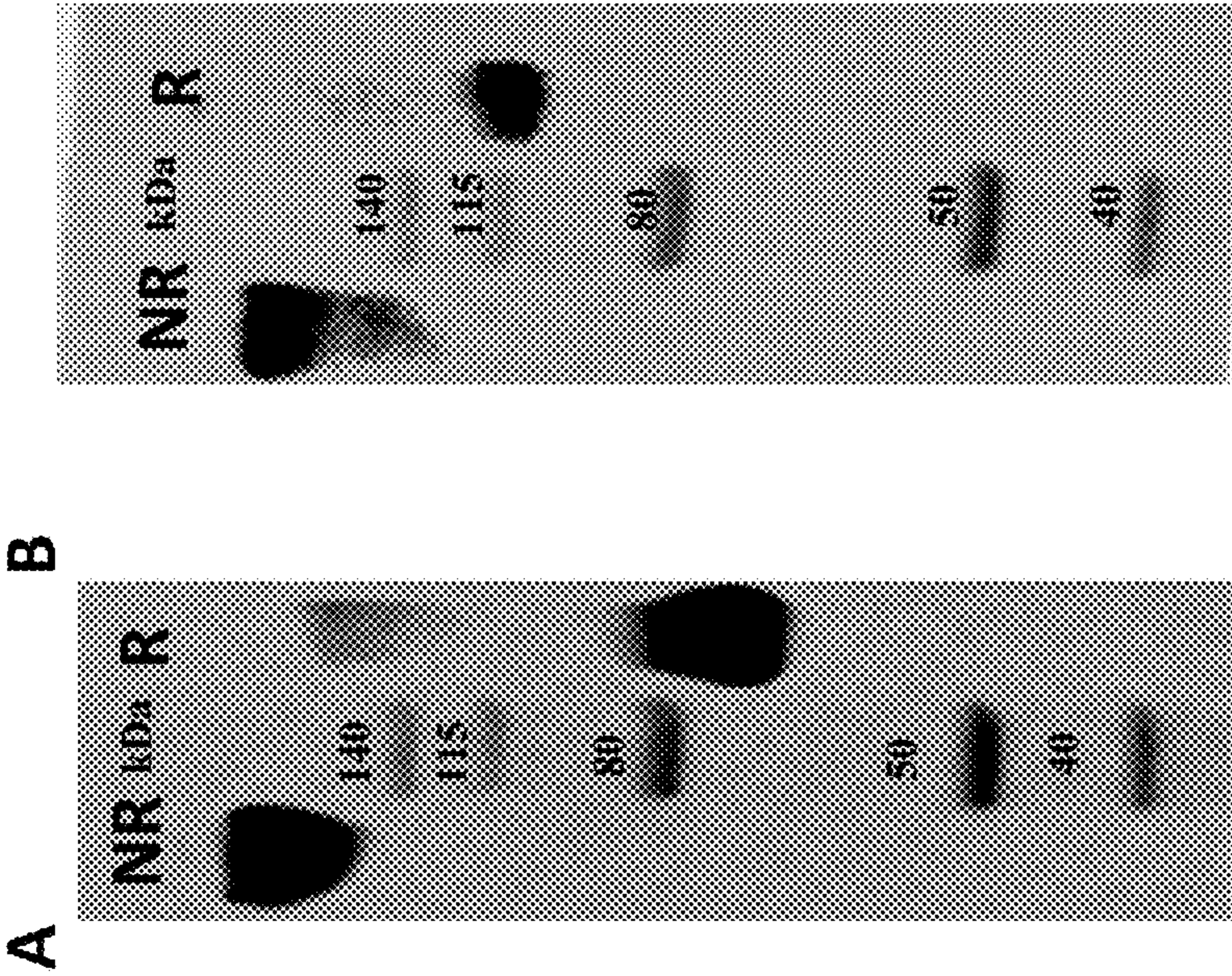


Figure 29

A

MG1	MG2	MG3	MG4	MG5	MG6	C5a	MG7	CUB	C5d	CUB	MG8	C345C
C5 β-chain							C5 α-chain					

B

MG1 :
QEQTYVISAPKIFRVGASENIVIQVGYTEAFDATISIKSYDPDKKFSYSSGHVHLSSENKFQNSAIL
TIQPKQLPGGQNPVSYYLEVVS KHFSKSRMPITYD (SEQ ID NO:84)

MG2 :
NGELFIHTDKPVYTPDQSVKVRVYSLNDDLKPAKRETVLTFIDPEGSEVDMVEEIDHIGIISFPDEFK
IPSNPRYGMWTIKAKYKEDFSTGTAYFEVKEYV (SEQ ID NO:85)

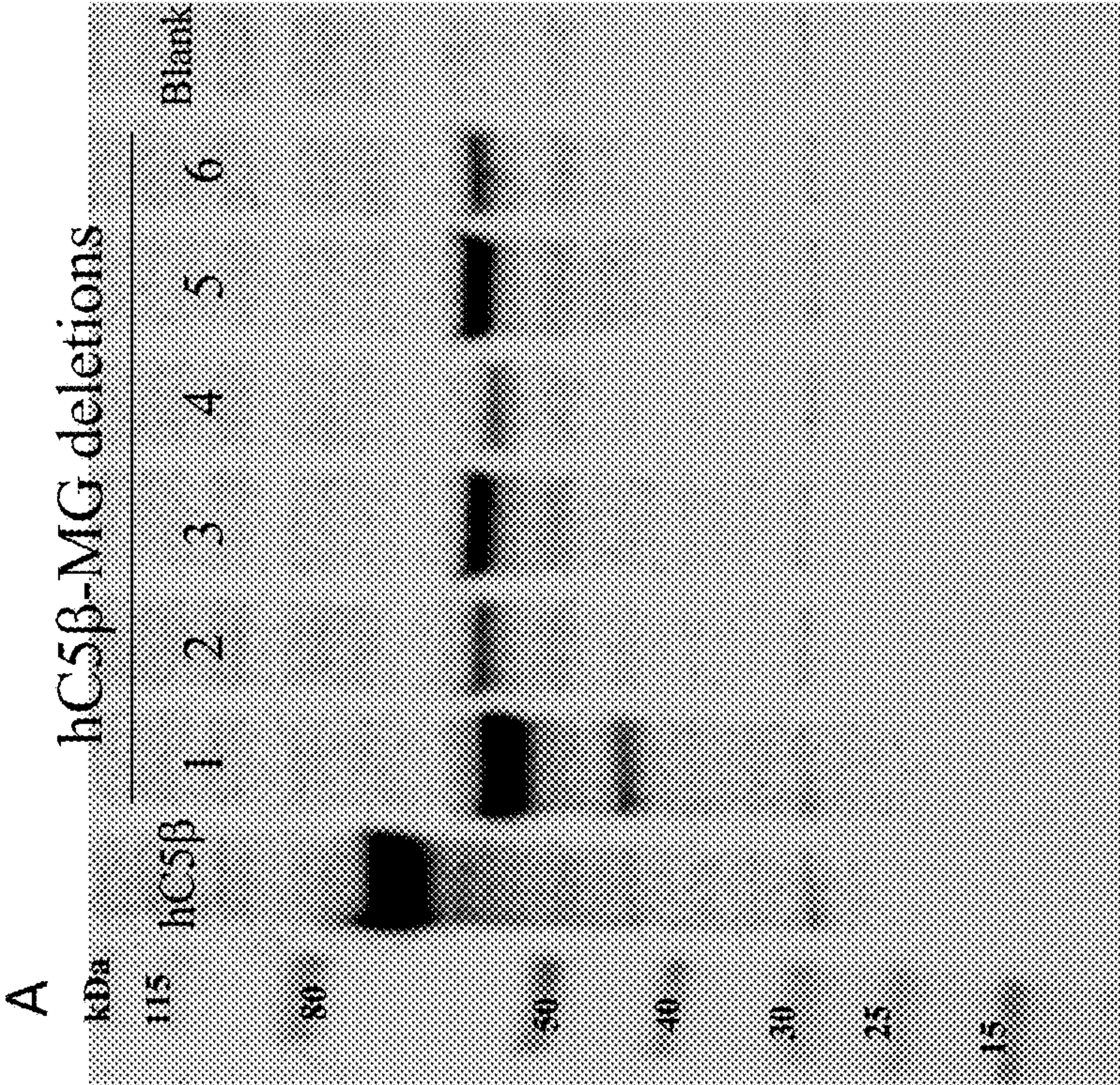
MG3 :
LPHFSVIEPEYNFIGYKNFKNFEITIKARYFYNKVVTEADVYITFGIREDLKDDQKEMMQTAMQNT
MLINGIAQVTFDSETAVKELSYSLEDLNNKYLVI AVTVIESTGGFSEEAEIPGIKYVLS (SEQ
ID NO:86)

MG4 :
PYKLNLVATPLFLKPGIPYPIKVQVKDSL DQLVGGVPVTLNAQTIDVNQETSDLDPSKSVTRVDDGV
ASFVLNLP SGVTVLEFNVKTDAPDLPEENQAREGYRAIAYS (SEQ ID NO:87)

MG5 :
SLSQSYLYIDWTDN HKALLVGEHLNII VTPKSPYIDKITHYNYLILSKGKIIHFGTREKFS DASYQS
INIPVTQNMVPSRLLVYYI VTGEQTAE LVSDSVWLNIEEK (SEQ ID NO:88)

MG6 :
CGNQLQVHLS PDADAYSPGQTVSLN MATGMDSWVALAAVDSAVYGVQRGAKKPLERV FQFLEKSDLG
CGAGGGLNNANVFHLAGLTLTNANADDSQENDEPCKEIL (SEQ ID NO:89)

Figure 30



B

hC5β and hC5β-MG deletions	Mol. Weight (kDa)
hC5β	73.2
hC5β-MG1 del	61.5
hC5β-MG2 del	61.6
hC5β-MG3 del	58.6
hC5β-MG4 del	61.6
hC5β-MG5 del	60.9
hC5β-MG6 del	62

Figure 31

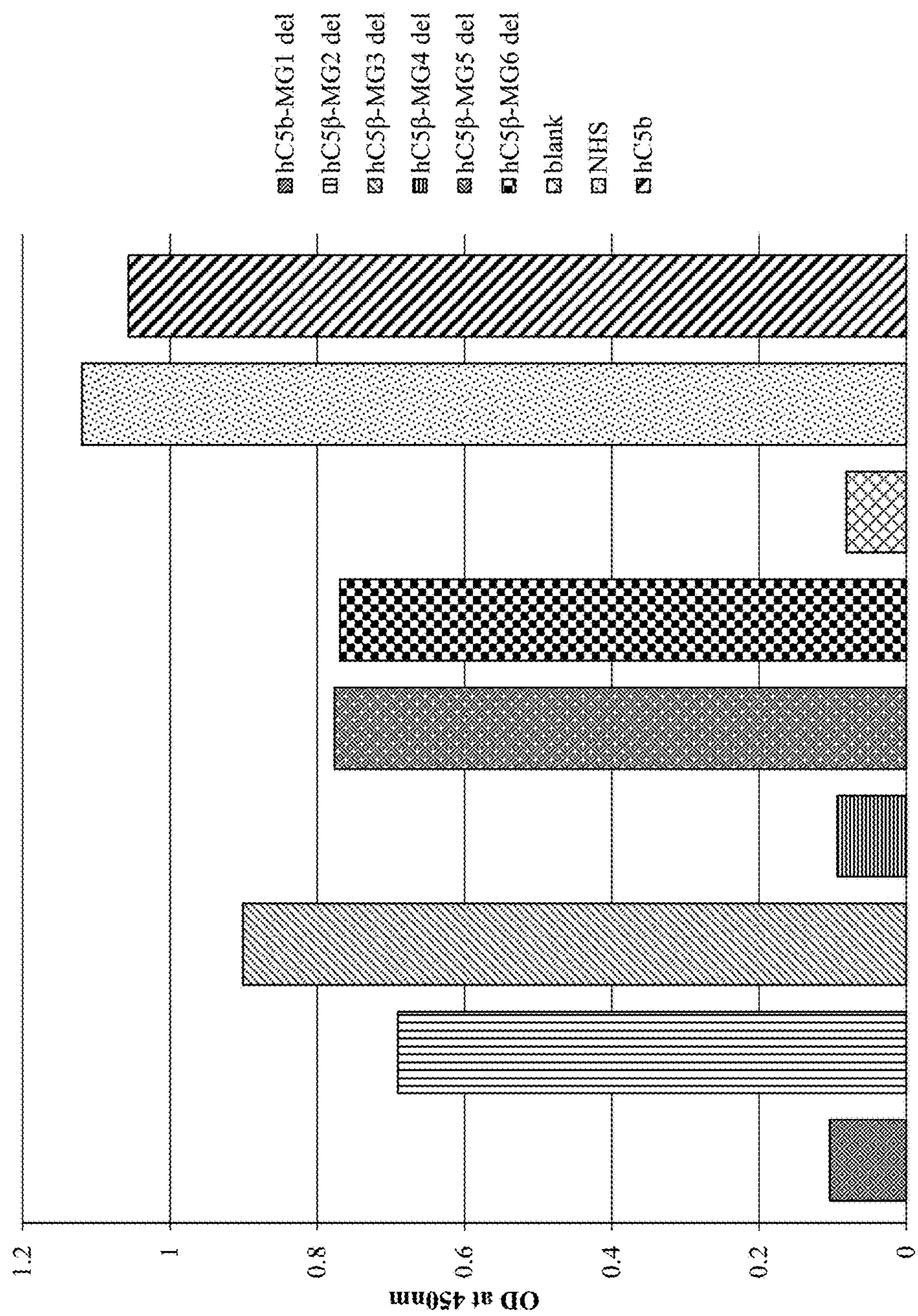


Figure 32

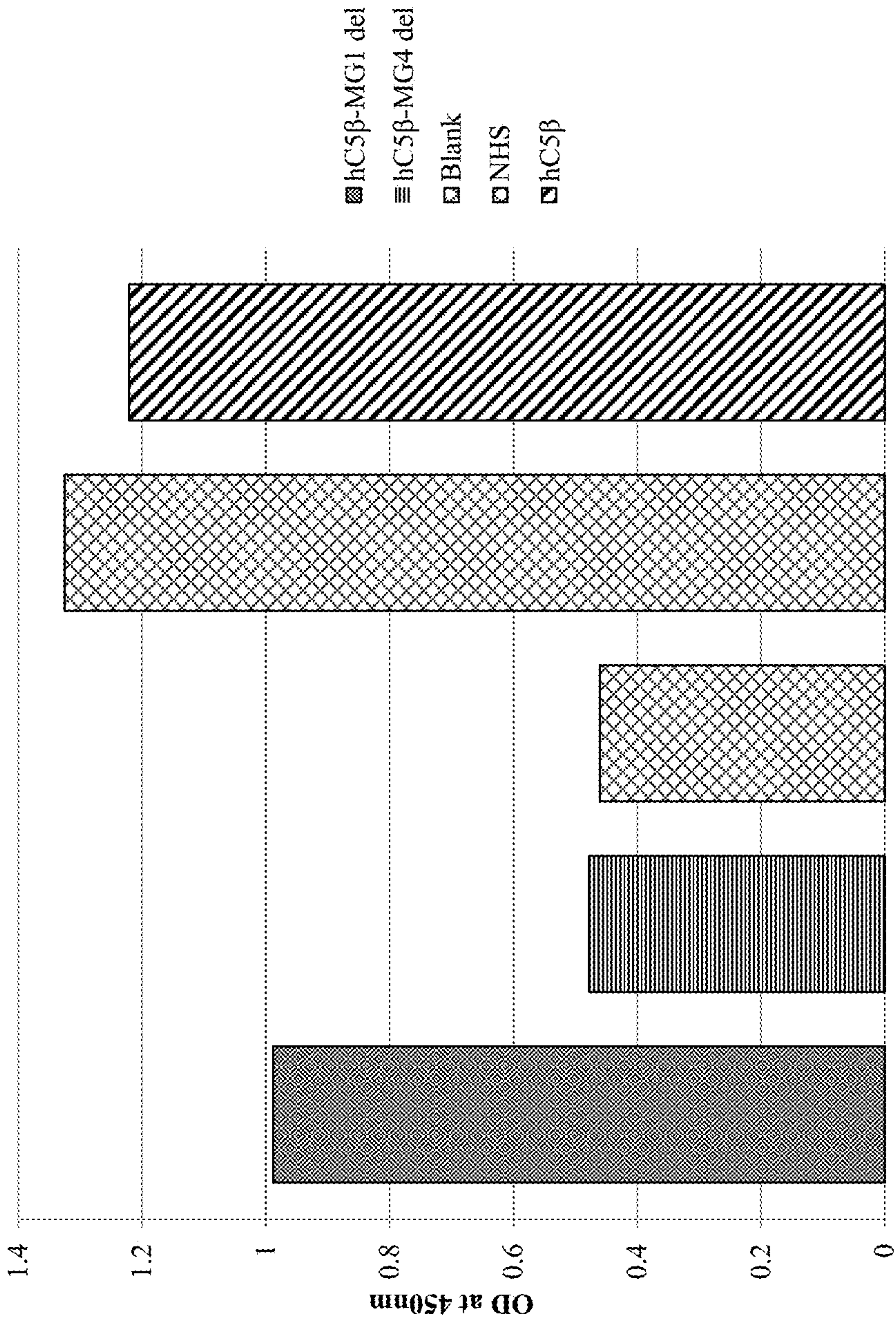


Figure 33

ANTI-C5 ANTIBODIES AND USES THEREOF**CROSS-REFERENCE TO RELATED APPLICATION**

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/467,498, filed Mar. 6, 2017, the contents of which are incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under NIH AI44970 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] The complement system is part of innate immunity that plays a key role in host defense. However, activated complement also has the potential to cause significant tissue injury and destruction and dysregulated complement activity has been found to be associated with a number of rare and common diseases such as paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome, rheumatoid arthritis, age-related macular degeneration etc. Thus, anti-complement therapy is a promising way of treating these human disorders.

[0004] Complement C5 is a critical protein in the terminal pathway of complement activation and is the precursor protein for generating the potent pro-inflammatory mediator C5a, as well as the cytolytic membrane attack complex (MAC).

[0005] A number of human inflammatory and autoimmune diseases are mediated by C5a and/or MAC, and blocking C5 activation should prevent C5a and MAC generation and be of therapeutic value. A humanized mouse anti-human C5 mAb, eculizumab, has been used to treat two complement-mediated diseases paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS). However, not all PNH patients are responsive to eculizumab treatments and one of the reasons for non-responsiveness is genetic polymorphism of human C5 with loss of epitope binding to eculizumab.

[0006] Thus, there is a need in the art for anti-human C5 mAbs that can inhibit terminal complement activity via different mechanisms and contact sites on C5 and thereby more effectively treat complement-dependent pathologies. The present invention addresses and meets these and other needs.

SUMMARY

[0007] In one embodiment, the invention comprises an antibody that specifically binds to C5. In one embodiment, the C5 is human C5. In one embodiment, the antibody is a monoclonal antibody. In one embodiment, the antibody is a humanized antibody. In one embodiment, the antibody is a chimeric antibody. In some embodiments, the antibody is a full-length antibody. In some embodiments, the antibody is an antibody fragment, which includes, but is not limited to, Fab, Fab', F(ab')₂, F(ab')₂, and scFv. In some embodiments, the antibody is part of a construct, for example a fusion construct comprising the antibody and a targeting moiety or

an effector moiety. In some embodiments, the antibody is part of a conjugate construct, such as an antibody drug conjugate construct.

[0008] In one embodiment, the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:3; VH-CDR2: SEQ ID NO:4; VH-CDR3: SEQ ID NO:5; VL-CDR1: SEQ ID NO:8; VL-CDR2: SEQ ID NO:9; and VL-CDR3: SEQ ID NO:10, or a variant or variants thereof. In one embodiment, the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:3; VH-CDR2: SEQ ID NO:4; VH-CDR3: SEQ ID NO:5; VL-CDR1: SEQ ID NO:8; VL-CDR2: SEQ ID NO:9; and VL-CDR3: SEQ ID NO:10, or a variant or variants thereof.

[0009] In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:2, or a variant thereof. In one embodiment, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:7, or a variant thereof. In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:2 and a light chain comprising the amino acid sequence of SEQ ID NO:7, or a variant or variants thereof.

[0010] In one embodiment, the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:13; VH-CDR2: SEQ ID NO:14; VH-CDR3: SEQ ID NO:15; VL-CDR1: SEQ ID NO:18; VL-CDR2: SEQ ID NO:19; and VL-CDR3: SEQ ID NO:20, or a variant or variants thereof. In one embodiment, the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:13; VH-CDR2: SEQ ID NO:14; VH-CDR3: SEQ ID NO:15; VL-CDR1: SEQ ID NO:18; VL-CDR2: SEQ ID NO:19; and VL-CDR3: SEQ ID NO:20, or a variant or variants thereof.

[0011] In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:12, or a variant thereof. In one embodiment, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:17, or a variant thereof. In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:12 and a light chain comprising the amino acid sequence of SEQ ID NO:17, or a variant or variants thereof.

[0012] In one embodiment, the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:23; VH-CDR2: SEQ ID NO:24; VH-CDR3: SEQ ID NO:25; VL-CDR1: SEQ ID NO:28; and VL-CDR2: SEQ ID NO:29, or a variant or variants thereof. In one embodiment, the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:23; VH-CDR2: SEQ ID NO:24; VH-CDR3: SEQ ID NO:25; VL-CDR1: SEQ ID NO:28; and VL-CDR2: SEQ ID NO:29, or a variant or variants thereof.

[0013] In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:22, or a variant thereof. In one embodiment, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:27, or a variant thereof. In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:22 and a light chain comprising the amino acid sequence of SEQ ID NO:27, or a variant or variants thereof.

[0014] In one embodiment, the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:32; VH-CDR2: SEQ ID NO:33; VH-CDR3: SEQ ID NO:34; VL-CDR1: SEQ ID NO:37;

VL-CDR2: SEQ ID NO:38, VL-CDR3: SEQ ID NO:39, or a variant or variants thereof. In one embodiment, the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:32; VH-CDR2: SEQ ID NO:33; VH-CDR3: SEQ ID NO:34; VL-CDR1: SEQ ID NO:37; VL-CDR2: SEQ ID NO:38, VL-CDR3: SEQ ID NO:39, or a variant or variants thereof.

[0015] In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31, or a variant thereof. In one embodiment, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:36, or a variant thereof. In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36, or a variant or variants thereof.

[0016] In one embodiment, the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:42; VH-CDR2: SEQ ID NO:43; VH-CDR3: SEQ ID NO:44; VL-CDR1: SEQ ID NO:47; VL-CDR2: SEQ ID NO:48, VL-CDR3: SEQ ID NO:49, or a variant or variants thereof. In one embodiment, the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:42; VH-CDR2: SEQ ID NO:43; VH-CDR3: SEQ ID NO:44; VL-CDR1: SEQ ID NO:47; VL-CDR2: SEQ ID NO:48, VL-CDR3: SEQ ID NO:49, or a variant or variants thereof.

[0017] In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof. In one embodiment, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:46, or a variant thereof. In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:41 and a light chain comprising the amino acid sequence of SEQ ID NO:46, or a variant or variants thereof.

[0018] In one embodiment, the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:52; VH-CDR2: SEQ ID NO:53; VH-CDR3: SEQ ID NO:54; VL-CDR1: SEQ ID NO:57; VL-CDR2: SEQ ID NO:58, VL-CDR3: SEQ ID NO:59, or a variant or variants thereof. In one embodiment, the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:52; VH-CDR2: SEQ ID NO:53; VH-CDR3: SEQ ID NO:54; VL-CDR1: SEQ ID NO:57; VL-CDR2: SEQ ID NO:58, VL-CDR3: SEQ ID NO:59, or a variant or variants thereof.

[0019] In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:51, or a variant thereof. In one embodiment, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:56, or a variant thereof. In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:51 and a light chain comprising the amino acid sequence of SEQ ID NO:56, or a variant or variants thereof.

[0020] In one embodiment, the antibody is at least one selected from the group consisting of 2G1, 8E1, 4E7, 9G6, 11C5 and 11D6.

[0021] In one embodiment, the present invention relates to a method of treating a complement pathway-mediated disease or disorder in an individual, comprising the step of administering to said individual the anti-C5 antibody of claim In one embodiment, the disease or disorder is at least selected from the group consisting of: macular degeneration (MD), age-related macular degeneration (AMD), ischemia reperfusion injury, arthritis, rheumatoid arthritis, asthma,

allergic asthma, lupus, ulcerative colitis, stroke, post-surgery systemic inflammatory syndrome, asthma, allergic asthma, chronic obstructive pulmonary disease (COPD), paroxysmal nocturnal hemoglobinuria (PNH) syndrome, myasthenia gravis, neuromyelitis optica, (NMO), multiple sclerosis, delayed graft function, antibody-mediated rejection, atypical hemolytic uremic (aHUS) syndrome, central retinal vein occlusion (CRVO), central retinal artery occlusion (CRAO), epidermolysis bullosa, sepsis, organ transplantation, inflammation (including, but not limited to, inflammation associated with cardiopulmonary bypass surgery and kidney dialysis), C3 glomerulopathy, membranous nephropathy, IgA nephropathy, glomerulonephritis (including, but not limited to, anti-neutrophil cytoplasmic antibody (ANCA)-mediated glomerulonephritis, lupus nephritis, and combinations thereof), ANCA-mediated vasculitis, Shiga toxin induced HUS, and antiphospholipid antibody-induced pregnancy loss, or any combinations thereof. In some embodiments, the AP-mediated disease is C3 glomerulopathy. In some embodiments, the AP-mediated disease is macular degeneration, such as age-related macular degeneration. In one embodiment, administration of the anti-C5 antibody inhibits the generation of a C5a or C5b protein.

[0022] In one embodiment, the present invention relates to a method of reducing the activity of a complement system of an individual, wherein the method comprises administering an antibody to the individual via a route of administration selected from the group consisting of enteral administration, parenteral administration, and a combination thereof, and wherein the antibody comprises six complementarity determining regions having the following amino acid sequences: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10, or a variant or variants thereof. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0023] In one embodiment, the present invention relates to a method of reducing the activity of a complement system of an individual, wherein the method comprises administering an antibody to the individual via a route of administration selected from the group consisting of enteral administration, parenteral administration, and a combination thereof, and wherein the antibody comprises six complementarity determining regions having the following amino acid sequences: SEQ ID NO: 13, SEQ ID NO: 14, and SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20, or a variant or variants thereof. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0024] In one embodiment, the present invention relates to a method of reducing the activity of a complement system of an individual, wherein the method comprises administering an antibody to the individual via a route of administration selected from the group consisting of enteral administration, parenteral administration, and a combination thereof, and wherein the antibody comprises six complementarity determining regions having the following amino acid sequences: SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 28, SEQ ID NO: 29, or a variant or variants thereof. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0025] In one embodiment, the present invention relates to a method of reducing the activity of a complement system of an individual, wherein the method comprises administering an antibody to the individual via a route of administration selected from the group consisting of enteral administration, parenteral administration, and a combination thereof, and wherein the antibody comprises six complementarity determining regions having the following amino acid sequences: SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39, or a variant or variants thereof. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0026] In one embodiment, the present invention relates to a method of reducing the activity of a complement system of an individual, wherein the method comprises administering an antibody to the individual via a route of administration selected from the group consisting of enteral administration, parenteral administration, and a combination thereof, and wherein the antibody comprises six complementarity determining regions having the following amino acid sequences: SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 47, SEQ ID NO: 48, and SEQ ID NO: 49, or a variant or variants thereof. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0027] In one embodiment, the present invention relates to a method of reducing the activity of a complement system of an individual, wherein the method comprises administering an antibody to the individual via a route of administration selected from the group consisting of enteral administration, parenteral administration, and a combination thereof, and wherein the antibody comprises six complementarity determining regions having the following amino acid sequences: SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 57, SEQ ID NO: 58, and SEQ ID NO: 59, or a variant or variants thereof. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0028] In some embodiments, the present invention is an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 2, or a variant thereof. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0029] In some embodiments, the present invention is an antibody against human C5, wherein the antibody has a light chain variable (vL) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 7, or a variant thereof. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0030] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region and a light chain variable (vL) region, wherein the vH region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 2, and wherein the vL region

has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 7. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0031] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 12. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0032] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a light chain variable (vL) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 17. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0033] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region and a light chain variable (vL) region, wherein the vH region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 12, and wherein the vL region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 17. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0034] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 22. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0035] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a light chain variable (vL) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 27. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0036] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region and a light chain variable (vL) region, wherein the vH region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 22, and wherein the vL region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, or 99%) identical to SEQ ID NO: 27. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0037] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 31. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0038] In one embodiment, the present invention relates to an antibody against human C5, wherein the antibody has a light chain variable (vL) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 36. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0039] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region and a light chain variable (vL) region, wherein the vH region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 31, and wherein the vL region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 36. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0040] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 41. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0041] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a light chain variable (vL) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 46. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0042] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region and a light chain variable (vL) region, wherein the vH region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 41, and wherein the vL region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 46. In one embodiment, the antibody is an antibody fragment

selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0043] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 51. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0044] In one embodiment, the present invention relates to an antibody against human C5, wherein the antibody has a light chain variable (vL) region that has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 56. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0045] In some embodiments, the present invention relates to an antibody against human C5, wherein the antibody has a heavy chain variable (vH) region and a light chain variable (vL) region, wherein the vH region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 51, and wherein the vL region has an amino acid sequence that is more than about 90% (such as more than any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NO: 56. In one embodiment, the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

[0046] In one embodiment, the present invention relates to a cell comprising at least one of the antibodies described elsewhere herein. In some embodiments, the cell produces the antibody of at least one of the antibodies described elsewhere herein. In one embodiment, the cell is a hybridoma.

[0047] In one embodiment, the present invention is a cell line comprising at least one of the antibodies described elsewhere herein. In some embodiments, the cell line produces at least one of the antibodies described elsewhere herein. In some embodiments, the cell line is a hybridoma cell line.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] The foregoing summary, as well as the following detailed description of exemplary embodiments of the invention, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings. In the drawings:

[0049] FIG. 1 depicts results from an ELISA assay demonstrating the binding of anti-human C5 mAbs 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1 to human C5. Direct antigen binding ELISA in which mAbs are serially diluted across microtiter plates coated with purified human C5. All six mAbs showed high reactivity with human C5.

[0050] FIGS. 2-7 depict the results of experiments measuring binding affinities of anti-C5 mAb 2G1, 8E1, 4E7, 9G6, 11C5 and 11D9 to C5. Purified α mRabbit IgG (RAMFc) mAb was coupled onto a CM4 chip using the

amine coupling method. Then, anti-C5 mAbs were captured on immobilized RAMFc. Biacore analyses were performed on a Biacore-2000 instrument.

[0051] FIG. 8 depicts dose-dependent inhibition of LPS-induced C5a production by anti-human C5 mAbs 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1. To assess the effect of anti-human C5 mAbs on LPS-induced C5a production, a combination of two assays was used: LPS-induced C5a production and human C5a sandwich ELISA. All six mAbs effectively inhibited LPS-induced C5a production when added to 10% normal human serum (NHS) at a final concentration of 12.5 $\mu\text{g/ml}$.

[0052] FIG. 9, comprising FIG. 9A and FIG. 9B, depicts the effects of anti-human C5 mAbs 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1 on complement-mediated hemolysis.

[0053] FIG. 9A illustrates red blood cells (RBC) lysis determined by measuring the absorbance at OD₄₀₅ after antibody-sensitized sheep RBCs were incubated with 50% NHS containing serial dilutions of each anti-C5 mAb at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD₄₀₅ nm. At 120 $\mu\text{g/ml}$, all mAbs inhibited 50% NHS-mediated sheep erythrocyte lysis. At lower doses (e.g., 30-60 $\mu\text{g/ml}$), 9G6 was less potent in preventing hemolysis than other mAbs. FIG. 9B illustrates that at 30 $\mu\text{g/ml}$, mAb 2G1 and 8E1 were more potent at inhibiting complement-mediated hemolysis than 4E7, 9G6, 11C5 and 11D9.

[0054] FIG. 10 depicts the results of experiments assessing the effects of anti-human C5 mAbs 4E7, 9G6, 11C5, 2G1, 11D9, 8E1 on complement-mediated hemolysis using sera from different animal species. Antibody-sensitized chicken RBCs were incubated with 50% NHS, normal rabbit serum, Rhesus monkey serum or Cynomolgus monkey serum containing each anti-C5 mAb (final concentration: 50 $\mu\text{g/ml}$) at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD₄₀₅ nm, and normalized against serum plus EDTA as negative control (0%), and against serum plus no antibody as positive control (100%). In the hemolytic assay using NHS, five of six anti-human C5 mAbs inhibited hemolysis significantly, i.e. by more than 50%. Especially, NHS samples containing 2G1 showed almost complete inhibition of hemolysis. When treated with mAb 9G6 or 2G1, hemolytic activity in rabbit serum was significantly reduced. On the other hand, all mAbs did not significantly inhibit complement-mediated hemolysis using monkey (Rhesus and Cynomolgus) sera.

[0055] FIG. 11 depicts the results of an experiment assessing the effects of anti-human C5 mAbs 2G1 and 8E1 on complement-mediated hemolysis. Antibody-sensitized chicken RBCs were incubated with 50% NHS containing serial dilutions of 2G1 or 8E1 or control mAb (MOPC, mouse IgG1) at 37° C. for 1 hr. RBC lysis was determined by measuring the absorbance at OD₄₀₅ nm. mAb 2G1 and 8E1 had similar activities in this assay.

[0056] FIG. 12 depicts the results of experiments assessing the effect of anti-human C5 mAb 2G1 on hemolysis of PNH RBCs. RBCs from paroxysmal nocturnal hemoglobinuria (PNH) patients were subjected to Ham's acidified serum test in the presence or absence of mAb 2G1. RBCs were incubated with 50% NHS containing serial dilutions of 2G1 at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD₄₀₅ nm. In the absence of mAb, about 35% of RBCs were lysed by acidified serum, while mAb

2G1 treatment caused 70% reduction of hemolytic activity at 25 $\mu\text{g/ml}$ and 85% reduction at 40 $\mu\text{g/ml}$.

[0057] FIG. 13 depicts the variable region sequences of heavy and light chains of mAb 2G1.

[0058] FIG. 14 depicts the variable region sequences of heavy and light chains of mAb 8E1.

[0059] FIG. 15 depicts the variable region sequences of heavy and light chains of mAb 4E7.

[0060] FIG. 16 depicts the variable region sequences of heavy and light chains of mAb 9G6.

[0061] FIG. 17 depicts the variable region sequences of heavy and light chains of mAb 11C5.

[0062] FIG. 18 depicts the variable region sequences of heavy and light chains of mAb 11D9.

[0063] FIG. 19 depicts amino acid sequences of human IgG4 heavy chain constant region, with a serine 228 to proline mutation (i.e., S228P) and human kappa light chain constant region. These sequences were used to construct chimeric (mouse variable region+human constant regions) and humanized (humanized mouse variable region+human constant regions) anti-human C5 antibody (2G1).

[0064] FIG. 20 depicts results from experiments evaluating the reactivity of 2G1 human IgG4 chimeric mAb with human C5. Chimeric 2G1 was made by joining the variable regions of mAb 2G1 with human IgG4 heavy chain constant region carrying a S228P mutation and human kappa light chain constant region. A plate was coated with human C5. After incubation with serially diluted chimeric 2G1 mAb, bound chimeric mAb was detected by HRP-conjugated anti-human IgG4. Chimeric 2G1 bound to human C5 in a dose-dependent manner.

[0065] FIG. 21 depicts results from experiments evaluating the effects of human IgG4-2G1 chimeric mAb on classical pathway complement-mediated hemolysis. Sensitized sheep RBCs were incubated with 50% NHS containing serially diluted chimeric 2G1 at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD₄₀₅ nm. The result shows that at 30 $\mu\text{g/ml}$ and higher concentrations, chimeric 2G1 mAb effectively inhibited NHS-mediated sheep erythrocyte lysis.

[0066] FIG. 22 depicts nucleotide and amino acid sequences of a humanized variable heavy chain (VH) of mAb 2G1 (humanized 2G1 VH-11801). Humanization was achieved by CDR grating from murine mAb 2G1 VH into a germline encoded human VH frame (11801). The amino acid sequence of signal peptide is underlined and that of CDR1, CDR2 and CDR3 is bolded and shaded.

[0067] FIG. 23 depicts nucleotide and amino acid sequences of another humanized VH of mAb 2G1 (humanized 2G1 VH-16901). Humanization was achieved by CDR grating from murine mAb 2G1 VH into a germline encoded human VH frame (16901). The amino acid sequence of signal peptide is underlined and that of CDR1, CDR2 and CDR3 is bolded shaded.

[0068] FIG. 24 depicts nucleotide and amino acid sequences of a humanized variable light chain (VL) of mAb 2G1 (humanized 2G1 VL-1901). Humanization was achieved by CDR grating from murine mAb 2G1 VL into a germline encoded human VL frame (1901). The amino acid sequence of signal peptide is underlined and that of CDR1, CDR2 and CDR3 is bolded and shaded.

[0069] FIG. 25 depicts results from experiments evaluating the reactivity of humanized 2G1 (VH-11801/VL-1901) with human C5. Humanized 2G1 (VH-11801/VL-1901) was

expressed as a human IgG4 mAb with S228P mutation in the Fc domain. A plate was coated with human C5. After incubation with serially diluted humanized 2G1 (VH-11801/VL-1901), bound mAb was detected by HRP-conjugated anti-human IgG4. Humanized 2G1 (VH-11801/VL-1901) bound human C5 in a dose-dependent manner.

[0070] FIG. 26 depicts results from experiments evaluating the reactivity of humanized 2G1 (VH-16901/VL-1901) with human C5. Humanized 2G1 (VH-16901/VL-1901) was expressed as a human IgG4 mAb with S228P mutation in the Fc domain. A plate was coated with human C5. After incubation with serially diluted humanized 2G1 (VH-16901/VL-1901), bound Ab was detected by HRP-conjugated anti-human IgG4. Humanized 2G1 (VH-16901/VL-1901) bound human C5 in a dose-dependent manner.

[0071] FIG. 27 depicts results from experiments evaluating the effects of the humanized 2G1 (VH-11801/VL-1901) on classical pathway complement-mediated hemolysis. Humanized 2G1 (VH-11801/VL-1901) was expressed as a human IgG4 mAb with S228P mutation in the Fc domain. Antibody-sensitized sheep RBCs were incubated with 10% NHS containing serially diluted humanized 2G1 (VH-11801/VL-1901) at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD₄₀₅ nm. Humanized 2G1 (VH-11801/VL-1901) significantly inhibited 10% NHS-mediated sheep erythrocyte lysis at 10 µg/ml and higher mAb concentrations.

[0072] FIG. 28 depicts results from experiments evaluating the effects of the humanized 2G1 (VH-16901/VL-1901) on classical pathway complement-mediated hemolysis. Humanized 2G1 (VH-16901/VL-1901) was expressed as a human IgG4 mAb with S228P mutation in the Fc domain. Antibody-sensitized sheep RBCs were incubated with 10% NHS containing serially diluted humanized 2G1 (VH-16901/VL-1901) at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD₄₀₅ nm. Humanized 2G1 (VH-16901/VL-1901) significantly inhibited 10% NHS-mediated sheep erythrocyte lysis at 10 µg/ml and higher mAb concentrations.

[0073] FIG. 29, comprising FIGS. 29A and 29B, depicts results from experiments using Western blotting to detect human C5 using mAb 2G1-3 (FIG. 29A) and a control mAb QDC5 (FIG. 29B) showing that mAb 2G1-3 binds to the β chain of human C5. Human C5 (1 µg was used per lane, from Comptech, cat #A120) was run on SDS-PAGE under non-reducing (NR) or reducing (R) conditions SDS-PAGE. The control mAb QDC5 is a recombinant human IgG4 mAb bearing VH and VL sequences of a humanized mouse anti-human C5 mAb as described in Thomas et al. (Mol Immunol. 1996 December; 33(17-18):1389-401). This mAb is known to binds to an epitope in the α chain of human C5. As expected, both mAb 2G1-3 and QDC5 bound to non-reduced human C5 similarly. Under reducing condition, QDC5 bound to the α chain of human C5 as expected, whereas mAb 2G1-3 bound to a different band corresponding to the β chain of human C5. After SDS-PAGE, proteins were transferred on to PVDF membrane and blocked with 5% non-fat dry milk in TBS for 1 h at room temperature. The membranes were incubated with 10 µg/ml of 2G1-3 or QDC5 for 1 h at room temperature. After washing with TBS with 0.1% Tween-20 (TBST) for 6×5 min, 1:4000 dilution of Rabbit α-mouse IgG-HRP or α-human IgG-HRP were added to the membranes and incubated for 1 h at room temperature. After final washing, proteins were detected

using Pierce™ ECL 2 Western Blotting substrate according to manufacturer's instructions.

[0074] FIG. 30, comprising FIGS. 30A and 30B, depicts the domain structure and sequences of human C5. Human C5 is composed of a and 13 chains separated by a small C5a segment that is released upon C5 activation (FIG. 30A). The human C5 β chain is in turn composed of 6 MG domains with amino acid sequences as listed (FIG. 30B).

[0075] FIG. 31, comprising FIGS. 31A and 31B, depicts the Western blot detection of human C5 β chain and human C5 β chain deletion mutants lacking individual MG domains. (FIG. 31A). Western blotting using a goat anti-human C5 polyclonal antibody detected the intact 13 chain and the 6 MG domain deletion mutants transiently expressed in HEK cells. Supernatants of transfected HEK cells were used for analysis. The numbers 1, 2, 3, 4, 5, 6 designates MG1, MG2, MG3, MG4, MG5 and MG6 deletion mutants. Supernatant from non-transfected HEK cells (Blank) was used as a negative control. These results demonstrated that all deletion mutants were expressed. (FIG. 31B) Calculated molecular weights in kDa of human C5 β chain and the 6 MG deletion mutants are consistent with the detected bands on Western blot.

[0076] FIG. 32 depicts the results of a Sandwich ELISA assay to assess the critical MG domains with human C513 chain for mAb 2G1-3 binding. mAb 2G1-3 was coated onto 96 well plate and supernatants from non-transfected or transfected HEK cells were added. After incubation and washing, the bound β chain or deletion mutant proteins were detected by a second anti-human C5 mAb SKY59 (Fukuzawa et al., Sci Rep. 2017 Apr. 24; 7(1):1080. doi: 10.1038/s41598-017-01087-7) which is known to bind sequences within the MG1 domain of human C5. The data demonstrated that signals for MG2, MG3, MG5 and MG6 deletion mutants were still detected, suggesting these domains were not involved in the binding of either 2G1-3 or SKY59. On the other hand, MG1 and MG4 deletion mutants lost binding, suggesting they are critical for human C5 binding by either 2G1-3 or SKY59 or both. Normal human serum (NHS) and intact β chain transfected cell supernatant were used as positive controls and non-transfected HEK cell supernatant (blank) was used as a negative control.

[0077] FIG. 32 depicts the results of a Sandwich ELISA assay using polyclonal anti-C5 antibody for detection demonstrating that MG4 domain within human C5 β chain is critical for mAb 2G1-3 binding. 2G1-3 was coated onto 96-well plate and supernatants from non-transfected or transfected HEK cells were added. After incubation and washing, the bound β chain or deletion mutant e proteins in transfection supernatants were detected by a goat anti-human C5 polyclonal antibody. Signals were detected in normal human serum (NHS) and supernatants of intact human β chain and MG1 deletion mutant but not MG4 deletion mutant, suggesting that MG4 but not MG1 within the β chain is critical for 2G1-3 binding.

DETAILED DESCRIPTION OF THE INVENTION

[0078] This invention relates to the inhibition of complement signaling using an anti-C5 antibody. In various embodiments, the invention is directed to compositions and methods for treating a complement-mediated disease or complement-mediated disorder in an individual by contacting the individual with an anti-C5 antibody. The comple-

ment-mediated pathologies and conditions that can be treated with the compositions and methods of the invention include, but are not limited to, macular degeneration (MD), age-related macular degeneration (AMD), ischemia reperfusion injury, arthritis, rheumatoid arthritis, lupus, ulcerative colitis, stroke, post-surgery systemic inflammatory syndrome, asthma, allergic asthma, chronic obstructive pulmonary disease (COPD), paroxysmal nocturnal hemoglobinuria (PNH) syndrome, myasthenia gravis, neuromyelitis optica, (NMO), multiple sclerosis, delayed graft function, antibody-mediated rejection, atypical hemolytic uremic (aHUS) syndrome, central retinal vein occlusion (CRVO), central retinal artery occlusion (CRAO), epidermolysis bullosa, sepsis, organ transplantation, inflammation (including, but not limited to, inflammation associated with cardiopulmonary bypass surgery and kidney dialysis), C3 glomerulopathy, membranous nephropathy, IgA nephropathy, glomerulonephritis (including, but not limited to, anti-neutrophil cytoplasmic antibody (ANCA)-mediated glomerulonephritis, lupus nephritis, and combinations thereof), ANCA-mediated vasculitis, Shiga toxin induced HUS, and antiphospholipid antibody-induced pregnancy loss, or any combinations thereof.

Definitions

[0079] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, exemplary methods and materials are described.

[0080] As used herein, each of the following terms has the meaning associated with it in this section.

[0081] The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0082] The terms “inhibit” and “inhibition,” as used herein, means to reduce, suppress, diminish or block an activity or function by at least about 10% relative to a control value. In some embodiments, the activity is suppressed or blocked by at least about 50% compared to a control value. In some embodiments, the activity is suppressed or blocked by at least about 75%. In some embodiments, the activity is suppressed or blocked by at least about 95%.

[0083] The terms “effective amount” and “pharmaceutically effective amount” refer to a sufficient amount of an agent to provide the desired biological result. That result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease or disorder, or any other desired alteration of a biological system. An appropriate effective amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

[0084] The terms “patient,” “subject,” “individual,” and the like are used interchangeably herein, and refer to any animal, in some embodiments a mammal, and in some embodiments a human, having a complement system, including a human in need of therapy for, or susceptible to, a condition or its sequelae. The individual may include, for example, dogs, cats, pigs, cows, sheep, goats, horses, rats, monkeys, and mice and humans.

[0085] The term “abnormal” when used in the context of organisms, tissues, cells or components thereof, refers to those organisms, tissues, cells or components thereof that differ in at least one observable or detectable characteristic (e.g., age, treatment, time of day, etc.) from those organisms, tissues, cells or components thereof that display the “normal” (expected/homeostatic) respective characteristic. Characteristics which are normal or expected for one cell, tissue type, or subject, might be abnormal for a different cell or tissue type.

[0086] A “disease” is a state of health of a subject wherein the subject cannot maintain homeostasis, and wherein if the disease is not ameliorated then the subject’s health continues to deteriorate.

[0087] In contrast, a “disorder” in a subject is a state of health in which the subject is able to maintain homeostasis, but in which the subject’s state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the subject’s state of health.

[0088] A disease or disorder is “alleviated” if the severity of a sign or symptom of the disease or disorder, the frequency with which such a sign or symptom is experienced by a patient, or both, is reduced.

[0089] An “effective amount” or “therapeutically effective amount” of a compound is that amount of compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered.

[0090] As used herein, an “instructional material” includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of a compound, composition, vector, or delivery system of the invention in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one or more methods of alleviating the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit of the invention can, for example, be affixed to a container which contains the identified compound, composition, vector, or delivery system of the invention or be shipped together with a container which contains the identified compound, composition, vector, or delivery system. Alternatively, the instructional material can be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

[0091] “Operably linked” or “operatively linked” as used herein may mean that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene may be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

[0092] A “therapeutic treatment” is a treatment administered to a subject who exhibits signs of disease or disorder, for the purpose of diminishing or eliminating those signs.

[0093] As used herein, “treating a disease or disorder” means reducing the frequency and/or severity of a sign and/or symptom of the disease or disorder is experienced by a patient.

[0094] The phrase “biological sample”, “sample” or “specimen” as used herein, is intended to include any sample comprising a cell, a tissue, or a bodily fluid in which expression of a nucleic acid or polypeptide can be detected. The biological sample may contain any biological material suitable for detecting the desired biomarkers, and may comprise cellular and/or non-cellular material obtained from the individual. Examples of such biological samples include but are not limited to blood, lymph, bone marrow, biopsies and smears. Samples that are liquid in nature are referred to herein as “bodily fluids.” Biological samples may be obtained from a patient by a variety of techniques including, for example, by scraping or swabbing an area or by using a needle to obtain bodily fluids. Methods for collecting various body samples are well known in the art.

[0095] The term “antibody,” as used herein, refers to an immunoglobulin molecule which is able to specifically bind to a specific epitope of an antigen. Antibodies can be intact immunoglobulins derived from natural sources, or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. The antibodies in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, intracellular antibodies (“intrabodies”), Fv, Fab, Fab', F(ab)2 and F(ab')2, as well as single chain antibodies (scFv), heavy chain antibodies, such as camelid antibodies, and humanized antibodies (Harlow et al., 1999, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, N.Y.; Houston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; Bird et al., 1988, *Science* 242:423-426).

[0096] By the term “synthetic antibody” as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and well known in the art.

[0097] As used herein, the term “heavy chain antibody” or “heavy chain antibodies” comprises immunoglobulin molecules derived from camelid species, either by immunization with a peptide and subsequent isolation of sera, or by the cloning and expression of nucleic acid sequences encoding such antibodies. The term “heavy chain antibody” or “heavy chain antibodies” further encompasses immunoglobulin molecules isolated from a subject with heavy chain disease, or prepared by the cloning and expression of VH (variable heavy chain immunoglobulin) genes from a subject.

[0098] A “chimeric antibody” refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

[0099] A “humanized antibody” refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see,

e.g., 1989, Queen et al., *Proc. Natl. Acad. Sci. USA*, 86:10029-10032; 1991, Hodgson et al., *Bio/Technology*, 9:421). A suitable human acceptor antibody may be one selected from a conventional database, e.g., the KABAT database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanized antibodies (see for example EP-A-0239400 and EP-A-054951).

[0100] The term “donor antibody” refers to an antibody (monoclonal, and/or recombinant) which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody.

[0101] The term “acceptor antibody” refers to an antibody (monoclonal and/or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but in some embodiments all) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. In certain embodiments a human antibody is the acceptor antibody.

[0102] “CDRs” are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., *Sequences of Proteins of Immunological Interest*, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, “CDRs” as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be so by a skilled person. See for example Chothia et al., (1989) *Conformations of immunoglobulin hypervariable regions*; *Nature* 342, p 877-883.

[0103] As used herein, an “immunoassay” refers to any binding assay that uses an antibody capable of binding specifically to a target molecule to detect and quantify the target molecule.

[0104] By the term “specifically binds,” as used herein with respect to an antibody, is meant an antibody which recognizes and binds to a specific target molecule, but does not substantially recognize or bind other molecules in a sample. In some instances, the terms “specific binding” or “specifically binding,” is used to mean that the recognition and binding is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the target molecule. If, for example, an antibody specifically binds to epitope “A,” the presence of an unlabelled molecule

containing epitope A (or free, unlabeled A) in a reaction containing labeled “A” and the antibody, will reduce the amount of labeled A bound to the antibody.

[0105] A “coding region” of a gene consists of the nucleotide residues of the coding strand of the gene and the nucleotides of the non-coding strand of the gene which are homologous with or complementary to, respectively, the coding region of an mRNA molecule which is produced by transcription of the gene.

[0106] A “coding region” of a mRNA molecule also consists of the nucleotide residues of the mRNA molecule which are matched with an anti-codon region of a transfer RNA molecule during translation of the mRNA molecule or which encode a stop codon. The coding region may thus include nucleotide residues comprising codons for amino acid residues which are not present in the mature protein encoded by the mRNA molecule (e.g., amino acid residues in a protein export signal sequence).

[0107] “Differentially decreased expression” or “down regulation” refers to biomarker product levels which are at least 10% or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% lower or less, and/or 2.0 fold, 1.8 fold, 1.6 fold, 1.4 fold, 1.2 fold, 1.1 fold or less lower, and any and all whole or partial increments therebetween than a control.

[0108] “Differentially increased expression” or “up regulation” refers to biomarker product levels which are at least 10% or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% higher or more, and/or 1.1 fold, 1.2 fold, 1.4 fold, 1.6 fold, 1.8 fold, 2.0 fold higher or more, and any and all whole or partial increments therebetween than a control.

[0109] “Complementary” as used herein to refer to a nucleic acid, refers to the broad concept of sequence complementarity between regions of two nucleic acid strands or between two regions of the same nucleic acid strand. It is known that an adenine residue of a first nucleic acid region is capable of forming specific hydrogen bonds (“base pairing”) with a residue of a second nucleic acid region which is antiparallel to the first region if the residue is thymine or uracil. Similarly, it is known that a cytosine residue of a first nucleic acid strand is capable of base pairing with a residue of a second nucleic acid strand which is antiparallel to the first strand if the residue is guanine. A first region of a nucleic acid is complementary to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide residue of the first region is capable of base pairing with a residue of the second region. In some embodiments, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, and or at least about 75%, or at least about 90%, or at least about 95% of the nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion. In some embodiments, all nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion.

[0110] The term “DNA” as used herein is defined as deoxyribonucleic acid.

[0111] “Encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides

(i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[0112] Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron (s).

[0113] “Isolated” means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in its normal context in a living subject is not “isolated,” but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural context is “isolated.” An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

[0114] The term “hybridoma,” as used herein refers to a cell resulting from the fusion of a B-lymphocyte and a fusion partner such as a myeloma cell. A hybridoma can be cloned and maintained indefinitely in cell culture and is able to produce monoclonal antibodies. A hybridoma can also be considered to be a hybrid cell.

[0115] An “isolated nucleic acid” refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, i.e., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, i.e., the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, i.e., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (i.e., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

[0116] In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. “A” refers to adenosine, “C” refers to cytosine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

[0117] The term “polynucleotide” as used herein is defined as a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are poly-

nucleotides, which can be hydrolyzed into the monomeric “nucleotides.” The monomeric nucleotides can be hydrolyzed into nucleosides.

[0118] As used herein polynucleotides include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR, and the like, and by synthetic means.

[0119] As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein’s or peptide’s sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

[0120] The term “progeny” as used herein refers to a descendent or offspring and includes the offspring of a mammal, and also included the differentiated or undifferentiated decedent cell derived from a parent cell. In one usage, the term progeny refers to a descendent cell which is genetically identical to the parent. In another use, the term progeny refers to a descendent cell which is genetically and phenotypically identical to the parent. In yet another usage, the term progeny refers to a descendent cell that has differentiated from the parent cell.

[0121] The term “RNA” as used herein is defined as ribonucleic acid.

[0122] The term “recombinant DNA” as used herein is defined as DNA produced by joining pieces of DNA from different sources.

[0123] The term “recombinant polypeptide” as used herein is defined as a polypeptide produced by using recombinant DNA methods.

[0124] As used herein, “conjugated” refers to covalent attachment of one molecule to a second molecule.

[0125] “Variant” as the term is used herein, is a nucleic acid sequence or a peptide sequence that differs in sequence from a reference nucleic acid sequence or peptide sequence respectively, but retains essential biological properties of the reference molecule. Changes in the sequence of a nucleic acid variant may not alter the amino acid sequence of a peptide encoded by the reference nucleic acid, or may result in amino acid substitutions, additions, deletions, fusions and truncations. Changes in the sequence of peptide variants are typically limited or conservative, so that the sequences of the reference peptide and the variant are closely similar overall and, in many regions, identical. A variant and reference peptide can differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A

variant of a nucleic acid or peptide can be a naturally occurring such as an allelic variant, or can be a variant that is not known to occur naturally. Non-naturally occurring variants of nucleic acids and peptides may be made by mutagenesis techniques or by direct synthesis. In various embodiments, the variant sequence is at least 99%, at least 98%, at least 97%, at least 96%, at least 95%, at least 94%, at least 93%, at least 92%, at least 91%, at least 90%, at least 89%, at least 88%, at least 87%, at least 86%, at least 85% identical to the reference sequence.

[0126] The term “regulating” as used herein can mean any method of altering the level or activity of a substrate. Non-limiting examples of regulating with regard to a protein include affecting expression (including transcription and/or translation), affecting folding, affecting degradation or protein turnover, and affecting localization of a protein. Non-limiting examples of regulating with regard to an enzyme further include affecting the enzymatic activity. “Regulator” refers to a molecule whose activity includes affecting the level or activity of a substrate. A regulator can be direct or indirect. A regulator can function to activate or inhibit or otherwise modulate its substrate.

[0127] A “scanning window,” as used herein, refers to a segment of a number of contiguous positions in which a sequence may be evaluated independently of any flanking sequence. A scanning window generally is shifted incrementally along the length of a sequence to be evaluated with each new segment being independently evaluated. An incremental shift may be of 1 or more than one position.

[0128] “Vector” as used herein may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

[0129] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Description

[0130] This invention relates to the inhibition of the complement signaling and complement-related disorders using an anti-human C5 antibody. In one embodiment, the invention is directed to inhibiting the complement signaling cascade by specifically targeting complement component C5 protein, or a fragment of the protein C5a or C5b. In one embodiment, the invention is directed to methods of treating and preventing inflammation and autoimmune diseases mediated by unwanted, uncontrolled, excessive complement activation. In one embodiment the invention is directed towards the treatment of complement-mediated disease or

complement-mediated disorder in an individual by contacting the individual with an anti-C5 antibody.

[0131] In one embodiment, the invention is a method of treating a complement-mediated disease or disorder in an individual, comprising the step of administering to said individual an anti-C5 antibody, thereby inhibiting the generation of a C5a or C5b protein, and formation of MAC. Examples of complement-mediated pathologies that can be treated using the methods of the invention include, but are not limited to macular degeneration (MD), age-related macular degeneration (AMD), ischemia reperfusion injury, arthritis, rheumatoid arthritis, lupus, ulcerative colitis, stroke, post-surgery systemic inflammatory syndrome, asthma, allergic asthma, chronic obstructive pulmonary disease (COPD), paroxysmal nocturnal hemoglobinuria (PNH) syndrome, myasthenia gravis, neuromyelitis optica, (NMO), multiple sclerosis, delayed graft function, antibody-mediated rejection, atypical hemolytic uremic (aHUS) syndrome, central retinal vein occlusion (CRVO), central retinal artery occlusion (CRAO), epidermolysis bullosa, sepsis, organ transplantation, inflammation (including, but not limited to, inflammation associated with cardiopulmonary bypass surgery and kidney dialysis), C3 glomerulopathy, membranous nephropathy, IgA nephropathy, glomerulonephritis (including, but not limited to, anti-neutrophil cytoplasmic antibody (ANCA)-mediated glomerulonephritis, lupus nephritis, and combinations thereof), ANCA-mediated vasculitis, Shiga toxin induced HUS, and antiphospholipid antibody-induced pregnancy loss, or any combinations thereof. In some embodiments, the compositions and methods of the invention are useful for treating subject, including subjects having PNH, who are not responsive to treatment with eculizumab. By way of non-limiting example, some subjects may have a mutation in the alpha chain of C5 that may render them resistant to treatment of eculizumab (see Genetic variants in C5 and poor response to eculizumab. Nishimura J, et al., *N Engl J Med.* 2014 Feb. 13; 370(7):632-9).

[0132] The ability of the immune system to discriminate between “self” and “non-self” antigens is vital to the functioning of the immune system as a specific defense against invading microorganisms. “Non-self” antigens are those antigens on substances entering or present in the body which are detectably different or foreign from the subject’s own constituents, whereas “self” antigens are those which, in the healthy subject, are not detectably different or foreign from its own constituents. In various embodiments of the methods, the complement activation that is inhibited is that which was triggered by at least one of the group consisting of a microbial antigen, a non-biological foreign surface, altered self-tissue, or combinations thereof. One example of a non-biological foreign surface is blood tubing such as that used in cardio-pulmonary bypass surgery or kidney dialysis. Examples of altered self-tissues include apoptotic, necrotic and ischemia-stressed tissues and cells, or combinations thereof.

[0133] In some embodiments, the anti-C5 antibodies of the invention inhibit the downstream effects of activation of the alternative complement pathway (AP), the classical pathway (CP), or the lectin pathway (LP). Generally, the CP is initiated by antigen-antibody complexes, the LP is activated by binding of lectins to sugar molecules on microbial surfaces, while the AP is constitutively active at a low level but can be quickly amplified on bacterial, viral, and parasitic cell surfaces due to the lack of regulatory proteins. Host cells

are usually protected from AP complement activation by regulatory proteins. But in some situations, such as when the regulatory proteins are defective or missing, the AP can also be activated uncontrollably on host cells, leading to complement-mediated disease or disorder. The CP consists of components C1, C2, C4 and converges with the AP at the C3 activation step. The LP consists of mannose-binding lectins (MBLs) and MBL-associated serine proteases (Masps) and shares with the CP the components C4 and C2. The AP consists of components C3 and several factors, such as factor B, factor D, properdin, C5 and the fluid phase regulator factor H. Complement activation consists of three stages: (a) recognition, (b) enzymatic activation, and (c) membrane attack leading to cell death. The first phase of CP complement activation begins with C1. C1 is made up of three distinct proteins: a recognition subunit, C1q, and the serine protease subcomponents, C1r and C1s, which are bound together in a calcium-dependent tetrameric complex, C1r₂ s₂. An intact C1 complex is necessary for physiological activation of C1 to result. Activation occurs when the intact C1 complex binds to immunoglobulin complexed with antigen. This binding activates C1s which then cleaves both the C4 and C2 proteins to generate C4a and C4b, as well as C2a and C2b. The C4b and C2a fragments combine to form the C3 convertase, C4b₂a, which in turn cleaves C3 to form C3a and C3b. Activation of the LP is initiated by MBL binding to certain sugars on the target surface and this triggers the activation of MBL-associated serine proteases (MASPs) which then cleave C4 and C2 in a manner analogous to the activity of C1s of the CP, resulting in the generation of the C3 convertase, C4b₂a. Thus, the CP and LP are activated by different mechanisms but they share the same components C4 and C2 and both pathways lead to the generation of the same C3 convertase, C4b₂a. The cleavage of C3 by C4b₂a into C3b and C3a is a central event of the complement pathway for two reasons. It initiates the AP amplification loop because surface deposited C3b is a central intermediate of the AP. Both C3a and C3b are biologically important. C3a is proinflammatory and together with C5a are referred to as anaphylatoxins. C3b and its further cleavage products also bind to complement receptors present on neutrophils, eosinophils, monocytes and macrophages, thereby facilitating phagocytosis and clearance of C3b-opsonized particles. Finally, C3b can associate with C4b₂a to form the C5 convertase of the CP and LP to activate the terminal complement sequence, leading to the production of C5a, a potent proinflammatory mediator, and the assembly of the lytic membrane attack complex (MAC), C5-C9.

[0134] In one embodiment, the activity of the complement pathway that is inhibited using a method of the invention is complement pathway activation induced by at least one of the group selected from a lipopolysacchride (LPS), lipooligosaccharide (LOS), pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). In another embodiment, the activity of complement signaling that is inhibited using a method of invention is the generation of C5a protein. In another embodiment, the activity of complement signaling that is inhibited using a method of invention is the generation of C5b protein. In another embodiment, the activity of complement signaling that is inhibited using a method of the invention is the formation of MAC. In another embodiment, the activity of the complement pathway that is inhibited using a method of the invention is C5 dependent.

[0135] In one embodiment, the invention is a method of inhibiting initiation of terminal complement activation in an individual, comprising the step of administering to said individual an anti-C5 antibody, thereby inhibiting initiation of terminal complement activation originating from CP, LP or AP activation in an individual. Examples of these embodiments are PNH patients who suffer from complement-mediated hemolysis and individuals suffering from complement-mediated aHUS, asthma, ischemic/reperfusion injury, rheumatoid arthritis and ANCA-mediated kidney diseases. In various embodiments of the invention, diseases and disorders that can be treated using the compositions and methods of the invention include, but are not limited to, complement-mediated hemolysis, complement-mediated aHUS, C3 glomerulopathy, neuromyelitis optica, myasthenia gravis, asthma, ischemic/reperfusion injury, rheumatoid arthritis and ANCA-mediated kidney diseases or disorders.

[0136] In various other embodiments, provided herein are methods of identifying a potential anti-C5 antibody having inhibitory effects on complement signaling. One such method includes the steps of: a) coating a plate with lipopolysaccharide (LPS); b) washing the plate to remove unbound LPS; c) adding bovine serum albumin (BSA) in phosphate buffered saline (PBS); d) washing the plate to remove unbound BSA; e) adding a mixture of a candidate anti-C5 antibody compound that has been pre-incubated with serum and is mixed into normal human serum; f) washing the plate; g) adding an HRP-conjugated anti-human C3 antibody; h) washing the plate to remove unbound antibody; i) adding HRP Substrate Reagent; j) adding sulphuric acid to stop the reaction; k) measuring the optical density at 450 nm; l) comparing the optical density of the plate containing the candidate anti-C5 antibody compound to the optical density of a positive comparator control and a negative comparator control; wherein when the optical density is diminished as compared with the positive comparator control, the anti-C5 antibody is identified.

Anti-C5 Antibodies

[0137] In some embodiments, the invention includes compositions comprising an antibody that specifically binds to C5. In one embodiment, the anti-C5 antibody is a polyclonal antibody. In another embodiment, the anti-C5 antibody is a monoclonal antibody. In some embodiments, the anti-C5 antibody is a chimeric antibody. In further embodiments, the anti-C5 antibody is a humanized antibody. In some embodiments, the antibody is an antibody fragment. In some embodiments, the C5 is human C5.

[0138] In some embodiments, binding of the antibody or the fragment of the antibody to human-C5 is associated with a reduction in the generation of C5a or C5b and the formation of MAC in the complement activation pathway in an intact organism. In some embodiments, the invention is a protein or a polypeptide capable of binding to human C5. In some embodiments, the antibody or antibody fragment; the protein or the polypeptide binds to a relevant portion or fraction or epitope of the human-C5; and the binding of the antibody, or the antibody fragment thereof, or the protein or the polypeptide to the relevant portion of the human-C5 is associated with a reduction in the generation of C5a or C5b and the formation of MAC in an intact organism.

[0139] In some embodiments, the human-C5 binding antibody or a C5 binding antibody fragment thereof, is further conjugated to a protein, a peptide or another compound. In

some embodiments, the human-C5 binding antibody, or an antibody fragment thereof, is conjugated to a protein, a peptide or other compound. In some embodiments, the protein, peptide or other compound to which the human-C5 binding antibody or antibody fragment thereof is conjugated is a targeting moiety (i.e., the targeting moiety specifically binds to a molecule other than human-C5). In some embodiments, the protein, peptide, or other compound to which the human-C5 binding antibody or antibody fragment thereof is conjugated to is an effector molecule (e.g., a cytotoxic molecule).

[0140] In one embodiment, the anti-C5 antibody or an antigen-binding fragment thereof comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:3; VH-CDR2: SEQ ID NO:4; VH-CDR3: SEQ ID NO:5; VL-CDR1: SEQ ID NO:8; VL-CDR2: SEQ ID NO:9; and VL-CDR3: SEQ ID NO:10, or a variant or variants thereof. In another embodiment, the anti-C5 antibody comprises all of the CDRs of the group consisting of: VH-CDR1: SEQ ID NO:3; VH-CDR2: SEQ ID NO:4; VH-CDR3: SEQ ID NO:5; VL-CDR1: SEQ ID NO:8; VL-CDR2: SEQ ID NO:9; and VL-CDR3: SEQ ID NO:10, or a variant or variants thereof.

[0141] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:3 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR1: SEQ ID NO: 8, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:3; and VL-CDR1: SEQ ID NO:8.

[0142] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:4, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR2: SEQ ID NO:9, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:4; and VL-CDR2: SEQ ID NO:9.

[0143] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:5, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3: SEQ ID NO:10, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:5; and VL-CDR3: SEQ ID NO:10.

[0144] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:3, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:4, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the

thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:20.

[0154] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:13; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:14; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:15; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:18; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:19; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:20.

[0155] In some embodiments, the anti-C5 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:12, or a variant thereof. In other embodiments, the anti-C5 antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:17, or a variant thereof. In another embodiment, the anti-C5 antibody is mAb 8E1, or a variant thereof. The monoclonal anti-C5 antibody mAb 8E1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:12 and a light chain comprising the amino acid sequence of SEQ ID NO:17. In some embodiments, the monoclonal anti-C5 antibody is humanized. In some embodiments the monoclonal anti-C5 antibody is a chimeric antibody.

[0156] In one embodiment, the anti-C5 antibody or an antigen-binding fragment thereof comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:23; VH-CDR2: SEQ ID NO:24; VH-CDR3: SEQ ID NO:25; VL-CDR1: SEQ ID NO:28; and VL-CDR3: SEQ ID NO:29, or a variant or variants thereof. In another embodiment, the anti-C5 antibody comprises all of the CDRs of the group consisting of: VH-CDR1: SEQ ID NO:23; VH-CDR2: SEQ ID NO:24; VH-CDR3: SEQ ID NO:25; VL-CDR1: SEQ ID NO:28; and VL-CDR3: SEQ ID NO:29, or a variant or variants thereof.

[0157] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:23 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR1: SEQ ID NO:28, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:23; and VL-CDR1: SEQ ID NO:28.

[0158] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:24, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR2: SEQ ID NO:29, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:24; and VL-CDR2: SEQ ID NO:29.

[0159] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:25, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodi-

ments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:25.

[0160] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:23, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:24, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:25, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:28, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:29, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[0161] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:23, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:24, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:25; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:28, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR2 comprising the amino acid sequence of SEQ ID NO:29, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[0162] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:23; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:24; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:25; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:28; and VL-CDR2 comprising the amino acid sequence of SEQ ID NO:29.

[0163] In some embodiments, the anti-C5 antibody or an antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:22, or a variant thereof. In other embodiments, the anti-C5 antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:27, or a variant thereof. In another embodiment, the anti-C5 antibody is mAb 4E7, or a variant thereof. The monoclonal anti-C5 antibody mAb 4E7 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:22 and a light chain comprising the amino acid sequence of SEQ ID NO:27. In some embodiments, the monoclonal anti-C5 antibody is humanized. In some embodiments the monoclonal anti-C5 antibody is a chimeric antibody.

[0164] In one embodiment, the anti-C5 antibody or an antigen-binding fragment thereof comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:32; VH-CDR2: SEQ ID NO:33; VH-CDR3: SEQ ID NO:34; VL-CDR1: SEQ ID NO:37; VL-CDR2: SEQ ID NO:38; and VL-CDR3: SEQ ID NO:39, or a variant or variants thereof. In another embodiment, the anti-C5

antibody comprises all of the CDRs of the group consisting of VH-CDR1: SEQ ID NO:32; VH-CDR2: SEQ ID NO:33; VH-CDR3: SEQ ID NO:34; VL-CDR1: SEQ ID NO:37; VL-CDR2: SEQ ID NO:38; and VL-CDR3: SEQ ID NO:39, or a variant or variants thereof.

[0165] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:32 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR1: SEQ ID NO:37, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:32; and VL-CDR1: SEQ ID NO:37.

[0166] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:33, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR2: SEQ ID NO:38, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:33; and VL-CDR2: SEQ ID NO:38.

[0167] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:34, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3: SEQ ID NO:39, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:34; and VL-CDR3: SEQ ID NO:39.

[0168] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:32, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:33, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:34, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:37, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:38, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:39, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[0169] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:32, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:33, or a variant

thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:34; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:37, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:38, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:39.

[0170] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:32; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:33; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:34; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:37; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:38; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:39.

[0171] In some embodiments, the anti-C5 antibody or an antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31, or a variant thereof. In other embodiments, the anti-C5 antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:36, or a variant thereof. In another embodiment, the anti-C5 antibody is mAb 9G6, or a variant thereof. The monoclonal anti-C5 antibody mAb 9G6 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36. In some embodiments, the monoclonal anti-C5 antibody is humanized. In some embodiments the monoclonal anti-C5 antibody is a chimeric antibody.

[0172] In one embodiment, the anti-C5 antibody or an antigen-binding fragment thereof comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO: 42; VH-CDR2: SEQ ID NO:43; VH-CDR3: SEQ ID NO:44; VL-CDR1: SEQ ID NO:47; VL-CDR2: SEQ ID NO:48; and VL-CDR3: SEQ ID NO:49, or a variant or variants thereof. In another embodiment, the anti-C5 antibody comprises all of the CDRs of the group consisting of: VH-CDR1: SEQ ID NO:42; VH-CDR2: SEQ ID NO:43; VH-CDR3: SEQ ID NO:44; VL-CDR1: SEQ ID NO:47; VL-CDR2: SEQ ID NO:48; and VL-CDR3: SEQ ID NO:49, or a variant or variants thereof.

[0173] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:42 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR1: SEQ ID NO:47, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:42; and VL-CDR1: SEQ ID NO:47.

[0174] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:43, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR2: SEQ ID NO:48, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-

binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:43; and VL-CDR2: SEQ ID NO:48.

[0175] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:44, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3: SEQ ID NO:49, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:44; and VL-CDR3: SEQ ID NO:49.

[0176] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:42, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:43, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:44, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:47, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:48, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:49, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[0177] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:42, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:43, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:44; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:47, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:48, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:49.

[0178] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:42; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:43; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:44; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:47; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:48; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:49.

[0179] In some embodiments, the anti-C5 antibody or an antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof. In other embodiments, the anti-C5 antibody comprises a light chain comprising the amino acid

sequence of SEQ ID NO:46, or a variant thereof. In another embodiment, the anti-C5 antibody is mAb 11C5, or a variant thereof. The monoclonal anti-C5 antibody designated mAb 11C5 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:41 and a light chain comprising the amino acid sequence of SEQ ID NO:46. In some embodiments, the monoclonal anti-C5 antibody is humanized. In some embodiments the monoclonal anti-C5 antibody is a chimeric antibody.

[0180] In one embodiment, the anti-C5 antibody or an antigen-binding fragment thereof comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:52; VH-CDR2: SEQ ID NO:53; VH-CDR3: SEQ ID NO:54; VL-CDR1: SEQ ID NO:57; VL-CDR2: SEQ ID NO:58; and VL-CDR3: SEQ ID NO:59, or a variant or variants thereof. In another embodiment, the anti-C5 antibody comprises all of the CDRs of the group consisting of: VH-CDR1: SEQ ID NO:52; VH-CDR2: SEQ ID NO:53; VH-CDR3: SEQ ID NO:54; VL-CDR1: SEQ ID NO:57; VL-CDR2: SEQ ID NO:58; and VL-CDR3: SEQ ID NO:59, or a variant or variants thereof.

[0181] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:52 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR1: SEQ ID NO:57, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:52; and VL-CDR1: SEQ ID NO:57.

[0182] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:53, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR2: SEQ ID NO:58, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:53; and VL-CDR2: SEQ ID NO:58.

[0183] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:54, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3: SEQ ID NO:59, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:54; and VL-CDR3: SEQ ID NO:59.

[0184] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:52, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:53, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:54, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3)

or 3) amino acid substitutions; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:76.

[0194] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:64; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:65; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:66; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:74; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:75; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:76.

[0195] In one embodiment, the anti-C5 antibody or an antigen-binding fragment thereof comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:69; VH-CDR2: SEQ ID NO:70; VH-CDR3: SEQ ID NO:71; VL-CDR1: SEQ ID NO:74; VL-CDR2: SEQ ID NO:75; and VL-CDR3: SEQ ID NO:76, or a variant or variants thereof. In another embodiment, the anti-C5 antibody comprises all of the CDRs of the group consisting of: VH-CDR1: SEQ ID NO:69; VH-CDR2: SEQ ID NO:70; VH-CDR3: SEQ ID NO:71; VL-CDR1: SEQ ID NO:74; VL-CDR2: SEQ ID NO:75; and VL-CDR3: SEQ ID NO:76, or a variant or variants thereof.

[0196] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:69 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR1: SEQ ID NO:74, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:69; and VL-CDR1: SEQ ID NO:74.

[0197] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:70, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR2: SEQ ID NO:75, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR2 comprising the amino acid sequence of SEQ ID NO:70; and VL-CDR2: SEQ ID NO:75.

[0198] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:71, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3: SEQ ID NO:76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-C5 antibody or an antigen-binding fragment thereof comprises: VH-CDR3 comprising the amino acid sequence of SEQ ID NO:71; and VL-CDR3: SEQ ID NO:76.

[0199] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:69, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:70, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the

amino acid sequence of SEQ ID NO:71, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:74, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:75, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[0200] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:69, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:70, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:71; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:74, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:75, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:76.

[0201] In some embodiments, the anti-C5 antibody or antigen-binding fragment thereof comprises: VH-CDR1 comprising the amino acid sequence of SEQ ID NO:69; VH-CDR2 comprising the amino acid sequence of SEQ ID NO:70; VH-CDR3 comprising the amino acid sequence of SEQ ID NO:71; VL-CDR1 comprising the amino acid sequence of SEQ ID NO:74; VL-CDR2 comprising the amino acid sequence of SEQ ID NO:75; and VL-CDR3 comprising the amino acid sequence of SEQ ID NO:76.

[0202] In some embodiments, the anti-C5 antibody or an antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:68, or a variant thereof. In other embodiments, the anti-C5 antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:73, or a variant thereof. In another embodiment, the anti-C5 antibody is mAb 2G1, or a variant thereof. The monoclonal anti-C5 antibody mAb 2G1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:68 and a light chain comprising the amino acid sequence of SEQ ID NO:73. In some embodiments, the monoclonal anti-C5 antibody is humanized. In some embodiments the monoclonal anti-C5 antibody is a chimeric antibody. In some embodiments, the anti-C5 antibody or an antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:63, or a variant thereof. In other embodiments, the anti-C5 antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:73, or a variant thereof. In another embodiment, the anti-C5 antibody is mAb 2G1, or a variant thereof. The monoclonal anti-C5 antibody mAb 2G1 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:63 and a light chain comprising the amino acid sequence of SEQ ID NO:73. In some embodiments, the monoclonal anti-C5 antibody is humanized. In some embodiments the monoclonal anti-C5 antibody is a chimeric antibody.

[0203] In some embodiments the antibodies are chimeric antibodies. In some embodiments the anti-human C5 antibody may comprise human light chain and human heavy chain constant regions in combination with the variable region CDR sequences described in the specification above. One of skill in the art would be able to prepare and obtain a chimeric antibody using known techniques of swapping relevant domains of specific antibodies of interest. Such an antibody is easily prepared by grafting heterogeneous antibody domains, incorporating one or more CDR sequences described in this application. Using known recombinant technology, it is possible to obtain and prepare a recombinant antibody comprising heavy and light chain constant regions encoded by nucleic acid sequences of human heavy and light chain constant regions; and the heavy and light chain variable regions comprising CDRs encoded by nucleic acid sequences corresponding to the CDR sequences set forth in the disclosure. One of skill in the art can prepare an anti-human C5 antibody comprises one or more CDR sequences described in this disclosure, wherein portions of the light chain alone or portions of the heavy chain alone are replaced with regions from an antibody belonging to another species, such as, for example, human. A human anti-human-C5 antibody comprising variable regions having one or more CDR sequences selected from SEQ ID NOs: 3-5, 8-10, 13-15, 18-20, 23-25, 28-29, 32-34, 37-39, 42-44, 47-49, 52-54 and 57-59, or a variant or variants thereof, in combination with murine or non-murine antibody structural elements outside the CDR regions can be prepared by routine methods known in the art. In some embodiments, the antibodies or antibody fragments are further humanized using known techniques in the art.

[0204] In various embodiments, any of the antibodies of the invention described herein, having any of the variable regions described herein, may comprise a human IgG4 constant heavy chain. SEQ ID NO: 104 is an example amino acid sequence of a human IgG4 constant heavy chain. In some embodiments, the antibody of the invention comprises a human IgG4 constant heavy chain having an S228P mutation. SEQ ID NO: 60 is an example amino acid sequence of a human IgG4 constant heavy chain having an S228P mutation.

[0205] In some embodiments the anti-C5 antibody comprises an antibody having at least about 85% (such as at least about any of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) amino acid identity with the CDR sequence described herein, listed in SEQ ID NOs 3-5, 8-10, 13-15, 18-20, 23-25, 28-29, 32-34, 37-39, 42-44, 47-49, 52-54 and 57-59.

[0206] In one embodiment, the current disclosure encompasses an anti-C5 antibody having CDR sequences of at least about 85% (such as at least about any of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the CDR sequences described above. In one embodiment, the antibody against human C5 has a heavy chain variable (vH) region and a light chain variable (vL) region, wherein the vH region has an amino acid sequence that is more than about 90% (such as more than about any of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to one selected from SEQ ID NOs 2, 12, 22, 31, 41, 51, 63, and 68 and wherein the vL region has an amino acid sequence that is more than about 90% (such as more than about any of 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to one selected from SEQ ID NOs 7, 17, 27, 36, 46, 56 and 73.

[0207] In some embodiments the antibody or the antibody fragment is modified. In some embodiments the modifications include fusion of the antibody or the antigen-binding fragment thereof with portions of another protein, or a protein fragment. In some embodiments the antibody or the antibody fragment thereof of the invention is modified to increase the circulating half-life of the same in vivo. For example, the antibody of the fragment may be fused with an FcRn molecule, which is also known as neonatal Fc receptor to stabilize the antibody in vivo. (Nature Reviews Immunology 7:715-725). In some embodiments, the antibody or antigen-binding fragment thereof is conjugated (e.g., fused) to an effector molecule and/or another targeting moiety (such as an antibody or antibody fragment recognizing a different molecule, different antigen or a different epitope).

[0208] One of skill in the art would be able to prepare human-C5 binding single chain variable fragment (scFv), comprising at least one specific CDR sequence selected from SEQ ID NOs 3-5, 8-10, 13-15, 18-20, 23-25, 28-29, 32-34, 37-39, 42-44, 47-49, 52-54 and 57-59, or a variant or variants thereof. An scFv may comprise heavy chain variable region sequences designated in SEQ ID NOs 3-5, or a variant or variants thereof, and light chain variable regions designated in SEQ ID NOs 8-10, or a variant or variants thereof. An scFv may comprise heavy chain variable region sequences designated in SEQ ID NOs 13-15, or a variant or variants thereof, and light chain variable regions designated in SEQ ID NOs 18-20, or a variant or variants thereof. An scFv may comprise heavy chain variable region sequences designated in SEQ ID NOs 23-25, or a variant or variants thereof, and light chain variable regions designated in SEQ ID NOs 28-29, or a variant or variants thereof. An scFv may comprise heavy chain variable region sequences designated in SEQ ID NOs 32-34, or a variant or variants thereof, and light chain variable regions designated in SEQ ID NOs 37-39, or a variant or variants thereof. An scFv may comprise heavy chain variable region sequences designated in SEQ ID NOs 42-44, or a variant or variants thereof, and light chain variable regions designated in SEQ ID NOs 47-49, or a variant or variants thereof. An scFv may comprise heavy chain variable region sequences designated in SEQ ID NOs 52-54, or a variant or variants thereof, and light chain variable regions designated in SEQ ID NOs 57-59, or a variant or variants thereof. CDR sequences incorporated within the scFv having amino acid sequence identity of at least about 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the CDR sequences described in the present disclosure are encompassed within the scope of the present disclosure.

[0209] In some embodiments, the isolated antibody binds to human C5, wherein the antibody binds to an epitope of human C5. In some embodiments, the human C5 antibody of the invention is one that binds to a specific epitope of human C5. In some embodiments, the epitope includes at least one amino acid in the α -chain of C5. In some embodiments, the epitope includes at least one amino acid in the β -chain of C5.

[0210] In some embodiments, the epitope includes at least one amino acid in the MG7 domain of the α -chain of C5. In some embodiments, the epitope includes at least one amino acid in the CUB domain of the α -chain of C5. In some embodiments, the epitope includes at least one amino acid in

the C5d domain of the α -chain of C5. In some embodiments, the epitope includes at least one amino acid in the MG8 domain of the α -chain of C5. In some embodiments, the epitope includes at least one amino acid in the C345C domain of the α -chain of C5.

[0211] In some embodiments, the epitope includes at least one amino acid in the MG1 domain of the β -chain of C5. In some embodiments, the epitope includes at least one amino acid in the MG2 domain of the β -chain of C5. In some embodiments, the epitope includes at least one amino acid in the MG3 domain of the β -chain of C5. In some embodiments, the epitope includes at least one amino acid in the MG4 domain of the β -chain of C5. In some embodiments, the epitope includes at least one amino acid in the MG5 domain of the β -chain of C5. In some embodiments, the epitope includes at least one amino acid in the MG6 domain of the β -chain of C5.

[0212] In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the MG7 domain of the α -chain of C5. In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the CUB domain of the α -chain of C5. In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the C5d domain of the α -chain of C5. In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the MG8 domain of the α -chain of C5. In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the C345C domain of the α -chain of C5.

[0213] In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the MG1 domain of the β -chain of C5. In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the MG2 domain of the β -chain of C5. In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the MG3 domain of the β -chain of C5. In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the MG4 domain of the β -chain of C5. In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the MG5 domain of the β -chain of C5. In some embodiments, the antibody, or binding portion thereof, binds to at least one amino acid in the MG6 domain of the β -chain of C5.

[0214] In one embodiment, at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 84 is in the epitope of the human C5 antibody. In one embodiment, at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 85 is in the epitope of the human C5 antibody. In one embodiment, at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 86 is in the epitope of the human C5 antibody. In one embodiment, at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 87 is in the epitope of the human C5 antibody. In one embodiment, at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 88 is in the epitope of the human C5 antibody. In one embodiment, at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 89 is in the epitope of the human C5 antibody.

[0215] In one embodiment, the antibody, or binding fragment thereof, of the invention binds to at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 84. In one embodiment, the antibody, or binding fragment

thereof, of the invention binds to at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 85. In one embodiment, the antibody, or binding fragment thereof, of the invention binds to at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 86. In one embodiment, the antibody, or binding fragment thereof, of the invention binds to at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 87. In one embodiment, the antibody, or binding fragment thereof, of the invention binds to at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 88. In one embodiment, the antibody, or binding fragment thereof, of the invention binds to at least one (such as at least 1, 2, 3, or 4) of the amino acids of SEQ ID NO: 89.

Screening Assays

[0216] The present invention has application in various screening assays, including, determining whether a candidate anti-C5 antibody can inhibit complement activity.

[0217] In some embodiments, the level of complement activity in the presence of the candidate anti-C5 antibody is compared with complement activity detected in a positive comparator control. The positive comparator control comprises complement activation in the absence of added test compound. In some embodiments, the candidate anti-C5 antibody is identified as an inhibitor of the complement when the complement activity in the presence of the candidate anti-C5 antibody is less than about 70% of the complement activity detected in a positive comparator control; this corresponds to greater than about 30% inhibition of complement activity in the presence of the test compound. In other embodiments, the candidate anti-C5 antibody is identified as an inhibitor of the complement when the complement activity in the presence of the candidate anti-C5 antibody is less than about 80% of the complement activity detected in a positive comparator control; this corresponds to greater than about 20% inhibition of complement activity in the presence of the test compound. In still other embodiments, the candidate anti-C5 antibody is identified as an inhibitor of the complement when the complement activity in the presence of the candidate anti-C5 antibody is less than about 90% of the complement activity detected in a positive comparator control; this corresponds to greater than about 10% inhibition of complement activity in the presence of the test compound. In some embodiments, the level of complement inhibition by the candidate anti-C5 antibody is compared with the level of inhibition detected in a negative comparator control.

[0218] A variety of immunoassay formats, including competitive and non-competitive immunoassay formats, antigen capture assays, two-antibody sandwich assays, and three-antibody sandwich assays are useful methods of the invention (Self et al., 1996, Curr. Opin. Biotechnol. 7:60-65). The invention should not be construed to be limited to any one type of known or heretofor unknown assay, provided that the assay is able to detect the inhibition of complement.

[0219] Enzyme-linked immunosorbent assays (ELISAs) are useful in the methods of the invention. An enzyme such as, but not limited to, horseradish peroxidase (HRP), alkaline phosphatase, beta-galactosidase or urease can be linked, for example, to an anti-C3 antibody or to a secondary antibody for use in a method of the invention. A horseradish-peroxidase detection system may be used, for example, with the chromogenic substrate tetramethylbenzidine (TMB),

which yields a soluble product in the presence of hydrogen peroxide that is detectable at 450 nm. Other convenient enzyme-linked systems include, for example, the alkaline phosphatase detection system, which may be used with the chromogenic substrate p-nitrophenyl phosphate to yield a soluble product readily detectable at 405 nm. Similarly, a beta-galactosidase detection system may be used with the chromogenic substrate o-nitrophenyl-beta-D-galactopyranoside (ONPG) to yield a soluble product detectable at 410 nm. Alternatively, a urease detection system may be used with a substrate such as urea-bromocresol purple (Sigma Immunochemicals, St. Louis, Mo.). Useful enzyme-linked primary and secondary antibodies can be obtained from any number of commercial sources.

[0220] Chemiluminescent detection is also useful for detecting the inhibition of the AP. Chemiluminescent secondary antibodies may be obtained from any number of commercial sources.

[0221] Fluorescent detection is also useful for detecting the inhibition of the AP. Useful fluorochromes include, but are not limited to, DAPI, fluorescein, Hoechst 33258, R-phycoerythrin, B-phycoerythrin, R-phycoerythrin, rhodamine, Texas red and lissamine-Fluorescein- or rhodamine-labeled antibodies.

[0222] Radioimmunoassays (RIAs) are also useful in the methods of the invention. Such assays are well known in the art, and are described for example in Brophy et al. (1990, *Biochem. Biophys. Res. Comm.* 167:898-903) and Guehot et al. (1996, *Clin. Chem.* 42:558-563). Radioimmunoassays are performed, for example, using Iodine-125-labeled primary or secondary antibody (Harlow et al., *supra*, 1999).

[0223] A signal emitted from a detectable antibody is analyzed, for example, using a spectrophotometer to detect color from a chromogenic substrate; a radiation counter to detect radiation, such as a gamma counter for detection of Iodine-125; or a fluorometer to detect fluorescence in the presence of light of a certain wavelength. Where an enzyme-linked assay is used, quantitative analysis is performed using a spectrophotometer. It is understood that the assays of the invention can be performed manually or, if desired, can be automated and that the signal emitted from multiple samples can be detected simultaneously in many systems available commercially.

[0224] The methods of the invention also encompass the use of capillary electrophoresis based immunoassays (CEIA), which can be automated, if desired. Immunoassays also may be used in conjunction with laser-induced fluorescence as described, for example, in Schmalzing et al. (1997, *Electrophoresis* 18:2184-2193) and Bao (1997, *J. Chromatogr. B. Biomed. Sci.* 699:463-480). Liposome immunoassays, such as flow-injection liposome immunoassays and liposome immunosensors, may also be used according to the methods of the invention (Rongen et al., 1997, *J. Immunol. Methods* 204:105-133).

[0225] Quantitative western blotting may also be used to determine the level of complement inhibition in the methods of the invention. Western blots are quantified using well known methods such as scanning densitometry (Parra et al., 1998, *J. Vasc. Surg.* 28:669-675).

Methods of Administration

[0226] The methods of the invention comprise administering a therapeutically effective amount of at least one anti-C5 antibody, or binding fragment thereof (such as any

of the antibodies or fragments thereof described elsewhere herein), to an individual identified as having a complement-mediated disease or disorder. In one embodiment the individual is a mammal having a complement system. In one embodiment the individual is a human. In various embodiments, the at least one anti-C5 antibody, or binding fragment thereof, is administered locally, regionally, or systemically.

[0227] In various embodiments, the disease or disorder is at least selected from the group consisting of: macular degeneration (MD), age-related macular degeneration (AMD), ischemia reperfusion injury, arthritis, rheumatoid arthritis, asthma, allergic asthma, lupus, ulcerative colitis, stroke, post-surgery systemic inflammatory syndrome, asthma, allergic asthma, chronic obstructive pulmonary disease (COPD), paroxysmal nocturnal hemoglobinuria (PNH) syndrome, myasthenia gravis, neuromyelitis optica, (NMO), multiple sclerosis, delayed graft function, antibody-mediated rejection, atypical hemolytic uremic (aHUS) syndrome, central retinal vein occlusion (CRVO), central retinal artery occlusion (CRAO), epidermolysis bullosa, sepsis, organ transplantation, inflammation (including, but not limited to, inflammation associated with cardiopulmonary bypass surgery and kidney dialysis), C3 glomerulopathy, membranous nephropathy, IgA nephropathy, glomerulonephritis (including, but not limited to, anti-neutrophil cytoplasmic antibody (ANCA)-mediated glomerulonephritis, lupus nephritis, and combinations thereof), ANCA-mediated vasculitis, Shiga toxin induced HUS, and antiphospholipid antibody-induced pregnancy loss, or any combinations thereof. In some embodiments, the AP-mediated disease is C3 glomerulopathy. In some embodiments, the AP-mediated disease is macular degeneration, such as age-related macular degeneration. In one embodiment, administration of the anti-C5 antibody inhibits the generation of a C5a or C5b protein. In some embodiments, the compositions and methods of the invention are useful for treating subject, including subjects having PNH, who are not responsive to treatment with eculizumab. By way of non-limiting example, some subjects may have a mutation in the alpha chain of C5 that may render them resistant to treatment of eculizumab (see Genetic variants in C5 and poor response to eculizumab. Nishimura J, et al., *N Engl J Med.* 2014 Feb. 13; 370(7): 632-9).

[0228] The methods of the invention can comprise the administration of at least one anti-C5 antibody, or binding fragment thereof, but the present invention should in no way be construed to be limited to the anti-C5 antibodies described herein, but rather should be construed to encompass any anti-C5 antibody, both known and unknown, that diminish and reduce complement activation.

[0229] The method of the invention comprises administering a therapeutically effective amount of at least one anti-C5 antibody, or binding fragment thereof, to an individual wherein a composition of the present invention comprising at least one anti-C5 antibody, or binding fragment thereof, either alone or in combination with at least one other therapeutic agent. The invention can be used in combination with other treatment modalities, such as, for example anti-inflammatory therapies, and the like. Examples of anti-inflammatory therapies that can be used in combination with the methods of the invention include, for example, therapies that employ steroidal drugs, as well as therapies that employ non-steroidal drugs.

[0230] The method of the invention comprises administering a therapeutically effective amount of an anti-C5 antibody, or an antigen-binding fragment thereof, to a subject. In some embodiments, the invention encompasses a method of treatment of C5 related diseases involving dysregulation of the complement signaling by administering a therapeutically effective amount of an antibody of the invention, or a therapeutically effective amount of an antibody fragment thereof, such that a reduction of C5a or C5b or MAC formation is effected in the subject. In some embodiments the invention encompasses a method of treatment of C5 related diseases involving dysregulation of complement signaling by administering a therapeutically effective amount of an antibody or an antibody fragment. In some embodiments the invention encompasses a method of treatment of C5 related diseases involving dysregulation of complement signaling by administering to a subject an effective amount of an antibody, an antibody fragment, a polypeptide, a peptide, a conjugated peptide, such that the complement activation pathway activation is reduced in the subject. In some embodiments, the method of treatment encompasses administering to a subject a systemically effective dose of an antibody or an antibody fragment, whereby systemic reduction of C5a or C5b or MAC formation is effected in the subject.

[0231] The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of at least about 1 ng/kg, at least about 5 ng/kg, at least about 10 ng/kg, at least about 25 ng/kg, at least about 50 ng/kg, at least about 100 ng/kg, at least about 500 ng/kg, at least about 1 µg/kg, at least about 5 µg/kg, at least about 10 µg/kg, at least about 25 µg/kg, at least about 50 µg/kg, at least about 100 µg/kg, at least about 500 µg/kg, at least about 1 mg/kg, at least about 5 mg/kg, at least about 10 mg/kg, at least about 25 mg/kg, at least about 50 mg/kg, at least about 100 mg/kg, at least about 200 mg/kg, at least about 300 mg/kg, at least about 400 mg/kg, and at least about 500 mg/kg of body weight of the subject. In one embodiment, the invention administers a dose which results in a concentration of the anti-C5 antibody of the present invention of at least about 1 µM, at least about 10 µM, at least about 100 µM, at least about 1 nM, at least about 10 nM, at least about 100 nM, at least about 1 µM, at least about 2 µM, at least about 3 µM, at least about 4 µM, at least about 5 µM, at least about 6 µM, at least about 7 µM, at least about 8 µM, at least about 9 µM and at least about 10 µM in an individual. In another embodiment, the invention envisions administration of a dose which results in a concentration of the anti-C5 antibody of the present invention between at least about 1 µM, at least about 10 µM, at least about 100 µM, at least about 1 nM, at least about 10 nM, at least about 100 nM, at least about 1 µM, at least about 2 µM, at least about 3 µM, at least about 4 µM, at least about 5 µM, at least about 6 µM, at least about 7 µM, at least about 8 µM, at least about 9 µM and at least about 10 µM in the plasma of an individual.

[0232] In some embodiments, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of no more than about 1 ng/kg, no more than about 5 ng/kg, no more than about 10 ng/kg, no more than about 25 ng/kg, no more than about 50 ng/kg, no more than about 100 ng/kg, no more than about 500 ng/kg, no more than about 1 µg/kg, no more than about 5 µg/kg, no more than about 10 µg/kg, no more than about 25 µg/kg, no more than about 50 µg/kg, no more than about 100 µg/kg, no more than about 1 mg/kg, no more than about 5 mg/kg, no more than about 10 mg/kg, no more than about 25 mg/kg, no more than about 50 mg/kg, no more than about 100 mg/kg, no more than about 200 mg/kg, no more than about 300 mg/kg, no more than about 400 mg/kg, and no more than about 500 mg/kg of body weight of the subject. In one embodiment, the invention administers a dose which results in a concentration of the anti-C5 antibody of the present invention of no more than about 1 µM, no more than about 10 µM, no more than about 100 µM, no more than about 1 nM, no more than about 10 nM, no more than about 100 nM, no more than about 1 µM, no more than about 2 µM, no more than about 3 µM, no more than about 4 µM, no more than about 5 µM, no more than about 6 µM, no more than about 7 µM, no more than about 8 µM, no more than about 9 µM and no more than about 10 µM in the plasma of an individual. Also contemplated are dosage ranges between any of the doses disclosed herein.

more than about 500 µg/kg, no more than about 1 mg/kg, no more than about 5 mg/kg, no more than about 10 mg/kg, no more than about 25 mg/kg, no more than about 50 mg/kg, no more than about 100 mg/kg, no more than about 200 mg/kg, no more than about 300 mg/kg, no more than about 400 mg/kg, and no more than about 500 mg/kg of body weight of the subject. In one embodiment, the invention administers a dose which results in a concentration of the anti-C5 antibody of the present invention of no more than about 1 µM, no more than about 10 µM, no more than about 100 µM, no more than about 1 nM, no more than about 10 nM, no more than about 100 nM, no more than about 1 µM, no more than about 2 µM, no more than about 3 µM, no more than about 4 µM, no more than about 5 µM, no more than about 6 µM, no more than about 7 µM, no more than about 8 µM, no more than about 9 µM and no more than about 10 µM in an individual. In another embodiment, the invention envisions administration of a dose which results in a concentration of the anti-C5 antibody of the present invention between no more than about 1 µM, no more than about 10 µM, no more than about 100 µM, no more than about 1 nM, no more than about 10 nM, no more than about 100 nM, no more than about 1 µM, no more than about 2 µM, no more than about 3 µM, no more than about 4 µM, no more than about 5 µM, no more than about 6 µM, no more than about 7 µM, no more than about 8 µM, no more than about 9 µM and no more than about 10 µM in the plasma of an individual. Also contemplated are dosage ranges between any of the doses disclosed herein.

[0233] Typically, dosages which may be administered in a method of the invention to a subject, in some embodiments a human, range in amount from 0.5 µg to about 50 mg per kilogram of body weight of the subject. While the precise dosage administered will vary depending upon any number of factors, including but not limited to, the type of subject and type of disease state being treated, the age of the subject and the route of administration. In some embodiments, the dosage of the compound will vary from about 1 µg to about 10 mg per kilogram of body weight of the subject. In other embodiments, the dosage will vary from about 3 µg to about 1 mg per kilogram of body weight of the subject.

[0234] The antibody may be administered to a subject as frequently as several times daily, or it may be administered less frequently, such as once a day, twice a day, thrice a day, once a week, twice a week, thrice a week, once every two weeks, twice every two weeks, thrice every two weeks, once a month, twice a month, thrice a month, or even less frequently, such as once every several months or even once or a few times a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the subject, etc. The formulations of the pharmaceutical compositions may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

[0235] Although the description of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the

skilled artisan that such compositions are generally suitable for administration to subjects of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various subjects is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Individuals to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as non-human primates, cattle, pigs, horses, sheep, cats, and dogs.

[0236] Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for ophthalmic, oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, intraocular, intravitreal, intramuscular, intradermal and intravenous routes of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

[0237] A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. A unit dose is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to an individual or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[0238] The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the individual treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient. In various embodiments, the composition comprises at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 13%, at least about 14%, at least about 15%, at least about 16%, at least about 17%, at least about 18%, at least about 19%, at least about 20%, at least about 21%, at least about 22%, at least about 23%, at least about 24%, at least about 25%, at least about 26%, at least about 27%, at least about 28%, at least about 29%, at least about 30%, at least about 31%, at least about 32%, at least about 33%, at least about 34%, at least about 35%, at least about 36%, at least about 37%, at least about 38%, at least about 39%, at least about 40%, at least about 41%, at least about 42%, at least about 43%, at least about 44%, at least about 45%, at least about 46%, at least about 47%, at least about 48%, at least about 49%, at least about 50%, at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least

about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% (w/w) active ingredient.

[0239] In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents.

[0240] Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

[0241] Parenteral administration of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of an individual and administration of the pharmaceutical composition through the breach in the tissue. Parental administration can be local, regional or systemic. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, intravenous, intraocular, intravitreal, subcutaneous, intraperitoneal, intramuscular, intradermal, intrasternal injection, and intratumoral.

[0242] Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

[0243] The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active

ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

[0244] A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers, and in some embodiments from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self-propelling solvent/powder-dispensing container such as a device comprising the active ingredient dissolved or suspended in a low-boiling propellant in a sealed container. In some embodiments, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. In some embodiments, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. In some embodiments, dry powder compositions include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

[0245] Low boiling propellants generally include liquid propellants having a boiling point of below 65° F. at atmospheric pressure. Generally, the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (in some embodiments having a particle size of the same order as particles comprising the active ingredient).

[0246] Pharmaceutical compositions of the invention formulated for pulmonary delivery may also provide the active ingredient in the form of droplets of a solution or suspension. Such formulations may be prepared, packaged, or sold as aqueous or dilute alcoholic solutions or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, or a preservative such as methylhydroxybenzoate. In some embodiments, the droplets provided by this route of administration have an average diameter in the range from about 0.1 to about 200 nanometers.

[0247] The formulations are also useful for intranasal delivery of a pharmaceutical composition of the invention.

[0248] Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers. Such a formulation is administered in the manner in which snuff is taken i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nares.

[0249] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more additional ingredients.

[0250] A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more additional ingredients. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. In some embodiments, such powdered, aerosolized, or aerosolized formulations, when dispersed, have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more additional ingredients.

[0251] As used herein, “additional ingredients” include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other “additional ingredients” which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Remington’s Pharmaceutical Sciences (1985, Genaro, ed., Mack Publishing Co., Easton, Pa.), which is incorporated herein by reference.

Cells Producing Antibodies and Antigen Binding Fragments Thereof

[0252] In some embodiments, the invention is a cell or cell line (such as host cells) that produces at least one of the anti-C5 antibodies, or antigen binding fragments, described herein. In one embodiment, the cell or cell line is a genetically modified cell that produces at least one of the anti-C5 antibodies, or antigen binding fragments, described herein. In one embodiment, the cell or cell line is a hybridoma that produces at least one of the anti-C5 antibodies, or antigen binding fragments, described herein.

[0253] Hybrid cells (hybridomas) are generally produced from mass fusions between murine splenocytes, which are highly enriched for B-lymphocytes, and myeloma “fusion partner cells” (Alberts et al., Molecular Biology of the Cell (Garland Publishing, Inc. 1994); Harlow et al., Antibodies. A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, 1988). The cells in the fusion are subsequently distributed into pools that can be analyzed for the production of antibodies with the desired specificity. Pools that test positive can be further subdivided until single cell clones are identified that produce antibodies of the desired specificity. Antibodies produced by such clones are referred to as monoclonal antibodies.

[0254] Also provided are nucleic acids encoding any of the antibodies, or antibody fragments, disclosed herein, as well as vectors comprising the nucleic acids. Thus, the antibodies and fragments of the invention can be generated by expressing the nucleic acid in a cell or a cell line, such as the cell lines typically used for expression of recombinant or humanized immunoglobulins. Thus, the antibodies and fragments of the invention can also be generated by cloning the nucleic acids into one or more expression vectors, and transforming the vector into a cell line such as the cell lines typically used for expression of recombinant or humanized immunoglobulins.

[0255] The genes encoding the heavy and light chains of immunoglobulins, or fragments thereof, can be engineered according to methods, including but not limited to, the polymerase chain reaction (PCR), known in the art (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor, N.Y., 1989; Berger & Kimmel, *Methods in Enzymology*, Vol. 152: *Guide to Molecular Cloning Techniques*, Academic Press, Inc., San Diego, Calif., 1987; Co et al., 1992, *J. Immunol.* 148:1149). For example, genes encoding heavy and light chains, or fragments thereof, can be cloned from an antibody secreting cell's genomic DNA, or cDNA is produced by reverse transcription of the cell's RNA. Cloning is accomplished by conventional techniques including the use of PCR primers that hybridize to the sequences flanking or overlapping the genes, or segments of genes, to be cloned.

[0256] Nucleic acids encoding the antibody of the invention, or the heavy chain or light chain or fragments thereof, can be obtained and used in accordance with recombinant nucleic acid techniques for the production of the specific immunoglobulin, immunoglobulin chain, or a fragment or variant thereof, in a variety of host cells or in an in vitro translation system. For example, the antibody-encoding nucleic acids, or fragments thereof, can be placed into suitable prokaryotic or eukaryotic vectors, e.g., expression vectors, and introduced into a suitable host cell by an appropriate method, e.g., transformation, transfection, electroporation, infection, such that the nucleic acid is operably linked to one or more expression control elements, e.g., in the vector or integrated into the host cell genome.

[0257] In some embodiments, the heavy and light chains, or fragments thereof, can be assembled in two different expression vectors that can be used to co-transfect a recipient cell. In some embodiments, each vector can contain two or more selectable genes, one for selection in a bacterial system and one for selection in a eukaryotic system. These vectors allow for the production and amplification of the genes in a bacterial system, and subsequent co-transfection of eukaryotic cells and selection of the co-transfected cells. The selection procedure can be used to select for the expression of antibody nucleic acids introduced on two different DNA vectors into a eukaryotic cell.

[0258] Alternatively, the nucleic acids encoding the heavy and light chains, or fragments thereof, may be expressed from one vector. Although the light and heavy chains are coded for by separate genes, they can be joined, using recombinant methods. For example, the two polypeptides can be joined by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single

chain Fv (scFv); see e.g., Bird et al., 1988, *Science* 242: 423-426; and Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883).

[0259] The invention provides for an isolated nucleic acid molecule comprising a nucleic acid sequence encoding a heavy chain and/or a light chain, as well as fragments thereof. A nucleic acid molecule comprising sequences encoding both the light and heavy chain, or fragments thereof, can be engineered to contain a synthetic signal sequence for secretion of the antibody, or fragment, when produced in a cell. Furthermore, the nucleic acid molecule can contain specific DNA links which allow for the insertion of other antibody sequences and maintain the translational reading frame so to not alter the amino acids normally found in antibody sequences.

[0260] In accordance with the present invention, antibody-encoding nucleic acid sequences can be inserted into an appropriate expression vector. In various embodiments, the expression vector comprises the necessary elements for transcription and translation of the inserted antibody-encoding nucleic acid so as to generate recombinant DNA molecules that direct the expression of antibody sequences for the formation of an antibody, or a fragment thereof.

[0261] The antibody-encoding nucleic acids, or fragments thereof, can be subjected to various recombinant nucleic acid techniques known to those skilled in the art such as site-directed mutagenesis.

[0262] A variety of methods can be used to express nucleic acids in a cell. Nucleic acids can be cloned into a number of types of vectors. However, the present invention should not be construed to be limited to any particular vector. Instead, the present invention should be construed to encompass a wide variety of vectors which are readily available and/or known in the art. For example, the nucleic acid of the invention can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[0263] In specific embodiments, the expression vector is selected from the group consisting of a viral vector, a bacterial vector and a mammalian cell vector. Numerous expression vector systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-vector based systems can be employed for use with the present invention to produce polynucleotides, or their cognate polypeptides. Many such systems are commercially and widely available.

[0264] Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2012), and in Ausubel et al. (1999), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In some embodiments, a murine stem cell virus (MSCV) vector is used to express a desired nucleic acid. MSCV vectors have been demonstrated to efficiently express desired nucleic acids in cells. However, the invention should not be limited to only using a MSCV vector, rather any retroviral expression method is included in the invention. Other examples of viral vectors are those based upon Moloney Murine Leukemia Virus (MoMuLV) and HIV. In some embodiments, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient

restriction endonuclease sites, and one or more selectable markers. (See, e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[0265] Additional regulatory elements, e.g., enhancers, can be used modulate the frequency of transcriptional initiation. A promoter may be one naturally associated with a gene or nucleic acid sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous." Similarly, an enhancer may be one naturally associated with a nucleic acid sequence, located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding nucleic acid segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a nucleic acid sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a nucleic acid sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," e.g., containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR, in connection with the compositions disclosed herein (U.S. Pat. Nos. 4,683,202, 5,928,906). Furthermore, it is contemplated the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

[0266] Naturally, it will be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the cell type, organelle, and organism chosen for expression. Those of skill in the art of molecular biology generally know how to use promoters, enhancers, and cell type combinations for protein expression, for example, see Sambrook et al. (2012). The promoters employed may be constitutive, tissue-specific, inducible, and/or useful under the appropriate conditions to direct high-level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins and fragments thereof.

[0267] An example of a promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, Moloney virus promoter, the avian leukemia virus promoter, Epstein-Barr virus immediate early promoter, Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the muscle creatine promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible

promoter in the invention provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter. Further, the invention includes the use of a tissue-specific promoter or cell-type specific promoter, which is a promoter that is active only in a desired tissue or cell. Tissue-specific promoters are well known in the art and include, but are not limited to, the HER-2 promoter and the PSA associated promoter sequences.

[0268] In order to assess the expression of the nucleic acids, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other embodiments, the selectable marker may be carried on a separate nucleic acid and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers are known in the art and include, for example, antibiotic-resistance genes, such as neo and the like.

[0269] Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. Reporter genes that encode for easily assayable proteins are well known in the art. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a protein whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells.

[0270] Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (see, e.g., Ui-Tei et al., 2000 FEBS Lett. 479:79-82). Suitable expression systems are well known and may be prepared using well known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[0271] Methods of introducing and expressing nucleic acids into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical or biological means.

[0272] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, laserporation and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2012) and Ausubel et al. (1999).

[0273] Biological methods for introducing a nucleic acid of interest into a host cell include the use of DNA and RNA

vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0274] Chemical means for introducing a nucleic acid into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

[0275] Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the nucleic acid of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, “molecular biological” assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; “biochemical” assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

Kits

[0276] The invention also includes a kit comprising an anti-C5 antibody, or combinations thereof, of the invention and an instructional material which describes, for instance, administering the anti-C5 antibody, or combinations thereof, to an individual as a therapeutic treatment or a non-treatment use as described elsewhere herein. In an embodiment, this kit further comprises a (optionally sterile) pharmaceutically acceptable carrier suitable for dissolving or suspending the therapeutic composition, comprising an anti-C5 antibody, or combinations thereof, of the invention, for instance, prior to administering the antibody to an individual. Optionally, the kit comprises an applicator for administering the antibody.

EXPERIMENTAL EXAMPLES

[0277] The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

[0278] Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore and are not to be construed as limiting in any way the remainder of the disclosure.

Example 1

[0279] The complement system is part of innate immunity that plays a key role in host defense. However, activated complement also has the potential to cause significant tissue injury and destruction and dysregulated complement activity has been found to be associated with a number of rare and

common diseases such as paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome, rheumatoid arthritis, age-related macular degeneration etc. Thus, anti-complement therapy is a promising way of treating these human disorders.

[0280] Complement C5 is a critical protein in the terminal pathway of complement activation and is the precursor protein for generating the potent pro-inflammatory mediator C5a, as well as the cytolytic membrane attack complex (MAC).

[0281] Six 6 murine anti-human C5 monoclonal antibodies have been generated, and have characterized and confirmed them to be function-blocking mAbs against human C5, they blocked both C5a production and MAC formation when complement is activated by either the classical, lectin or alternative pathways. The identities of these mAbs are as follows: mAbs 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1.

[0282] Furthermore, the Ig subtypes of the heavy and light chains of the above mentioned antibodies have been determined, and have cloned the cDNAs of the variable regions of the heavy chains of the 6 mAbs.

The methods and material used in this example are now described.

Affinity Analysis of Anti-C5 mAbs:

[0283] Surface Plasmon resonance analysis was used to measure the association and dissociation rate constant for binding of human C5 to anti-C5 mAbs using BIAcore 3000 instrument (Biacore AB, Uppsala, Sweden) and all Biacore experiments were performed at 25° C. The carboxylated dextran matrix of a CM5 sensor chip was used to couple the purified α mRabbit IgG (RAMFc) mAb by amine coupling chemistry to obtain 1000 RU surface density, anti-C5 mAbs were captured on immobilized RAMFc. After stabilization of anti-C5 mAb binding, varying concentration of human C5 ranging from 100, 50, 25, 12.5, 6.25 and 0 nM was injected on to the surface in HBSET (HEPES buffer saline EDTA with Tween 20) buffer and the samples were injected on the antibody surface at 30 μ l/min (60 μ l injection) for 180 s and dissociation of bound analyte was allowed to proceed for 900 s. The data were analyzed by the BIA evaluation software 3.2 assuming 1:1 binding model. Regeneration of the surface was achieved with a 50 μ l injection (50 μ l/min) of 10 mM Glycine HCl pH 1.5.

LPS-Induced C5a Production Assay:

[0284] 10% normal human serum (NHS) was pre incubated with different concentration of 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1 at 4° C. for 1 hr. The antibody-treated samples were incubated with LPS in the Mg-EGTA GVB2+ at 37 °C for 1 hour, and then stopped with 40 mM EDTA in PBS.

Sandwich ELISA for Detection of Human C5a:

[0285] 96-well plates were coated with a mouse anti-human C5a-specific antibody (R&D systems #MAB2037) at a final concentration of 1 μ g/ml at 4° C. overnight. Following three washes with PBS containing 0.05% Tween-20, the plates were incubated with 1/8 diluted serum samples (from LPS-induced C5a production assay) at room temperature for 1 hr. After another wash, the plates were incubated with the corresponding biotinylated anti-human C5a mAb (R&D systems #BAM20371) at room temperature for 1 h, washed again and incubated with streptavidin conjugated to horseradish peroxidase (BD pharmagen #554058) at room tem-

perature for 1 hr. After final washing, the plate was developed with HRP substrate for 6-10 min. The reaction was stopped with 2N H₂SO₄ and plate was read at 450 nm in a micro plate

Human C5 and mAb Binding Assay:

[0286] Polystyrene microtiter plates were coated with purified human C5 (50 ng/well) in PBS at 37° C. for 1 hr. After aspirating the C5 solution, wells were blocked with PBS containing 1% BSA in PBS at room temperature for 1 hr. Wells without C5 coating served as background controls. Different concentration of 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1, 50 µl/well in blocking solution, were added to the wells. Following a 1 hour incubation at room temperature, the wells were extensively washed with PBST. Human C5-bound mAb was detected by the addition of anti-mouse IgG HRP or anti-human IgG4 HRP 1:4000 dilution in blocking solution, which was allowed to incubate for 1h at RT. After washing with PBST, the plate was developed with HRP substrate for 6-10 min. The reaction was stopped with 2N H₂SO₄ and plate was read at 450 nm in a micro plate reader.

Generation of Anti-Human C5 mAbs:

[0287] B10.D2/oSnJ female (Stock #000461, Jackson laboratory) mice were immunized with 30 µg of purified human C5 (#A120, Complement Technology Inc) emulsified with adjuvant. At day 14, the mice were again immunized with 30 µg of purified human C5 emulsified with adjuvant. Mice were boosted with 33 µg of purified human C5 three times before fusion. Then, mice were sacrificed by cervical dislocation and spleen was isolated for preparation of single cell suspension by mechanical disruption. The spleen cell suspension was washed once with HYB-SFM (Invitrogen)+10% FBS medium and cells were counted, and mixed with X63-Ag8.653 myeloma cells (ATCC) in a 2:1 ratio. Cell mixture was again washed with HYB-SFM medium, and the cell pellet was prepared by centrifugation (1000 rpm×5 min). The cell pellet was gently disturbed and loosened and then cell fusion was induced by slowly adding poly ethylene glycol (PEG 1500) (1.5 ml PEG for 3×10⁸ cells). The cells were left for 1 min at 37° C. and then 20 ml HYB-SFM medium were added to the cells in 3 min (1 ml for the first minute, 3 ml for the second minute and 16 ml for the third minute). The mixture was centrifuged at 1000 rpm for 5 min and the cells were plated in 24 well plates in HAT medium (10 ml HAT [Sigma H0262], 5 ml Pen/Strep, 500 µl Gentamicin and 10% FBS in 500 ml HYB-SFM medium). After 2 weeks, supernatants from wells with visible colonies were withdrawn for screening of reactivity with purified human C5 by ELISA. Positive clones were picked up and plated in 96 well plates by limiting dilution method to obtain single clones after second round screening by ELISA. Positive clones were expanded in HT-medium (10 ml HT, 5 ml Pen/Strep 500 µl Gentamicin and 10% FBS in 500 ml HYB-SFM medium). Before antibody collection, the hybridoma cells were switched to serum-free medium (HYB-SFM) for 2-3 days. Cell culture medium was collected for mAb purification by protein G affinity chromatography.

Mab Cloning:

[0288] To clone the cDNAs of 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1, total RNAs were isolated from the

hybridoma cells by TRizol reagent (Sigma). First-strand cDNAs were synthesized by reverse transcription using Oligo(dT) primer. To amplify the heavy chain cDNAs (for IgG1, IgG2a/b), the following primers were used in PCR reactions: 5'-GAGGTGAAGCTGGTGGAG(T/A)C(T/A)GG-3' (SEQ ID NO: 77) and 5'-GGGGCCAGTGGATAGAC-3' (SEQ ID NO:78). To amplify the k light chain, the following primers were used: mixture of 4 upstream primers: 5'-CCAGTTCCGAGCTCCAGATGACCCA-GACTCCA-3' (SEQ ID NO: 79); 5'-CCAGTTCCGAGCTCGTGCTCACCCAGTCTCCA-3' (SEQ ID NO:80); 5'-CCAGTTCCGAGCTCCA-GATGACCCAGTCTCCA-3' (SEQ ID NO:81); 5'-CCAGTTCCGAGCTCGTGATGACACAGTCTCCA-3' (SEQ ID NO:82); downstream primer: 5'-GTTGGTGCAG-CATCAGC-3, (SEQ ID NO:83). The PCR amplicons were cloned into pCR TOPO TA 2.1 vector (Invitrogen) and sequenced. To obtain the signal peptide (leader) sequence of the mAbs, the 5'-RACE method was used with a kit (GeneRacer) from Invitrogen. The complete variable region cDNAs were amplified using specific primers determined from the 5'-RACE and the initial sequencing data.

Construction and Expression of Chimeric 2G1 mAb

[0289] Chimeric 2G1 heavy chain cDNA was constructed by cloning the variable region of mAb 2G1 into the pFUSE-CHIg-hG4 vector (from InvivoGen, containing the human IgG4 heavy chain constant region, with Serine 229 mutated to Proline (SEQ ID NO:60) using EcoRI/NheI sites. Chimeric 2G1 light chain cDNA was constructed by cloning the variable region of mAb 2G1 into the pFUSE2-CLIg-hk vector (from invivoGen, containing the human k light chain constant region (SEQ ID NO 61)) using AgeI/BsiWI sites. CHO cells were co-transfected with chimeric heavy and light chains of 2G1 or humanized heavy and light chains of 2G1 (the two humanized heavy chains were paired with the same humanized light chain) using Lipofectamine reagent. After transfection, CHO cells were selected with Geocine (1 mg/ml) and Blastcidine (10 µg/ml) for approximately 7 days. Drug-resistant cell colonies were picked up, trypsinized and subjected to limited dilution culture in 96-well plates in the presence of the same selection drugs. After cells became confluent in the 96-well plates, the medium was tested for reactivity with human C5 by ELISA and positive clones were expanded. For antibody production, stable lines of transfected CHO cells were grown in DMEM: F 12 medium with 10% FBS in 150 cm culture flasks and after reaching confluence, they were switched to serum free CD-CHO medium (Invitrogen). After 3 days, the medium was collected and Abs were purified by protein G chromatography. Aliquots of the purified Abs were analyzed by SDS-PAGE.

Construction and Expression of Humanized 2G1 mAb

[0290] mAb 2G1 CDRs of VH and VL were grafted onto the frameworks of germline variable and joining (V, J) gene segments of human Ig heavy and light chains, respectively by which were chosen based on the CDR similarities between human immunoglobulins and mAb 2G1 (Hwang et al, Method, 2005). The advantage of this approach is to generate humanized Abs that retain their binding affinity to their cognate antigen and is to highly reduce the potential immunogenicity of non-human Abs since all residues in

frameworks are from human Ab germline sequences. Humanized 2G1 heavy chain cDNAs were constructed by cloning the humanized heavy chain variable region of 2G1 (Synthesized by Genescript) into the pFUSE-CHlg-hG4 vector (from InvivoGen, containing the human IgG4 heavy chain constant region, with Serine 229 mutated to Proline (SEQ ID NO 60)) using EcoRI/NheI sites. Humanized 2G1 light chain cDNA was constructed by cloning the humanized light chain variable region of 2G1 (Synthesized by Genescript) into the pFUSE2-CLlg-hk vector (from InvivoGen, containing the human kappa light chain constant region (SEQ ID NO 61)) using AgeI/BsiWI sites. CHO cells were co-transfected with chimeric heavy and light chains of 2G1 or humanized heavy and light chains of 2G1 (the two humanized heavy chains were paired with the same humanized light chain) using Lipofectamine reagent. After transfection, CHO cells were selected with Geocine (1 mg/ml) and Blastidine (10 μ l g/ml) for approximately 7 days. Drug-resistant cell colonies were picked up, trypsinized and subjected to limited dilution culture in 96-well plates in the presence of the same selection drugs. After cells became confluent in the 96-well plates, the medium was tested for reactivity with human C5 by ELISA and positive clones were expanded. For antibody production, stable lines of transfected CHO cells were grown in DMEM: F 12 medium with 10% FBS in 150 cm culture flasks and after reaching confluence, they were switched to serum free CD-CHO medium (Invitrogen). After 3 days, the medium was collected and Abs were purified by protein G chromatography. Aliquots of the purified Abs were analyzed by SDS-PAGE.

Hemolysis Assay:

[0291] Sheep red blood cell (RBCs) lysis assay: Sheep RBCs (1×10^7 or 1×10^8 cells, Complement Technology Inc) were incubated at 37° C. for 20 min with 10% or 50% NHS (Complement Technology Inc) in gelatin veronal buffer (GVB2+, Sigma). Before addition to the sheep RBCs, NHS was pre-incubated with mAb (4E7, 9G6, 11C5, 2G1, 11D9, 8E1 and a control mAb 7A12) or chimeric 2G1 and 7A12, or humanized 2G1 for 1 hour at 4° C. Lysis reaction was stopped by addition of ice-cold 40 mM EDTA in PBS. The incubation mixtures were centrifuged for 5 min at 1500 rpm and the supernatant was collected and measured for OD₄₀₅ nm. Samples without NHS or with EDTA added were used as negative lysis controls, and a sample of sheep RBCs lysed completely with distilled water was used as a positive control (100% lysis) against which % lysis in other samples was normalized.

[0292] Chicken RBCs lysis assay: Chicken RBCs (1×10^7 or 1×10^8 cells, Complement Technology Inc) were incubated at 37° C. for 1 hour with 50% serum (Complement Technology Inc) in GVB2+. Before addition to the chicken RBCs, sera from different animal species including human, dog, rabbit hamster, goat, pig, sheep, Rhesus monkey or Cynomolgus monkey, was pre-incubated with 50 μ g/ml of 4E7, 9G6, 11C5, 2G1, 11D9, or 8E1 for 1 hour at 4° C. In some experiments, NHS was pre-incubated with serial diluted 2G1 or 8E1 for 1 hour at 4° C. Lysis reaction was stopped by addition of ice-cold 40 mM EDTA in PBS. The incubation mixtures were centrifuged for 5 min at 1500 rpm and the supernatant was collected and measured for OD₄₀₅ nm. The collected data were normalized against serum plus EDTA as negative control (0%) and against serum plus no antibody as positive control (100%).

[0293] PNH RBCs lysis assay: NHS were diluted with GVB2+ buffer and acidified to pH 6.4 (acidified normal human serum: aNHS) and used for assays with PNH RBCs. 5×10^6 cells were added in 50% aNHS. Incubated at 37° C., and after 30 min, reaction was stopped by adding 200 μ l of cold 20 mM EDTA in PBS. mAb 2G1 were incubated with aNHS for 30 min in 4C before adding to the PNH RBCs. The RBCs were pelleted by centrifugation, and the optical density at 405 nm of an aliquot of the recovered supernatant was used to calculate the percentage lysis. PNH RBCs lysed in water used as 100% lysis standard.

Expression of Human C5 β Chain and Domain Deletion Mutants:

[0294] Human C5 (hC5) cDNA in pGEM-T vector was purchased from Sino Biologicals Inc. (Cat #HG13416-G). The C5 nucleotide sequence obtained from the Sino Biologicals Inc. was confirmed with gene and vector specific primers. hC5 β -chain nucleotide and protein sequence was identified with PubMed (NM_001735.2) and Uniprot Id-P01031. β -chain from hC5 was amplified and cloned into the pCAGGS vector. Macroglobulin (MG) domain deletion mutants (MG1, MG, MG3, MG4, MG5 and MG6) of β -chain were carried out in hC5-pGEM-T vector by inverse PCR and deletion was confirmed by sequencing. MG domain mutants of hC5 β chain were similarly cloned into the pCAGGS vector. Primers used for MG deletion mutants construction and cloning are listed in Table 1 below.

TABLE 1

Primers used for MG mutants construction and cloning		
SEQ ID NO:	Primer	Sequence (5'-3')
90	hC5 β -MG1delReverse	TCCCCAGGTTTTCCCCAGGAAGAT
91	hC5 β -MG1delForward	AATGGATTCTCTTCATTCATAC
92	hC5 β -MG2delReverse	GTCATAGGTTATTGGCATTCTT
93	hC5 β -MG2delForward	TTGCCACATTTTTCTGTCTCAATC
94	hC5 β -MG3delReverse	GACATATTCTTTAACTTCAAAATATG

TABLE 1-continued

Primers used for MG mutants construction and cloning		
SEQ ID NO:	Primer	Sequence (5'-3')
95	hC5β-MG3delForward	CCCTACAACTGAATTTGGTTG
96	hC5β-MG4delReverse	AGAGAGGACATATTTGATGCCAG
97	hC5β-MG4delForward	TCTCTCAGCCAAAGTTACCTT
98	hC5β-MG5delReverse	TGAGTATGCTATTGCTCGGTAAC
99	hC5β-MG5delForward	TGTGGCAACCAGCTCCAGGTTC
100	hC5β-MG6delReverse	TTTTTCTTCAATATTTAACCAG
101	hC5β-MG6delForward	AGGCCAAGAAGAACGCTGCAAAAG
102	hC5infF	TTTTGGCAAAGAATTCGCCACCATGGG CCTTTTGGGAATAC
103	hC5pinfR	CCTGAGGAGTGAATTCTTAATGGTGATGG TGATGGTGGAGAATTTCTTTACAAGGTTC

Transfection of hC5β and hC5β-MG Deletions:

[0295] The intact β chain and various MG domain deletion mutant cDNAs were transfected into HEK cells using Lipofectamine 2000 (Invitrogen). After 48 hours, supernatants were collected and used for assay.

SDS-PAGE and Western Blotting:

[0296] For Western blot analysis, 40 μl supernatant from hC5 β chain- and MG domain deletion mutant-transfected HEK cells or non-transfected HEK cell supernatant (negative control) was added to the tubes containing sample buffer and β-mercaptoethanol. Purified human C5 was used as a positive control. The samples were boiled at 100° C. for 7 min and loaded onto 4-12% gradient gel and proteins were transferred onto 0.2 μm pore size PVDF membrane. The membrane was blocked with 5% non-fat dry milk in TBS for 1 hour at room temperature. The membrane was then incubated with primary antibody (goat anti-hC5 polyclonal from Comptech, cat #A220 or biotinylated mAb 2G1-3 or QDC5) in 5% non-fat dry milk in TBST at 4° C. overnight. The membrane was washed with TBS with 0.1% Tween-20 (TBST) for 6×5 min and incubated with 1:4000 dilution of rabbit anti-goat-HRP (Bio-Rad, Cat #172-1034) for 1 hr at room temperature. Proteins were detected using Pierce™ ECL 2 Western Blotting substrate according to manufacturer's instructions. The mAb QDC5 is a recombinant human IgG4 mAb bearing VH and VL sequences of a humanized mouse anti-human C5 mAb as described in Thomas et al. (Mol Immunol., 1996 December; 33(17-18):1389-401). It was expressed in Expi-CHO cells (Invitrogen) and purified by protein A affinity chromatography.

Sandwich ELISA for Detection of hC5 β Chain and MG Deletion Mutants:

[0297] For Sandwich ELISA, 96-well plates were coated with 2G1-3 mAb at a final concentration of 2 μg/ml at 4° C. overnight. Following three washes with PBS containing 0.05% Tween-20, the plates were blocked with 3% bovine serum albumin (BSA) for 1 hour at room temperature. After

washing, the plates were incubated with 200 μl of transfected HEK cell supernatants or 20% NHS for 1 h at room temperature. After another wash, the plates were incubated with the detection antibody SKY59 at a final concentration of 2 μg/ml at room temperature for 1 h, washed again and incubated with HRP-conjugated secondary human IgG4-specific antibody (Invitrogen #MA1-34437) at room temperature for 1 hr. The mAb SKY59 was expressed as an IgG4 recombinant mAb based on published VH/VL sequences (Fukuzawa et al., Sci Rep., 2017 Apr. 24; 7(1): 1080. doi: 10.1038/s41598-017-01087-7). In the case of Western blotting using a polyclonal goat anti-human C5 antibody, the antibody was used at 1:500 dilution at room temperature for 1 hr. After further washing, the wells were incubated with rabbit anti-goat IgG HRP (1:4000) for 1 hr. Plates were developed with HRP substrate for 6-10 min. The reaction was stopped with 2N H2SO4 and plate was read at 450 nm in a micro plate reader.

The results of this example are now described.

[0298] Results from an ELISA assay demonstrating the binding of anti-human C5 mAbs 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1 to human C5 are shown in FIG. 1. Direct antigen binding ELISA in which mAbs are serially diluted across microtiter plates coated with purified human C5. All six mAbs showed high reactivity with human C5.

[0299] Results of experiments demonstrating binding affinities of anti-C5 mAbs 2G1, 8E1, 4E7, 9G6, 11C5 and 11D9 to C5 are shown in FIG. 2-7. Purified αmRabbit IgG (RAMFc) mAb was coupled onto a CM4 chip using the amine coupling method. Then, anti-C6 mAbs were captured on immobilized RAMFc. Biacore analyses were performed on a Biacore-2000 instrument.

[0300] The dose-dependent inhibition of LPS-induced C5a production by anti-human C5 mAbs 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1 is illustrated in FIG. 8. To assess the effect of anti-human C5 mAbs on LPS-induced C5a production, a combination of two assays was used: LPS-induced C5a production and human C5a sandwich ELISA. All six mAbs effectively inhibited LPS-induced C5a pro-

duction when added to 10% normal human serum (NHS) at a final concentration of 12.5 $\mu\text{g/ml}$.

[0301] The effects of anti-human C5 mAbs 4E7, 9G6, 11C5, 2G1, 11D9 and 8E1 on complement-mediated hemolysis are shown in FIG. 9. FIG. 9A illustrates RBC lysis determined by measuring the absorbance at OD405 nm after sheep RBCs were incubated with 50% NHS containing serial dilutions of each anti-C5 mAb at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD405 nm. At 120 $\mu\text{g/ml}$, all mAbs inhibited 50% NHS-mediated sheep erythrocyte lysis. At lower doses (e.g., 30-60 $\mu\text{g/ml}$), 9G6 was less potent in preventing hemolysis than other mAbs. FIG. 9B illustrates that at 30 $\mu\text{g/ml}$, mAb 2G1 and 8E1 were more potent at inhibiting complement-mediated hemolysis than 4E7, 11C5 and 11D9.

[0302] The effects of anti-human C5 mAbs 4E7, 9G6, 11C5, 2G1, 11D9, 8E1 on complement-mediated hemolysis using sera from different animal species are shown in FIG. 10. Chicken RBCs were incubated with 50% NHS, normal rabbit serum, Rhesus monkey serum or Cynomolgus monkey serum containing each anti-C5 mAb (final 50 $\mu\text{g/ml}$) at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD405 nm, and normalized against serum plus EDTA as negative control (0%), and against serum plus no antibody as positive control (100%). In the hemolytic assay using NHS, five of six anti-human C5 mAbs inhibited hemolysis significantly, i.e. by more than 50%. Especially, NHS samples containing 2G1 showed almost complete inhibition of hemolysis. When treated with mAb 9G6 or 2G1, hemolytic activity in rabbit serum was significant reduced. On the other hand, all mAbs failed to inhibit complement-mediated hemolysis using sera of monkey (Rhesus and Cynomolgus), goat, pig and sheep.

[0303] The effects of anti-human C5 mAbs 2G1 and 8E1 on complement-mediated hemolysis are depicted in FIG. 11. Chicken RBCs were incubated with 50% NHS containing serial dilutions of 2G1 or 8E1 or control mAb (MOPC, mouse IgG1) at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD405 nm.

[0304] The effect of anti-human C5 mAb 2G1 on hemolysis of PNH RBCs is presented in FIG. 12. RBCs from paroxysmal nocturnal hemoglobinuria (PNH) patients were subjected to Ham's acidified serum test in the presence or absence of mAb 2G1. RBCs were incubated with 50% NHS containing serial dilutions of 2G1 at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD405 nm. In the absence of mAb, about 35% of RBCs were lysed by acidified serum, while mAb 2G1 treatment caused 70% reduction of hemolytic activity at 25 $\mu\text{g/ml}$ and 85% reduction at 40 $\mu\text{g/ml}$.

[0305] Antibody sequences for the six antibodies are shown in FIG. 13 through FIG. 18. FIG. 13 depicts the variable region sequences of heavy and light chains of mAb 2G1. FIG. 14 depicts the variable region sequences of heavy and light chains of mAb 8E1. FIG. 15 depicts the variable region sequences of heavy and light chains of mAb 4E7. FIG. 16 depicts the variable region sequences of heavy and light chains of mAb 9G6. FIG. 17 depicts the variable region sequences of heavy and light chains of mAb 11C5. FIG. 18 depicts the variable region sequences of heavy and light chains of mAb 11D9.

[0306] The amino acid sequences of human IgG4 constant heavy region, with a serine 228 to proline mutation (i.e., S228P) and human kappa constant light region are shown in

FIG. 19. These sequences were used to construct chimeric (mouse variable region+human constant region) and humanized (humanized mouse variable region+human constant region) anti-human C5 antibody (2G1).

[0307] The reactivity of 2G1 human IgG4 chimeric mAb with human C5 is shown in FIG. 20. Chimeric 2G1 was made by joining the variable regions of mAb 2G1 with human IgG4 heavy chain constant region carrying a S228P mutation and human kappa light chain constant region. A plate was coated with human C5. After incubation with serially diluted chimeric 2G1, bound chimeric mAb was detected by HRP-conjugated anti-human IgG4. Chimeric 2G1 bound to human C5 in a dose dependent manner.

[0308] The effects of 2G1 human IgG4 chimeric mAb on classical pathway complement-mediated hemolysis is shown in FIG. 21. Sensitized sheep RBCs were incubated with NHS containing serially diluted chimeric 2G1 at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD405 nm. The result shows that at 30 $\mu\text{g/ml}$ and higher concentrations, chimeric 2G1 mAb inhibited 50% of NHS-mediated sheep erythrocyte lysis.

[0309] The nucleotide and amino acid sequences of a humanized variable heavy chain (VH) of mAb 2G1 (humanized 2G1 VH-11801) are illustrated in FIG. 22. Humanization was achieved by CDR grating from murine mAb 2G1 VH into a germline encoded human VH frame (11801). The amino acid sequence of signal peptide is underlined and that of CDR1, CDR2 and CDR3 is bolded and shaded.

[0310] The nucleotide and amino acid sequences of another humanized VH of mAb 2G1 (humanized 2G1 VH-16901) are illustrated in FIG. 23. Humanization was achieved by CDR grating from murine mAb 2G1 VH into a germline encoded human VH frame (16901). The amino acid sequence of signal peptide is underlined and that of CDR1, CDR2 and CDR3 is bolded shaded.

[0311] The nucleotide and amino acid sequences of a humanized variable light chain (VL) of mAb 2G1 (humanized 2G1 VL-1901) are illustrated in FIG. 24. Humanization was achieved by CDR grating from murine mAb 2G1 VL into a germline encoded human VL frame (1901). The amino acid sequence of signal peptide is underlined and that of CDR1, CDR2 and CDR3 is bolded and shaded.

[0312] The reactivity of humanized 2G1 (VH-11801/VL-1901) with human C5 is shown in FIG. 25. Humanized 2G1 (VH-11801/VL-1901) was expressed as a human IgG4 mAb with S228P mutation in the Fc domain. A plate was coated with human C5. After incubation with serially diluted humanized 2G1 (VH-11801/VL-1901), bound mAb was detected by HRP-conjugated anti-human IgG4. Humanized 2G1 (VH-11801/VL-1901) bound human C5 in a dose dependent manner.

[0313] The reactivity of humanized 2G1 (VH-16901/VL-1901) with human C5 is shown in FIG. 26. Humanized 2G1 (VH-16901/VL-1901) was expressed as a human IgG4 mAb with S228P mutation in the Fc domain. A plate was coated with human C5. After incubation with serially diluted humanized 2G1 (VH-16901/VL-1901), bound Ab was detected by HRP-conjugated anti-human IgG4. Humanized 2G1 (VH-16901/VL-1901) bound human C5 in a dose dependent manner.

[0314] The effects of the humanized 2G1 (VH-11801/VL-1901) on classical pathway complement-mediated hemolysis is shown in FIG. 27. Humanized 2G1 (VH-11801/VL-1901) was expressed as a human IgG4 mAb with S228P

mutation in the Fc domain. Sensitized sheep RBCs were incubated with 10% NHS containing serially diluted humanized 2G1 (VH-11801/VL-1901) at 37° C. for 1 hour. RBC lysis was determined by measuring the absorbance at OD405 nm. Humanized 2G1 (VH-11801/VL-1901) significantly inhibited 10% NHS-mediated sheep erythrocyte lysis at 10 µg/ml and higher mAb concentrations.

[0315] The effects of the humanized 2G1 (VH-16901/VL-1901) on classical pathway complement-mediated hemolysis are shown in FIG. 28. Humanized 2G1 (VH-16901/VL-1901) was expressed as a human IgG4 mAb with S228P mutation in the Fc domain. Sensitized sheep RBCs were incubated with 10% NHS containing serially diluted humanized 2G1 (VH-16901/VL-1901) at 37° C. for 1 hour. RBC

lysis was determined by measuring the absorbance at OD405 nm. Humanized 2G1 (VH-16901/VL-1901) significantly inhibited 10% NHS-mediated sheep erythrocyte lysis at 10 µg/ml and higher mAb concentrations.

[0316] The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

[0317] While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

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	organism = synthetic construct	
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tgcaaggctt ctggatacac aatcacagac tacaatttgg actgggtgaa gcagagccat	180	
ggaaagagcc ttgagtggat tggagatatt agtcctaact atggttatac tatctacaac	240	
cagaaattca aggacaaggc cacattgact gtagacaggt cctccagcac agcctacatg	300	
gagctccgca gcctgacatc tgaggacact gcagtttatt actgtgcaag aagggacatt	360	
cgttactccg gtaattccta caaatggtac ttcgatgtct ggggcacagg gaccacggtc	420	
accgtctcct ca	432	
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source	1..144	
	mol_type = protein	
	organism = synthetic construct	
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GKSLEWIGDI SPNYGYTIYN QKFKDKATLT VDRSSSTAYM ELRSLTSEDV AVYYCARRDI	120	
RYSGNSYKWKY FDVWGTGTTV TVSS	144	
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source	1..10	
	mol_type = protein	
	organism = synthetic construct	
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SEQ ID NO: 4	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
REGION	1..17	
	note = Amino acid sequence of CDR2 of VH of mAb 2G1	
source	1..17	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 4		
DISPNYGYTI YNQKFKD	17	
SEQ ID NO: 5	moltype = AA length = 16	
FEATURE	Location/Qualifiers	
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	note = Amino acid sequence of CDR3 of VH of mAb 2G1	
source	1..16	
	mol_type = protein	
	organism = synthetic construct	

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SEQUENCE: 5
RDIRYSGNSY KWFYFDV 16

SEQ ID NO: 6 moltype = DNA length = 381
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note = Nucleotide sequence of VL of mAb 2G1
source 1..381
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 6
atgagggttcc aggttcaggt tctggggctc cttctgctct ggatatcagg tgcccagtggt 60
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attaattgca ggacaagtaa gagcataagc aaatatcttag cctgggtatca agagaaacct 180
gggaaaacta ataagcttct tatctactct ggatccacct tgcaatctgg aattccatca 240
aggttcaggg gcagtggatc tggtagacat ttcactctca ccatcagtag cctggagcct 300
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source 1..127
mol_type = protein
organism = synthetic construct

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GTKLEIK 127

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source 1..11
mol_type = protein
organism = synthetic construct

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SEQ ID NO: 9 moltype = AA length = 7
FEATURE Location/Qualifiers
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source 1..7
mol_type = protein
organism = synthetic construct

SEQUENCE: 9
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SEQ ID NO: 10 moltype = AA length = 9
FEATURE Location/Qualifiers
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source 1..9
mol_type = protein
organism = synthetic construct

SEQUENCE: 10
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SEQ ID NO: 11 moltype = DNA length = 414
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note = Nucleotide sequence of VH of mAb 8E1
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mol_type = other DNA
organism = synthetic construct

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source	1..138		
	mol_type = protein		
	organism = synthetic construct		
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GKGLEWLAVI WSDGRTTYNS	ALNSRLSISK DNSKSQVFFK	MNSLQTDDTA MYICARHDGR	120
GDYSMDYWGQ GTSVTVSS			138
SEQ ID NO: 13	moltype = AA	length = 10	
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source	1..10		
	mol_type = protein		
	organism = synthetic construct		
SEQUENCE: 13			
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SEQ ID NO: 14	moltype = AA	length = 16	
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	mol_type = protein		
	organism = synthetic construct		
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source	1..11		
	mol_type = protein		
	organism = synthetic construct		
SEQUENCE: 15			
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SEQ ID NO: 16	moltype = DNA	length = 390	
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	organism = synthetic construct		
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ggtactatct acaaccagaa gttcaaggac aaggccacat tgactgtaaa caagtctctc	300	
agcacagcct acatggaatt ccgcagcctg acatcggagg attctgcagt ctatttctgt	360	
tcaagagggg gggtttatta ccggggggtt gcttactggg gccaaaggac actgggcact	420	
gtctctgca	429	
SEQ ID NO: 22	moltype = AA length = 143	
FEATURE	Location/Qualifiers	
REGION	1..143	
	note = Amino acid sequence of VH of mAb 4E7	
source	1..143	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 22		
MSSPQTLNLT TLTMGWSWIF LFLLSETAGV LSEVQLQQGA SVKMSCKTSR YSFTDYIMHW	60	
WVKLSHGKSLE WIGYINPNNG GTIYNQKFKD KATLTVNKSS STAYMEFRSL TSEDSAVYFC	120	
SRGGVYYRGF AYWGQGTLLVT VSA	143	
SEQ ID NO: 23	moltype = AA length = 9	
FEATURE	Location/Qualifiers	
REGION	1..9	
	note = Amino acid sequence of CDR1 of VH of mAb 4E7	
source	1..9	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 23		9
RYSFTDYIM		
SEQ ID NO: 24	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
REGION	1..17	
	note = Amino acid sequence of CDR2 of VH of mAb 4E7	
source	1..17	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 24		17
YINPNNGGTI YNQKFKD		
SEQ ID NO: 25	moltype = AA length = 10	
FEATURE	Location/Qualifiers	

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REGION 1..10
note = Amino acid sequence of CDR3 of VH of mAb 4E7
source 1..10
mol_type = protein
organism = synthetic construct

SEQUENCE: 25
GGVYYRGFAY 10

SEQ ID NO: 26 moltype = DNA length = 411
FEATURE Location/Qualifiers
misc_feature 1..411
note = Nucleotide sequence of VL of mAb 4E7
source 1..411
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 26
atggcctgga tttcacttat actctctctc ctggctctca gctcaggggc catttcccag 60
gctgttgtga ctcaggaatc tgcactcacc acatcacctg gtgaaacagt cacattcact 120
tgctcgtcaa gtactggggc tgttacaaac agtaactatg ccaactgggt ccaagaaaaa 180
ccagatcatt ttttactagg tctaataagg gttaccaaca accgaggtcc aggtgttcct 240
gcccgattct caggctccct gattggagac aaggctgccc tcaccatcac aggggcacag 300
actgaggatg aggcaatata tttctgtgct ctatgggtaca gcaaccactt ggggtgttcgg 360
tgagggaacc aaactgactg tcctaggcca gcccaagtct tcgccatcag t 411

SEQ ID NO: 27 moltype = AA length = 137
FEATURE Location/Qualifiers
REGION 1..137
note = Amino acid sequence of VL of mAb 4E7
source 1..137
mol_type = protein
organism = synthetic construct

SEQUENCE: 27
MAWISLILSL LALSSGAISQ AVVTQESALT TSPGETVTFT CRSSTGAVTN SNYANWVQEK 60
PDHFFTGLIG VTNNRGP GVP ARFSGSLIGD KAALTITGAQ TEDEAIYFCA LWYSNHLGVR 120
WRNQTD CPRP AQVFAIS 137

SEQ ID NO: 28 moltype = AA length = 14
FEATURE Location/Qualifiers
REGION 1..14
note = Amino acid sequence of CDR1 of VL of mAb 4E7
source 1..14
mol_type = protein
organism = synthetic construct

SEQUENCE: 28
RSSTGAVTNS NYAN 14

SEQ ID NO: 29 moltype = AA length = 7
FEATURE Location/Qualifiers
REGION 1..7
note = Amino acid sequence of CDR2 of VL of mAb 4E7
source 1..7
mol_type = protein
organism = synthetic construct

SEQUENCE: 29
VTNNRGP 7

SEQ ID NO: 30 moltype = DNA length = 423
FEATURE Location/Qualifiers
misc_feature 1..423
note = Nucleotide sequence of VH of mAb 9G6
source 1..423
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 30
atggattggg tgtggacctt ggtattcctg atagcagctg cccaaagtgc ccaagcacag 60
atccagttgg tgcagtctgg acctgagctg aagaagcctg gagagacagt caagatctcc 120
tgcaaggctt ctgggtatac cttcacagaa tatccaatgc actgggtgaa gcaggctcca 180
ggaaagagtt tcaagtggat gggcatccta tacaccgaca ctggagagcc aacatatgct 240
gaagagtcca ggggacggtt tgccttctct ttggagacct ctgccagcac tgcctatttg 300
cagatcaaca acctcaaaaa tgaggacacg gctacatatt tctgtgttca ctcaggctac 360
gtaggctact ggggccaaag caccactctc acagtctcat cagccaaaac aacaccccca 420
tca 423

SEQ ID NO: 31 moltype = AA length = 134
FEATURE Location/Qualifiers
REGION 1..134

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source	note = Amino acid sequence of VH of mAb 9G6 1..134 mol_type = protein organism = synthetic construct	
SEQUENCE: 31		
MDWVWTLVFL IAAQSAQAQ IQLVQSGPEL KKPGETVKIS CKASGYTFTE YPMHWVKQAP	60	
GKSFKWMGIL YTDTEPTYA EEFRGRFAFS LETSASTAYL QINNLIKNET ATYFCVHSGY	120	
VGWGGQTTL TVSS	134	
SEQ ID NO: 32	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
REGION	1..10	
	note = Amino acid sequence of CDR1 of VH of mAb 9G6	
source	1..10 mol_type = protein organism = synthetic construct	
SEQUENCE: 32		
GYTFTEYPMH		10
SEQ ID NO: 33	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
REGION	1..17	
	note = Amino acid sequence of CDR2 of VH of mAb 9G6	
source	1..17 mol_type = protein organism = synthetic construct	
SEQUENCE: 33		
ILYTDTEPT YAEFRG		17
SEQ ID NO: 34	moltype = AA length = 6	
FEATURE	Location/Qualifiers	
REGION	1..6	
	note = Amino acid sequence of CDR3 of VH of mAb 9G6	
source	1..6 mol_type = protein organism = synthetic construct	
SEQUENCE: 34		
SGVVG		6
SEQ ID NO: 35	moltype = DNA length = 384	
FEATURE	Location/Qualifiers	
misc_feature	1..384	
	note = Nucleotide sequence of VL of mAb 9G6	
source	1..384 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 35		
atgagtgtgc ccactcaggt cctgggggtg ctgctgctgt ggcttacagg tgccagatgt	60	
gacatccaga tgactcagtc tccagcctcc ctatcagcat ctgtgggaga aactgtcacc	120	
ttcacatgtc gggcaagtga gattatttac agttatttag ttggtatca gcagaaacag	180	
ggaaaatctc ctcagctcct ggtctctaata gcaaaaacct tagcagaggg tgtgccatca	240	
aggttcagtgc gcagtggatc aggcacacag ttttctctga agatcaatag cctgcagcct	300	
gaagattttg ggagttatta ctgtcaacat tattatggta atcctccac gttcggaggg	360	
gggaccaagc tggaaataaa acgg	384	
SEQ ID NO: 36	moltype = AA length = 128	
FEATURE	Location/Qualifiers	
REGION	1..128	
	note = Amino acid sequence of VL of mAb 9G6	
source	1..128 mol_type = protein organism = synthetic construct	
SEQUENCE: 36		
MSVPTQVLGL LLLWLTGARC DIQMTQSPAS LSASVGETVT FTCRASEIIY SYLVWYQQKQ	60	
GKSPQLLVSN AKTLAEGVPS RFGSGSGTQ FSLKINSLOP EDFGSYYCQH YYGNPPTFGG	120	
GTKLEIKR	128	
SEQ ID NO: 37	moltype = AA length = 11	
FEATURE	Location/Qualifiers	
REGION	1..11	
	note = Amino acid sequence of CDR1 of VL of mAb 9G6	
source	1..11 mol_type = protein organism = synthetic construct	
SEQUENCE: 37		
RASEIIYSYL V		11

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SEQ ID NO: 38	moltype = AA	length = 7	
FEATURE	Location/Qualifiers		
REGION	1..7		
	note = Amino acid sequence of CDR2 of VL of mAb 9G6		
source	1..7		
	mol_type = protein		
	organism = synthetic construct		
SEQUENCE: 38			
NAKTLAE			7
SEQ ID NO: 39	moltype = AA	length = 9	
FEATURE	Location/Qualifiers		
REGION	1..9		
	note = Amino acid sequence of CDR3 of VL of mAb 9G6		
source	1..9		
	mol_type = protein		
	organism = synthetic construct		
SEQUENCE: 39			
QHYYGNPPT			9
SEQ ID NO: 40	moltype = DNA	length = 447	
FEATURE	Location/Qualifiers		
misc_feature	1..447		
	note = Nucleotide sequence of VH of mAb 11C5		
source	1..447		
	mol_type = other DNA		
	organism = synthetic construct		
SEQUENCE: 40			
atgagcactg aacacggacc cctcaccatg aacttcgggc tcagcttgat ttcccttgtc			60
cttggttttaa aaggtgtcca gtgtgaagtg cagctagtgg agtctggggg aggccttagtg			120
aagcctggag ggtccctgaa acttcctctg gcagcctctg gattcacttt cactacctat			180
gccatgtctt gggttcgcca gactccggaa aagaggctgg agtgggtcgc aaccattagt			240
gctggtggta cttacaccta ctattcagac aatgtaaagg gccgattcac catctccaga			300
gacaatgcc aagaacaacct gtacctgcaa atgagccatc tgaagtctga ggacacagcc			360
atgttttact gtgcaagaga tcccgattac tacggtagaa gcccgtttgc ttactggggc			420
caagggactc tggtcactgt ctctgca			447
SEQ ID NO: 41	moltype = AA	length = 149	
FEATURE	Location/Qualifiers		
REGION	1..149		
	note = Amino acid sequence of VH of mAb 11C5		
source	1..149		
	mol_type = protein		
	organism = synthetic construct		
SEQUENCE: 41			
MSTEHGPLTM NFGLSLIFLV LVLKGVQCEV QLVESGGGLV KPGGSLKLSC AASGFTFTTY			60
AMSWVRQTPE KRLEWVATIS AGGTYYTYSD NVKGRFTISR DNAKNNLYLQ MSHLKSEDTA			120
MFYCARDPDY YGRSPFAYWG QGTLVTVSA			149
SEQ ID NO: 42	moltype = AA	length = 10	
FEATURE	Location/Qualifiers		
REGION	1..10		
	note = Amino acid sequence of CDR1 of VH of mAb 11C5		
source	1..10		
	mol_type = protein		
	organism = synthetic construct		
SEQUENCE: 42			
GFTFTTYAMS			10
SEQ ID NO: 43	moltype = AA	length = 17	
FEATURE	Location/Qualifiers		
REGION	1..17		
	note = Amino acid sequence of CDR2 of VH of mAb 11C5		
source	1..17		
	mol_type = protein		
	organism = synthetic construct		
SEQUENCE: 43			
TISAGGTYTY YSDNVKG			17
SEQ ID NO: 44	moltype = AA	length = 12	
FEATURE	Location/Qualifiers		
REGION	1..12		
	note = Amino acid sequence of CDR3 of VH of mAb 11C5		
source	1..12		
	mol_type = protein		

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organism = synthetic construct

SEQUENCE: 44
DPDYYGRSPF AY 12

SEQ ID NO: 45 moltype = DNA length = 384
FEATURE Location/Qualifiers
misc_feature 1..384
note = Nucleotide sequence of VL of mAb 11C5
source 1..384
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 45
atgggttttca cacctcagat acttggactt atgctttttt ggatttcagc ctccagaggt 60
gaaattgtgc tcactcagtc tccagccacc ctgtctgtga ctccaggaga gaggctcagt 120
ctttcctgta gggccagcca aagtattagc aacaacctac agtggtatca acaaaaatca 180
catgagtctc caagacttct catcaaatat gcttcccagt ccatctctgg gatccccctcc 240
aggttcagtg gcagtggatc agggacagat ttcactctca gtatcaacag tgtggagact 300
gaagattttg gaatgtattt ctgtcaacag agtaacagct ggctctcac gttegggtgg 360
gggaccaagg tggagctgaa acgg 384

SEQ ID NO: 46 moltype = AA length = 128
FEATURE Location/Qualifiers
REGION 1..128
note = Amino acid sequence of VL of mAb 11C5
source 1..128
mol_type = protein
organism = synthetic construct

SEQUENCE: 46
MVFTPQILGL MLFWISASRG EIVLTQSPAT LSVTPGESVS LSCRASQSIG NNLQWYQOKS 60
HESPRLLIKY ASQSIGSIPS RFSGSGSGTD FTLSINSVET EDFGMYFCQQ SNSWPLTFGG 120
GTKVELKR 128

SEQ ID NO: 47 moltype = AA length = 11
FEATURE Location/Qualifiers
REGION 1..11
note = Amino acid sequence of CDR1 of VL of mAb 11C5
source 1..11
mol_type = protein
organism = synthetic construct

SEQUENCE: 47
RASQSIGNNL Q 11

SEQ ID NO: 48 moltype = AA length = 7
FEATURE Location/Qualifiers
REGION 1..7
note = Amino acid sequence of CDR2 of VL of mAb 11C5
source 1..7
mol_type = protein
organism = synthetic construct

SEQUENCE: 48
YASQSIG 7

SEQ ID NO: 49 moltype = AA length = 9
FEATURE Location/Qualifiers
REGION 1..9
note = Amino acid sequence of CDR3 of VL of mAb 11C5
source 1..9
mol_type = protein
organism = synthetic construct

SEQUENCE: 49
QQSNSWPLT 9

SEQ ID NO: 50 moltype = DNA length = 465
FEATURE Location/Qualifiers
misc_feature 1..465
note = Nucleotide sequence of VH of mAb 11D9
source 1..465
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 50
atgtcctcac cacagacact gaacacactg actctaacca tgggatggag ctggatcttt 60
ctcttttctcc tgtcaggaaac tgcaggtgtc ctctctgagg tccagctgca acaatctgga 120
cctgaggtgg tgaagcctgg ggcttcagtg aagatatcct gtaaggcttc tggatacacg 180
ttcactgact tctacatgaa ctgggtgaaa cagagccatg gaaagagcct tgagtggatt 240
ggatatatta atcctaacaa tgggtgatact agttacaacc agaagttaa gggcaaggcc 300
acatcgactg tagacaggtc ctccaacaca gcctacatgg agctccgcag cctgacatct 360

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gaggactctg cagtctatta ctgtgcaaga ctcatcttct atggtaactg gtactttgat		420
gtctggggca cagggaccac ggtcaccgtc tcctcagcca aaaca		465
SEQ ID NO: 51	moltype = AA length = 155	
FEATURE	Location/Qualifiers	
REGION	1..155	
	note = Amino acid sequence of VH of mAb 11D9	
source	1..155	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 51		
MSSPQTLNTL TLTMGWSWIF LFLLSGTAGV LSEVQLQQSG PEVVKPGASV KISCKASGYT		60
FTDFYMNWVK QSHGKSLEWI GYINPNNGDT SYNQKFKGKA TSTVDRSSNT AYMELRSLTS		120
EDSAVYYCAR LIFYGNWYFD VWGTGTTTVT SSAKT		155
SEQ ID NO: 52	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
REGION	1..10	
	note = Amino acid sequence of CDR1 of VH of mAb 11D9	
source	1..10	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 52		
GYTFTDFYMN		10
SEQ ID NO: 53	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
REGION	1..17	
	note = Amino acid sequence of CDR2 of VH of mAb 11D9	
source	1..17	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 53		
YINPNNGDTS YNQKFKG		17
SEQ ID NO: 54	moltype = AA length = 11	
FEATURE	Location/Qualifiers	
REGION	1..11	
	note = Amino acid sequence of CDR3 of VH of mAb 11D9	
source	1..11	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 54		
LIFYGNWYFD V		11
SEQ ID NO: 55	moltype = DNA length = 393	
FEATURE	Location/Qualifiers	
misc_feature	1..393	
	note = Nucleotide sequence of VL of mAb 11D9	
source	1..393	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 55		
atggattttc agatgcagat tatcagcttg ctgctaata ggtgcacagt catagtgtct		60
aatggagaaa ttgtgtcac ccagtcctcca cccaccatgg ctgcatctcc cggggagaag		120
atcactatca cctgcagtgc cagctcaagt ataagttcca attacgtgca ttggtatcag		180
cagaagccag gattctccc taaactcttg atttatagga catccaatct ggcttctgga		240
gtcccagttc gcttcagtgg cagtgggtct gggacctctt actctctcac aattggcacc		300
atggaggctg aagatgttgc cacttactac tgccagcagg gtactagtat accgtggacg		360
ttcgtgggag gcaccaaggt ggaaattaat cgg		393
SEQ ID NO: 56	moltype = AA length = 131	
FEATURE	Location/Qualifiers	
REGION	1..131	
	note = Amino acid sequence of VL of mAb 11D9	
source	1..131	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 56		
MDFQMQUIISL LLISVTIVIS NGEIVLTQSP PTMAASPGEK ITITCSASSS ISSNYVHWYQ		60
QKPGFSPKLL IYRTSNLASG VPVRFSGSGS GTSYSLTIGT MEAEDVATYY CQQGTSIPWT		120
FGGGTKVEIN R		131
SEQ ID NO: 57	moltype = AA length = 12	
FEATURE	Location/Qualifiers	
REGION	1..12	

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source	note = Amino acid sequence of CDR1 of VL of mAb 11D9 1..12 mol_type = protein organism = synthetic construct	
SEQUENCE: 57 SASSSISSNY VH		12
SEQ ID NO: 58 FEATURE REGION	moltype = AA length = 7 Location/Qualifiers 1..7 note = Amino acid sequence of CDR2 of VL of mAb 11D9	
source	1..7 mol_type = protein organism = synthetic construct	
SEQUENCE: 58 RTSNLAS		7
SEQ ID NO: 59 FEATURE REGION	moltype = AA length = 9 Location/Qualifiers 1..9 note = Amino acid sequence of CDR3 of VL of mAb 11D9	
source	1..9 mol_type = protein organism = synthetic construct	
SEQUENCE: 59 QQGTSIPWT		9
SEQ ID NO: 60 FEATURE REGION	moltype = AA length = 327 Location/Qualifiers 1..327 note = Amino acid sequence of human IgG4 Constant Heavy chain regionwith S228P mutation	
source	1..327 mol_type = protein organism = synthetic construct	
SEQUENCE: 60 ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGKTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPDSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDSD DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSPGK	60 120 180 240 300 327	
SEQ ID NO: 61 FEATURE REGION	moltype = AA length = 106 Location/Qualifiers 1..106 note = Amino acid sequence of human Kappa Constant Light region	
source	1..106 mol_type = protein organism = synthetic construct	
SEQUENCE: 61 TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYLSST LTLKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC	60 106	
SEQ ID NO: 62 FEATURE misc_feature	moltype = DNA length = 432 Location/Qualifiers 1..432 note = Nucleotide sequence of Humanized 2G1 VH-11801	
source	1..432 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 62 atggactgga cctgggtctt tctcttctctc ctgtcagtaa ctgcaggtgt ccaactcccag gttcagctgg tgcagtctgg agctgaggtg aagaagcctg gggcctcagt gaaggtctcc tgcaaggctt ctggatacac aatcacagac tacaatttgg actgggtgcg acaggcccct ggacaagggc ttgagtggat gggagatatt agtcctaact atgggttatac tatctacaac cagaaattca aggacagagt caccatgacc acagacacat ccacgagcac agcctacatg gagctgagga gcctgagatc tgacgacacg gccgtgtatt actgtgagag aagggacatt cgttactccg gtaattccta caaatggtac ttcgatgtct ggggcccaagg gacaatggtc accgtctctt ca	60 120 180 240 300 360 420 432	
SEQ ID NO: 63 FEATURE REGION	moltype = AA length = 144 Location/Qualifiers 1..144 note = Amino acid sequence of Humanized 2G1 VH-11801	

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source 1..144
mol_type = protein
organism = synthetic construct

SEQUENCE: 63
MDWTWVFLFL LSVTAGVHSQ VQLVQSGAEV KKPGASVKVS CKASGYTITD YNLDWVRQAP 60
GQGLEWMGDI SPNYGYTIYN QKFKDRVMT TDTSTSTAYM ELRSLRSDDT AVYYCARRDI 120
RYSGNSYKWKY FDVWGQGTMTV TVSS 144

SEQ ID NO: 64 moltype = AA length = 10
FEATURE Location/Qualifiers
REGION 1..10
note = Amino acid sequence of CDR1 of Humanized 2G1 VH-11801

source 1..10
mol_type = protein
organism = synthetic construct

SEQUENCE: 64
GYTITDYNLD 10

SEQ ID NO: 65 moltype = AA length = 18
FEATURE Location/Qualifiers
REGION 1..18
note = Amino acid sequence of CDR2 of Humanized 2G1 VH-11801

source 1..18
mol_type = protein
organism = synthetic construct

SEQUENCE: 65
GDISPNYGYT IYNQKFKD 18

SEQ ID NO: 66 moltype = AA length = 16
FEATURE Location/Qualifiers
REGION 1..16
note = Amino acid sequence of CDR3 of Humanized 2G1 VH-11801

source 1..16
mol_type = protein
organism = synthetic construct

SEQUENCE: 66
RDIRYSGNSY KWFYFDV 16

SEQ ID NO: 67 moltype = DNA length = 432
FEATURE Location/Qualifiers
misc_feature 1..432
note = Nucleotide sequence of Humanized 2G1 VH-16901

source 1..432
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 67
atggactgga cctgggtctt tctcttcctc ctgtcagtaa ctgcaggtgt ccactcccag 60
gtgcagctgg tgcagtctgg ggctgaggtg aagaagcctg ggtcctcggg gaagggtctcc 120
tgcaaggctt ctggatacac aatcacagac tacaatttgg actgggtgcg acaggcccct 180
ggacaagggc ttgagtggat gggagatatt agtcctaact atggttatac tatctacaac 240
cagaaattca aggacagagt cagcattacc gcggacgaat ccacgagcac agcctacatg 300
gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgagag aagggacatt 360
cgttactcgg gtaattccta caaatggtag ttcgatgtct ggggcccaagg gacaatgggc 420
accgtctctt ca 432

SEQ ID NO: 68 moltype = AA length = 144
FEATURE Location/Qualifiers
REGION 1..144
note = Amino acid sequence of Humanized 2G1 VH-16901

source 1..144
mol_type = protein
organism = synthetic construct

SEQUENCE: 68
MDWTWVFLFL LSVTAGVHSQ VQLVQSGAEV KKPGSSVKVS CKASGYTITD YNLDWVRQAP 60
GQGLEWMGDI SPNYGYTIYN QKFKDRVIT ADESTSTAYM ELSSLRSEDV AVYYCARRDI 120
RYSGNSYKWKY FDVWGQGTMTV TVSS 144

SEQ ID NO: 69 moltype = AA length = 10
FEATURE Location/Qualifiers
REGION 1..10
note = Amino acid sequence of CDR1 of Humanized 2G1 VH-16901

source 1..10
mol_type = protein
organism = synthetic construct

SEQUENCE: 69
GYTITDYNLD 10

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SEQ ID NO: 76      multype = AA   length = 9
FEATURE            Location/Qualifiers
REGION            1..9
                  note = Amino acid sequence of CDR3 of Humanized 2G1 VL-1901
source            1..9
                  mol_type = protein
                  organism = synthetic construct

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SEQUENCE: 76
QQHNEYPYT 9

SEQ ID NO: 77 moltype = DNA length = 24
FEATURE Location/Qualifiers
misc_feature 1..24
note = PCR primer
misc_feature 19
note = A or T
misc_feature 21
note = A or T
source 1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 77
gaggtgaagc tggaggagc nagg 24

SEQ ID NO: 78 moltype = DNA length = 17
FEATURE Location/Qualifiers
misc_feature 1..17
note = PCR primer
source 1..17
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 78
ggggccagtg gatagac 17

SEQ ID NO: 79 moltype = DNA length = 32
FEATURE Location/Qualifiers
misc_feature 1..32
note = PCR primer
source 1..32
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 79
ccagttccga gctccagatg acccagactc ca 32

SEQ ID NO: 80 moltype = DNA length = 32
FEATURE Location/Qualifiers
misc_feature 1..32
note = PCR primer
source 1..32
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 80
ccagttccga gctcgtgctc acccagtctc ca 32

SEQ ID NO: 81 moltype = DNA length = 32
FEATURE Location/Qualifiers
misc_feature 1..32
note = PCR primer
source 1..32
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 81
ccagttccga gctccagatg acccagtctc ca 32

SEQ ID NO: 82 moltype = DNA length = 32
FEATURE Location/Qualifiers
misc_feature 1..32
note = PCR primer
source 1..32
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 82
ccagttccga gctcgtgatg acacagtctc ca 32

SEQ ID NO: 83 moltype = DNA length = 17
FEATURE Location/Qualifiers
misc_feature 1..17
note = PCR primer
source 1..17
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 83
gttggtgcag catcagc 17

-continued

SEQ ID NO: 84 moltype = AA length = 104
FEATURE Location/Qualifiers
source 1..104
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 84
QEQTYSVISAP KIFRVGASEN IVIQVYGYTE AFDATISIKS YPDKKFSYSS GHVHLSSSENK 60
FQNSAILTIQ PKQLPGGQNP VSYVYLEVVS KHFSKSKRMP ITYD 104

SEQ ID NO: 85 moltype = AA length = 101
FEATURE Location/Qualifiers
source 1..101
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 85
NGFLFIHTDK PVYTPDQSVK VRVYSLNDDL KPAKRETVLT FIDPEGSEVD MVEEIDHIGI 60
ISFPDFKIPS NPYRGMWTIK AKYKEDFSTT GTAYFEVKEY V 101

SEQ ID NO: 86 moltype = AA length = 127
FEATURE Location/Qualifiers
source 1..127
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 86
LPHFSVSIIEP EYNFIGYKNF KNFEITIKAR YFYNKVVTEA DVYITFGIRE DLKDDQKEMM 60
QTAMQNTMLI NGIAQVTFDS ETAVKELSY YSLEDLNNKYL YIAVTVIEST GGFSEEAIEP 120
GIKYVLS 127

SEQ ID NO: 87 moltype = AA length = 108
FEATURE Location/Qualifiers
source 1..108
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 87
PYKLNLVATP LFLKPGIPYP IKVQVKDSLQ QLVGGVPVTL NAQTIDVNQE TSDLDPSKSV 60
TRVDDGVASF VLNLPSTGTV LEFNVKTDAP DLPEENQARE GYRAIAYS 108

SEQ ID NO: 88 moltype = AA length = 108
FEATURE Location/Qualifiers
source 1..108
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 88
SLSQSYLYID WTDNHNKALLV GEHLNIIIVTP KSPYIDKITH YNYLILSKGK IIHFGTREKF 60
SDASYQSINI PVTQNMVPSS RLLVYYIVTG EQTAEVLSDS VVLNIEEK 108

SEQ ID NO: 89 moltype = AA length = 107
FEATURE Location/Qualifiers
source 1..107
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 89
CGNQLQVHLS PDADAYSPGQ TVSLNMTATM DSWVALAAVD SAVYGVQRGA KKPLERVFQF 60
LEKSDLGCGA GGGLNNANVF HLAGLTFLTQ ANADDSQEND EPCKEIL 107

SEQ ID NO: 90 moltype = DNA length = 24
FEATURE Location/Qualifiers
misc_feature 1..24
 note = PCR Primer
source 1..24
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 90
tccccaggtt ttccccagga agat 24

SEQ ID NO: 91 moltype = DNA length = 23
FEATURE Location/Qualifiers
misc_feature 1..23
 note = PCR primer
source 1..23
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 91
aatggatttc tcttcattca tac 23

-continued

SEQ ID NO: 92	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
misc_feature	1..22	
	note = PCR primer	
source	1..22	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 92		
gtcatagggtt attggcattc tt		22
SEQ ID NO: 93	moltype = DNA length = 24	
FEATURE	Location/Qualifiers	
misc_feature	1..24	
	note = PCR primer	
source	1..24	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 93		
ttgccacatt tttctgtctc aatc		24
SEQ ID NO: 94	moltype = DNA length = 26	
FEATURE	Location/Qualifiers	
misc_feature	1..26	
	note = PCR primer	
source	1..26	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 94		
gacatattct ttaacttcaa aatatg		26
SEQ ID NO: 95	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
misc_feature	1..22	
	note = PCR primer	
source	1..22	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 95		
ccctacaaac tgaatttggt tg		22
SEQ ID NO: 96	moltype = DNA length = 23	
FEATURE	Location/Qualifiers	
misc_feature	1..23	
	note = PCR primer	
source	1..23	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 96		
agagaggaca tatttgatgc cag		23
SEQ ID NO: 97	moltype = DNA length = 21	
FEATURE	Location/Qualifiers	
misc_feature	1..21	
	note = PCR primer	
source	1..21	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 97		
tctctcagcc aaagttacct t		21
SEQ ID NO: 98	moltype = DNA length = 23	
FEATURE	Location/Qualifiers	
misc_feature	1..23	
	note = PCR primer	
source	1..23	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 98		
tgagtatgct attgctcggc aac		23
SEQ ID NO: 99	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
misc_feature	1..22	
	note = PCR primer	
source	1..22	
	mol_type = other DNA	

-continued		
organism = synthetic construct		
SEQUENCE: 99		
tgtggcaacc agctccaggt tc		22
SEQ ID NO: 100	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
misc_feature	1..22	
	note = PCR primer	
source	1..22	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 100		
tttttcttca atatttaacc ag		22
SEQ ID NO: 101	moltype = DNA length = 24	
FEATURE	Location/Qualifiers	
misc_feature	1..24	
	note = PCR primer	
source	1..24	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 101		
aggccaagaa gaacgctgca aaag		24
SEQ ID NO: 102	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
misc_feature	1..41	
	note = PCR primer	
source	1..41	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 102		
ttttggcaaa gaattcgcca ccatgggcct tttgggaata c		41
SEQ ID NO: 103	moltype = DNA length = 58	
FEATURE	Location/Qualifiers	
misc_feature	1..58	
	note = PCR primer	
source	1..58	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 103		
cctgaggagt gaattcttaa tggatgatggt gatggtggag aatttcttta caagggttc		58
SEQ ID NO: 104	moltype = AA length = 327	
FEATURE	Location/Qualifiers	
REGION	1..327	
	note = Amino acid sequence of human IgG4 Constant Heavy chain region	
source	1..327	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 104		
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS	60	
GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPSCP APEFLGGPSV	120	
FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY	180	
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK	240	
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDLS DGSFFLYSRL TVDKSRWQEG	300	
NVFSCSVMHE ALHNHYTQKS LSLSPGK	327	

- What is claimed is:
1. An antibody that specifically binds to human C5.

2. The antibody of claim 1, wherein the antibody is a chimeric antibody.

3. The antibody of claim 1, wherein the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:3; VH-CDR2: SEQ ID NO:4; VH-CDR3: SEQ ID NO:5; VL-CDR1: SEQ ID NO:8; VL-CDR2: SEQ ID NO:9; and VL-CDR3: SEQ ID NO:10.

4. The antibody of claim 1, wherein the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:3; VH-CDR2: SEQ ID NO:4; VH-CDR3: SEQ ID NO:5; VL-CDR1: SEQ

ID NO:8; VL-CDR2: SEQ ID NO:9; and VL-CDR3: SEQ ID NO:10, or a variant or variants thereof.

5. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:2, or a variant thereof.

6. The antibody of claim 1, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:7, or a variant thereof.

7. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:2 and a light chain comprising the amino acid sequence of SEQ ID NO:7, or a variant or variants thereof.

8. The antibody of claim 1, wherein the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:13; VH-CDR2: SEQ ID NO:14; VH-CDR3: SEQ ID NO:15; VL-CDR1: SEQ ID NO:18; VL-CDR2: SEQ ID NO:19; and VL-CDR3: SEQ ID NO:20, or a variant or variants thereof.

9. The antibody of claim 1, wherein the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:13; VH-CDR2: SEQ ID NO:14; VH-CDR3: SEQ ID NO:15; VL-CDR1: SEQ ID NO:18; VL-CDR2: SEQ ID NO:19; and VL-CDR3: SEQ ID NO:20, or a variant or variants thereof.

10. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:12, or a variant thereof.

11. The antibody of claim 1, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:17, or a variant thereof.

12. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:12 and a light chain comprising the amino acid sequence of SEQ ID NO:17, or a variant or variants thereof.

13. The antibody of claim 1, wherein the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:23; VH-CDR2: SEQ ID NO:24; VH-CDR3: SEQ ID NO:25; VL-CDR1: SEQ ID NO:28; and VL-CDR2: SEQ ID NO:29, or a variant or variants thereof.

14. The antibody of claim 1, wherein the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:23; VH-CDR2: SEQ ID NO:24; VH-CDR3: SEQ ID NO:25; VL-CDR1: SEQ ID NO:28; and VL-CDR2: SEQ ID NO:29, or a variant or variants thereof.

15. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:22, or a variant thereof.

16. The antibody of claim 1, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:27, or a variant thereof.

17. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:22 and a light chain comprising the amino acid sequence of SEQ ID NO:27, or a variant or variants thereof.

18. The antibody of claim 1, wherein the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:32; VH-CDR2: SEQ ID NO:33; VH-CDR3: SEQ ID NO:34; VL-CDR1: SEQ ID NO:37; VL-CDR2: SEQ ID NO:38; VL-CDR3: SEQ ID NO:39, or a variant or variants thereof.

19. The antibody of claim 1, wherein the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:32; VH-CDR2: SEQ ID NO:33; VH-CDR3: SEQ ID NO:34; VL-CDR1: SEQ ID NO:37; VL-CDR2: SEQ ID NO:38; VL-CDR3: SEQ ID NO:39, or a variant or variants thereof.

20. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31, or a variant thereof.

21. The antibody of claim 1, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:36, or a variant thereof.

22. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36, or a variant or variants thereof.

23. The antibody of claim 1, wherein the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:42; VH-CDR2: SEQ ID NO:43; VH-CDR3: SEQ ID NO:44; VL-CDR1: SEQ ID NO:47; VL-CDR2: SEQ ID NO:48; VL-CDR3: SEQ ID NO:49, or a variant or variants thereof.

24. The antibody of claim 1, wherein the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:42; VH-CDR2: SEQ ID NO:43; VH-CDR3: SEQ ID NO:44; VL-CDR1: SEQ ID NO:47; VL-CDR2: SEQ ID NO:48; VL-CDR3: SEQ ID NO:49, or a variant or variants thereof.

25. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof.

26. The antibody of claim 1, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:46, or a variant thereof.

27. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:41 and a light chain comprising the amino acid sequence of SEQ ID NO:46, or a variant or variants thereof.

28. The antibody of claim 1, wherein the antibody comprises at least one of the CDRs selected from the group consisting of: VH-CDR1: SEQ ID NO:52; VH-CDR2: SEQ ID NO:53; VH-CDR3: SEQ ID NO:54; VL-CDR1: SEQ ID NO:57; VL-CDR2: SEQ ID NO:58; VL-CDR3: SEQ ID NO:59, or a variant or variants thereof.

29. The antibody of claim 1, wherein the antibody comprises the CDRs: VH-CDR1: SEQ ID NO:52; VH-CDR2: SEQ ID NO:53; VH-CDR3: SEQ ID NO:54; VL-CDR1: SEQ ID NO:57; VL-CDR2: SEQ ID NO:58; VL-CDR3: SEQ ID NO:59, or a variant or variants thereof.

30. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:51, or a variant thereof.

31. The antibody of claim 1, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:56, or a variant thereof.

32. The antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:51 and a light chain comprising the amino acid sequence of SEQ ID NO:56, or a variant or variants thereof.

33. A method of treating a complement pathway-mediated disease or disorder in an individual, comprising the step of administering to said individual the anti-C5 antibody of any one of claims 1-32.

34. The method of claim 33, wherein the disease or disorder is at least selected from the group consisting of: macular degeneration (MD), age-related macular degeneration (AMD), ischemia reperfusion injury, arthritis, rheumatoid arthritis, lupus, ulcerative colitis, stroke, post-surgery systemic inflammatory syndrome, asthma, allergic asthma, chronic obstructive pulmonary disease (COPD), paroxysmal nocturnal hemoglobinuria (PNH) syndrome, myasthenia gravis, neuromyelitis optica, (NMO), multiple sclerosis, delayed graft function, antibody-mediated rejection, atypical hemolytic uremic (aHUS) syndrome, central retinal vein occlusion (CRVO), central retinal artery occlusion (CRAO), epidermolysis bullosa, sepsis, organ transplantation, inflammation (including, but not limited to, inflammation associated with cardiopulmonary bypass surgery and kidney dialysis), C3 glomerulopathy, membranous nephropathy, IgA nephropathy, glomerulonephritis (including, but not limited to, anti-neutrophil cytoplasmic antibody (ANCA)-mediated

glomerulonephritis, lupus nephritis, and combinations thereof), ANCA-mediated vasculitis, Shiga toxin induced HUS, and antiphospholipid antibody-induced pregnancy loss, or any combinations thereof.

35. A method of reducing the activity of a complement system of an individual, wherein the method comprises administering an antibody to the individual via a route of administration selected from the group consisting of enteral administration, parenteral administration, and a combination thereof, and wherein the antibody is the antibody of any one of claims **1-32**.

36. The method of claim **35**, wherein the antibody is an antibody fragment selected from the group consisting of a Fab, Fab', F(ab)2, F(ab')2, scFv, and combinations thereof.

37. A cell comprising the antibody of at least one of claims **1-32**.

38. The cell of claim **37**, wherein the cell produces the antibody of at least one of claims **1-32**.

39. The cell of claim **37**, wherein the cell is a hybridoma.

* * * * *