MHC-PEPTIDE COMPLEX BINDING LIGANDS

Inventors: Henricus Renerus Jacobus Mattheus HOOGENBOOM, Maastricht (NL); Yoram Reiter, Haifa (IL)

Correspondence Address:
MARTIN D. MOYNIHAN d/b/a PRTSI, INC.
P.O. BOX 16446
ARLINGTON, VA 22215 (US)

Assignee: Technion Research & Development Foundation Ltd., Haifa (IL)

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ABSTRACT
Disclosed are protein ligands comprising an immunoglobulin heavy chain variable (VH) domain and an immunoglobulin light chain variable (VL) domain, wherein the proteins bind a complex comprising an MHC and a peptide, do not substantially bind the MHC in the absence of the bound peptide, and do not substantially bind the peptide in the absence of the MHC. The peptide is a peptide fragment of gp100, MUC1, TAX, or hTERT. Also disclosed are methods of using and identifying such ligands.
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone lall

1  GACATCCAGTTGACCCAGTCTCCATCTCCCTGCTCTGCTCTGTGTTAGGAGACAGGACATCACC
1  D I Q L T Q S P S S L S A S V G D R V T
21  ATCAGTGGCCGCGCAGTCAAGACCATTTAGGTGTTATCAACACAAGACA
21  I T C R A S Q S I S Y L N W Y Q H R P
41  GTCTCAAGC (SEQ ID NO: 9)
41  GTCTCAAGC
61  W S S (SEQ ID NO: 10) . FG. 1B
61  W S S
81  GGGAAAGCCCTAAGCTCTGGATCTCATTCCACATGGAGCTGCAGTCAGCTCCACCT
81  G K A P K L L I Y S A S S L Q S G V P S
101  FIG. 1A
101

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone lall

1  CAGGTACAGCTGGACAGATGCTCCAGGAGCTGGAGGGCCCTGCAGGCCAGCCCCTCTACCTC
1  Q V Q L Q Q S G P G L V K P S Q T L S L
21  CAGGTACAGCTGGACAGATGCTCCAGGAGCTGGAGGGCCCTGCAGGCCAGCCCCTCTACCTC
21  Q V Q L Q Q S G P G L V K P S Q T L S L
21
41  ACCTGGCCGTCATCTCAGGAGACATCTCTAGATCAAGCTGGGTGGCTGGAGCTGCAGTCAGCTCCACCT
41  T C A I S G D S I S S N S V W N W I R
61  CAGTCCCATGGAGAGGCTGTCGTCTGGAGACATCTACTATAGTCTGAGATGGATAT
61  Q S P S R G E W L G R Y T Y R S K W Y
81  N D Y A S V K S R I T I N P D T S K N
201  FIG. 1B
201
301  CAGGTACAGCTGGACAGATGCTCCAGGAGCTGGAGGGCCCTGCAGGCCAGCCCCTCTACCTC
301  Q V Q L Q Q S G P G L V K P S Q T L S L
361  GICCTCAAGC (SEQ ID NO: 9)
361  GICCTCAAGC
361
361  V S S (SEQ ID NO: 10)
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 1A7

1
CAGTCTGTCGTCGACGACGCGCCTCTGCAGCTGCTGGGCCCCAGGGCAGAGGGTCACCACTC
Q S V V T Q P P S V S G A P G Q R V T I

CDR1
61
TCCTGCACCTGGGCCCGCCTCCACATCCACAGGCTGTTGATGATGACTGACGTACCAGCAG
S C T G S S S N I G A G Y D V H W Y Q Q

CDR2
121
CTCCAGAGACGACCCCAACACCTCCACATCTATGTAACGACAGAACAGGCGCCCTCAAGGTCG
L F G P K L L I Y G N S R P S G V

CDR3
181
CCCTGGACGGATTTCTTGCCACACCTCCACAGGCGGCTCCCTTCGACATCCTGGGGCTC
P D R F S G S K T S A S L A I T G L

241
CAGGCTAGGAGGGTGGCTATATTACTGCCAGGTCTCCCTATGACAGAGCAGCTGTGACGTCCCTA
Q A E D E A D Y C Q S Y D S S L S A L

301
TTCCGCGGACGGACCAAGTCGAGCGTCCTTA (SEQ ID NO:11)
101
FIG. 2A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 1A7

1
CAGGTACATCGCCGATGCCGCGACCGCCGAGCTGGTGAGCCCTCTGGCGAGACCCGCTCTG
Q V Q L Q Q S G P F G L V K P S E T L S L

CDR1
61
ACTTGACGTCTGCTGGCCCGCCTCCACATCCACAGGCTGTTGATGATGACTGACGTACCAGCAG
T C T V S G G S S I R N Y W S W I R Q F

CDR2
121
CCAGGGAAGGGACGACGCGGCTGGTATATATCAGTGGGGGACGGGACGGCCCAATACACAC
P G K G L E W I G Y M Y Y S G G A N Y N

181
CCCTCCCTCAAGACCTGAGTCCACCAATATCCACTAGCAGGCTGAGGCTCCAAGACGACATCCCGTCTCG
P S L N S R V T I S L D T S K N Q F S L

241
AAACCTGACCTCTGTGACCCTGCGGACGCGCCGCTGTTATTATTCTGGGAGAAATTCCCAAC
K L T S V T A A D T A V Y Y C A R I P N

CDR3
301
TACTATAGGATAGGTTATATTCTCCGCTTACCGTACCTGGTAGCAGCGGGGGGCGTGGG
Y Y D R S G Y Y P G Y W Y F D L W G R G

121
TLTVTVSS (SEQ ID NO:14) FIG. 2B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone IA9

1         GATGTTGTGATGACGTCTCCCTCCCTCTCCCCGTGGCCTCAGGAGGAGGCCCTCTCC
1         D V V M T Q S P L S P V T P G E P A S

CDR1
61        ATCTCTCTGCCAGGCTCTAGCTCCAGGCTCTCCCCGATTAAGGATCAGTAAGGTGATTTG
21        I S C R S S Q S L L H S N G Y K Y V N W

CDR2
121       TACCTGCAAGGCCGGGGGCGATGCTCCACAGTTCTGGATAGATTCTGTTATCGGGGCC
41        Y L Q K P G Q S P Q L L I Y F G S Y R A

CDR3
181       TCCGGGCTCCCTGACAGCAGTCAGTGGCAGTGGGATACGCGACAGTAAAATTCACAGAATAC
61        S G V P D R F S G S G S G T D F T L K I

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone IA9

1         CAGGTGCACGTGGTGCAGGCTCGGGGCGGCTGGTCCAGGCTCCCTCTGAGACTC
1         Q V Q L V Q S G G V V Q P G R S L R L

CDR1
61        TGGCTGGAGGTCCATCAGGCTCTGGAGGTATGCTGGTGGGATAGATTCTGTTATCGGGGCC
21        S C A A S G F T F S S Y G M H W V R Q A

CDR2
121       CAGGCAAGGGCAGCGTGGGTGGGAGGTATGCTGGTGGGATAGATTCTGTTATCGGGGCC
41        F G K G L E W V A V I S Y D G S N K Y Y

CDR3
181       CAGACTCCCTCTGAGGCGCCATAGCCACAGCAGGCTAGAGTGCTGGTGGGATAGATTCTGTTATCGGGGCC
61        A D S V K G R F T I S R D N S K N T L Y

241       CTGCAATGAAGACAGCCTGGAGAGCGGACCGCAGGCTGTGAATTCTGTTGAGAGATTAC
81        L Q M N S L R A E D T A V Y Y C A R D Y

(SEQ ID NO: 15) (SEQ ID NO: 16) FIG. 3A

(SEQ ID NO: 17) (SEQ ID NO: 18) FIG. 3B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone IC8

1  GACATCCAGTGGACCCAGACTTCTCCTGCATCTGCTTGCTCTTGAGGAGACAGAGTCACC
1  DIQLTQSPSSLASAVGDRT

CDR1
61  ATCAGTGGACGGCAGTCCAGAGCAGCTTATCCATTAAGGGATCTACACAGCACT
21  ITCRASLSILNYQHP

CDR2
121  GGGAAAGCCCCCTAGCTCCTGAGATCTGCTCTCCATCTCCAGAGCTCTGCCCACTCC
41  GKPQKLSASSLQGSP

181  AGGGTCACTGCGACGCTCTGGAGCCAGCTTACCTCCACTGCAGCTCTCACGGTCTCC
61  RFSGSGDFTLTITLQLP

CDR3
241  GAGGATTTCGCAACCTACTGCTCAAGGCTAGTCAATCCGCTCTCTCAGCGCCGGA
81  EDFAFYQQSDITFLTG

301  GGGACAAAGGTGAGTCACCAAGCA (SEQ ID NO:19)
101  GTKVEINR (SEQ ID NO:20) FIG. 4A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone IC8

1  CAGGTTCAGCCAGCTGCAAGGAGATGGGAGACGCCCTCTGGAGAGACAGCCCTC
1  QVQLQPGLVPSQTL

CDR1
61  ACCTGGCCATCTCCAGGGACAGTATCTGAGTACCCAGTATGTTTGGAAACTGGCTCAGG
21  TCAISSNSVWNVWIR

CDR2
121  CAGTCCCCATCGAGAGGGCTGGGTGGGAGGCAACTACATGACTGAGCTCAACG
41  QSSFRELWRGRTYRKS

181  AATTATATGCCAGTACTCGAAAGAGCTGAGTACACATCAACACCAGACAGTCACAGAC
61  NDYAVSVEKSKRINTIPDTSKN

241  CAGTTCTCCCTGCAACTGTCCTGCTCCTGACCCAGGACACGGCTCTCTCTATGCTTCC
81  QFSLQLNSVTDPDDTALYYCA

CDR3
301  AGAGGATCATTTTGGGACAGGCGGAAAATTCTGACGACTGGGGCCAGGACACCTCTGGCTCAGG
101  RASFGETSDKDDWQGTLVT

361  GTCTCAAGC (SEQ ID NO:21)
121  VSS (SEQ ID NO:22) FIG. 4B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 1D7

1  GAAACGACACCTCAGCAGCAGTCTCCAGGCAACCCGTTTGGCTCCAGGACAGAGG GCC
1  E T T L T Q S F G T L S L S P G E R A T

CDR1
61  CTCTCCGAGGCCAGGTCTCTGATATTTAGCCCAAGTTAGCTCTGCTGGTACCCAGCAGAA
21  L S C R A S R Y I N A N F L A W Y Q Q K

CDR2
121  CCTGAGCAGGCTCCAGGCTCTCAGGCTCTGATATTTAGCCCAAGTTAGCTCTGCTGGTACCCAGCAGAA
41  P G Q A P R L L I Y D A S T R A T G I P

CDR3
181  GACAGGGTCAGGCTGCAAGGTCAGGCTGCAAGGTCAGGCTGCAAGGTCAGGCTGCAAGGTC
61  D R F S G S G S G T D F T L T I S R L E

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 1D7

1  CAGGGCCAGGCTCCAGGCTCTCAGGCTCTGATATTTAGCCCAAGTTAGCTCTGCTGGTACCCAGCAGAA
1  Q V Q L V Q S G A E V K K P G S S V K V

CDR1
61  TCCTGCAAGGGCTCTGAGAGGCTCAGAGGCTCTGATATTTAGCCCAAGTTAGCTCTGCTGGTACCCAGCAGAA
21  S C K A S G G T F S S Y A I S W V R Q A

CDR2
121  CCTGAGCAGGCTCCAGGCTCTCAGGCTCTGATATTTAGCCCAAGTTAGCTCTGCTGGTACCCAGCAGAA
41  P G Q A P R L L I Y D A S T R A T G I P

CDR3
181  GCACAGGAAGTCCAGGAGAGTCCAGGAGAGTCCAGGAGAGTCCAGGAGAGTCCAGGAGAGTCC
61  A Q K F Q G R V T I T A D E S T T A Y

FIG. 5A

FIG. 5B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 1G2

1 21 31 41 51 61
GAAAATGCTGCTGACTAGTCATGTCAGCAACCCCTGTTCAGGGGAGAGACAC
EIVLTSQP DTLSLSPGERAT

CDR1

61 21 LSCRAQS QSVSHS YLAWYQK

121 41 CTCYCTGACGGAGGCTCCAGATGGTTACGACGTACCTAAGCTGTTACGCACAGAGAA
PGQAPRL LIXDSSRATDIP

CDR2

181 61 GACAGTCTCAGTGCCAGTGCTTGAGAGAAGAGAGCACTGCTAAGGAGAG
DFSSGSGSTDFTLTISRLE

241 81 CTTGACGATCTGACATGGTTACCTCAGACGTGTTAGCTACCCCTCTCTGCTTGGC
FEDSAYYCCQYYVSSFFPG

301 101 CAGGGGACAGCTGAGACATCGACAGA (SEQ ID NO:27) QCJKLEIKR (SEQ ID NO:28)

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 1G2

1 1 21 41 61
CAGGTCCAGCTCAGTCATGTCAGCAACCCCTGTTCAGGGGAGAGACAC
QVQLVPSGGGLVQPGDRSRSLRL

CDR1

61 21 SCCAAASQFTGSTYGLHWSVRQ

121 41 CCCGCTAGGGCTGGATGGCTGATTATTATCTATGAGTGTAATATATACCT
FKGLMWVAPISYDGNSKYY

181 61 CACAGACTCCTGTACAGAGCCCATCAGCAGAAGAAATCCAGACAGCTGTA
ADSVKGRFTISRDSNKNTLY

241 81 CTTGAAATGACGGCCTAGGAGACAGACAGTCGTTATTACGCTGAGAGACTGTG
LQMNGLRAEDTAIYYCAKTTV

CDR3

301 101 GTGGATACATTTCTCGGATCTTTTGGATATATGAGTGTAATATATACCT
GVTFSWSDPDIWGQGTVTMVTV

361 121 TCAAGC (SEQ ID NO:29) SS (SEQ ID NO:30)
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 2B2

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>GATGTGTGATAGCAGCTCTCCAGCGCCACCTGCTCTGCTCTCCGGGG2RMCGCCGAC</td>
<td>DVMQSTPGLSVPAGDSAT</td>
</tr>
<tr>
<td>CTCTCTCTGGGCACTGAGCTCTCAGCTGAGCTCTACGGCTCTGGTAACGACAGAAG</td>
<td>LSCWASQSLDSYVWSYQK</td>
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<td>CCGGGCCAGCCCGGCTCTCGCTAAATAGACTGGCGGGCGGCTGTCAGGCCCTGGCCATCCG</td>
<td>PCQAPRRILHSAASIKAFGIP</td>
</tr>
<tr>
<td>GACAGGCTTCAGTGCAGTGCAGTGGTGGCAGACACATTCACTTGGACCATCAGGCTGGAG</td>
<td>DRSFSGVSCTEFTLTLISGEL</td>
</tr>
<tr>
<td>CCGGAGTTGCGGGATGCGTGAGATCTCTTGACCAGATCGTGTGCGTGGGACHTTGG</td>
<td>PEDFAVYSCHOQYGFPLWTFG</td>
</tr>
<tr>
<td>CAAAGGACCAAGGGTGAGATCAGACAGA (SEQ ID NO:31)</td>
<td>FIG. 7A</td>
</tr>
<tr>
<td>QGTⅤYEBRR (SEQ ID NO:32)</td>
<td></td>
</tr>
</tbody>
</table>

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 2B2

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<thead>
<tr>
<th>Nucleotide</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAGGTCCGGGCTCTGATAGTACACAGCTCGGGCGCTGAGAGGAAGCTCGGGGCTCCAGGGAAGTCC</td>
<td>QVQLVQSGGERVKKPGASVKKV</td>
</tr>
<tr>
<td>TCTGCAAGGGCTCTGCTGGTACACTTACAGTATGGTTATATAGCTGGGAGGCGTACGACGCCC</td>
<td>SCKASGFYTVGSWQRQ</td>
</tr>
<tr>
<td>CCGAACAGGCGCTAGAGCTGGGAGGCTGACTTCTCCATGGTCTACACACTAGATAT</td>
<td>PGQGLEWWMGWISSSNSGYTKY</td>
</tr>
<tr>
<td>GCACGAGATCTCCAGGGCAGCAGCTACCCGACCCAGACACGACATCCACCGGGGCGCACCCATAC</td>
<td>AQNLQQRLLTLTDTSTGTAY</td>
</tr>
<tr>
<td>AGGGACTGAGGCGGAGGGCTAGCTGAGGCGGCTGAGGACGGCGCCCTTTATTACTGCGAGGATGAT</td>
<td>MEILRSLRSEDTALYYCARYD</td>
</tr>
<tr>
<td>ATTAGGCTGATGTTTGGATATTGCGGCCAGGCGCAAAGAGCAGAAGCTGCGCTTTCCCAAGGC</td>
<td>FIG. 7B</td>
</tr>
<tr>
<td>SLGDCFDIWGQGTMVTVSS (SEQ ID NO:33)</td>
<td></td>
</tr>
<tr>
<td>(SEQ ID NO:34)</td>
<td></td>
</tr>
</tbody>
</table>
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 2C5

1
GAAACGACACTCACGCACTGCTGGATTACAGGCTCTGGTTCTCTCACGCTGGGAGTGGGGACCCACC
ETTLTQSGPGTLSSLSPGERAT

CDR1
CTCTCCTGCCAGGCCCCAGTCTGAGGTGGAGCCACACTTACTTACCTGCTGCTGCTCAGGCCAGGAA
LSCRASQSVSVSNYNALAWYQQK

CDR2
CCCTGGCCAGGCTCCGAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
PGQLPRLIYAASSRATGIP

CDR3
GAGATGGTCAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGT
DSRSASGFDSTLTLTISRL

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 2C5

1
CAGGCTGACGCTGACGCTGACGCTGACGCTGACGCTGACGCTGACGCTGACGCTGACGCTGACGCTG
QVQLOQESGGGLVKGPGGSLRL

CDR1
TCTGGGGGAGCTCTGGGAGCTCTGGGAGCTCTGGGAGCTCTGGGAGCTCTGGGAGCTCTGGGAGCT
SCASSGFTPSYSTMNMWVRQA

CDR2
CCACAGGACACTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT
PGKGLEWVYISSSGStYY

CDR3
GAGACCTCGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGG
ADSVRGRFTISRDNKNTLY

Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 2C5

1
AGC (SEQ ID NO:37)

S (SEQ ID NO:38)
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 2D1

1  GACATCCAGTGACCCAGCTCCATTCTCCCTGGTCTGCTTCAGGAGACAGATCTAC
1  D I Q L T Q S P S S L S A S V G D R V I

CDR1
61  ATCACTTGGCCGGGCAACTCAAGACCACTCACTCTAAATGTGGATACGCAGAGACCA
21  I T C R A T Q S I S T H L N W Y Q Q K P

CDR2
121  GGGAAAGCGCCCTAAGCTCTCTCTTCTCTCTCTGTCATCCAGTACCAAAAGTGGGCTCCACATCT
41  G K A P K L I Y S S A S S L Q S G V P S

CDR3
181  AGTTTCAGTGCCAGTGCTGGACAGATTCACACTCCTCACTAACACAGTCCATTCCACCTCGACAC
61  R F S G S G S G T G D F T L T I S S L Q P

241  GGACAGATATTCTAAAGCTGTTAGCAAGAAGCTGACGATCTCCTCACCGGACGATCTGGGC
81  E D F A T Y Y C Q Q S Y S S P P I T P G

301  CAAGGGGACACGACTGGAATTAAACGA (SEQ ID NO: 39)
101  Q G T R L E I K R (SEQ ID NO: 40) FIG. 9A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 2D1

1  CAGGTGGCAGCTGGAGAGGAGTTCCCGGCGCCGGACTGTTGAGGACACCTTGTCGCCCTC
1  Q V Q L Q E S G F G L V K P S E T L S L

CDR1
61  AGCTGCACGTCTGCTGTTGCTGCTACAGTACGAACTGCTGGCTGCTGCTGCC
21  T C T V S G G S I S S N M Y Y W G W R

CDR2
121  CAGCCCCCAGGGAGGGCGCTGAGCTCTGGAGATTCTGGAGTTGAGAAGCTACCCCTAC
41  Q P P G K G L E W I G S I D Y S G S T Y

181  TACAAATCCGCTCCCATGGAGATGCAGATCTCCATGACAGACGCCTCAAGAGACAGATTC
61  Y N P S L R S R V T M S V D T S K K Q F

241  TCCTGGAGAGTTGACCCGGAGCTCGGGACAGCCGCTGCTGGATTACTGCTGGAGAGAA
81  S L K H T S V T A A D T A V Y Y C A R E

CDR3
301  TCCGGAACATCTACTTTGGACTGGCCAGGCCACCCCTGTGCACCGTCTCAAGC
101  S G S P Y Y F D Y W G Q G T L V T V S S (SEQ ID NO: 41)
 (SEQ ID NO: 42) FIG. 9B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 2F1

1  CAGTCTGTTTGGACGACGGCCCTCTCGGTGTCGGCCAGGAAGACAGTACACCTCT
1  QSVLTQPSPSVSAAPGQTVTI

CDR1

61  TCTGCTGTTGAAGCAAGTCCTCCACCATGIGGAAGAATTTCTCTGTCGGTCCAAACAGT
61  SCSSSSSAINRHYVSWFQV

CDR2

121  CCAGGCCAGGCAACCCACACTCTCAATTTAGCACAATATATGACGAGCAGGATACCT
121  PGRAPKLLIYDNNQRPSSGF

181  GGCACGATTCTGACCTCCAACTGACACCGCCACCTTCAGCATGTCCAGATACCTCCAAG
181  GRFSASKSDTSAKLTDITGLQ

CDR3

241  AGTGCGGCGCGTGTTGACACCTTCCCTTCGACCTTTATGTC
241  SGEAVYYCGTWDSTLDLTV

301  TCGGCGCGGACCATGCCCCTCCTCT (SEQ ID NO:43)
301  FGGGTHVPVL (SEQ ID NO:44) FIG. 10A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 2F1

1  GAGGTCACCTGCTGCTGGGCTGAGTGAAGACGAGCTCTGGGCTTCTCGGCTCAAGGCTTT
1  EYQLVQGSGAVKPKPGASVXKV

CDR1

61  TCTGCAAGGCACTGACTGCACCTCACCAGCTACTPMTTCACACTGTGGAGCGAGCC
61  SCKASGYTFYTSYIHWVQRAG

CDR2

121  CCCTGGACAAAGTCTGACTGTGATTGACCACTACCACTCCCGGASTGCTGAGACACCT
121  PGGQGLEWMGAINPSGSGSTPY

181  GACACAAGTTTGGCCAGAAGTCCACCATGACAGGCGAGACATCGCAGACCACTACCTAC
181  AQKFQGRVTMRDTSTSTVT

241  AMGAGGCGGACCTGACTGCACCTGACTGACTGAGCGCCTGGTATTACTTCGGAGAGATGG
241  MELSSLRSEDFTAVYYCARDG

CDR3

301  ACCTCGTGGGGAGCTTATCTCTAATCTACTACGTAGTATGGACATCCTGGGCGCA
301  TYGSGSYPYYYGGMDVWGWQ FIG. 10B

361  GGGACCACGCTGCCCTCCCTCAACGC (SEQ ID NO:45)
121  GTTVVTVSS (SEQ ID NO:46)
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone GID12

1
mFmlTqPShvSBSPGKT

61
TCCCTCACCCGCAACGGGTCCAGCATCTGCACAGACAGCTATTTAGTGAACCAGCT

21
SCTGGSGSDDNNYVHWWYQR

121
CCTGGAAGAGCTGGACTGACTGACTCTGGTGGTGGGGCTCTT

41
PGSAPTVMFEDNQPFSGPV

181
QATCCGGTCTCTCCTCCTGAGAGCTCCTCCACTCCCTCCCTCTGCTCCATCTCTG

61
DFSGSIDSNSHSSASLVSQ

241
CTAGAACTGAAAGGACGGGGACAGACTACTACTGCTACCTCCTGTGGAAGCTAAGTGTC

81
LKTEDGYYCQSSDSGKS

301
TTCCGCCGAGGGACACGGTACCTGAGCTTCGTCAG (SEQ ID NO:47)

101
FGGGTKLTVDGLQ (SEQ ID NO:49) FIG. 11A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone GID12

1
GAGCTCACCAGCTGGTCCAGTCTGGCCAGAGGTGCTGCCCTGGAGCTCCCTCCCTGAGCT

1
EVQLVQSGGGYQQPGRLTL

61
TCCCTCACCCGCAACGGGTCCAGCATCTGCACAGACAGCTATTTAGTGAACCAGCT

21
SCTGGSGSDDNNYVHWWYQR

121
CCTGGAAGAGCTGGACTGACTGACTCTGGTGGTGGGGCTCTT

41
PGSAPTVMFEDNQPFSGPV

181
QATCCGGTCTCTCCTCCTGAGAGCTCCTCCACTCCCTCCCTCTGCTCCATCTCTG

61
DFSGSIDSNSHSSASLVSQ

241
CTAGAACTGAAAGGACGGGGACAGACTACTACTGCTACCTCCTGTGGAAGCTAAGTGTC

81
LKTEDGYYCQSSDSGKS

301
TTCCGCCGAGGGACACGGTACCTGAGCTTCGTCAG (SEQ ID NO:49)

101
FGGGTKLTVDGLQ (SEQ ID NO:49) FIG. 11B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone G3F12

1
1

GAAACGCACCTCAAGCACTGTCCACAACCCTGTGGCTTCTTCTCCAGGGAAAGGACCCGCC
ETTTLQSPGTLSSLPSGERAT

CDR1

61
61

CTCTCTGCAAGGCCGCATTGAGTTACAGAACTCTACTTTGCTGGTACCACAGGACAA
LSCRAASQSVSSSYLAWYQQK

CDR2

121
121

CTTGCCAGATCTGCCACAGTGTACATCTACCTCACTGACAGCACAGATGGAG
PGQAPRLILXGASSRATGI

181
181

GAAGGGTGATGCAAGTGGGCTGCAGATGGTACAGAGATCTATCAGACAGACAGTGGAG
DKFSGSCTSGSGTDPTLTLTSRL

CDR3

241
241

CTGGAAATTTGCCAGAGTGTACATCTACCTCACTGACAGACAGATGGAG
PEFDANYCQQHDSSPPRTFG

301
301

CAAGGAGACCAGTGAGATCAACAGRA (SEQ ID NO:51)
QGTKVEIKR (SEQ ID NO:52)

361
361

FIG. 12A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone G3F12

1
1

CACTGGCAAGCGGTACCTGAGTTACAGAACTCTACTTTGCTGGTACCACAGGACAA
QVQLVQSGGGCVCVQPGSRSLRL

CDR1

61
61

CTCTCTGCAAGGCCGCATTGAGTTACAGAACTCTACTTTGCTGGTACCACAGGACAA
SACASGFTFSYGMHWWVRQA

CDR2

121
121

CCAGGCAAGGGGTGAGTTACAGAACTCTACTTTGCTGGTACCACAGGACAA
PGREGLWVAFYSYDGSKDKNF

181
181

CAGATGGAAATTTGCCAGAGTGTACATCTACCTCACTGACAGACAGATGGAG
ADSVKGRFTISRDNSKNTLY

CDR3

241
241

CTGGAAATTTGCCAGAGTGTACATCTACCTCACTGACAGACAGATGGAG
LQNNSLRAEDTAVYVCAKDS

301
301

FIG. 12B

361
361

ACG (SEQ ID NO:53)
S (SEQ ID NO:54)
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone G3F3

![Sequence](image)

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone G3F3

![Sequence](image)
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone G3G4

1  GAAGACGACACTACGCAGTTCTCAGGCACCCCTTGTCCTTCTCCAGGGAGAAAGGCCA
1  ETTLTQSPGTLSLSPLGERAT

CDR1

61  CTCTCCCTGACGCGCCAGTACAGTGTTACGCCAGCTCTACCTGCTGGTACCCAGCAGAAA
21  LSCRASQSVSSSYLAWYQQK

CDR2

121  CCGCCAGGGCTCCAGGGCTCCTCATCTTATGTGCTAGCATCCCAGGGCCACCTGGCCATCCC
41  FQQAPRLLILYGASSRATGIP

CDR3

181  GAACAGTCCAGTGACGGGCTGGGCAGACTCCACTCCACCATCCACATCCAGCAGTGAG
61  DRFSFTDLTLTISRLE

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone G3G4

1  CAGGTCCAGCTGGCAGTCTCAGGGGQGAGGCTGGTCCAGCTGAGCTGGCCATGCAGACT
1  QVQLIVSGLVPSQPVPSRGSLRL

CDR1

61  TCTCTGCAGGGCTCCAGGGCTCCTCATCTTATGTGCTAGCATCCCAGGGCCACCTGGCCATCCC
21  SCAASGPTFFTSSYGMHVRQA

CDR2

121  CCAAGCCAGGGCCAGGGCCATGCCCATCTGGGATCTCGGATCTCGGAGAAAGCTGAAAGTGAT
41  PGKGLEWVAFISYDGDSDKNF

CDR3

181  GCAAGCTCTGCTGACAGTCTCAGGGGQGAGGCTGGTCCAGCTGAGCTGGCCATGCAGACT
61  ADKVGRFTISRDNSKNTLY

241  CTGCAAAATGACAGCCAGCTGAGGCTGGACAGGCGGCTGTTACTCGGAGAAAGACTGCTGAT
81  LQMNSLRAEDTVYVCKDS

CDR3

301  TACTGATAATAGTTCTTCAGGCCAGACTGGGCGACCCCTGGTACCCGCTCAA
101  YYDNSAFQADWQGQGTLVTVS

FIG. 14A

FIG. 14B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone M11A1

1
GAACAGCACTOCGAGAATTTTGCTCTCCAGTCCTGGAGGAAAGAGACCC
1
ETTLTQ5PSGTLGSLPSGFRAT

CDR1
51
CTCTCTCTCCAGGCTGTACAGAAGGAAGAACTGAGCTTTAGACACGAGGAT
21
LSCRAQSBSVSLSLYWQCK

CDR2
121
CGCGCTCTTCCAGGCTGTACAGAAGGAAGAACTGAGCTTTAGACACGAGGAT
41
FGQAPPLILLYGASSRTAGIP

CDR3
181
GACCAGCTCTACGAGGCTGTACAGAAGGAAGAACTGAGCTTTAGACACGAGGAT
61
DRFSGSAGSGTDFTLTLTISREL

FIG. 15A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone M11A1

1
GAACAGCACTOCGAGAATTTTGCTCTCCAGTCCTGGAGGAAAGAGACCC
1
EVLQVLQ5PSGTVKKGPGSSSVKV

CDR1
61
CTCTCTCTCCAGGCTGTACAGAAGGAAGAACTGAGCTTTAGACACGAGGAT
21
SCKASGAGCTAISVWQKA

CDR2
121
CGCGCTCTTCCAGGCTGTACAGAAGGAAGAACTGAGCTTTAGACACGAGGAT
41
FGQGQLEWMGGGIGIFGTANY

181
GCACAGAGCTCTACGAGGCTGTACAGAAGGAAGAACTGAGCTTTAGACACGAGGAT
61
AQKFQTGCVRTITADESTSTAY

CDR3
241
AATCAAGCTGAGAATTTTGCTCTCCAGTCCTGGAGGAAAGAGACCC
81
WELSSLSREDTAAYCAREF

301
GATCTCAAGC (SEQ ID NO:65)
101
EYCINGVCSLDVWGGQGTTVT

FIG. 15B

361
VSS (SEQ ID NO:66)
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone M388

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAAATTGGAGATGACAGCACAGTCTCCAGGACCCACCCTGGCTTTTGTCTTCAGGGGAAAGGAGGCCCC</td>
<td>EIVMTQSPATLSLSPGERAT</td>
</tr>
<tr>
<td>CTCTCCTGCAAGCGTACTGGAAGTTAGCAGCCTACTTACCTGGTACCAACGAAAGCCTT</td>
<td></td>
</tr>
<tr>
<td>LSCRASQSVSSLAWYQQQP</td>
<td></td>
</tr>
<tr>
<td>GCCGACGCTCTCAGGCTACCTCTCATCTTATGTGATCCACAGGCGGCACTGGCAATCCCAGCCG</td>
<td></td>
</tr>
<tr>
<td>GQAPRLILYDASNRATGIFA</td>
<td></td>
</tr>
<tr>
<td>AGGTTCAGTGCGAGGTCCTCAGTGAACCTGAGAAGCTGACCCATCTACCCCTGAAAGGCGCTTACCT</td>
<td></td>
</tr>
<tr>
<td>RFSCTCGSTDFTLTLTESELPE</td>
<td></td>
</tr>
<tr>
<td>GAAGATTTCGATGGATGGTATCTGCTACCAATGTCTGCTACACCAAACTGGTGCTGGGCCAAG</td>
<td></td>
</tr>
<tr>
<td>EDPAVYCHQYGSSSFQTFGGQ</td>
<td></td>
</tr>
<tr>
<td>GGGACCAAGTGAGGAAAATCAAACGA (SEQ ID NO:67)</td>
<td></td>
</tr>
<tr>
<td>GTKVEIKR (SEQ ID NO:68)</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 16A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone M388

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAACTCCGAGCTGTTGCTACGCTGGGTGTAGTGAGAAGCGCTCTGTCCTGGTGAACGGTC</td>
<td>EQLVQSGAEVKKPFGSSYVKV</td>
</tr>
<tr>
<td>CTCTGCAAGGCCTTCTGGAAGCGCAGCTCCAGCACTTTGCACTCTGGTGAGCCACGGCC</td>
<td></td>
</tr>
<tr>
<td>SCKASGGTFFSSYAIASWVRQA</td>
<td></td>
</tr>
<tr>
<td>CCTGGACAAAGGGCTCTGGAGAGCTCAGCTCACTCTCTATCTTTGCTACAGAAGACTAC</td>
<td></td>
</tr>
<tr>
<td>PGGGLEWNGIGIPFGTANY</td>
<td></td>
</tr>
<tr>
<td>GCAAGAAGTTCCAGGCGAGAGGCTACTGAGATTACGGCGAGCAATCCACAGGCACAGCCCTAC</td>
<td></td>
</tr>
<tr>
<td>AQFQGRVTITADESTSTAY</td>
<td></td>
</tr>
<tr>
<td>ATGGAAGCTGGAGGGTCGTTGAGAACACGCGGCTGTATTCTGACCTGGTGCCACTAC</td>
<td></td>
</tr>
<tr>
<td>MELSSLRSBETAVYCACVHT</td>
<td></td>
</tr>
<tr>
<td>GTGACGTACGTTTCTCTCTTGTAGTGGACCTGCTGGGCGCGCGGCTGGATATTCTGCGGCGTCCACTAC</td>
<td></td>
</tr>
<tr>
<td>GDVDYFSMSMDVWGGSTTVTVS</td>
<td></td>
</tr>
<tr>
<td>AGC (SEQ ID NO:69)</td>
<td></td>
</tr>
<tr>
<td>S (SEQ ID NO:70)</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 16B
**Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone T3E3**

1. GAAATTGCTGACTCTAGCTCCAGGCACTGCTCTCTCCAGGGGGAAAGGACACCC
2. EIVLTQSPATLSLSFGERAT

**CDR1**

1. CTCTCCGCCAGGGCCAGTCAGAGGTGGAGCGCTACTCTTGCTCCACACGAGACCT
2. LSCRASQSGSYGQAYVQKQP

**CDR2**

1. GCCTAGCGCTCCAGAAGCTCTCTACCTCTAGTAGCCATCCACAGGGCAGACTGCTCCACAGCC
2. G*APRLILYDASHRATGIPA

**CDR3**

1. AGGCATTTTACTTAGTGCAAGCTCGATGCACTGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCT
2. KPSGSSTDFTLTISSLPS

**Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone T3E3**

1. GAGGCCTCGTGGCTGACTCTGTGCGAGGCGAGCTGCTCTCTCCAGGGGGAAAGGACACCC
2. EVQLVQSGAEEVKPGSSVTKV

**CDR1**

1. TCTCTCCAGGGCCAGTCAGAGGTGGAGCGCTACTCTTGCTCCACACGAGACCT
2. SCKASGGFSSYTISSWVRQA

**CDR2**

1. CTTGACCAAGGGCTTAGGACCGCACTCAGCAGCTACTCTACTCATCAGCTGGCTGAGCAGGCC
2. FGQGLEWIMGGIIPIFGTANY

**CDR3**

1. GCACAGAAGCTCCAGGCGAGACTCGAGTACCGCGGCTGGACATTACACGAGCGACCTAGCTAC
2. AQPQGQGRVITADKSTSTAY

**FIG. 17A**

**FIG. 17B**
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone T3P1

1   GAAATTTCGTCGACGAAGGAGCCACGAGACAGAAGCCCTCCGAGGGGCAAAGAACCCACCC
   EIVLTQSPATLSLSFGERAT
   ↓

CDR1

61   CTTCCTTGCCAGGAGCCAGTCAAGATGGTTAGGTGCAAAGCTACTTGAACCTGGTACC
   LSCRAASQVSYSLYAYWYEQQK
   ↓

121  GCCCTAGGTCCGACATCTCCTCATATGCTACCCACAGGAGAGAGCTCTGAC
   G*APRLIYDASHRATGIPA
   ↓

181  AGGTTCAGGAGGATGCTGCTGGCTGGACAGAATCATTCCACACTGGACGCTGGTAC
   RPSGSGSGTDFDTLTISSLPE
   ↓

241  GAGATTTTGACGTTATATTCTGAGGACGCTTGCACGCTGGTACACTGCG
   EDFAVYYCQQRSHWPMPMYTF
   ↓

301  GCCAGGAGGACCCAGCTGGAGATCAACGA (SEQ ID NO:75)
   GQTKEIXR (SEQ ID NO:76)
   ↓

FIG. 18A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone T3P1

1   GAGCTCCAGCGTGCTGCTGCTCCGGCTGGCCGCTCAGAAGCCCTGCTCGGCTGAAGGTC
   EVQLVGSCAEYVKPGSSKVVK
   ↓

CDR1

61   TCCTCAAGGCTTGCGGCTGGCACCTCACTTGTCTGCATGGGTTGAAGAGCC
   SCKASGSFTSSYTSWVRQA
   ↓

121  GCTGGAACAGCGCTCTGAGGGGAAGATACATCGCCTATCAGGATCATAGAC
   PFGQLEGWMCIGIFIFCTANY
   ↓

181  GCACAGAGTCCACGCGCCAGAAGATACATTCCGCGCCAAATCCACGACGCCCTAC
   AQKPGRVITITADKSTSTAY
   ↓

241  ATGGACTGACGAGCGCTGGAGATCAGACTGGAGGCTCTGATCTGGCCGGGATACG
   MELSSLRSEDVTAYYCAMGDT
   ↓

CDR3

301  GATAGTAGTGCTTATTACGGGCACGGTGGGACTACCGGCGGCGCCAGCCTGCCGCCCTC
   DSGYGAVDYWCGQTGLVTV
   ↓

361  TCAAGC (SEQ ID NO:77)
   SS (SEQ ID NO:78)
   ↓

FIG. 18B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone T3F2

GAAATGTCGCTGACTGTCACTCCACCTCTGCGCCGCTGTAACGCCCTGCCGAGGCGGGCCCTCC
E I V L T Q S F L S L P V T P G E P A S

CDR1
ATCTCCGGTCAGTCTAGTTAGGGCTGCTGCTGATCAAGACTCTTGAATTTGATGGGGTG
I S C R S Q S L L S H S N G Y N Y L D W

CDR2
TACCTGCAGAGCCAGGGGTCGCTGCTGCTGATCAAGACTCTTGAATTTGATGGGGTG
Y L Q K P G S Q P L L I Y L G S N R A

CDR3
TCCGGCGCCCTGACAGGTCTCCCTGCCGAGGCTGCTGATCAAGACTCTTGAATTTGATGGGGTG
S G V P D R F S G S G S G T D F T L K I

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone T3F2

CAGGTGCAGCTGCTGCAGTCTGCAATTCTGGGAGGCCCTGCCGAGGTCTGCCTCTGAGACTC
Q V Q L V Q S G G G V Q P G R S L R L

CDR1
TCCCTGACGCTCTGCCAGTCTCATGCTGCTGATCAAGACTCTTGAATTTGATGGGGTG
S C A A S G F T P S S Y G M H W V R Q A

CDR2
CACGAGAAGCGGCTGAGGTGCTGCTGATCAAGACTCTTGAATTTGATGGGGTG
P G K G L E W V A V I S Y D G S N K Y Y

CDR3
CGAGACTCTGCTGAGGCTGCTGATCAAGACTCTTGAATTTGATGGGGTG
A D S V K G R F T I S R D N S K N T L Y

FIG. 19A

CGGGACTGTCGGACGGAGACAGGGCAGAAGGGTGAAATCAAACGA (SEQ ID NO:79)
RTFGQGKTVEIKR (SEQ ID NO:80)

FIG. 19B

ACCGTCTCAAGC (SEQ ID NO:81)
TVSS (SEQ ID NO:82)
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 4A9

1  GACACAGAGATCACCAGACCTCTCCATCTCGCTGAGATGGCTGAGAGAGTTCT
1  D I Q M T G S P S I L S A S V G D R V T

CDR1

61  ATCCATGCGCAGGCTGATTTGAGGTACCTGGCCTGAAACGAAACAA
21  I T C R A S Q R F G D Y L A W Y Q Q K F

CDR2

121  GGGCAAGGCTCTTGAGCTCTGGAGTACCTGGGCTGAAAGCTGAGCT
41  G Q A P K L L I Y G A S T L Q S G V P S

181  AGTGGCTCAGGCAAGGTGCTCTGGAGTACCTGGGCTGAAAGCTGAGCT
61  R F S G S G T E P T L L T I S G L Q P

CDR3

241  GAGAATTTCTACACTACATGGTCAGAGCTCAAATGTTTCCATCCATCCTCGGAA
81  E D F A T Y Y C Q Q A N S F P I T P G K

 FIG. 20A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 4A9

1  CAGGTCCAGCTGCTGAGCTCTGAGTTTTOAGAGCTGGCTCTCAAGCTGAGCT
1  Q V Q L V Q S G A E V X X K P G S S V K V

CDR1

61  TGGGGAGGGCTCTGGAGCTCTGGAGTACCTGGGCTGAAAGCTGAGCT
21  S C K A S G T P F S S Y A T S W V R Q A

CDR2

121  CTTGGACAGGCTCTGGAGTACCTGGGCTGAAAGCTGAGCT
41  P G Q C L E W M G W I N V G N G N I Y

181  TCACAGAGACCTTTAGGACACGTCAACACCTAGGACAGACACAGCAGCTAC
61  S Q K P Q G R V T I T R D T S A T T A Y

241  ATGGGACACTGAGCAGCAGCTCTGAAGACAGCAAGGACAGACAGACAGC
81  K E L S S L R S E D T A V Y Y C A R D G

CDR3

301  GAGACAGCTGGGACACCTTTAGGACACGTCAACACCTAGGACAGACAGC
101  E R A W D L D Y W G Q G T L V T V S S
(SEQ ID NO: 85)
(SEQ ID NO: 86)

 FIG. 20B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 4B4:

1
AATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGGGGAAGACCGTAACCATC
N F M L T Q P H S V S E S P G K T V T I
CDR1
61
TCCTGCACCGGCAAGCGATGTCAGGCACCTTCTGCGGGAAGACCGTAACCATC
S C T G S G G S I A T N Y V Q W Y Q Q R
CDR2
121
CGGGCCAGCTGCCGCCGCACCTGTGGCTATGACAGCCAAAGACCTCTGTTGACTGT
P G S A P A T V I Y E D D Q R P S G V P
181
GATCGGTCTCTGGCCTACGGGATGGGCACCTTCTGCTCCCTACCACTCTCGGGA
D R F S G S I D S S S N S A S L I S G
CDR3
241
CTGAAGACTCTGGAGGAGCGCTGACCTCTATCTCAGCTCGGACCAGACAAAGAC
L K T E D E A D Y Y C Q S Y D S S N Q V
301
TTGGGGAGGGGACCCGGGCTCCCTCA (SEQ ID NO:87)
101
FGGKTKLTVL (SEQ ID NO:88)

FIG. 21A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 4B4:

1
CAGGGCCAGCTCACCGGCTCCGAGGAGCTGAGCCACCTGCGGAAAGCGGAGGGTCGGTCCCTC
Q V Q L Q Q W G A C L L K P S T E L S L
CDR1
61
ACCTGGGGTCCTTTGGGTGGGCTCTTCCATGGAGGCACCTGCTGAGGCCACGGACCCCGCCG
T C A V Y G S P S G Y Y W S W I R Q P
CDR2
121
CCAGGGGAGGGGCTCCAGGGTGGGTGGGGAAGACCTGACAGCCCACTCCAGGAGCACAC
P G K S L E W I G E I N H S G S T N Y N
181
CCCTCCCTCCCAGAGCTGACCTACCATAGAGTAGCAACGGTACCCGAGACCCGCAGGGCTG
P S L K S R V T I S V D T S K N Q F S L
241
AAGCTGACCTTGAGCCGCGGCAAGGCTCTCTTGGGTGGGGAAGACCTGACAGCCGCAAC
K L S S V T A A D T A V Y C A R M V R
CDR3
301
TACCTCGGGCATGGTGCTTGCTTCGCGCGCCCTGGGGAAGACCTGACAGCCGCAACCTG
Y Y Y G M D V W G Q G T T V T V V S S (SEQ ID NO:90)
101

FIG. 21B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 4C2

1

1
S Y V L T Q P P S V S E A P G K T A R I

CDR1

61
21
T C E G I T I G R K S V H W Y Q Q K P G

CDR2

121
41
Q A P V L V V V Y D D T V R P S G V F R

161
61
F S G S N S G N T A T L I I S G V E A G

CDR3

241
81
D E A D Y Y C Q V W D S S T D P Q V V F

301
102
G G G T K V V T V L (SEQ ID NO:92)

FIG. 22A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 4C2

1

1
Q V Q L Q Q Q S G P G L V K P S Q T L S L

CDR1

61
21
T C A I S G D S V S S K N S S W N I R

CDR2

121
41
Q S P S R G L E W L G R T Y R S K N Y

181
61
Y D Y A V S V K G R I T F T F T P D T S K N

CDR3

241
81
O V S L E H L N A V T P E D T A N Y Y C V

301
102
R G S I F D V W G Q G T H V V T V S S (SEQ ID NO:94)

FIG. 22B
Nucleotide (top) and amino acid (bottom) sequence of the light
chain variable region of antibody clone 4G9

1  GACATCCAGATGACCCAGTCTTCTCTACACCTGTCTCTAGAGACAGACGCTAC
1  D I Q M T Q S P S I L S A S V G D R V T

CDR1

61  ATCACTTGGCGGCGCGAGTCAAGATTTGGTCTACTTGGCTGCTGGATCAGACAGACCA
21  I T C R A S Q R F G D Y L A W Y Q Q K P

CDR2

121  GGGCRAAGCCCTPAAGCTCTGGAGAAGAATTTGGGTAGCTTGGCCTTCCTCTGCA
41  G Q A P K L L I Y G A S T L Q S G V P S

181  AGGTTGACGGCAGATCAGGCTAGGCTACCTCTACAGCAGAGCTACAGGTGAC
61  R F S G G S G S G T E F T L T I S G L Q P

CDR3

241  GAAAGATTTGACCACTTACTTCTAGTGGCAGAGCTACAGGCTACAGGTGAC
81  E D F A T Y C Q Q A N S F P I T F G K

301  GGGACACGCGCTGGACATCAGACGA (SEQ ID NO: 95)  
101  G T R L D I R R (SEQ ID NO: 96)  

FIG. 23A

Nucleotide (top) and amino acid (bottom) sequence of the heavy
chain variable region of antibody clone 4G9

1  CAGGTCAAGCTCTGGAGACAGGCTCTGGGGCAGGCTCTGGGTGACTGCAAGAC
1  Q V Q L V Q S G A E V K T K F G G S S V K V

CDR1

61  TGGTGCAAGGCTCTGGGACAGGCTCTGGGGCAGGCTCTGGGTGACTGCAAGAC
21  S C K A S G G T F S S Y A I S W V R Q A

CDR2

121  CCTGGACACAGGGCTTAGGAGATGGATGGATGGATGGATGCACATGGAAGCTTATATAT
41  P G Q G L E W M G W I N V G N G N A I Y

181  TCAGAGATTTGACAGAGCTAGAGCTACAGGCTACAGGTGAC
61  S Q K F Q G R V T I T R D S T A T T A Y

241  ATGGAATCTGAGACGCGCTGGGACATCAGGCTCTGGGTGACTGCAAGACGAAGCC
81  M E L S S L R S E D T A V Y Y C A R D G

CDR3

301  GAGAGAGCCTGGGACCTTTGACTGCGGGGCCAGGGACACCTTGGGTGACTCAAGG
101  E R A W D L D Y W G Q G T L V T V S S  
(SEQ ID NO: 97)  
(SEQ ID NO: 98)  

FIG. 23B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 3A12

1  GAAACGACACTCACGCAGTTCTCCAGCGGCGTCTCCTCTTCTGCTTCCAGGAAAGGACCCAC
   ETTILTQSPGSTLSLSPPGERAT

CDR1

61  CTCTTCCTGACGGCGAGTGCTAGCTACGCTATAGCTGCTATTACTGCTACGATGGACAA
   LSCRASQSVSSRYPWLYYQQK

CDR2

121  CTTGACCAGGCTCCAGGCTCCTCATTCTCTATGGGATCAGCCGACGTGGGAGCTGGAG
   PGQAPRLLYGASSRATGIP

181  GACAGGTCTGATTGGAGCTGGGATCTGGTCTGGGACAGCTTACGTACCACGACTGGGAG
   DRFSGSSTSGTDTFTLTTISRELE

CDR3

241  CTCGAGATTTCTTCAGGATTTACGTCTGATGACGCTATGGGATACGCTAAGACCTTTGGCCAG
   PEDFAYYCYQYGYSSNFTGQ

301  GGGACCAGCTGAAGATCAAAAGCA (SEQ ID NO:99)
   GTKLEIKR (SEQ ID NO:100)

FIG. 24A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 3A12

1  CAGGTGCAGCTCCAGGACCTGGAGGCGCGGCGAAGCTTGGAGGACCCTTGGGGCTGGCTCCCT
   QVQLQESGPGLVKPSLETLS

CDR1

61  ACCGGACTCTGTCCTGCTGAGCCGCTCCTCAGTACGCTTAGGACTGGAGGGCTGGAGCCGC
   TCTVSAGSSSYYWGWR

CDR2

121  GACGCCCCAGGGAAAGGGGGTGGAGGGATGGGATTAGGGGAGCTTAGTTATATTAGGGGAGACACC
   QPGKGLWGICEYYSYSTY

181  TACACCAGGCTCTCAAGAGCTGCACTCAACCATATTCCGTACAGCAGCGCAAGAACCAGCTTC
   YNPSLKSRTIVSDTSKQNF

241  TCCCTGAGCTGAGCTGACGCGGCGCGACAGACTGGAGGCTGATTACTGCTGCGAGAG
   SLKLSSVTADAATAVYYCAR

CDR3

301  AGGAGTGGGAGCCTCCAGTATGTTGTTGGATCTGTGTTGCTGATGCTGAGACAG
   RSGSYLNNDAPDIWGGQGTMVT

361  GTCTCAAGC (SEQ ID NO:101)
   VSS (SEQ ID NO:102)

FIG. 24B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 3B1

1  GAAACGACACTCAGACAGCTCTCCAGCAACCTGTCTTGTCTCCAGGGGAAAGAGCC
   ETTLTQSPCTLSLPGERAT

CDR1
61  CTCTCCCTGAGGSGGCAATGAGGTGTCAAGAGCAGCTACTCTAGGCTGAGCCGAGAA
21  LSCRASQSVSYLAWYQQK

CDR2
121  CCTGCCAGGGCTCTCGAGGCTCATTCTACATCTGTCAGGCAATCCAGGCCAGTCGGCAGACG
41  PGQPAPRLILYGASSRATGIP

181  GACAGGTCAGCGGCTGCGAGCTTGAGCCAGACAGCTCTCGACAGGAGCTGGAG
61  DRFSGSGGSDFTTLSRTE

CDR3
241  CCTGAAAGATTTTGCAAGTGATTACTGTCACAGATGAGCTACTGACGCTGGAGTGGCC
81  PEDFAPVYCYQQYGSSSGTFG

301  CAAGGGACCAAGGTGAAATAATCAACGA (SEQ ID NO:103)
101  QGTKVEIKR (SEQ ID NO:104)

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 3B1

1  CAGGTACAGCTCAAGCTAGCTGAGGTGATAGAGCATGGCGTCCCTGGTGAAGCTC
1  QVQLQSGAEVKKPGSSVKV

CDR1
61  TCCTGCAAGCTCTCTGGACGCCATCAGCAGCTGGCTCTGGAGATCG
21  SKASGGTFFSSYAIWVRQA

CDR2
121  CCTGGACATGGCTTGAGAATCTCTTGATCTGGATAGCATGACGCGCAGCC
41  PGGGLEGWNGRIPILGYANY

181  GCAAGGGAGTCCAGGAGCTAGCGCTGTACCTGACAGTACGCAGCTGAGGAGCTC
61  AQKFQGRVTITADKSTSTAY

241  ATGGAAGCTGACGGCAGCTGAGATTCTGACAGGCAGGGATATTACTGCGAGATGGTTC
81  MELSLSRSEDTAYYCARGF

CDR3
301  CGCTTGGATCTACTGAGCTCTGAGGCTTGAGGGCCAAGGGACACAGTCAGGCTACTC
101  RPYYYYGMDVWGGTGVVTVS

361  AGC (SEQ ID NO:105)
121  S (SEQ ID NO:106)

FIG. 25A

FIG. 25B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 3F5

1  GAAAAGACACTACGCACGCTTCGCCACCCCCTGCTTTGTCTCCAGGGAAAGAGGACC
   ETTLTQSPGTLSLSPGERAT

CDR1
61  CTCTGGCGAGCCAGTCTACAGTGGTCTGGGACATCTGCTGATCCAAGAGGACCT
21  LSCNASQSVGSGNLAWYQQRP

CDR2
121  GGGCCAGCTCGCCAGCTCGCTCTGATATGCTGCACTAGCGCTGAGCCACTGCTGACTG
41  GQAPSLLYYGASSRATGVPD

181. AGGTTCAGTGACATCCTTCTTCATGGAGACACTCTCCTCCTCAGACACTCGAGGCT
61  RFSGSGTDFTLTISRLF

CDR3
241  GAAGATTTTGCTGTATATCCTACTGCCACAGCTGATCTTCCTCGCTTTGCTAGACTT
81  EDFAVYYCQYGDSPRLYTF

301  GGCAGGGAGAAGGCTTGAGATCAAAGGCAGATAACGAAGAAGCAGCTACACAC
101  GQGTKLEIKR (SEQ ID NO: 108) FIG. 26A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 3F5

1  CAGGTGCACCTACGCCAGCGTGCGGCGAGACCTAGGTGAGGCTCCTCCTG
1  QVQLQQWSLAGLKLPSETLSL

CDR1
61  ACCYCTGCTGCTACTTTGATGGGTCTCAGGACTGCTGGATCCGGGCCAGCCC
21  TCAYVGSSGYSWYSWIRQP

CDR2
121  CGCGGAAGGGCGCTGGATGCTGGAAATACATCACTGATGGAAGCGCAGCAACTGAC
41  PGKGLWIEGINHSGSTNYN

181  CCGTCCTCAGAGATGCACCTCAGACACTCTAGCTAGACGCTGCTCCTCGT
61  FLSLKSRVTISVDTSKKNQFSL

CDR3
241  AAGCTGAGCTCTGCAGCCGCGGACACGCTGCTGGATAGTCTCTCGAGAGGCTTAC
81  KLSSTVAAADTVAYCARYAVAY

301  TAGTACGTGATTCTACCTCCTGACGTATCTGCTGTTCTTGGGACAGAGGACAATG
101  YDSGYYPYDAFDFIEEEWQGTM

FIG. 26B

361  GTACCGCTCTCAAAGC (SEQ ID NO: 109)
121  VTVSS (SEQ ID NO: 110)
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 3G3.

1
GAAACGCACCTCAGGCACTGTCCTCCAGGGGAAAGAGAGGACACC
ETTLTQSPGTLSLSPGERAT

CDR1
61
CTCTCCTGCAAGGGCAAGTGTCCTCTCTACCTAGCTGGGTACCCGACGAAA
LSCRASQSVSSYLYWQQK

CDR2
121
CCTGGCCAGGCTCCAGGGCTCCACTATATTTGATCGTCCAGGGCCACTGGCATCCAGA
PGQAPRLIYGRASSRATGI

CDR3
181
GACAGTTCTCTGCTGACTGCTCTCCAGCTCTCCTCAGTCACTCCAGGCTGCCAGCTTGAG
DRFSGSCSCTGDFTLTISSLRE

CTCTGAAGATTTTCCATGCTATTACCTGACCCATATTTGCTGAGCGAGCACTTTG
PGQAPRLIYGRASSRATGI

PEDFAVYVCQYYGSSPTFG

301
CAGGGGACCAAGCTGGAATCAAGCA (SEQ ID NO:111)
QGTKLEIKR (SEQ ID NO:112) FIG. 27A

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 3G3.

1
CAGGGGCACGCTCTGCTACATCTGGAGAGGCGCTGTCCTGAGGCTGGGCCTAGACTC
QVLVQSGCCGVQPGRSRLRL

CDR1
61
TTCTGACAGCTCTGCACTACCTGGCTCCCACGTGCTCGAGGCTGGCCCTGGC
SCASGFTFSYAMHWVRQA

CDR2
121
CAGGGGACGCTCTGCTACATCTGGCTCCCACGTGCTCGAGGCTGGCCCTGGC
PGKGLEWVAVISYDGSNRY

181
GCAGACTCTGCTGACAGGGCCGATCTCACTTCAGAGACATCTCCAGGAGAAGAGCAGCTG
ADSVKGRFTISRDNSKNTLY

241
CTCGAATGGAACCATCCTGCTGAGGCTGGCCAGAGACATCTCCAGGAGAAGAGCAGCTG
LMNLSRAEDTAVYYCAREL

CDR3
301
CGATTTTTGAGGTCTGCCTCGAGATTTTCTATCTGGGCAAGGACCACATGTCACC
RFLEWSDDAFIDWGGQGTMVT

361
GTCTCAAGC (SEQ ID NO:113)
VSS (SEQ ID NO:114) FIG. 27B
Nucleotide (top) and amino acid (bottom) sequence of the light chain variable region of antibody clone 3H2

1
GAAACGCACACTGCGACGCTCCAGGCGACCTGCTTCCTCCAGGGAAAGAGCCACCC
1
ETTTLTQSPGTLSSLSPGERAT

CDR1

61
CTCTCCCGACGAGCGCCAGCTGGAGGTAGATAGGGTAGCCGACCACTATATCTGCTGTAACGAGAC
21
LSCLRSAQSVEVSSSYLAWVQQK

CDR2

121
CTGCGACACTGCGACGCTCCAGGCGACCTGCTTCCTCCAGGGAAAGAGCCACCC
41
FGQAPRLILIGASTRATGV

181
GAGCATGGTACATGGGACGAGAGTTCACTGACACATATTACACACACACATGGGACGAGAC
61
DRFSGSSGSGTDFTLTISRLE

CDR3

241
CTGAAAGATTTTGAGCTATTACACTGCAACAGTGCTACTGACCTTACACTGACCTGACG
81
PEFDAYVCQYGTSLTWTF

301
GGCCAGGGACCAGGTGGAACTCAAGACCA (SEQ ID NO:115)
101
GQGTKVEIKR (SEQ ID NO:116)

Nucleotide (top) and amino acid (bottom) sequence of the heavy chain variable region of antibody clone 3H2

1
CAGTTGCCAAGCTGAAGCTGGAGGCCCAAGACGAGCTGCTGGACGGAGACCCCTGGCTTC
1
QVQLQESGPGGLVPSQETLSL

CDR1

61
ACCTGCACCTGCTCTTGGCTGGAGCTACGACAGTAAAGTTTACTACTGCGCTGGGTGGGATC
21
TCTVSGCGSISSSSYYWAWIR

CDR2

121
CAAGCCGACCAGGGACAGGGGGCTGGAGTATTGGGAAATCTAGCATATGIGAGACACCCACACAC
41
QPPGGKGLWEIGEINHSGSTFN

181
TCACACCCTCCCTCAAGAGTGGAGTTCATACATGACAGCTCCAGAGACAGCACTCTTG
61
YNPSLKSRVTISVDTSKNQF

241
TCCTGGACTGCTGCTGCTGCCGACGGGAGAGCGGATGATTCTACTGCGCTGGAGAGATA
81
SLNLNSVVTADTAVYYCARV

CDR3

301
GTAGCAGAGCTGGTACACTGCTACTGCTACTGCTACTGCTAGCATGCGACTCCGAGGCTCCGAG
101
VAAYGHYFYYFMDVWGKGGTT

361
GTACCCGCTCAAGC (SEQ ID NO:117)
121
VTSS (SEQ ID NO:118)

FIG. 28A

FIG. 28B
O.D. 450 nm

Competing MHC-peptide complex

FIG. 31D
### Selection of Recombinant Fab Antibodies with TCR-like Specificity

<table>
<thead>
<tr>
<th>Cycle No.</th>
<th>Phage Input</th>
<th>Phage Output</th>
<th>Ratio (OI)</th>
<th>Enrichment</th>
<th>MHCpeptide binders</th>
<th>TCR-like Binders</th>
<th>Finger Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A T540</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6x10^13</td>
<td>1x10^9</td>
<td>2x10^7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5x10^12</td>
<td>1x10^9</td>
<td>3x10^6</td>
<td></td>
<td>2494 (24%)</td>
<td>1324 (13%)</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1x10^13</td>
<td>1x10^10</td>
<td>9x10^4</td>
<td>1200</td>
<td>6054 (65%)</td>
<td>4154 (41%)</td>
<td>3</td>
</tr>
<tr>
<td><strong>B T856</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6x10^9</td>
<td>2x10^7</td>
<td>3x10^7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2x10^12</td>
<td>1x10^7</td>
<td>2x10^6</td>
<td></td>
<td>1794 (18%)</td>
<td>594 (6%)</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>4x10^2</td>
<td>6x10^6</td>
<td>2x10^3</td>
<td>600</td>
<td>5854 (58%)</td>
<td>2154 (22%)</td>
<td>3</td>
</tr>
</tbody>
</table>

**FIG. 34A**

![Graph showing OD 450nm vs Clones](image)

**FIG. 34B**

![Graph showing OD 450nm vs Clones](image)

**FIG. 34C**

![Graph showing OD 450nm vs Clones](image)
Selection of Recombinant Fab Antibodies with TCR-like specificity to MUC1-D6 peptide.

<table>
<thead>
<tr>
<th>Panning Cycle</th>
<th>Phage Input</th>
<th>Phage Output</th>
<th>Ratio (O/D)</th>
<th>Enrichment</th>
<th>MHC-peptide binders</th>
<th>TCR-like binders</th>
<th>Finger Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.2x10^{12}</td>
<td>5.4x10^{8}</td>
<td>7.5x10^{-4}</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>5x10^{13}</td>
<td>3x10^{7}</td>
<td>6x10^{-2}</td>
<td>55</td>
<td>46/90 (51%)</td>
<td>41/90 (45%)</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>4.9x10^{12}</td>
<td>1.7x10^{10}</td>
<td>3.5x10^{-4}</td>
<td>580</td>
<td>76/90 (84%)</td>
<td>72/90 (80%)</td>
<td>16</td>
</tr>
</tbody>
</table>

FIG. 38A

FIG. 38B
FIG. 39
**FIG. 40A**

Graph with the following axes:
- **OD (450 nm)**
  - Scale: 0, 0.5, 1, 1.5, 2, 2.5, 3
- **Fab (ng)**
  - Values: 1, 5, 25, 100, 500

Graph line labeled **M3A1**

**FIG. 40B**

Graph with the following axes:
- **OD (450 nm)**
  - Scale: 0, 0.5, 1, 1.5, 2, 2.5, 3
- **Fab (ng)**
  - Values: 1, 5, 25, 100, 500

Graph line labeled **M3B8**
FIG. 42
MHC-PEPTIDE COMPLEX BINDING
LIGANDS

RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 11/582,416 filed on Oct. 18, 2006, which is a divisional of U.S. patent application Ser. No. 10/371,942 filed on Feb. 20, 2003, now abandoned, which claims the benefit of priority from U.S. Provisional Patent Application No. 60/358,994 filed on Feb. 20, 2002. The contents of the above applications are incorporated herein by reference in their entirety.

FIELD AND BACKGROUND OF THE INVENTION

[0002] In recent years, major advances in tumor immunology have led to an increased understanding of the immune responses against tumors. For example, with respect to melanoma, human melanoma and other tumor cells express antigens that are recognized by cytotoxic T lymphocytes (CTL) derived from cancer patients (Boon and van der Bruggen (1996) J Exp Med 183:725-9; Rosenberg (2001) Nature 411:380-4; Renkvist and Parmiani (2001) Cancer Immunol Immunother 50:3-15). The cascade of molecular recognition events associated with these tumor-associated immune responses involve the expression of specific peptides in complex with MHC class I molecules on the cancer cells. For example, human melanomas express tumor-associated peptides that are presented to the immune system in a complex with class I HLA-A2 molecules (Anichini et al. (1995) J. Exp. Med. 177:989-98; Coulie et al. (1994) J. Exp. Med. 180:35-42). Several categories of cancer-associated antigens have been reported as targets for CTLs in vitro and in vivo such as “cancer-testis” antigens that are expressed in different tumors and in normal testis, melanocyte differentiation antigens, point mutations of normal genes, antigens that are overexpressed in malignant tissues, and viral antigens (Renkvist and Parmiani (2001) Cancer Immunol Immunother 50:3-15). Although there is strong experimental evidence demonstrating the presence of these antigens on a variety of tumors, they are apparently unable to elicit a strong enough anti-tumor immune response (Rivoltini et al. (1998) Crit. Rev Immunol 18:55-63).

[0003] Therefore many modern cancer immunotherapy approaches are now designed to induce and enhance T cell reactivity against these tumor antigens. Intensive research on cancer peptides has culminated in many clinical trials involving therapeutic vaccination of cancer patients with antigenic peptides or proteins (Rosenberg (2001) Nature 411:380-4; Offringa and Melief (2000) Curr Opin Immunol 12:576-82). Moreover, several studies demonstrated that the inability of the patient’s immune system to elicit an effective immune response against the tumor is often due to poor antigen presentation (Restifo et al. (1993) J. Exp. Med. 177:265-72; Seliger and Ferrone (2000) Immunol. Today 21:455-64). Nevertheless, these studies have encouraged the development of new immunotherapeutic strategies that employ vaccination protocols with tumor cells, tumor extracts, RNA-loaded dendritic cells, or tumor cell-dendritic cell hybrid vaccination (Esche (1999) Curr Opin Mol Ther 1:72-81; Kugler et al. (2000) Nat. Med. 6:332-36). Tumor-specific MHC-peptide complexes present on the surface of tumor cells may also offer a unique and specific target for an antibody-based therapeutic approach. To develop such a strategy, targeting moieties such as recombinant antibodies that will specifically recognize peptide-MHC complexes must be isolated.

[0004] The recent advent of MHC-peptide tetramers has provided a novel tool for studying antigen-specific T cell populations in health and disease, even when they are very rare, by monitoring tetramer-T cell binding via flow cytometry (Allman et al. (1996) Science 274:94-96; Lee et al. (1998) Nat. Med. 5:677-85; Oggi et al. (1998) Science 279:2105-06). However, to date there are very few tools available to detect, visualize, count, and study antigen (MHC-peptide) presentation. Indeed, several studies demonstrated that the inability of the patient’s immune system to elicit an effective immune response against the tumor is often due to poor antigen presentation (Restifo et al. (1993) J. Exp. Med. 177:265-72; Seliger and Ferrone (2000) Immunol. Today 21:455-64). Antibodies with T cell receptor-like specificity could enable measuring the antigen presentation capabilities of such tumor or antigen-presenting cells, for example by direct visualization of the specific MHC-peptide complex on the cell surface. Some attempts to use recombinant soluble T cell receptors for this purpose have largely failed because of their inherent low affinity for their target as well as their instability as recombinant-engineered molecules (Wulffing and Flickhuhn (1994) J Mol Biol 242:655-69). Therefore, in addition to being used as targeting agents, TCR-like antibodies would serve as a valuable tool to obtain precise information about the presence, expression pattern, and distribution of the target tumor antigen, i.e., the MHC-peptide complex, on the tumor cell surface, on tumor metastases, in lymphoid organs, and on professional antigen-presenting cells.


SUMMARY OF THE INVENTION

[0006] This invention provides, in part, protein ligands that bind to MHC-peptide complexes. The peptide component of a complex can be, e.g., a tumor associated antigen (TAA). As used herein, “TAA” refers to a peptide fragment presented on a MHC molecule, wherein the peptide fragment or the polypeptide that it is processed from is associated with a tumorous or cancerous state. Renkvist and Parmiani (2001) Cancer Immunol Immunother 50:3-15 provides a list of exemplary TAAAs. A ligand of the invention can specifically bind to a TAA, e.g., a TAA listed in Renkvist (supra).

[0007] In a preferred embodiment, the protein ligands are antibodies, or antigen-binding fragments thereof. In another preferred embodiment, the protein ligands are modified scaffold polypeptides (or peptides). In still another preferred embodiment, the protein ligands are cyclic peptides or linear peptides, e.g., of less than 25 amino acids. Whereas many examples described herein refer to antibody ligands or fragments thereof, it is understood that the invention can be
practiced using any protein ligand (e.g., antibody and non-antibody ligand) provided herein.

The anti-(MHC-peptide complex) ligands bind to MHC-peptide complexes with high affinity and specificity for the peptide moiety within the complex, and thus can be used as diagnostic, prophylactic, or therapeutic agents in vivo and in vitro. Preferably the ligands specifically bind to the MHC-peptide complex with a partial or complete peptide-specificity.

“MHC” is a major histocompatibility complex (MHC) protein that includes at least two subunits. The identity of the subunits depends on the class of MHC molecule. For example, a Class I MHC includes a α subunit and β2-microglobulin. In another example, a Class II MHC includes a α subunit and a β subunit.

“MHC-peptide complex” is complex that includes at least an MHC and a peptide. The peptide is bound in the peptide binding groove of the MHC. The peptide can be added exogenously, or can be assembled into the complex within a cell, e.g., in a TAP2 dependent process. For example, the peptide can be produced by the processing of an antigen by the proteasome.

As used herein, “specific binding” refers to the property of the antibody: (1) to bind to MHC-peptide complex with an affinity of at least 1x10^6 M^-1, and (2) to preferentially bind to MHC-peptide complex, with an affinity that is at least two-fold, 50-fold, 100-fold, or greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than a MHC-peptide complex.

In one aspect, the invention features a protein that includes: an immunoglobulin heavy chain variable (VH) domain and an immunoglobulin light chain variable (VL) domain. The protein binds a complex comprising an MHC and a peptide, does not substantially bind the MHC in the absence of the bound peptide, and does not substantially bind the peptide in the absence of the MHC. The term “does not substantially bind” means that the binding affinity is less than 2% of the binding affinity of the protein for complex. Typically, the protein is isolated. The peptide can be a peptide fragment of gp100, MUC1, TAX, or hTERT, e.g., a peptide fragment listed in Table 1.

In one embodiment, the protein includes a label or signaling entity, e.g., a label or signaling entity described herein, or at least a component of a label or signaling entity. In another embodiment, the protein includes a cytokine or at least a component of a cytokine. In still another embodiment, the protein is attached to an insoluble support, e.g., a solid support. For example, the solid support can be a surface of a multi-well container or a planar array.

In one embodiment, the protein is attached to a cell. For example, the protein can include a transmembrane domain that is inserted to the plasma membrane of the cell. The cell can be, e.g., an immune cell, e.g., a T cell, a cytotoxic T lymphocyte (CTL).

The VH and VL domains of the protein can be components of the same polypeptide chain or of different polypeptide chains. In a particular embodiment, the different polypeptide chains are attached by a disulfide bond.

The protein can include an effector domain, e.g., an Fe domain or a non-immunoglobulin effector domain, e.g., a synthetic peptide that specifically binds to a target. In another implementation, the effector domain includes an antigen binding domain (e.g., specific for a target other than an MHC-peptide complex or for a different epitope of an MHC-peptide complex), e.g., an scFv antigen binding domain.

The association constant for binding of the protein to the complex can be at least 10^6 M^-1, 10^6 M^-1, 10^8 M^-1, or 10^10 M^-1. In one embodiment, the protein binds to the complex if the peptide fragment is in the complex, but not if a non-overlapping peptide fragment that differs by at least 3 amino acids from the peptide fragment is in the complex.

The invention also provides a pharmaceutical composition that includes the protein, and a pharmaceutical carrier. For example, the protein can include a cytokine or a label (e.g., an imaging component).

In yet another aspect, the invention features a cytotoxic entity that includes a moiety that (1) binds a complex that includes an MHC and a peptide, does not substantially bind the MHC in the absence of the bound peptide, and does
not substantially bind the peptide in the absence of the MHC and/or (2) binds an epitope of the MHC-peptide complex that includes a moiety of the peptide and a moiety of the MHC. [0027] In one embodiment, the component of the moiety that binds the complex includes an immunoglobulin variable domain. In another embodiment, the complex binding moiety includes a modified scaffold domain (e.g., a non-immunoglobulin scaffold domain), a disulfide loop, or linear peptide. [0028] The cytotoxic entity can include, for example, a radioisotopic compound or a polypeptide (e.g., peptide) toxin, or at least a component thereof. In another example, the cytotoxic entity includes a heterologous immune cell. [0029] The association constant for binding of the cytotoxic entity to the complex can be at least $10^{-7} \text{M}^{-1}$, $10^{-8} \text{M}^{-1}$, $10^{-9} \text{M}^{-1}$, or $10^{-10} \text{M}^{-1}$. The peptide of the complex can be, e.g., a peptide fragment of gp100, MUC1, TAX, or hTERT, e.g., a fragment listed in Table 1. The invention also provides a pharmaceutical composition that includes the cytotoxic entity and a pharmaceutical carrier. [0030] In another aspect, the invention features a cytotoxic T cell that includes one or more nucleic acids for expressing a heterologous protein that (1) binds a complex that includes an MHC and a peptide, does not substantially bind the MHC in the absence of the bound peptide, and does not substantially bind the peptide in the absence of the MHC and/or (2) binds an epitope of the MHC-peptide complex that includes a moiety of the peptide and a moiety of the MHC. The protein can include one or more polypeptide chains. Multiple chains can be encoded by a single nucleic acid, e.g., by different segments of the single nucleic acid, or by a plurality of nucleic acids. The protein functions to bind to the MHC complex. [0031] In one embodiment, the heterologous protein includes an immunoglobulin variable domain that binds the complex, independently or in cooperation with other factors. In another embodiment, the heterologous protein includes a modified scaffold domain (e.g., a non-immunoglobulin scaffold domain), a disulfide loop, or linear peptide, that binds the complex, independently or in cooperation with other factors. [0032] In one embodiment, the heterologous protein includes a cell surface attachment signal that anchors the protein on a surface of the cell. For example, the attachment signal can include a transmembrane domain, a glyco-phosphatidyl-inositol anchor signal, or another cell surface attachment sequence. [0033] The cytotoxic T cell can have a cytotoxic activity that is specific for a cell that displays the MHC and peptide components of the complex on its cell surface. [0034] The association constant for binding of the cytotoxic T cell to the complex can be at least $10^{-7} \text{M}^{-1}$, $10^{-8} \text{M}^{-1}$, $10^{-9} \text{M}^{-1}$, or $10^{-10} \text{M}^{-1}$. The peptide of the complex can be, e.g., a peptide fragment of gp100, MUC1, TAX, or hTERT, e.g., a fragment listed in Table 1. The invention also provides a pharmaceutical composition that includes the cytotoxic T cell and a pharmaceutical carrier. [0035] In another aspect, the invention features an isolated nucleic acid that includes a segment that encodes an immunoglobulin variable domain such that a protein that includes the immunoglobulin variable domain and a second immunoglobulin variable domain: (1) binds a complex that includes an MHC and a peptide, does not substantially bind the MHC in the absence of the bound peptide, and does not substantially bind the peptide in the absence of the MHC and/or (2) binds an epitope of the MHC-peptide complex that includes a moiety of the peptide and a moiety of the MHC. The peptide can be, e.g., a peptide fragment of gp100, MUC1, TAX, or hTERT. [0036] The nucleic acid can include a second segment that encodes the second immunoglobulin variable domain, e.g., as a polypeptide region of the same polypeptide chain as the first immunoglobulin variable domain or as a second polypeptide chain. [0037] In one embodiment, the nucleic acid includes a region that encodes a label or signaling entity, e.g., a label or signaling entity described herein, or at least a component of a label or signaling entity. In another embodiment, the nucleic acid includes region that encodes a cytotoxin or at least a component of a cytotoxin. [0038] In one embodiment, the nucleic acid includes a region that encodes a cell surface attachment signal operably linked to the binding immunoglobulin variable domain. The protein encoded by the nucleic acid is attached to a cell. For example, the cell attachment signal can include a transmembrane domain that is inserted to the plasma membrane of the cell. [0039] In another embodiment, the nucleic acid includes a region that encodes an effector domain, e.g., an Fc domain or a non-immunoglobulin effector domain, e.g., a synthetic peptide that specifically binds to a target. The effector domain is translationally fused, or otherwise operably linked to the immunoglobulin variable domain. In another implementation, the effector domain includes an antigen binding domain (e.g., specific for a target other than an MHC-peptide complex or for a different epitope of an MHC-peptide complex), e.g., an scFv antigen binding domain. [0040] In yet another aspect, the invention features a host cell that includes heterologous nucleic acid sequences that encode a protein comprising an immunoglobulin heavy chain variable domain and an immunoglobulin light chain variable domain. The protein binds to an MHC-peptide complex if the peptide present in the complex. The peptide can be a peptide fragment of gp100, MUC1, TAX, or hTERT, e.g., a fragment listed in Table 1. The host cell can be a mammalian cell, e.g., an immune cell, or a non-mammalian cell, e.g., another eukaryotic cell such as a yeast cell or a prokaryotic cell. The nucleic acid can encode a protein or protein variant described herein. [0041] In another aspect, the invention features a transgenic animal whose genome includes heterologous nucleic acid sequences that encode a protein comprising an immunoglobulin heavy chain variable domain and an immunoglobulin light chain variable domain, wherein the protein binds to an MHC-peptide complex if the peptide present in the complex. The peptide can be a peptide fragment of gp100, MUC1, TAX, or hTERT, e.g., a fragment listed in Table 1. [0042] In one embodiment, the heterologous nucleic acid sequences include a region that encodes a label or signaling entity, e.g., a label or signaling entity described herein, or at least a component of a label or signaling entity. In another embodiment, the nucleic acid includes a region that encodes a cytotoxin or at least a component of a cytotoxin. [0043] In one embodiment, the heterologous nucleic acid sequences includes a region that encodes a cell surface attachment signal operably linked to one or more of the immunoglobulin variable domains. The protein encoded by the heterologous nucleic acid sequences is attached to a cell. For
example, the cell attachment signal can include a transmembrane domain that is inserted to the plasma membrane of the cell.

[0044] In another embodiment, the heterologous nucleic acid sequences include a region that encodes an effector domain, e.g., an Fc domain or a non-immunoglobulin effector domain, e.g., a synthetic peptide that specifically binds to a target. The effector domain is translationally fused, or otherwise operably linked to the immunoglobulin variable domain. In another implementation, the effector domain includes an antigen binding domain (e.g., specific for a target other than an MHC-peptide complex or for a different epitope of an MHC-peptide complex), e.g., an scFv antigen binding domain.

[0045] In one embodiment, the heterologous nucleic acid sequences are operably linked to a regulatory element, e.g., an element which directs tissue or cell specific expression, e.g., expression in immune cells, e.g., cytotoxic immune cells.

[0046] The invention also features a kit that includes a nucleic acid, a protein, a cell, or transgenic animal described herein and instructions for use of the protein to treat, prevent, or detect a disorder, e.g., a neoplastic disorder.

[0047] In one aspect, the invention features a method that includes providing a protein library that comprises a plurality of proteins, e.g., each protein comprising a immunoglobulin variable domain that includes a CDR sequence from a subject with a preselected MHC allele; optionally selecting an MHC complex known to be the same allele as the preselected allele; contacting the library to an MHC-peptide complex, wherein the MHC component of the complex is the same allele as the given MHC allele; and isolating a member of the library that binds the complex and specifically recognizes the peptide in the complex. The CDR sequence from the subject can be a germline CDR sequence or a somatic mutant thereof. For example, the CDR sequences of the subject are isolated from nucleic acid encoding affinity matured immunoglobulin domains.

[0048] In one embodiment, the isolated member binds the complex with an affinity of 100 nM, 50 nM, 10 nM or less. In another embodiment, a plurality of members (e.g., at least two, five, ten, 20, or 50) of the library are isolated, and each member of the plurality binds the complex and specifically recognizes the peptide in the complex, the binding of the complex having an affinity of 100 nM, 50 nM or less.

[0049] In one embodiment, the library includes a first plurality of at least 10^3, 10^4, 10^5, 10^6, or 10^7 proteins, e.g., between 10^7 and 10^{12} proteins. In an embodiment, each protein of the first plurality is a single chain antibody or a Fab fragment. The library can include a second plurality of protein, e.g., proteins that differ from the first plurality.

[0050] In an embodiment, each protein of the library is attached to an array.

[0051] In another embodiment, the protein library is a display library. For example, each protein is displayed on a replicable genetic package, e.g., a viral particle or a cell. In another example, the protein is directly attached to a nucleic acid that encodes it, or its complement.

[0052] In one embodiment, the library further includes a second plurality of proteins. Each protein of the second plurality includes an immunoglobulin variable domain that includes a CDR from a second subject with the preselected MHC allele. In another embodiment, each protein of the second plurality includes an immunoglobulin variable domain that includes a CDR from a second subject with an MHC allele, other than the preselected MHC allele.

[0053] In one embodiment, the MHC-peptide complex is a single-chain MHC-peptide complex. For example, the method can further include expressing the single-chain MHC-peptide complex in a prokaryotic or eukaryotic cell.

[0054] In another embodiment, the MHC component of the complex is tagged, e.g., biotinylated (e.g., via a biotin tag). The tag can be bound to a support, e.g., a magnetic particle, an array, or other support, e.g., a solid or semi-porous support.

[0055] In another embodiment, the MHC-peptide complex is attached to the surface of a cell. The MHC-peptide complex can be assembled within the cell and the peptide can be processed by a cellular proteasome. The protein from which the peptide is derived can be overexpressed.

[0056] In another embodiment, the MHC-peptide complex is assembled in vitro. The complex can be attached to a support, e.g., a magnetic particle, an array, or other support, e.g., a solid or semi-porous support.

[0057] In one embodiment, the peptide component of the complex is a peptide fragment of MUC1, hTERT, TAX, or gp100, e.g., a fragment listed in Table 1 or a peptide fragment described in Renkvist et al. (supra).

[0058] In another embodiment, the MHC-peptide complex is attached to a cell surface, e.g., a living cell surface. The library is contacted to the cell. The cell can present a plurality of peptide complexes. The cell can be loaded with the peptide, e.g., exogenous peptide, the cell can overexpress a protein that includes the peptide, and so forth.

[0059] The isolated protein can be formulated as a pharmaceutical composition. The composition can be administered to a subject, e.g., a test subject, or a subject identified as having a disorder, e.g., a neoplastic or autoimmune disorder.

[0060] The formulation can include attaching a toxic entity or label to the isolated protein. The invention also provides proteins identified by the method and pharmaceutical compositions that include the identified protein.

[0061] In another aspect, the invention features a method that includes: contacting members of a protein library to a single-chain MHC-peptide complex; and isolating one or more members that (1) bind to the single-chain MHC-peptide complex, does not substantially bind the MHC in the absence of the peptide, and does not substantially bind the peptide in the absence of the MHC and/or (2) bind to an epitope that includes the MHC component of the complex and that includes the peptide component of the complex.

[0062] In one embodiment, the isolated member binds the complex with an affinity of 100 nM, 50 nM, 10 nM or less. In another embodiment, a plurality of members (e.g., at least two, five, ten, 20, or 50) of the library are isolated, and each member of the plurality binds the complex and specifically recognizes the peptide in the complex, the binding of the complex having an affinity of 100 nM, 50 nM or less.

[0063] In one embodiment, the library includes a first plurality of at least 10^3, 10^4, 10^5, 10^6, or 10^7 proteins, e.g., between 10^7 and 10^{12} proteins. The library can include a second plurality of protein, e.g., proteins that differ from the first plurality.

[0064] In one embodiment, each protein of the first plurality is a modified scaffold domain protein (e.g., an immunoglobulin scaffold domain, a non-immunoglobulin scaffold domain, such as a domain of less than 70 or 50 amino acids). Each protein of the first plurality can include a synthetic
peptide. In another embodiment, each protein of the first plurality is a single chain antibody or a Fab fragment.

In an embodiment, each protein of the library is attached to an array.

In another embodiment, the protein library is a display library. For example, each protein is displayed on a replicable genetic package, e.g., a viral particle or a cell. In another example, the protein is directly attached to a nucleic acid that encodes it, or its complement.

The isolated protein can be formulated as a pharmaceutical composition. The composition can be administered to a subject, e.g., a test subject, or a subject identified as having a disorder, e.g., a neoplastic or autoimmune disorder.

The formulating can include attaching a toxic entity or label to the isolated protein. The invention also provides proteins identified by the method and pharmaceutical compositions that include the identified protein.

In still another aspect, the invention features a method that includes: contacting a protein library to a first mixture of MHC-peptide complexes; isolating a plurality of members of the library, wherein each isolated member of the plurality displaying an antigen binding domain that binds to an MHC- and the epitope recognized by the antigen binding domain comprising a moiety of the MHC and a moiety of the peptide; and identifying members of the plurality that do not substantially bind to a second mixture of MHC-peptide complexes. The first and/or second mixture can include complexes having different MHC alleles and/or different peptides. In one example, the first and/or second mixture includes a cell that presents a plurality of different MHC-peptide complexes. In another example, the first and/or second mixture includes complexes isolated from one or more cells or displayed on one or more cells.

The peptide component of the each complex in the first and/or second mixture can be a peptide that is endogenously processed by the cell. The first mixture can include complexes from one or more indicated cells, and the second mixture can include complexes from one or more normal cells.

The method can further including, after the identifying, purifying MHC-peptide complexes with one of the identified members. The purified complexes can be characterized, e.g., to identify the peptide component of the purified MHC-peptide complexes (e.g., by mass spectroscopy) and/or the MHC allele.

For example, the indicated cells can be cancer cells, or cells of individual with an immune disorder. The first and/or second mixture can include a cell, e.g., a living cell, a mammalian cell, and/or a cancer cell. The cell can have TAP1 or TAP2 activity. The cell can be attached to a magnetic particle.

In one embodiment, the identified member binds to a complex of the first mixture with an affinity of 100 nM, 50 nM, 10 nM or less. In another embodiment, a plurality of members (e.g., at least two, five, ten, 20, or 50) of the library are isolated, and each member of the plurality binds the complex and specifically recognizes the peptide in the complex, the binding of the complex having an affinity of 100 nM, 50 nM or less.

In one embodiment, the MHC component of the complex can be a class I MHC. In another embodiment, the MHC component of the complex can be a class II MHC. The MHC allele can be, e.g., any of the HLA-A11 types described in Schreuder et al., The HLA Dictionary 2001: a summary of HLA-A, -B, -C, -DRB1/3/4/5, -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR and -DQ antigens. Human Immunology 2001:62:826-849. For example, the allele is A80201. In one embodiment, the library includes a first plurality of at least 103, 104, 105, or 106 proteins, e.g., between 104 and 105 proteins. The library can include a second plurality of protein, e.g., proteins that differ from the first plurality.

In one embodiment, each protein of the first plurality is a modified scaffold domain protein (e.g., an immunoglobulin g scaffold domain, a non-immunoglobulin scaffold domain, such as a domain of less than 70 or 50 amino acids). Each protein of the first plurality can include a synthetic peptide. In another embodiment, each protein of the first plurality is a single chain antibody or a Fab fragment.

In an embodiment, each protein of the library is attached to an array.

In another embodiment, the protein library is a display library. For example, each protein is displayed on a replicable genetic package, e.g., a viral particle or a cell. In another example, the protein is directly attached to a nucleic acid that encodes it, or its complement.

The identified protein can be formulated as a pharmaceutical composition. The composition can be administered to a subject, e.g., a test subject, or a subject identified as having a disorder, e.g., a neoplastic or autoimmune disorder.

The formulating can include attaching a toxic entity or label to the identified protein. The invention also provides proteins identified by the method and pharmaceutical compositions that include the identified protein.

In another aspect, the invention features a collection that includes a plurality of proteins. Each protein of the plurality: (1) binds a complex comprising an MHC and a peptide, does not substantially bind the MHC in the absence of the bound peptide, and does not substantially bind the peptide in the absence of the MHC and/or (2) binds to an epitope that includes a component of an MHC and a component of the peptide. The peptide of the bound complex can differ for each protein of the plurality, or can be the same for each or at least some proteins of the plurality. The MHC of the bound complex can be the same allele for each protein of the plurality or a different allele for each protein of the plurality. The plurality can include at least 1, 2, 10, or 20 proteins.

The peptide can be a peptide described in Renkvist et al. (supra) and/or a peptide from a viral antigen, MUC1, TAX, gp100, or hTERT, e.g., a peptide in Table 1.

Each protein of the plurality can be attached to a support (e.g., an array), a display package, or a cell.

The invention also provides a method that includes providing a collection of proteins as described above, contacting a cell to each protein of the collection; and determining if the cell is bound by a protein of the collection. A related method includes eluting peptides from surfaces of cells; binding the peptides to an MHC protein to form complexes; determining if one or more proteins of the collection binds to one of the complexes.

In another aspect, the invention features a method that includes: providing a first nucleic acid segment encoding a heavy chain variable region and a second nucleic acid segment encoding a light chain variable region, wherein the heavy chain variable region and the light chain variable region form an antigen binding protein that binds an MHC-
target peptide complex if the target peptide is present; introducing said first and second nucleic acid segments into a cytotoxic cell; and maintaining the cytotoxic cell under conditions that allow expression and assembly of said antigen-binding protein. [0085] The introducing can include providing a virus that includes the first and second nucleic acid segments and infecting the cytotoxic cell with the virus. The introducing can be effected in vivo (e.g., in a subject animal) or ex vivo. The method can further include, after the introducing, administering the cytotoxic cell to a subject, e.g., a test animal, a patient, or a subject identified for a disorder, e.g., a neoplastic or autoimmune disorder.

[0086] In another aspect, the invention features a method of ablating or killing a target cell that displays a peptide on a surface MHC molecule. The method includes: contacting the target cell with a protein described herein, the protein specifically recognizing the displayed peptide on the surface MHC molecule of the target cell, and ablating or killing the target cell. For example, the target cell is a cancer cell.

[0087] The protein can include a cytotoxic agent. The protein can be attached to an effector cell, e.g., prior to contacting the protein to the target cell, during or after contacting the protein to the target cell.

[0088] In still another aspect, the invention features a method of treating or preventing a cancerous disorder in a subject. The method includes administering to the subject a cytotoxic entity or cytotoxic cell described herein in an amount effective to treat or prevent the disorder.

[0089] In another aspect, the invention features a method for in vivo imaging a subject. The method includes: administering to a subject a protein described herein, wherein the protein further comprises a label that can be in vivo imaged, and detecting distribution of the protein in the subject.

[0090] In still another aspect, the invention features a method for detecting an MHC-peptide complex in a sample. The method includes contacting the sample with a protein described herein; and detecting binding of the protein and the sample, wherein detection of binding indicates presence of the MHC-peptide complex in the sample.

[0091] The sample can include cells. The method can further include sorting the cells bound by the protein from cells not bound by the protein. For example, the protein is fluorescently labeled and the sorting comprises fluorescently activated cell sorted. In another example, the protein is attached to an insoluble support, e.g., a column matrix or a magnetic particle.

[0092] In another aspect, the invention features a method that includes: providing a first nucleic acid segment encoding a heavy chain variable region and a second nucleic acid segment encoding a light chain variable region, wherein the heavy chain variable region and the light chain variable region form an antigen binding protein that binds an MHC-target peptide complex if the target peptide is present; introducing said first and second nucleic acid segments into a host cell; and maintaining the host cell under conditions that allow expression and assembly of said antigen-binding protein. The target peptide can be a peptide fragment of gp100, MUC1, TAX, or hTERT, e.g., a peptide listed in Table 1.

[0093] The first and second nucleic acid segments can be segments of the same nucleic acid or of different nucleic acids. In one embodiment, the first and second nucleic acid segments are in frame and are translated as a single polypeptide. The nucleic acid can include a third segment that encodes a linker is located between the first and second nucleic acid segments. In another embodiment, the first and second nucleic acid segments are translated as separate polypeptide chains. The separate polypeptide chains can be covalently bond by a non-peptide bond.

[0094] The antigen binding protein can be soluble and secreted, or attached to a surface of the host cell. In the latter case, for example, the antigen binding protein can include a polypeptide that includes a transmembrane domain inserted into the host cell membrane, and optionally a cytoplasmic domain, e.g., a T cell receptor cytoplasmic domain.

[0095] The host cell can be a bacterial cell or a eukaryotic cell, e.g., a yeast, insect, plant, or mammalian cell (e.g., a human, rodent, dairy mammal cell). For example, the mammalian cell is a COS cell, or a T cell.

[0096] The introducing can occur in vitro or in vivo. The maintaining can occur in vitro or in vivo in a subject, e.g., the host cell is a cell of the subject, cell of a blood relative of an individual for treatment (e.g., shares a grandparent), a cell of a subject having the same MHC alleles as the individual for treatment.

[0097] In an embodiment, the T cell mediates a cytotoxic activity against a cell that includes a cell-surface MHC-peptide complex in which the cell-surface peptide is the target peptide. For example, the T cell is mediates a cytotoxic activity against a cancer cell.

[0098] The antigen binding protein can include a purification tag.

[0099] The method can further include purifying the antigen binding protein from media surrounding the cell, and/or from a lysate or membranes of the cell. The method can further include modifying the purified protein. The method can further include contacting the host cell to a cell that includes a cell-surface MHC-peptide complex in which the cell-surface peptide is the target peptide.

[0100] In still another aspect, the invention features a method that includes: providing a host cell that expresses a first nucleic acid segment encoding a heavy chain variable region and a second nucleic acid segment encoding a light chain variable region, wherein the expressed heavy chain variable region and the expressed light chain variable region assemble as an antigen binding protein that binds an epitope of a MHC-peptide complex, wherein the epitope includes a moiety of the MHC and a moiety of the peptide, and the peptide is a fragment of hTERT, MUC1, TAX or gp100; and harvesting the antigen-binding protein from the host cell. For example, the host cell is a cell of a transgenic animal, e.g., a mammal. The host cell can be a fibroblast, a mammary cell, an immune cell. In an embodiment, the antigen-binding protein further includes a purification tag. The method can further include purifying the harvested antigen-binding protein to at least 50, 70, 80, 90, 95, or 99% purity.

[0101] Further, the invention provides anti-(MHC-peptide complex) antibodies, antibody fragments, and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such antibodies and fragments. Methods of using the antibodies of the invention to detect a MHC-peptide complex, or to ablate or kill a cell that presents a particular MHC-peptide complex either in vitro or in vivo, are also encompassed by the invention. For example, the peptide is a cancer associated antigen.

[0102] The protein ligands of the invention interact with, e.g., bind to a MHC-peptide complex, preferably a human MHC-peptide complex, with high affinity and specificity.
Preferably, the protein ligand does not substantially bind the MHC in the absence of the bound peptide, and does not substantially bind the peptide in the absence of the MHC. The epitope bound by the protein ligand can include, e.g., a moiety of the MHC and a moiety of the bound peptide. The bound peptide can be a TAA.

[0103] For example, the protein ligand binds to a human MHC-peptide complex with an affinity constant of at least 10^10 M^-1, preferably, at least 10^10 M^-1, at least 10^9 M^-1, or at least 10^8 M^-1. In one embodiment, the anti-(MHC-peptide complex) ligand binds all or part of the epitope of an antibody described herein, e.g., an anti-(gp100 peptide-MHC complex) antibody (such as: 1A11, 1A7, 1A9, 1C8, 1D7, 1G2, 2B2, 2C5, 2D1, 2F1, 2G2D12, 3E21, 4C3, 5G3, or 5H2), an anti-(hTERT peptide-MHC complex) antibody (such as: 4A4, 4B4, 4C2, 4G9, 3A12, 3B1, 3F5, 3G3, or 3H2), an anti-(MUC1 peptide MHC complex) antibody (such as: M3A1 or M3B8), or an anti-(TAX peptide MHC complex) antibody (such as: T3E3, T3F1, or T3F2) (e.g., in which TAX is derived from HTLV-1). The anti-(MHC-peptide complex) ligand can inhibit, e.g., competitively inhibit, the binding of an antibody described herein, e.g., an anti-(gp100 peptide-MHC complex) antibody (such as: 1A11, 1A7, 1A9, 1C8, 1D7, 1G2, 2B2, 2C5, 2D1, 2F1, 2G2D12, 3E21, 4C3, 5G3, or 5H2), an anti-(hTERT peptide-MHC complex) antibody (such as: 4A4, 4B4, 4C2, 4G9, 3A12, 3B1, 3F5, 3G3, or 3H2), an anti-(MUC1 peptide MHC complex) antibody (such as: M3A1 or M3B8), or an anti-(TAX peptide MHC complex) antibody (such as: T3E3, T3F1, or T3F2). An anti-(MHC-peptide complex) ligand may bind to an epitope, e.g., a conformational or a linear epitope, which epitope when bound prevents binding of an antibody described herein, e.g., an anti-(gp100 peptide-MHC complex) antibody (such as: 1A11, 1A7, 1A9, 1C8, 1D7, 1G2, 2B2, 2C5, 2D1, 2F1, 2G2D12, 3E21, 4C3, 5G3, or 5H2), an anti-(hTERT peptide-MHC complex) antibody (such as: 4A4, 4B4, 4C2, 4G9, 3A12, 3B1, 3F5, 3G3, or 3H2), an anti-(MUC1 peptide MHC complex) antibody (such as: M3A1 or M3B8), or an anti-(TAX peptide MHC complex) antibody (such as: T3E3, T3F1, or T3F2). The epitope can be in close proximity spatially or functionally-associated, e.g., an overlapping or adjacent epitope in linear sequence or conformationally to the one recognized by an antibody described above or elsewhere herein. Preferably, the epitope includes a moiety from the peptide, e.g., from a peptide fragment of gp100, MUC1, or hTERT.

[0104] MHC-peptide complexes that include a TAA can identify a cancer cell. The antibodies of the invention bind to the cell surface of these cells, and in particular, to the cell surface of the living cells. Preferably, the protein ligands of the present invention are also internalized with the MHC-peptide complex, which permits the intracellular delivery of an agent conjugated to the antibody, e.g., a cytotoxic or a labeling agent. Accordingly, the protein ligands of the invention can be used to target living normal, benign hyperplastic, and cancerous cells that display on their surfaces TAA in an MHC-peptide complex.

[0105] In a preferred embodiment, the protein ligand is an antibody. As used herein, the term “antibody” refers to a protein comprising at least one, and preferably two, heavy (H) chain variable regions (abbreviated herein as VH), and at least one and preferably two light (L) chain variable regions (abbreviated herein as VL). The VH and VL regions can be further subdivided into regions of hypervariability, termed “complementarity determining regions” (“CDR”), interspersed with regions that are more conserved, termed “framework regions” (FR). The extent of the framework region and CDR’s has been precisely defined (see, Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, which are incorporated herein by reference). Each VH and VL is composed of three CDR’s and four FR’s, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0106] The VH or VL chain of the antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The term “antibody” includes intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof), wherein the light chains of the immunoglobulin may be of types kappa or lambda.

[0107] As used herein, the term “immunoglobulin” refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin “light chains” (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH- terminus (about 110 amino acids). Full-length immunoglobulin “heavy chains” (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

[0108] The term “antigen-binding fragment” of an antibody (or simply “antibody portion,” or “fragment”), as used herein, refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to a MHC-peptide complex (e.g., a human MHC-peptide complex, e.g., a complex wherein the peptide is a TAA). Examples of binding fragments encompassed within the term “antigen-binding fragment” of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes,
they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv or 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). Such single chain antibodies are also encompassed within the term “antigen-binding fragment” of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

**0109** The antibody is preferably monospecific, e.g., a recombinant antibody, a monoclonal antibody, or an antigen-binding fragment thereof. The term “monospecific antibody” refers to an antibody that displays a single binding specificity and affinity for a particular target, e.g., epitope, regardless of method of identification or synthesis. This term includes a “monoclonal antibody” or “monoclonal antibody composition,” which as used herein refer to a preparation of antibodies or fragments thereof of single molecular composition. The term also includes a “recombinant antibody” which is described below.

**0110** The anti-(MHC-peptide complex) antibodies can be full-length (e.g., an IgG (e.g., an IgG1, IgG2, IgG3, IgG4), IgM, IgA (e.g., IgA1, IgA2), IgD, and IgE) or can include only an antigen-binding fragment (e.g., a Fab, F(ab')2, or scFv fragment). The antibody, or antigen-binding fragment thereof, can include two heavy chain immunoglobulins and two light chain immunoglobulins, or can be a single chain antibody. The antibodies can, optionally, include a constant region chosen from a kappa, lambda, alpha, gamma, delta, epsilon or a mu constant region gene. A preferred anti-(MHC-peptide complex) antibody includes a heavy and light chain constant region substantially from a human antibody, e.g., a human IgG1 constant region or a portion thereof. As used herein, “isotype” refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

**0111** In a preferred embodiment, the antibody (or fragment thereof) is a recombinant or modified anti-(MHC-peptide complex) antibody, e.g., a chimeric, a humanized, a deimmunized, or an in vitro generated antibody. The term “recombinant” or “modified” human antibody, as used herein, is intended to include all antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial antibody library, antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant antibodies include humanized, CDR grafted, chimeric, deimmunized, in vitro generated antibodies, and may optionally include constant regions derived from human germline immunoglobulin sequences. In other embodiments, the anti-(MHC-peptide complex) antibody is a human antibody.

**0112** Also within the scope of the invention are antibodies, or antigen-binding fragments thereof, which bind overlapping epitopes, adjacent epitopes, and/or substantially identical epitopes (e.g., identical epitopes) of antibodies disclosed herein, e.g., an anti-(gp100 peptide-MHC complex) antibody (such as: 1A11, 1A7, 1A9, 1C8, 1D7, 1G2, 2B2, 2C5, 2D1, 2F1, G2D12, G3F12, G3F3, or G3G4), an anti-(hTERT peptide-MHC complex) antibody (such as: 4A9, 4B4, 4C2, 4G9, 3A12, 3B1, 3F5, 3G3, or 3H12), an anti-(MUC1 peptide MHC complex) antibody (such as: M3A1 or M3B8), or an anti(TAX peptide MHC) complex antibody (such as: T3E3, T3F1, or T3F2).

**0113** Also within the scope of the invention are antibodies, or antigen-binding fragments thereof, which competitively inhibit or compete with the binding of the anti-(MHC-peptide complex) antibodies disclosed herein, e.g., antibodies which competitively inhibit or compete with the binding of monospecific antibodies, e.g., an anti-(gp100 peptide-MHC complex) antibody (such as: 1A11, 1A7, 1A9, 1C8, 1D7, 1G2, 2B2, 2C5, 2D1, 2F1, G2D12, G3F12, G3F3, or G3G4), an anti-(hTERT peptide-MHC complex) antibody (such as: 4A9, 4B4, 4C2, 4G9, 3A12, 3B1, 3F5, 3G3, or 3H12), an anti-(MUC1 peptide MHC complex) antibody (such as: M3A1 or M3B8), or an anti(TAX peptide MHC) complex antibody (such as: T3E3, T3F1, or T3F2).

**0114** Any combination of anti-(MHC-peptide complex) antibodies is within the scope of the invention, e.g., two or more antibodies that bind to different regions of MHC-peptide complex, e.g., antibodies that bind to two different epitopes on the MHC-peptide complex, e.g., a bispecific antibody.

**0115** In one embodiment, the anti-(MHC-peptide complex) antibody, or antigen-binding fragment thereof, includes at least one light or heavy chain immunoglobulin (or preferably, at least one light chain immunoglobulin and at least one heavy chain immunoglobulin). Preferably, each immunoglobulin includes a light or a heavy chain variable region having at least one, two and, preferably, three complementarity determining regions (CDR’s) substantially identical to a CDR from an anti-(MHC-peptide complex) light or heavy chain variable region, respectively, i.e., from a variable region of one of an anti-(gp100 peptide-MHC complex) antibody (such as: 1A11, 1A7, 1A9, 1C8, 1D7, 1G2, 2B2, 2C5, 2D1, 2F1, G2D12, G3F12, G3F3, or G3G4), an anti-(hTERT peptide-MHC complex) antibody (such as: 4A9, 4B4, 4C2, 4G9, 3A12, 3B1, 3F5, 3G3, 3E7, 3C10, or 3H12), an anti-(MUC1 peptide MHC complex) antibody (such as: M3A1, M3C8, M2B1, or M3B8), or an anti(TAX peptide MHC) complex antibody (such as: T3E3, T3F1, or T3F2).

**0116** In a preferred embodiment, the antibody (or fragment thereof) includes at least one, two and preferably three CDR’s from the light or heavy chain variable region of antibodies listed in FIGS. 1 to 28 having an amino acid sequence chosen from the sequences in FIGS. 1A to 28A (light chain CDR’s, i.e., FIGS. 1A, 2A, 3A, . . . 38A), or FIGS. 1B to 28B (heavy chain CDR’s, i.e., FIGS. 1B, 2B, 3B, . . . 38B), or a sequence substantially identical thereto. The SEQ ID NO’s correspond to heavy and light CDR1, CDR2, or CDR3 of an antibody also listed in Table 2 to 5. In other embodiments, the antibody (or fragment thereof) can have at least one, two and preferably three CDR’s from the light or heavy chain variable region of an antibody listed in FIGS. 1 to 28 or listed above. In one preferred embodiment, the antibody, or antigen-binding fragment thereof, includes all six CDR’s from the human anti-(MHC-peptide complex) antibody, e.g., an antibody listed in FIGS. 1 to 28. In those embodiments, the CDR’s have the amino acid sequences in FIGS. 1A to 28A (light chain CDR’s, i.e., FIGS. 1A, 2A, 3A, . . . 38A), or FIGS. 1B to 28B (heavy chain CDR’s, i.e., FIGS. 1B, 2B, 3B, . . . 38B).
embodiment, the antibody heavy and light chain amino acid sequences are related (e.g., substantially identical to or variants of) respective heavy and light chain amino acid sequences of an antibody described herein.

[0117] In another preferred embodiment, the antibody (or fragment thereof) includes at least one, two and preferably three CDR’s from the light or heavy chain variable region of an antibody listed in FIGS. 1 to 28 having an amino acid sequence that differs by no more than 3, 2.5, 2, 1.5, 1, or 0.5 substitutions, insertions or deletions for every 10 amino acids relative to the amino acid sequences in FIGS. 1A to 28A (light chain CDR’s, i.e., FIGS. 1A, 2A, 3A, ... 38A), or FIGS. 1B to 28B (heavy chain CDR’s, i.e., FIGS. 1B, 2B, 3B, ... 38B). Further, the antibody, or antigen-binding fragment thereof, can include six CDR’s, each of which differs by no more than 3, 2.5, 2, 1.5, 1, or 0.5 substitutions, insertions or deletions for every 10 amino acids relative to the corresponding CDR’s of the human anti-(MHC-peptide complex) antibody, e.g., an antibody list in FIGS. 1 to 28.

[0118] In another embodiment, the light or heavy chain immunoglobulin of the anti-(MHC-peptide complex) antibody, or antigen-binding fragment thereof, can further include a light or a heavy chain variable framework that has no more than 3, 2.5, 2, 1.5, 1, or 0.5 substitutions, insertions or deletions for every 10 amino acids in FR1, FR2, FR3, or FR4 relative to the corresponding frameworks of an antibody listed in FIGS. 1 to 28. In a preferred embodiment, the light or heavy chain immunoglobulin of the anti-(MHC-peptide complex) antibody, or antigen-binding fragment thereof, further includes a light or a heavy chain variable framework, e.g., FR1, FR2, FR3, or FR4, that is identical to a framework of an antibody listed in FIGS. 1 to 28.

[0119] In one embodiment, the light or the heavy chain variable framework can be chosen from: (a) a light or heavy chain variable framework including at least 80%, 90%, 95%, or preferably 100% of the amino acid residues from a human light or heavy chain variable framework, e.g., a light or heavy chain variable framework residue from a human mature antibody or a human germline sequence, or a consensus sequence; (b) a light or heavy chain variable framework including from 20% to 80%, 40% to 80%, or 60% to 90% of the amino acid residues from a human light or heavy chain variable framework, e.g., a light or heavy chain variable framework residue from a human mature antibody or a human germline sequence, or a consensus sequence; (c) a non-human framework (e.g., a rodent framework); or (d) a non-human framework that has been modified, e.g., to remove antigenic or cytotoxic determinants, e.g., deimmunized, or partially humanized.

[0120] In one embodiment, the heavy or light chain framework includes an amino acid sequence, which is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or higher identical to a sequence listed in FIGS. 1 to 28 (A and B); or which differs at least 1 or 5 but less than 40, 30, 20, or 10 residues from the amino acid sequence listed in FIGS. 1 to 28 (A and B).

[0121] Preferred anti-(MHC-peptide complex) antibodies include at least one, preferably two, light and at least one, preferably two, heavy chain variable regions having the amino acid sequence shown in FIGS. 1 to 28 (A and B), the heavy and light chain combination being a combination shown.

[0122] In other embodiments, the light or heavy chain variable framework of the anti-(MHC-peptide complex) antibody, or antigen-binding fragment thereof, includes at least one, two, three, four, five, six, seven, eight, nine, ten, fifteen, sixteen, or seventeen amino acid residues from a human light or heavy chain variable framework, e.g., a light or heavy chain variable framework residue from a human mature antibody, a human germline sequence, or a consensus sequence. In one embodiment, the amino acid residue from the human light chain variable framework is the same as the residue found at the same position in a human germline. Preferably, the amino acid residue from the human light chain variable framework is the most common residue in the human germline at the same position.

[0123] An anti-(MHC-peptide complex) ligand described herein can be used alone, e.g., can be administered to a subject or used in vitro in non-derivatized or unconjugated forms. In other embodiments, the anti-(MHC-peptide complex) ligand can be derivatized, modified or linked to another functional molecule, e.g., another peptide, protein, isotope, cell, or insoluble support (e.g., a bead, a matrix, or a planar support such as an array). For example, the anti-(MHC-peptide complex) ligand can be functionally linked (e.g., by chemical coupling, genetic fusion, non-covalent association or otherwise) to one or more other molecular entities, such as an antibody (e.g., if the ligand is an antibody to form a bispecific or a multispecific antibody), a toxin, a radioisotope, a therapeutic (e.g., a cytotoxic or cytostatic) agent or moiety, among others. For example, the anti-(MHC-peptide complex) ligand can be coupled to a radioactive ion (e.g., a β-, γ-, or β-emitter), e.g., iodine (131I or 125I), yttrium (89Y), lutetium (177Lu), actinium (225Ac), rhenium (186Re), or bismuth (212Bi or 213Bi).

[0124] In another aspect, the invention provides compositions, e.g., pharmaceutical compositions, which include a pharmaceutically acceptable carrier, excipient or stabilizer, and at least one of the anti-(MHC-peptide complex) ligands (e.g., antibodies or fragments thereof) described herein. Preferably, the anti-(MHC-peptide complex) ligand does not substantially bind the MHC in the absence of the peptide, and does not substantially bind the peptide in the absence of the MHC. In one embodiment, the compositions, e.g., the pharmaceutical compositions, comprise a combination of two or more of the aforesaid anti-(MHC-peptide complex) ligands.

[0125] In another aspect, the invention features a kit that includes an anti-(MHC-peptide complex) antibody (or fragment thereof), e.g., an anti-(MHC-peptide complex) antibody (or fragment thereof) as described herein, for use alone or in combination with other therapeutic modalities, e.g., a cytotoxic or labeling agent, e.g., a cytotoxic or labeling agent as described herein, along with instructions on how to use the anti-(MHC-peptide complex) antibody or the combination of such agents to treat, prevent or detect cancerous lesions. Preferably, the antibody does not substantially bind the MHC in the absence of the peptide, and does not substantially bind the peptide in the absence of the MHC.

[0126] The invention also features nucleic acid sequences that encode a heavy and light chain immunoglobulin or immunoglobulin fragment described herein. For example, the invention features, a first and second nucleic acid encoding a heavy and light chain variable region, respectively, of an anti-(MHC-peptide complex) antibody molecule as described herein. In another aspect, the invention features host cells and vectors containing the nucleic acids of the invention.

[0127] In another aspect, the invention features, a method of producing a anti-(MHC-peptide complex) antibody, or antigen-binding fragment thereof. The method includes: providing a first nucleic acid encoding a heavy chain variable
region, e.g., a heavy chain variable region as described herein; providing a second nucleic acid encoding a light chain variable region, e.g., a light chain variable region as described herein; and expressing said first and second nucleic acids in a host cell under conditions that allow assembly of said light and heavy chain variable regions to form an antigen binding protein. The first and second nucleic acids can be linked or unlinked, e.g., expressed on the same or different vector, respectively.

[0128] The host cell can be a eukaryotic cell, e.g., a mammalian cell, an insect cell, a yeast cell, or a prokaryotic cell, e.g., E. coli. For example, the mammalian cell can be a cultured cell or a cell line. Exemplary mammalian cells include lymphocytic cell lines (e.g., NSO), Chinese hamster ovary cells (CHO), COS cells, oocyte cells, and cells from a transgenic animal, e.g., mammary epithelial cell. For example, nucleic acids encoding the antibodies described herein can be expressed in a transgenic animal. In one embodiment, the nucleic acids are placed under the control of a tissue-specific promoter (e.g., a mammary specific promoter) and the antibody is produced in the transgenic animal. For example, the antibody molecule is secreted into the milk of the transgenic animal, such as a transgenic cow, pig, horse, sheep, goat or rodent.

[0129] The invention also features a method of treating, e.g., ablating or killing, a cell, e.g., a normal, benign or hyperplastic cell (e.g., a cell found in pulmonary, breast, renal, urothelial, colonic, prostatic, or hepatic cancer and/or metastasis). Methods of the invention include contacting the cell with a (MHC-peptide complex) ligand, in an amount sufficient to treat, e.g., ablate or kill, the cell. The ligand can include another entity, e.g., a cytotoxic entity. The anti-(MHC-peptide complex) ligand can also be displayed on a cell surface, e.g., the surface of cytotoxic T lymphocytes that have been transfected with the genes encoding the ligand fused to a membrane anchor, thereby programming these T cells with the ligand’s specificity. Methods of the invention can be used, for example, to treat or prevent a disorder, e.g., a cancerous (e.g., a malignant or metastatic disorder), or non-cancerous disorder (e.g., a benign or hyperplastic disorder) by administering to a subject a anti-(MHC-peptide complex) ligand in an amount effective to treat or prevent such disorder.

[0130] The subject method can be used on cells in culture, e.g., in vitro or ex vivo. For example, cancerous or metastatic cells (e.g., pulmonary, breast, renal, urothelial, colonic, prostatic, or hepatic cancer or metastatic cells) can be cultured in vitro in culture medium and the contacting step can be effected by adding the anti-(MHC-peptide complex) ligand to the culture medium. The method can be performed on cells (e.g., cancerous or metastatic cells) present in a subject, as part of an in vivo (e.g., therapeutic or prophylactic) protocol. For in vivo embodiments, the contacting step is effected in a subject and includes administering the anti-(MHC-peptide complex) ligand to the subject under conditions effective to permit both binding of the ligand to the cell, and the treating, e.g., the killing or ablating of the cell.

[0131] The method of the invention can be used to treat or prevent cancerous disorders, e.g., including but are not limited to, solid tumors, soft tissue tumors, and metastatic lesions. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary tract (e.g., renal, urothelial cells), pharynx, as well as adenocarcinomas which include malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Metastatic lesions of the aforementioned cancers can also be treated or prevented using the methods and compositions of the invention.

[0132] The subject can be a mammal, e.g., a primate, preferably a higher primate, e.g., a human (e.g., a patient having, or at risk of, a disorder described herein, e.g., cancer).

[0133] The anti-(MHC-peptide complex) antibody or fragment thereof, e.g., an anti-(MHC-peptide complex) antibody or fragment thereof as described herein, can be administered to the subject systemically (e.g., orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intranasally, transdermally, or by inhalation), topically, or by application to mucous membranes, such as the nose, throat and bronchial tubes.

[0134] The methods of the invention can further include the step of monitoring the subject, e.g., for a reduction in one or more of: a reduction in tumor size; reduction in cancer markers, e.g., levels of cancer specific antigen (i.e., TAA) levels of a cancer specific MHC-peptide complex; reduction in the appearance of new lesions, e.g., in a bone scan; a reduction in the appearance of new disease-related symptoms; or decreased or stabilization of size of soft tissue mass; or any parameter related to improvement in clinical outcome. The subject can be monitored in one or more of the following periods: prior to beginning of treatment; during the treatment; or after one or more elements of the treatment have been administered. Monitoring can be used to evaluate the need for further treatment with the same anti-(MHC-peptide complex) ligand or for additional treatment with additional agents. Generally, a decrease in one or more of the parameters described above is indicative of the improved condition of the subject.

[0135] The anti-(MHC-peptide complex) ligand can be used alone in unconjugated form to thereby ablate or kill cells that present a TAA. For example, if the ligand is an antibody, the ablation or killing can be mediated by an antibody-dependent cell killing mechanisms such as complement-mediated cell lysis and/or effector cell-mediated cell killing. In other embodiments, the anti-(MHC-peptide complex) ligand can be bound to a substance, e.g., a cytotoxic agent or moiety, effective to kill or ablate the cells. For example, the anti-(MHC-peptide complex) ligand can be coupled to a radiopharmaceutical (e.g., an α-, γ-, or β-emitter), e.g., iodine (131I) or (125I), yttrium (89Y), lutetium (177Lu), actinium (225Ac), or bismuth (213Bi). The methods and compositions of the invention can be used in combination with other therapeutic modalities. In one embodiment, the methods of the invention include administering to the subject a anti-(MHC-peptide complex) ligand, e.g., a anti-(MHC-peptide complex) antibody or fragment thereof, in combination with a cytotoxic agent, in an amount effective to treat or prevent said disorder. The ligand and the cytotoxic agent can be administered simultaneously or sequentially. In other embodiments, the methods and compositions of the invention are used in combination with surgical and/or radiation procedures.

[0136] In another aspect, the invention features methods for detecting the presence of a particular MHC-peptide complex, in a sample, in vitro (e.g., a biological sample, a tissue biopsy, e.g., a cancerous lesion). The subject method can be used to evaluate, e.g., diagnose or stage a disorder described herein, e.g., a cancerous disorder. The method includes: (i) contact-
ing the sample (and optionally, a reference, e.g., control, sample) with an anti-(MHC-peptide complex) ligand, as described herein, under conditions that allow interaction of the anti-(MHC-peptide complex) ligand and the MHC-peptide complex protein to occur; and (ii) detecting formation of a complex between the anti-(MHC-peptide complex) ligand, and the sample (and optionally, the reference, e.g., control, sample). Formation of the complex is indicative of the presence of MHC-peptide complex protein, and can indicate the suitability or need for a treatment described herein. E.g., a statistically significant change in the formation of the complex in the sample relative to the reference sample, e.g., the control sample, is indicative of the presence of MHC-peptide complex in the sample.

In yet another aspect, the invention provides a method for detecting the presence of a particular MHC-peptide complex in vivo (e.g., in vivo imaging in a subject). The subject method can be used to evaluate, e.g., diagnose, localize, or stage a disorder described herein, e.g., a cancerous disorder. The method includes: (i) administering to a subject (and optionally a control subject) an anti-(MHC-peptide complex) ligand (e.g., an antibody or antigen binding fragment thereof), under conditions that allow interaction of the anti-(MHC-peptide complex) ligand and the MHC-peptide complex protein to occur; and (ii) detecting formation of a complex between the ligand and MHC-peptide complex, wherein a statistically significant change in the formation of the complex in the subject relative to the reference, e.g., the control subject or subject's baseline, is indicative of the presence of the particular MHC-peptide complex.

In other embodiments, a method of diagnosing or staging, a disorder as described herein, e.g., a cancerous disorder, is provided. The method includes: (i) identifying a subject having, or at risk of having, the disorder; (ii) obtaining a sample of a tissue or cell affected with the disorder; (iii) contacting said sample or a control sample with an anti-(MHC-peptide complex) ligand, under conditions that allow interaction of the ligand and the MHC-peptide complex to occur, and (iv) detecting the interaction. Preferably, the anti-(MHC-peptide complex) ligand does not substantially bind the MHC in the absence of the bound peptide, and does not substantially bind the peptide in the absence of the MHC. For example, the peptide can be a TAA. A statistically significant increase in the formation of the complex between the ligand with respect to a reference sample, e.g., a control sample, is indicative of the disorder or the stage of the disorder.

Preferably, the anti-(MHC-peptide complex) ligand used in the in vivo and in vitro diagnostic methods is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound binding agent. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. In one embodiment, the anti-(MHC-peptide complex) ligand is coupled to a radioactive ion. In another embodiment, the ligand is labeled with an NMR contrast agent.

The invention also provides polypeptides and nucleic acids that encompass a range of amino acid and nucleic acid sequences. The term “polypeptide” refers to a linear polymer of two or more amino acid residues linked with peptide bonds, and the term “peptide” is used herein to refer to short polypeptides that have fewer than about 30 amino acids.

Plasmids encoding proteins described herein may be deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209 under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

An “isolated” or “purified” polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. “Substantially free” means that a preparation of a protein is at least 10% pure. In a preferred embodiment, the preparation of the protein has less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of another protein (also referred to herein as a “contaminating protein”), or of chemical precursors. When the protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium and/or containing cellular contents (e.g., endogenous proteins of the recombinant cell), i.e., the other material represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

As used herein, a “naturally-occurring” nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in Nature. For example a naturally occurring nucleic acid molecule can encode a natural protein. Likewise, a “naturally-occurring” protein refers to a protein having an amino acid sequence that occurs in Nature.

A “heterologous” sequence refers to a sequence which is introduced into a cell or into the context of a nucleic acid by artifice. A heterologous sequence may be a copy of an endogenous gene, but, for example, inserted into an exogenous plasmid or into a chromosomal site at a position other than its endogenous position.

The term “isolated nucleic acid molecule” or “purified nucleic acid molecule” includes nucleic acid molecules that are separated from other nucleic acid molecules present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term “isolated” includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated.

As used herein, the term “substantially identical” (or “substantially homologous”) is used herein to refer to a first amino acid or nucleotide sequence that contains a sufficient number of identical or equivalent (e.g., with a similar side chain, e.g., conserved amino acid substitutions) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have similar activities. In the case of antibodies, the second antibody has the same specificity and has at least 50% of the affinity of the same.

Sequences similar or homologous (e.g., at least about 85% sequence identity) to the sequences disclosed herein are also part of this application. In some embodiments, the sequence identity can be about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher. Alternatively, substantial identity exists when the nucleic acid segments will hybridize under selective hybridization conditions (e.g., highly stringent hybridization conditions), to the
complement of the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

[0148] Calculations of “homology” or “sequence identity” between two sequences (the terms are used interchangeably herein) are performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid “identity” is equivalent to amino acid or nucleic acid “homology”). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0149] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ([1970] J. Mol. Biol. 48:444-453) algorithm which has been incorporated into the GAP program in the GCG® software package (available from Accelrys, San Diego Calif.), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GTP program in the GCG® software package, using a NWSgapdna. CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0150] As used herein, the term “homologous” is synonymous with “similarity” and means that a sequence of interest differs from a reference sequence by the presence of one or more amino acid substitutions (although modest amino acid insertions or deletions) may also be present. Presently preferred means of calculating degrees of homology or similarity to a reference sequence are through the use of BLAST algorithms (available from the National Center of Biotechnology Information (NCBI), National Institutes of Health, Bethesda Md.), in each case, using the algorithm default or recommended parameters for determining significance of calculated sequence relatedness. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of E. Meyers and W. Miller ([1989] CABIOS: 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0151] As used herein, the term “hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions” describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6x sodium chloride/sodium citrate (SSC) at about 45° C., followed by two washes in 0.2xSSC, 0.1% SDS at least at 50° C. (the temperature of the washes can be increased to 55° C. for low stringency conditions); 2) medium stringency hybridization conditions in 6xSSC at about 45° C., followed by one or more washes in 0.2xSSC, 0.1% SDS at 60° C.; 3) high stringency hybridization conditions in 6xSSC at about 45° C., followed by one or more washes in 0.2xSSC, 0.1% SDS at 65° C.; and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65° C., followed by one or more washes at 0.2xSSC, 1% SDS at 65° C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified.

[0152] It is understood that the binding agent polypeptides of the invention may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on the polypeptide functions. Whether or not a particular substitution will be tolerated, i.e., will not adversely affect desired biological properties, such as binding activity can be determined as described in Bowie et al. (1990) Science 247:1306-1310. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0153] A “non-essential” amino acid residue is a residue that can be altered from the wild-type sequence of the binding agent, e.g., the antibody, without abolishing or more preferably, without substantially altering a biological activity, whereas an “essential” amino acid residue results in such a change.

[0154] Other features and advantages of the instant invention will become more apparent from the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0155] FIGS. 1A and 1B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 1A11, respectively.

[0156] FIGS. 2A and 2B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 1A7, respectively.
FIGS. 3A and 3B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 1A9, respectively.

FIGS. 4A and 4B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 1C8, respectively.

FIGS. 5A and 5B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 1D7, respectively.

FIGS. 6A and 6B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 1G2, respectively.

FIGS. 7A and 7B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 2B2, respectively.

FIGS. 8A and 8B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 2C5, respectively.

FIGS. 9A and 9B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 2D1, respectively.

FIGS. 10A and 10B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 2F1, respectively.

FIGS. 11A and 11B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody G2D12, respectively.

FIGS. 12A and 12B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody G3F12, respectively.

FIGS. 13A and 13B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody G3F3, respectively.

FIGS. 14A and 14B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody G3G4, respectively.

FIGS. 15A and 15B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody G3M1, respectively.

FIGS. 16A and 16B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody G3M3, respectively.

FIGS. 17A and 17B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody T3F3, respectively.

FIGS. 18A and 18B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody T3F1, respectively.

FIGS. 19A and 19B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody T3F2, respectively.

FIGS. 20A and 20B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 4A9, respectively.

FIGS. 21A and 21B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 4B4, respectively.

FIGS. 22A and 22B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 4C2, respectively.

FIGS. 23A and 23B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 4C9, respectively.

FIGS. 24A and 24B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 3A12, respectively.

FIGS. 25A and 25B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 3B1, respectively.

FIGS. 26A and 26B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 3F5, respectively.

FIGS. 27A and 27B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 3G3, respectively.

FIGS. 28A and 28B list the coding nucleic acid and predicted amino acid sequence of the light chain and heavy chain variable region of antibody 3F12, respectively.

FIGS. 29A-29D. Functional characterization of recombinant sCHLA-A2-peptide complexes. (A-D) Functional analysis of sCHLA-A2/gp100-derived complexes showing the ability of tetramers to stain a CTL clone, R6C12, specific for the gp100-derived peptide G9-209 in complex with HLA-A2 (A). Staining of more than 70% of the cell population is observed in comparison with control tetramers containing the G9-280 gp100-derived peptide (B) and TAP-derived peptide (C) that did not stain the CTLs. The cells were double-stained with PE-labeled tetramers (y-axis) and FITC-labeled anti-CD8 antibody (x-axis). In (D) the histogram overlay of tetramer staining in A-C is shown to demonstrate the specificity pattern of the recombinant HLA-A2-peptide complexes.

FIGS. 30A-30C. Specificity analysis of phage clones selected on gp100-derived HLA-A2-restricted peptides. Phage clones (10^6 plaques/well) derived mainly from the third round of selection on gp100-derived peptides G9-154 (A), G9-209 (B), and G9-280 (C) were tested for binding specificity on the various immobilized sCHLA-A2/peptide complexes as indicated. Clones 1G2(II) and 2DII(I) are from the second round of panning. Shown is the specificity pattern of phage clones with the MHC-peptide complex to which they were selected for but not with control MHC-peptide complexes containing either a different gp100-derived epitope or other control HLA-A2-restricted peptides.

FIGS. 31A-31D. Binding in ELISA of soluble purified Fabs to recombinant sCHLA-A2-peptide complexes. Binding of soluble purified Fab clones specific for the gp100-derived epitopes G9-154 (A), G9-209 (B), and G9-280 (C) to immobilized sCHLA-A2/peptide complexes as indicated. Shown are the specificities of several Fab clones to the gp100-derived epitopes to which they were selected for but not to the indicated control MHC-peptide complexes containing other gp100 and telomerase-derived HLA-A2-restricted epitopes. (D) The ELISA binding specificity results were confirmed in competition experiments, in which excess specific and control soluble sMHC-peptide complexes were present in solution and competed for binding to the immobilized complex. Competition was observed with the specific soluble MHC-peptide complex but not with control complexes. An example for this type of assay is shown in Fig. 31D, in which soluble G9280 containing HLA-A2 but not G9154/HLA-A2 complexes in solution competed and inhibited the binding of Fab 2F1 to the immobilized G9280/HLA-A2 complexes.

FIGS. 32A-32F. Binding characteristics of three TCR-like Fabs. (A-C) Titration ELISA of purified soluble Fab antibodies G2D12 (A), 1A9 (B), and 2F1 (C) directed to sCHLA-A2 containing the G9-154, G9-209, and G9-280 pep-
tides, respectively. Wells were coated with the corresponding MHC-peptide complexes as described M&M. (D-F) Competitive binding analysis of the ability of purified Fab G2D12 (D), IAG (E), and 2F1 (F) directed against sHLA-A2-peptide complexes containing the G9-154, G9-209, and G9-280 gp100-derived peptides, respectively to inhibit the binding of 125I-labeled G2D12, IAG or 2F1 to the corresponding HLA-A2-peptide complex. The apparent binding affinity of the recombiant Fab was determined as the concentration of competitor (soluble purified Fab) required for 50% inhibition of the binding of the 125I-labeled tracer.

[0187] FIGS. 33A-33H. Detection of MHC-peptide complexes on the surface of tumor cells. Melanoma FM3D(A) and YU ZA6(C) which express HLA-A2 (B and D) as determined by reactivity with MAb BB7.2 were stained with 5, 10, and 20 μg of Fab G2D12 specific for the melanoma gp100-derived G9-154 epitope or with a Fab TCR-like antibody specific for the viral epitope TAP. Detection of binding was with FITC-labeled anti-human Fab. The melanoma HLA-A2-M2-MEL.3 cells were not stained with G2D12 (E) or BB7.2 (F) (indication for HLA-A2*). MCF7-HLA-A2* breast carcinoma cells were stained with BB7.2 (E) but neither with Fab G2D12 or the TAP-specific Fab (G). Control cells are cells incubated with the secondary FITC-labeled antibody.

[0188] FIGS. 34A-34C. Frequency (A) and specificity (B,C) of recombiant Fab antibodies selected on telomerase-derived HLA-A2-restricted peptides. ELISA with phage particles was performed on immobilized sHLA-A2/peptide complexes as described in Materials and Methods. (A) Summary of mapping against hTERT T cell epitope T540 and T865 in complex with sHLA-A2. (B) Phage ELISA of clones selected against sHLA-A2/T540 peptide. (clones 4C2(II), 4B4(II) and 4E7(II) are from the second round of panning and clones 4A9 and 4G9 are from the third round). (C) Phage ELISA of clones selected against sHLA-A2/T865. (clones 3F5(II), 3B1(II) and 3C10(II) are from the second round of panning and clones 3H2, 3G3, and 3A12 are from the third round).

[0189] FIGS. 35A-35B. Binding of soluble purified Fab antibodies with TCR-like specificity in ELISA (A+B) Binding of soluble Fab’s to immobilized MHC-peptide complexes containing various HLA-A2-restricted peptides. In (A) Fab clones selected against sHLA-A2/T540 complexes; in (B) Fab clones selected against sHLA-A2/T865 complexes.

[0190] FIGS. 36A-36D. Binding characteristics of two recombinant TCR-like Fab antibodies. (A+B) Titration ELISA of purified soluble Fab antibodies 4A9 (A) and 3H2 (B) directed to sHLA-A2/T540 and sHLA-A2/T865, respectively. Wells were coated with the corresponding MHC-peptide complexes as described M&M. (C+D) Competitive binding analysis of the ability of purified Fab 4G9 (C) or 3G3 (D) to inhibit the binding of 125I-labeled Fab to the corresponding HLA-A2-peptide complex. The apparent binding affinity of the recombinant Fab was determined as the concentration of competitor (soluble purified Fab) required for 50% inhibition of the binding of the 125I-labeled tracer.

[0191] FIGS. 37A-37T. Detection of HLA-A2/Telomerase-derived peptide complexes on tumor cells. HLA-A2 positive FM3D melanoma, LnCap prostate carcinoma, HeLa epithelial carcinoma cells or hTERT-transfected human foreskin fibroblasts and control non-transfected cells (10^6) expressing telomerase were incubated with Fab antibodies 4A9 and 3H2 specific for the HLA-A2/T540 and HLA-A2/T865 complexes, respectively. Binding was detected using FITC-labeled anti-human Fab. The HLA-A2 negative but hTERT-positive prostate carcinoma PC3 cells are used as control. FM3D cells stained with 4A9, 3H2, and control Fab directed against a mucin peptide in complex with HLA-A2. Cells stained with secondary FITC-labeled anti-human Fab are in black throughout. LnCap cells stained with 4A9, or 3H2; HeLa cells stained with 3H2, or control Fab directed to a melanoma gp100-derived peptide in complex with HLA-A2; PC3 cells stained with 4A9, or 3H2; hTERT-transfected human fibroblasts stained with 4A9, 3H2, or a control melanoma specific Fab; Control non-transfected fibroblasts stained with 4A9, 3H2, or control Fab.
acids), or a multi-chain protein (e.g., including at least two peptides or polypeptides). For example, the protein can be, e.g., a small peptide or modified protein scaffold. The invention also provides a variety of methods of using such proteins, e.g., for research, diagnostic, therapeutic, and prophylactic applications.

0198. The identified proteins that recognize these MHC-peptide complexes can discriminate between different peptide sequences bound in the complex. In some embodiments, the identified proteins also are specific or at least partially specific for the allele of the MHC component of the complex.

0199. The invention also provides particular antibodies that bind to particular MHC-peptide complexes.

0200. MHC-Peptide Complexes

0201. MHC-peptide complexes include two components: the peptide component and the MHC component. The peptide component is bound in an extended conformation in the groove of the MHC component. The peptide component is typically of less than 30 amino acids.

0202. The MHC component is a major-histocompatibility complex. There are two principal classes of MHC complexes: Class I and Class II. Each complex includes a heterodimer of two polypeptide chains.

0203. Class I complexes are formed from an α polypeptide and β2-microglobulin. The α polypeptide is a transmembrane protein with three extracellular globular domains, α1, α2, and α3. Each chain is non-covalently associated with a small extracellular protein, β2-microglobulin. The α chain is also highly polymorphic. Class I molecules are present on the surfaces of almost all nucleated cells.

0204. The three-dimensional crystal structure of the Class I complex with peptide bound has been described, e.g., in Bjorkman et al. (1987) Nature 329:506-512. Peptides of about eight to ten amino acids are bound in an extended conformation in the peptide binding site.

0205. Class II molecules are formed from two chains, α and β. Both chains include a transmembrane domain, an immunoglobulin domain, and an amino-terminal peptide binding domain. The peptide binding domain of both chains is polymorphic. Class II molecules are present on the surfaces of a restricted number of antigen-presenting cells, such as B lymphocytes and macrophages.

0206. The three-dimensional crystal structure of the Class II complex with peptide bound has been described, e.g., in Fremont et al. (1998) Immunity 8:305-17. The peptide binding site of Class II molecules resembles that of the Class I molecules. However, it can bind longer (e.g., 15 to 24 amino acids) and more heterogeneous peptides.

0207. The peptide component is the product of intracellular processing of an antigen. The TAP pathway insures that MHC complexes that are secreted to the cell surface include a peptide component from a processed antigen. Thus, processed antigens are displayed on the surface of the cell, indicating to the immune system if any intracellular contents are foreign or aberrant.

0208. T cells include T cell receptors that specifically recognize MHC-peptide complexes. Each T cell receptor has its own specificity for an MHC-presented peptide. An engaged T cell receptor activates the T cell, in the case of cytotoxic T lymphocytes (CTLs) to kill the cell presenting the recognized antigenic peptide.

MHC Complexes and Cancer.

0209. Tumor cells can be identified by antigens that are differentially expressed in tumor cells relative to non-tumor cells. Some of these antigens are processed by the proteasome into peptide fragments that are assembled with an MHC molecule and displayed on the surface of the cell as a complex with the MHC. These antigens, termed “tumor-associated antigens” or TAs, present epitopes that can be specifically recognized by T cells. Renkvist et al. (2001) Cancer Immunol Immunother 50:3-15 tabulate many known T-cell defined epitopes. The methods described here can be used to identify protein ligands that specifically recognize these T-cell defined epitopes, e.g., immuno-globulins that specifically recognize the peptide component of the epitope when bound to an MHC molecule.

0210. In therapeutic applications, tumor-specific T-cell defined epitopes distinguish a tumor cell from surrounding normal cells. Accordingly, a protein ligand that specifically recognizes one of these epitopes can specifically deliver a cytoytic activity to the tumor cell but not to normal cells, particularly, surrounding normal cells. In diagnostic and research applications, recognition of the tumor-specific T-cell defined epitopes by a protein ligand identifies that a tumor cell is present.

0211. In some instances, natural T cell mediated reactivity against tumors has been observed (Boon and van der Bruggen (1996) J Exp Med 185:725-9; Rosenberg (2001) Nature 411:380-4; Renkvist et al. (2001) Cancer Immunol Immunother 50:3-15). Hence, it is desirable to devise T-cell mediated cancer therapies. In particular, protein ligands that specifically recognize particular peptide-MHC complexes are used to direct T cell cytoxicity against cancer cells. (See also, “T-Cell Reprogramming,” below).

MHC Complexes and Pathogens

0212. MHC complexes also present peptide fragments from antigens of pathogens, particularly intracellular pathogens, e.g., viruses, intracellular bacteria, and other organisms. Thus, MHC proteins provide a natural defense against pathogens that attempt to avoid immune surveillance by spending, in some cases, substantial portions of their life cycle within an infected cell. Further, in many cases, the pathogens can remain latent within the cell for extended times.

0213. A protein ligand that specifically recognizes a peptide derived from a pathogen when presented on an MHC protein can be used in therapeutic and diagnostic modes. As described for applications for cancer cells, the protein ligand can be used to deliver a cytoxic to kill the infected cell. In addition, the protein ligand can be used for in vivo imaging to locate infected cells within a subject and in vitro to assay a sample for an infected cell or for a processed peptide that originated from the pathogen.

0214. Identification of MHC-Peptide Binding Proteins

0215. The invention provides methods for identifying protein ligands that bind to MHC-peptide complexes. The methods can be used to identify protein ligands that bind only if the particular peptide is present in the complex, and not if the particular peptide is absent or if another, non-overlapping or unrelated peptide is present. In many cases, the identified proteins are at least partially specific. An exemplary identified protein may bind to MHC-peptide complex if the particular peptide is present, and also bind if a related peptide that has two substitutions relative to the particular peptide is present.

0216. The identified protein may be a small peptide (e.g., a peptide of between 7 and 20 amino acids), a polypeptide
(e.g., a polypeptide of at least 20 amino acids), or a multi-chain protein (e.g., including at least two peptides or polypeptides).

[0217] The inventors unexpectedly discovered numerous human Fab fragments that bind to MHC-peptide complexes from a display library prepared from mRNA of B-cells expressing immunoglobulin genes that predominantly have no or few mutations with respect to germline (see "EXAMPLES" below). Among other features, these discoveries indicate the use of a single-chain MHC complex for peptide presentation during screening and the use of a display library constructed from an immunized subject, particularly a subject having the same MHC allele as the MHC-peptide complex that is the target.

[0218] The methods include: providing a library (e.g., an expression library, e.g., a display library) and screening the library to identify a member whose polypeptide component binds to an MHC-peptide complex.

[0219] The screening can be performed in a number of ways.

[0220] In one embodiment, a bacteriically prepared MHC class Ia polypeptide and β2-microglobulin are purified, e.g., from bacterial inclusion bodies. These proteins are denatured and refolded in vitro in the presence of the peptide component of the MHC-peptide complex. Further, a chain and the β2-microglobulin can be covalently linked, e.g., by an approximately 15 amino acid linker, e.g., as described in Denkberg and Reiter (2000) Eur. J. Immunol. 30:3522-32. One of the chains, e.g., the α chain, can include a purification handle such as the BirA sequence that is biotinylated or the hexahistidine tag. This purified complex can be screened against the display library to identify members of the library the bind the MHC-peptide complex.

[0221] Bacterial purification and refolding improve the homogeneity of the MHC-peptide complex. The particular peptide of interest which is incorporated in vitro into the complex does not have to compete with a large number of cellular peptides for binding to the MHC complex and, e.g., results in a homogenous target for binding the display library against.

[0222] In another embodiment, cells of interest (e.g., cancer cells or infected cells) are attached to a support, and a display library is contacted to the cells. Members of the library that bind to the cells are isolated and characterized. For example, the cells can be isolated from a patient or prepared using a laboratory model for a disease.

[0223] In still another embodiment, tissue culture cells that are deficient in TAP2 activity are used. For example, MASH-HHD cells can be used. The cells are transfected with a nucleic acid that expresses an MHC protein having an allele of interest. The transfected cells are loaded with a peptide of interest. Then, the display library is contacted to the cell to identify display library members that specifically bind to the cells. In another embodiment, a gene encoding the polypeptide of interest is co-transfected into a cell and expressed therein. The cell naturally processes the polypeptide and displays processed peptides in the MHC-Class I context.

[0224] The methods include: providing a library and screening the library to identify a member that encodes a protein that binds to the MHC-peptide complex. Preferably, the protein does not substantially bind the MHC in the absence of the bound peptide, and does not substantially bind the peptide in the absence of the MHC. The peptide can be, e.g., a TAA. The screening can be performed in a number of ways. For example, the library can be a display library.

[0225] The MHC component of the complex can be tagged and recombinantly expressed. The recombinant MHC is reconstituted with the peptide, e.g., that is produced synthetically. The MHC-peptide complex is attached to a support, e.g., to paramagnetic beads or other magnetically responsive particle.

[0226] The MHC complex can also be expressed on the surface of a cell. The display library can be screened to identify members that specifically bind to the cell, e.g., only if the MHC complex displays the peptide of interest.

[0227] Display Libraries

[0228] A display library is used to identify proteins that bind to the MHC-peptide complex and recognize the peptide moiety of the complex. A display library is a collection of entities; each entity includes an accessible variable protein component and a recoverable component that encodes or identifies the variable protein component. The protein component can be of any length, e.g., from three amino acids to over 300 amino acids. In a selection, the variable protein component of each member of the library is probed with the MHC-peptide complex and if the variable protein component binds to the MHC-peptide complex, then the display library member is identified, typically by retention on a support.

[0229] Retained display library members are recovered from the support and analyzed. The analysis can include amplification and a subsequent selection under similar or dissimilar conditions. For example, positive and negative selections can be alternated. The analysis can also include determining the amino acid sequence of the selected protein component and purification of the polypeptide component for detailed characterization.

[0230] A variety of formats can be used for display libraries. Examples include the following.


[0232] Phage display systems have been developed for filamentous phage (phage II, fd, and M13) as well as other bacteriophage (e.g., T7 bacteriophage and lambdoid phages; see, e.g., Santini (1998) J. Mol. Biol. 282:125-135; Rosenberg et al. (1996) Innovations 6:1-6; Househ et al. (1999) Anal Bio-
The filamentous phage display systems typically use fusions to a minor coat protein, such as gene III protein, and gene VIII protein, a major coat protein, but fusions to other coat proteins such as gene VI protein, gene VII protein, gene IX protein, or domains thereof can also be used (see, e.g., WO 00/71694). In a preferred embodiment, the fusion is to a domain of the gene III protein, e.g., the anchor domain or “stump,” (see, e.g., U.S. Pat. No. 5,658,727 for a description of the gene III protein anchor domain).

The valency of the varied protein component can also be controlled. Cloning of the sequence encoding the varied protein component into the complete phage genome results in multivalent display since all replicates of the gene III protein are fused to the varied protein component. For reduced valency, a phagemid system can be utilized. In this system, the nucleic acid encoding the varied protein component fused to gene III is provided on a plasmid, typically of length less than 700 nucleotides. The plasmid includes a phage origin of replication so that the plasmid is incorporated into bacteriophage particles when bacterial cells bearing the plasmid are infected with helper phage, e.g., M13K01. The helper phage provides an intact copy of gene III and other phage genomes required for phage replication and assembly. The helper phage has a defective origin such that the helper phage genome is not efficiently incorporated into phage particles relative to the plasmid that has a wild type origin.

Bacteriophage displaying the varied protein component can be grown and harvested using standard phage preparatory methods, e.g., PEG precipitation from growth media.

After selection of individual display phages, the nucleic acid encoding the selected varied protein components, by infecting cells using the selected phages. Individual colonies or plaques can be picked, the nucleic acid isolated and sequenced.

Cell-based Display. In still another format the library is a cell-display library. Proteins are displayed on the surface of a cell, e.g., a eukaryotic or prokaryotic cell. Exemplary prokaryotic cells include E. coli cells, B. subtilis cells, spores (see, e.g., Lu et al. (1995) Biotechnology 13:366). Exemplary eukaryotic cells include yeast (e.g., Saccharomyces cerevisiae, Schizosaccharomyces pombe, Hansenula, or Pichia pastoris). Yeast surface display is described, e.g., in Bodor and Wittrup (1997) Nat. Biotechnol. 15:553-557. U.S. Provisional Patent Application Ser. No. 60/326,320, filed Oct. 1, 2001, describes a yeast display system that can be used to display immunoglobulin proteins such as Fab fragments.

In one embodiment, nucleic acid encoding immunoglobulin variable domains are cloned into a vector for yeast display. The cloning joins the nucleic acid encoding at least one of the variable domains with nucleic acid encoding a fragments of a yeast cell surface protein, e.g., Flo1, a-agglutinin, α-agglutinin, or, fragments derived thereof e.g., Aga2p, Aga1p. A domain of these proteins can anchor the polypeptide encoded by the diversified nucleic acid sequence by a GPI-anchor (e.g. a-agglutinin, α-agglutinin, or, fragments derived thereof e.g. Aga2p, Aga1p), by a transmembrane domain (e.g., Flo1). The vector can be configured to express two polypeptide chains on the cell surface such that one of the chains is linked to the yeast cell surface protein. For example, the two chains can be immunoglobulin chains.


Peptide-Nucleic Acid Fusions. Another format utilizes peptide-nucleic acid fusions. Polypeptide-nucleic acid fusions can be generated by the in vitro translation of mRNA that include a covalently attached puromycin group, e.g., as described in Roberts and Sostak (1997) Proc. Natl. Acad. Sci. USA 94:12297-12302, and U.S. Pat. No. 6,207,446. The mRNA can then be reverse transcribed into DNA and crosslinked to the polypeptide.

Other Display Formats. Yet another display format is a non-biological display in which the varied protein component is attached to a non-nucleic acid tag that identifies the polypeptide. For example, the tag can be a chemical tag attached to a bead that displays the polypeptide or a radiofrequency tag (see, e.g., U.S. Pat. No. 5,874,214).

Scaffolds. Criteria for evaluating a scaffolding domain can include: (1) amino acid sequence, (2) sequences of several homologous domains, (3) 3-dimensional structure, and/or (4) stability data over a range of pH, temperature, salinity, organic solvent, oxidant concentration. In one embodiment, the scaffolding domain is a small, stable protein domains, e.g., a protein of less than 100, 70, 50, 40 or 30 amino acids. The domain may include one or more disulfide bonds or may chelate a metal, e.g., zinc.

Scaffolds for display can include: antibodies (e.g., Fab fragments, single chain Fv molecules (scFv), single domain antibodies, camelid antibodies, and camelized antibodies); T-cell receptors; MHC proteins themselves; extracellular domains (e.g., fibronectin Type III repeats, EGF repeats); protease inhibitors (e.g., Kunitz domains, ecotin, BPTI, and so forth); TPR repeats; tristil structures; zinc finger domains; DNA-binding proteins; particularly monomeric DNA binding proteins; RNA binding proteins; enzymes, e.g., proteases (particularly inactivated proteases), RNases; chaperones, e.g., thioredoxin, and heat shock proteins; and intracellular signaling domains (such as SH2 and SH3 domains).

Examples of small scaffolding domains include: Kunitz domains (58 amino acids, 3 disulfide bonds), Cucurbita maxima trypsin inhibitor domains (31 amino acids, 3 disulfide bonds), domains related to guanylin (14 amino acids, 2 disulfide bonds), domains related to heat-stable enterotoxin IA from gram negative bacteria (18 amino acids, 3 disulfide bonds), EGF domains (50 amino acids, 3 disulfide bonds), kringle domains (60 amino acids, 3 disulfide bonds), fungal carbohydrate-binding domains (35 amino acids, 2 disulfide bonds), endothelin domains (18 amino acids, 2 disulfide bond), and Streptococcal C IgG-binding domain (35 amino acids, no disulfide bonds). Examples of small intracellular scaffolding domains include SH2, SH3, and EVH domains. Generally, any modular domain, intracellular or extracellular, can be used.

The scaffold domain can include a synthetic peptide. A “synthetic peptide” is an artificial peptide of 30 amino acids or less. The synthetic peptide can include one or more disulfide bonds. Other synthetic peptides, so-called “linear peptides,” are devoid of cysteines. Synthetic peptides may have little or no structure in solution (e.g., unstructured), heterogeneous structures (e.g., alternative conformations or “loosely structured,” or a singular native structure (e.g., cooperatively folded). Some synthetic peptides adopt a particular
structure when bound to a target molecule. Some exemplary synthetic peptides are so-called “cyclic peptides” that have one disulfide bond, and a loop of about 4 to 12 non-cysteine residues, e.g., a sequence of

\[ \text{Xaa-Xaa-Xaa-Cys-(Xaa)_1-Cys-Xaa-Xaa-Xaa} \]

[0245] where Xaa is any non-cysteine amino acid, and n is an integer between 4 and 12. The selection of amino acids can be varied at each position, e.g., to a mixture of 18 or fewer amino acids. U.S. Pat. No. 5,223,409 also describes a variety of other disulfide bonded peptides and polypeptides that can function as scaffolds.

[0246] Another useful type of scaffolding domain is the immunoglobulin (lg) domain. Methods using immunoglobulin domains for display are described below (see, e.g., “Antibody Display Libraries”).

[0247] Display technology can also be used to obtain ligands, e.g., antibody ligands, particular epitopes of a target. This can be done, for example, by using competing non-target molecules that lack the particular epitope or are mutated within the epitope, e.g., with alanine. Such non-target molecules can be used in a negative selection procedure as described below, as competing molecules when binding a display library to the target, or as a pre-elution agent, e.g., to capture in a wash solution dissociating display library members that are not specific to the target.

[0248] Iterative Selection. In one preferred embodiment, display library technology is used in an iterative mode. A first display library is used to identify one or more ligands for a target. These identified ligands are then varied using a mutagenesis method to form a second display library. Higher affinity ligands are then selected from the second library, e.g., by using higher stringency or more competitive binding and washing conditions.

[0249] In some implementations, the mutagenesis is targeted to regions known or likely to be at the binding interface. If, for example, the identified ligands are antibodies, then mutagenesis can be directed to the CDR regions of the heavy or light chains as described herein. Further, mutagenesis can be directed to framework regions near or adjacent to the CDRs. In the case of antibodies, mutagenesis can also be limited to one or a few of the CDRs, e.g., to make precise step-wise improvements. Likewise, if the identified ligands are enzymes, mutagenesis can be directed to the active site and vicinity.


[0251] In one example of iterative selection, the methods described herein are used to first identify a protein ligand from a display library that binds a MHC-peptide complex with at least a minimal binding specificity for the varied protein component or a minimal activity, e.g., an equilibrium dissociation constant for binding of greater than 1 nM, 10 nM, or 100 nM. The nucleic acid sequence encoding the initial identified protein ligand are used as a template nucleic acid for the introduction of variations, e.g., to identify a second protein ligand that has enhanced properties (e.g., binding affinity, kinetics, or stability) relative to the initial protein ligand.

[0252] Off-Rate Selection. Since a slow dissociation rate can be predictive of high affinity, particularly with respect to interactions between polypeptides and their targets, the methods described herein can be used to isolate ligands with a desired kinetic dissociation rate (i.e., reduced) for a binding interaction to a target.

[0253] To select for slow dissociating ligands from a display library, the library is contacted to an immobilized target. The immobilized target is then washed with a first solution that removes non-specifically or weakly bound biomolecules. Then the immobilized target is eluted with a second solution that includes a saturation amount of free target, i.e., replicates of the target that are not attached to the particle. The free target binds to biomolecules that dissociate from the target. Rebinding is effectively prevented by the saturating amount of free target relative to the much lower concentration of immobilized target.

[0254] The second solution can have solution conditions that are substantially physiological or that are stringent. Typically, the solution conditions of the second solution are identical to the solution conditions of the first solution. Fractions of the second solution are collected in temporal order to distinguish early from late fractions. Later fractions include biomolecules that dissociate at a slower rate from the target than biomolecules in the early fractions.

[0255] Further, it is also possible to recover display library members that remain bound to the target even after extended incubation. These can either be dissociated using chaotropic conditions or can be amplified while attached to the target. For example, phage bound to the target can be contacted to bacterial cells.

[0256] Selecting or Screening for Specificity. The display library screening methods described herein can include a selection or screening process that discards display library members that bind to a non-target molecule. Examples of non-target molecules include: (i) a TAA peptide that is not bound to an MHC; (ii) a MHC which is not bound by a peptide; (iii) a MHC which is bound by a peptide that differs from the peptide of interest; and (iv) a MHC which is bound by the peptide of interest, but has a different allele from the MHC of interest.

[0257] In one implementation, a so-called “negative selection” step is used to discriminate between the target MHC-peptide complex and related non-target molecule and a related, but distinct non-target molecules. The display library or a pool thereof is contacted to the non-target molecule. Members of the sample that do not bind the non-target are collected and used in subsequent selections for binding to the target molecule or even for subsequent negative selections. The negative selection step can be prior to or after selecting library members that bind to the target MHC-peptide complex.

[0258] In another implementation, a screening step is used. After display library members are isolated for binding to the target MHC-peptide complex, each isolated library member is tested for its ability to bind to a non-target molecule (e.g., a non-target listed above). For example, a high-throughput ELISA screen can be used to obtain this data. The ELISA screen can also be used to obtain quantitative data for binding of each library member to the target. The non-target and target
binding data can be compared (e.g., using a computer and software) to identify library members that specifically bind to the target MHC-peptide complex.

[0259] Diversity

[0260] Display libraries include variation at one or more positions in the displayed polypeptide. The variation at a given position can be synthetic or natural. For some libraries, both synthetic and natural diversity are included.

[0261] Synthetic Diversity. Libraries can include regions of diverse nucleic acid sequence that originate from artificially synthesized sequences. Typically, these are formed from degenerate oligonucleotide populations that include a distribution of nucleotides at each given position. The inclusion of a given sequence is random with respect to the distribution. One example of a degenerate source of synthetic diversity is an oligonucleotide that includes NNN wherein N is any of the four nucleotides in equal proportion.

[0262] Synthetic diversity can also be more constrained, e.g., to limit the number of codons in a nucleic acid sequence at a given trinucleotide to a distribution that is smaller than NNN. For example, such a distribution can be constructed using less than four nucleotides at some positions of the codon. In addition, trinucleotide addition technology can be used to further constrain the distribution.

[0263] So-called “trinucleotide addition technology” is described, e.g., in Wells et al. (1985) Gene 34:315-323, U.S. Pat. Nos. 4,760,025 and 5,869,644. Oligonucleotides are synthesized on a solid phase support, one codon (i.e., trinucleotide) at a time. The support includes many functional groups for synthesis such that many oligonucleotides are synthesized in parallel. The support is first exposed to a solution containing a mixture of the set of codons for the first position. The unit is protected so additional units are not added. The solution containing the first mixture is washed away and the solid support is deprotected so a second mixture containing a set of codons for a second position can be added to the attached first unit. The process is iterated to sequentially assemble multiple codons. Trinucleotide addition technology enables the synthesis of a nucleic acid that at a given position can encode a number of amino acids. The frequency of these amino acids can be regulated by the proportion of codons in the mixture. Further the choice of amino acids at the given position is not restricted to quadrants of the codon table as is the case if mixtures of single nucleotides are added during the synthesis.

[0264] Natural Diversity. Libraries can include regions of diverse nucleic acid sequence that originate (or are synthesized based on) from different naturally-occurring sequences. An example of natural diversity that can be included in a display library is the sequence diversity present in immune cells (see also below). Nucleic acids are prepared from these immune cells and are manipulated into a format for polypeptide display. Another example of naturally diversity is the diversity of sequences among different species of organisms. For example, diverse nucleic acid sequences can be amplified from environmental samples, such as soil, and used to construct a display library.

[0265] Antibody Display Libraries

[0266] In one embodiment, the display library presents a diverse pool of polypeptides, each of which includes an immunoglobulin domain, e.g., an immunoglobulin variable domain. Display libraries are particular useful, for example for identifying human or “humanized” antibodies that recognize human antigens. Such antibodies can be used as therapeutics to treat human disorders such as cancer. Since the constant and framework regions of the antibody are human, these therapeutic antibodies may avoid themselves being recognized and targeted as antigens. The constant regions are also optimized to recruit effector functions of the human immune system. The in vitro display selection process surmounts the inability of a normal human immune system to generate antibodies against self-antigens.

[0267] A typical antibody display library displays a polypeptide that includes a VH domain and a VL domain. An “immunoglobulin domain” refers to a domain from the variable or constant domain of immunoglobulin molecules. Immunoglobulin domains typically contain two β-sheets formed of about seven β-strands, and a conserved disulphide bond (see, e.g., A. F. Williams and A. N. Barclay 1988. Ann. Rev. Immunol. 6:381-405). The display library can display the antibody as a Fab fragment (e.g., using two polypeptide chains) or a single chain Fv (e.g., using a single polypeptide chain). Other formats can also be used.

[0268] As in the case of the Fab and other formats, the displayed antibody can include a constant region as part of a light or heavy chain. In one embodiment, each chain includes one constant region, e.g., as in the case of a Fab. In other embodiments, additional constant regions are displayed.

[0269] Antibody libraries can be constructed by a number of processes (see, e.g., WO 00/70023). Further, elements of each process can be combined with those of other processes. The processes can be used such that variation is introduced into a single immunoglobulin domain (e.g., VH or VL) or into multiple immunoglobulin domains (e.g., VH and VL). The variation can be introduced into an immunoglobulin variable domain, e.g., in the region of one or more of CDR1, CDR2, CDR3, FR1, FR2, FR3, and FR4, referring to such regions of either and both of heavy and light chain variable domains. In one embodiment, variation is introduced into all three CDRs of a given variable domain. In another preferred embodiment, the variation is introduced into CDR1 and CDR2, e.g., of a heavy chain variable domain. Any combination is feasible. In one process, antibody libraries are constructed by inserting diverse oligonucleotides that encode CDRs into the corresponding regions of the nucleic acid. The oligonucleotides can be synthesized using monomeric nucleotides or trimucleotides. For example, Kuittik et al. (2000) J. Mol. Biol. 296: 57-86 describes a method for constructing CDR encoding oligonucleotides using trinucleotide synthesis and a template with engineered restriction sites for accepting the oligonucleotides.

[0270] In another process, an animal, e.g., a rodent, is immunized with the MHC-peptide complex that includes a specific peptide or with a cell that presents a specific peptide on its surface bound to the MHC. The cell can have a particular allele of the MHC protein. The animal is optionally boosted with the antigen to further stimulate the response. Then spleen cells are isolated from the animal, and nucleic acid encoding VH and/or VL domains is amplified and cloned for expression in the display library. Of course, a display library may not be needed to screen out nucleic acids that encode antibodies specific for the target in this case.

[0271] In yet another process, antibody libraries are constructed from nucleic acid amplified from native germline immunoglobulin genes. The amplified nucleic acid includes nucleic acid encoding the VH and/or VL domain. Sources of immunoglobulin-encoding nucleic acids are described below.
Amplification can include PCR, e.g., with primers that anneal to the conserved constant region, or another amplification method.

[0272] Nucleic acid encoding immunoglobulin domains can be obtained from the immune cells of, e.g., a human, a primate, mouse, rabbit, camel, or rodent. In one example, the cells are selected for a particular property. B cells at various stages of maturity can be selected. In another example, the B cells are naïve.

[0273] In one embodiment, fluorescent-activated cell sorting (FACS) is used to sort B cells that express surface-bound IgM, IgD, or IgG molecules. Further, B cells expressing different isotypes of IgG can be isolated. In another preferred embodiment, the B or T cell is cultured in vitro. The cells can be stimulated in vitro, e.g., by culturing with feeder cells or by adding mitogens or other modulatory reagents, such as anti-bodies to CD40, CD40 ligand or CD20, phorbol myristate acetate, bacterial lipopolysaccharide, concanavalin A, phytohemagglutinin or pokeweed mitogen.

[0274] In still another embodiment, the cells are isolated from a subject that has an immunological disorder, e.g., systemic lupus erythematosus (SLE), rheumatoid arthritis, vasculitis, Sjogren syndrome, systemic sclerosis, or anti-phospholipid syndrome. The subject can be a human, an animal, e.g., an animal model for the human disease, or an animal having an analogous disorder. In yet another embodiment, the cells are isolated from a transgenic non-human animal that includes a human immunoglobulin locus.

[0275] In one preferred embodiment, the cells have activated a program of somatic hypermutation. Cells can be stimulated to undergo somatic mutagenesis of immunoglobulin genes, for example, by treatment with anti-immunoglobulin, anti-CD40, and anti-CD38 antibodies (see, e.g., Bergthorsdóttir et al. (2001) J. Immunol. 166:2228). In another embodiment, the cells are naïve.

[0276] The nucleic acid encoding an immunoglobulin variable domain can be isolated from a natural repertoire by the following exemplary method. First, RNA is isolated from the immune cell. Full length (i.e., capped) mRNAs are separated (e.g., by degrading uncapped RNAs with calf intestinal phosphatase). The cap is then removed with tobacco acid pyrophosphatase and reverse transcription is used to produce the cDNAs.

[0277] The reverse transcription of the first (antisense) strand can be done in any manner with any suitable primer. See, e.g., de Haard et al. (1999). J. Biol. Chem. 274:18218-30. The primer binding region can be constant among different immunoglobulins, e.g., in order to reverse transcribe different isotypes of immunoglobulin. The primer binding region can also be specific to a particular isotype of immunoglobulin. Typically, the primer is specific for a region that is 3' to a sequence encoding at least one CDR. In another embodiment, poly-dT primers may be used (and may be preferred for the heavy-chain genes).

[0278] A synthetic sequence can be ligated to the 3' end of the reverse transcribed strand. The synthetic sequence can be used as a primer binding site for binding of the forward primer during PCR amplification after reverse transcription. The use of the synthetic sequence can obviate the need to use a pool of different forward primers to fully capture the available diversity.

[0279] The variable domain-encoding gene is then amplified, e.g., using one or more rounds. If multiple rounds are used, nested primers can be used for increased fidelity. The amplified nucleic acid is then cloned into a display library vector.

[0280] Any method for amplifying nucleic acid sequences may be used for amplification. Methods that maximize, and do not bias, diversity are preferred. A variety of techniques can be used for nucleic acid amplification. The polymerase chain reaction (PCR; U.S. Pat. Nos. 4,683,195 and 4,683,202, Saiki et al. (1985) Science 230, 1350-1354) utilizes cycles of varying temperature to drive rounds of nucleic acid synthesis. Transcript-based methods utilize RNA synthesis by RNA polymerases to amplify nucleic acid (U.S. Pat. No. 6,066,457; U.S. Pat. No. 6,132,997; U.S. Pat. No. 5,716,785; Sarkar et al., Science (1989) 244: 331-34; Stoller et al., Science (1988) 239: 491). NASBA (U.S. Pat. Nos. 5,130,238; 5,409,818; and 5,554,517) utilizes cycles of transcription, reverse-transcription, and RNAseH-based degradation to amplify a DNA sample. Still other amplification methods include rolling circle amplification (RCA; U.S. Pat. Nos. 5,854,033 and 6,143,495) and strand displacement amplification (SDA; U.S. Pat. Nos. 5,455,166 and 5,624,825).

[0281] Secondary Screening Methods

[0282] After selecting candidate display library members that bind to a target, each candidate display library member can be further analyzed, e.g., to further characterize its binding properties for the MHC-peptide complex. Each candidate display library member can be subjected to one or more secondary screening assays. For example, the assays can determine relative binding to different MHC-peptide complexes, e.g., to assess specificity for the peptide moiety and/or the MHC allele. The assay can be for a binding property, a catalytic property, a physiological property (e.g., cytotoxicity, renal clearance, immunogenicity), a structural property (e.g., stability, conformation, oligomerization state) or another functional property. The same assay can be used repeatedly, but with varying conditions, e.g., to determine pH, ionic, or thermal sensitivities.

[0283] Exemplary assays for binding properties include the following.

[0284] ELISA. Polypeptides encoded by a display library can also be screened for a binding property using an ELISA assay. For example, each polypeptide is contacted to a microtitre plate whose bottom surface has been coated with the target, e.g., a limiting amount of the target. The plate is washed with buffer to remove non-specifically bound polypeptides. Then the amount of the polypeptide bound to the plate is determined by probing the plate with an antibody that can recognize the polypeptide, e.g., a tag or constant portion of the polypeptide. The antibody is linked to an enzyme such as alkaline phosphatase, which produces a colorimetric product when appropriate substrates are provided. The polypeptide can be purified from cells or assayed in a display library format, e.g., as a fusion to a filamentous bacteriophage coat. In another version of the ELISA assay, each polypeptide of a library is used to coat a different well of a microtitre plate. The ELISA then proceeds using a constant target molecule to query each well.

[0285] Homogeneous Binding Assays. The binding interaction of candidate polypeptide with a target can be analyzed using a homogeneous assay, i.e., after all components of the assay are added, additional fluid manipulations are not required. For example, fluorescence resonance energy transfer (FRET) can be used as a homogeneous assay (see, for example, Lakowicz et al., U.S. Pat. No. 5,631,169; Stavrian-
opoulos, et al., U.S. Pat. No. 4,868,103). A fluorophore label on the first molecule (e.g., the molecule identified in the fraction) is selected such that its emitted fluorescent energy can be absorbed by a fluorescent label on a second molecule (e.g., the target) if the second molecule is in proximity to the first molecule. The fluorescent label on the second molecule fluoresces when it absorbs to the transferred energy. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the ‘acceptor’ molecule label in the assay should be maximal. A binding event that is configured for monitoring by FRET can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorometer). By titrating the amount of the first or second binding molecule, a binding curve can be generated to estimate the equilibrium binding constant.

[0286] Another example of a homogenous assay is ALPHASCREEN™ (Packard Bioscience, Meriden Conn.). ALPHASCREEN™ uses two labeled beads. One bead generates singlet oxygen when excited by a laser. The other bead generates a light signal when singlet oxygen diffuses from the first bead and collides with it. The signal is only generated when the two beads are in proximity. One bead can be attached to the display library member, the other to the target. Signals are measured to determine the extent of binding.

[0287] The homogenous assays can be performed while the candidate polypeptide is attached to the display library vehicle, e.g., a bacteriophage.

[0288] Surface Plasmon Resonance (SPR). The binding interaction of a molecule isolated from a display library and a target can be analyzed using SPR. SPR or Biomolecular Interaction Analysis (BIA) detects biospecific interactions in real time, without labeling any of the interactants. Changes in the mass at the binding surface (indicative of a binding event) of the BIA chip result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)). The changes in the refractivity generate a detectable signal, which are measured as an indication of real-time reactions between biological molecules. Methods for using SPR are described, for example, in U.S. Pat. No. 5,641,640, Raether (1988) Surface Plasmons Springer Verlag, Sjolander and Urbaniczky (1991) Anal. Chem. 63:2338-2345; Szabo et al. (1995) Curr. Opin. Struct. Biol. 5:699-705 and on-line resources provide by Biacore International AB (Uppsala, Sweden).

[0289] Information from SPR can be used to provide an accurate and quantitative measure of the equilibrium dissociation constant (Kₐ), and kinetic parameters, including Kᵦₐ and Kₐₑ, for the binding of a biomolecule to a target. Such data can be used to compare different biomolecules. For example, proteins encoded by nucleic acid selected from a display library can be compared to identify individual proteins that have high affinity for the target or that have a slow Kₐₑ. This information can also be used to develop structure-activity relationships (SAR). For example, the kinetic and equilibrium binding parameters of matured versions of a parent protein can be compared to the parameters of the parent protein. Variant amino acids at given positions can be identified that correlate with particular binding parameters, e.g., high affinity and slow Kₐₑ. This information can be combined with structural modeling (e.g., using homology modeling, energy minimization, or structure determination by crystallography or NMR). As a result, an understanding of the physical interaction between the protein and its target can be formulated and used to guide other design processes.

[0290] Protein Arrays. Polypeptides identified from the display library can be immobilized on a solid support, for example, on a bead or an array. For a protein array, each of the polypeptides is immobilized at a unique address on a support. Typically, the address is a two-dimensional address. Protein arrays are described below (see, e.g., Diagnostics).

[0291] Cellular Assays. Candidate polypeptides (e.g., previously identified by a display library or otherwise) can be screened for biological or other functional activity, e.g., using a cellular assay. For example, in the case of an antibody that binds to the MHC-peptide complex, the activity may be cell- or complement-mediated cytotoxicity toward a cell that present the peptide on a surface MHC group. An antibody can be expressed in a mammalian cell, harvested, and then tested for cell- or complement-mediated cytotoxicity.

[0292] The Cr-release assay, for example, can be used to assay cell-mediated cytotoxicity. Peripheral blood lymphocytes (PBL) are prepared as effector cells, while target cells that express the targeted MHC-peptide complex are loaded with ⁵¹Cr. The target cells are washed and then seeded into a flat bottom microtitre plate. PBL (50 Tl) are added to the target cells in combination with the ligand (e.g., a known anti-MHC-peptide complex) ligand or a candidate ligand). Maximum release is determined by the addition of TWEEN-20 to target cells, whereas minimal release is determined in the absence of PBLs. After overnight incubation, ⁵¹Cr released into the supernatant is counted in a K scintillation counter.

[0293] In another embodiment, the library of cells is in the form of a cellular array. The cellular array can likewise be screened for any appropriate detectable activity.

[0294] Ligand Production

[0295] Standard recombinant nucleic acid methods can be used to express a protein ligand that binds to a MHC-peptide complex and recognizes the peptide moiety. Generally, a nucleic acid sequence encoding the protein ligand is cloned into a nucleic acid expression vector. Of course, if the protein includes multiple polypeptide chains, each chain must be cloned into an expression vector, e.g., the same or different vectors, that are expressed in the same or different cells. If the protein is sufficiently small, i.e., the protein is a peptide of less than 50 amino acids, the protein can be synthesized using automated organic synthetic methods. Methods for producing antibodies are also provided below.

[0296] The expression vector for expressing the protein ligand can include, in addition to the segment encoding the protein ligand or fragment thereof, regulatory sequences, including for example, a promoter, operable linked to the nucleic acid(s) of interest. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBluescript, PstI714, pBluescript SK, pBluescript KS, pN118a, pN116a, pN118a, pN146a (Stratagene); pTc99A, pKK232-3, pKK233-3, pOR540, and pIT15 (Pharmacia). Eukaryotic: pWLANeo, pSV2cat, pOG44, PXT1, pOG (Stratagene) pSVK3, pBPV, pMSG, and pSVL (Pharmacia). One preferred class of preferred libraries is the display library, which is described below.
Methods well known to those skilled in the art can be used to construct vectors containing a polynucleotide of the invention and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Sambrook & Russell, Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory, N.Y. (2001) and Ausubel et al., Current Protocols in Molecular Biology (Greene Publishing Associates and Wiley Interscience, N.Y. (1989). Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, mouse metallothionein-1, and various art known tissue specific promoters.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae auxotrophic markers (such as UR3, LEU2, HIS3, and TRP1 genes), and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The polynucleotide of the invention is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionnally, a nuclear acid of the invention can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Useful expression vectors for bacteria are constructed by inserting a polynucleotide of the invention together with suitable translation initiation and termination signals, optionally in operable reading phase with a functional promoter. The vector will comprise one or more phenotype selectable markers and a vector origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacteria can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pH132 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmac Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega, Madison, Wis., USA).

The present invention further provides host cells containing the vectors of the present invention, wherein the nucleic acid has been introduced into the host cell using known transformation, transfection or infection methods. For example, the host cells can include members of a library or a nucleic acid encoding components of a poly-(MHC-peptide complex) ligand. The host cell can be a eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected, for example, by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. et al., Basic Methods in Molecular Biology (1986)).

Any host/vector system can be used to identify one or more of the target elements of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, CV-1 cell, COS cells, and SF9 cells, as well as prokaryotic host such as E. coli and B. subtilis. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level.


The host of the invention may also be a prokaryotic cell such as E. coli, other enterobacteria such as Serratia marcescens, bacilli, various pseudomonads, or other prokaryotes which can be transformed, transfected, infected.

The present invention further provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using transformation, transfection or infection methods. The present invention still further provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell.

Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. et al., Basic Methods in Molecular Biology (1986)). The host cells containing one of polynucleotides of the invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF).

Any host/vector system can be used to express one or more of the anti-(MHC-peptide complex) ligands. These include, but are not limited to, eukaryotic hosts such as HeLa cells, CV-1 cell, COS cells, and SF9 cells, as well as prokaryotic host such as E. coli and B. subtilis. The most preferred cells are those which do not normally express the polypeptide or protein or which expresses the polypeptide or protein at low natural level. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the
control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., in Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y. (1989), the disclosure of which is hereby incorporated by reference.

Examples of mammalian cell culture systems can also be employed to express recombinant protein.

Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Ghuzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and also any necessary ribosome-binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' and 3' flanking non-transcribed sequences.

DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Recombinant polypeptides and proteins produced in bacterial culture are usually isolated by initial extraction from cell pellets, followed by one or more saltings-out, aqueous ion exchange or size exclusion chromatography steps. In some embodiments, the template nucleic acid also encodes a polypeptide tag, e.g., penta- or hexa-histidine. The recombinant polypeptides can then be purified using affinity chromatography.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. A number of types of cells may act as suitable host cells for expression of the protein. Scopes (1994) Protein Purification: Principles and Practice, New York: Springer-Verlag provides a number of general methods for purifying recombinant (and non-recombinant) proteins. The method includes, e.g., ion-exchange chromatography, size-exclusion chromatography, affinity chromatography, selective precipitation, dialysis, and hydrophobic interaction chromatography. These methods can be adapted for devising a purification strategy for the anti-MHC-peptide complex protein ligand.

Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HeK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, Candida, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include Escherichia coli, Bacillus subtilis, Salmonella typhimurium, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods. In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods.

Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the function or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting, including polyadenylation signals. mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

Antibody Production. Some antibodies, e.g., Fabs, can be produced in bacterial cells, e.g., E. coli cells. For example, if the Fab is encoded by sequences in a phage display vector that includes a suppressible stop codon between the display entity and a bacteriophage protein (or fragment thereof), the vector nucleic acid can be shuffled into a bacterial cell that cannot suppress a stop codon. In this case, the Fab is not fused to the gene III protein and is secreted into the media.

Antibodies can also be produced in eukaryotic cells. In one embodiment, the antibodies (e.g., scFv's) are expressed in a yeast cell such as Pichia (see, e.g., Powers et al. (2001) J Immunol Methods. 251:123-35), Hanseula, or Saccharomyces.

In one preferred embodiment, antibodies are produced in mammalian cells. Preferred mammalian host cells for expressing the clone antibodies or antigen-binding fragments thereof include Chinese Hamster Ovary (CHO) cells (including dhfr- CHO cells, described in Uralba and Chasin (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp (1982) Mol. Biol. 159:601-621), lymphocytic cell lines, e.g., NS0 myeloma cells and SP2 cells, COS cells, and a cell from a transgenic animal, e.g., a transgenic mammal. For example, the cell is a mammary epithelial cell.

In addition to the nucleic acid sequence encoding the diversified immunoglobulin domain, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

In an exemplary system for recombinant expression of an antibody, or antigen-binding portion thereof, of the
invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus, and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfet the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G.

[0325] A “pharmaceutically acceptable salt” refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see e.g., Berge, S. M., et al. (1977) J. Pharm. Sci. 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N-dibenzylethylenediamine, N-methylglycine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[0326] The compositions of this invention may be in a variety of forms. These include, for example, liquid, semisolid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for administration of humans with antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the anti-(MHC-peptide complex) ligand is administered by intravenous infusion or injection. In another preferred embodiment, the anti-(MHC-peptide complex) ligand is administered by intramuscular or subcutaneous injection.

[0327] The phrases “parenteral administration” and “administered parenterally” as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, intrathoracic, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intraskeletal injection and infusion.

[0328] Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. A pharmaceutical composition can also be tested to insure it meets regulatory and industry standards for administration. For example, endotoxin levels in the preparation can be tested using the Limulus amebocyte lysate assay (e.g., using the kit from Bio Whittaker lot #72L3790, sensitivity 0.125 EU/mL) according to the USP 24/NF 19 methods. Sterility of pharmaceutical compositions can be determined using thiglycollate medium according to the USP 24/NF 19 methods. For example, the preparation is used to inoculate the thiglycollate medium and incubated at 35 °C for 14 or more days. The medium is inspected periodically to detect growth of a microorganism.

[0329] The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active com-
ound (i.e., the ligand) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, suspensions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0330] The anti-(MHC-peptide complex) protein ligands of the present invention can be administered by a variety of methods known in the art, although for many applications, the preferred route/type of administration is intravenous injection or infusion. For example, for therapeutic applications, the anti-(MHC-peptide complex) ligand can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m² or 7 to 25 mg/m². The route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyhydroxy alcohols, polyglycolic acid, collagen, polyurethanes, and polyactic acid. Many methods for the preparation of such formulations are patented or generally known. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0331] In certain embodiments, the ligand may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject’s diet. For oral therapeutic administration, the compositions may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

[0332] Pharmaceutical compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a pharmaceutical composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Pat. Nos. 5,399,163, 5,383,851, 5,312,355, 5,064,413, 4,941,880, 4,790,824, or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Pat. No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Pat. No. 4,486,194, which discloses a therapeutic device for administering medicaments through the skin; U.S. Pat. No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Pat. No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Pat. No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Pat. No. 4,475,196, which discloses an osmotic drug delivery system. Of course, many other such implants, delivery systems, and modules are also known.

[0333] In certain embodiments, the compounds of the invention can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., V. V. Ranade (1989) J. Clin. Pharmacol. 29:685).

[0334] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0335] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody of the invention is 0.1-20 mg/kg, more preferably 1-10 mg/kg. The anti-(MHC-peptide complex) antibody can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m² or about 5 to 30 mg/m². For ligands smaller in molecular weight than an antibody, appropriate amounts can be proportionally less. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0336] The pharmaceutical compositions of the invention may include a “therapeutically effective amount” or a “prophylactically effective amount” of an anti-(MHC-peptide complex) ligand of the invention. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age,
sex, and weight of the individual, and the ability of the protein ligand to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition is outweighed by the therapeutically beneficial effects. A “therapeutically effective dosage” preferably inhibits a measurable parameter, e.g., tumor growth rate by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit a measurable parameter, e.g., cancer, can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition in vitro by assays known to the skilled practitioner.

A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Also within the scope of the invention are kits comprising the protein ligand that binds to a MHC-peptide complex and instructions for use, e.g., treatment, prophylactic, or diagnostic use. In one embodiment, the instructions for diagnostic applications include the use of the anti-(MHC-peptide complex) ligand (e.g., antibody or antigen-binding fragment thereof, or other polypeptide or peptide) to detect a MHC-peptide complex, in vitro, e.g., in a sample, e.g., a biopsy or cells from a patient having a cancer or neoplastic disorder, or in vivo. In another embodiment, the instructions for therapeutic applications include suggested dosages and/or modes of administration in a patient with a cancer or neoplastic disorder. The kits can further contain a least one additional reagent, such as a diagnostic or therapeutic agent, e.g., a diagnostic or therapeutic agent as described herein, and/or one or more additional anti-(MHC-peptide complex) ligands, formulated as appropriate, in one or more separate pharmaceutical preparations.

Treatments

Protein ligands that bind to a MHC-peptide complex and/or identified by the method described herein have therapeutic and prophylactic utilities. For example, these ligands independently or as part of a therapeutic entity can be administered to cells in culture, e.g., in vitro or ex vivo, or in a subject, e.g., in vivo, to treat, prevent, and/or diagnose a variety of disorders, such as cancers. In another example, the ligands are expressed on cells, e.g., cytotoxic cells. The ligand expressing cells are used to treat, prevent, and/or diagnose a disorder.

As used herein, the term “treat” or “treatment” is defined as the application or administration of an anti-(MHC-peptide complex) antibody, alone or in combination with, a second agent to a subject, e.g., a patient, or application or administration of the agent to an isolated tissue or cell, e.g., cell line, from a subject, e.g., a patient, who has a disorder (e.g., a disorder as described herein), a symptom of a disorder or a predisposition toward a disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. Treating a cell refers to the inhibition, ablation, killing of a cell in vitro or in vivo, or otherwise reducing capacity of a cell, e.g., an aberrant cell, to mediate a disorder, e.g., a disorder as described herein (e.g., a cancerous disorder). In one embodiment, “treating a cell” refers to a reduction in the activity and/or proliferation of a cell, e.g., a hyperproliferative cell. Such reduction does not necessarily indicate a total elimination of the cell, but a reduction, e.g., a statistically significant reduction, in the activity or the number of the cell. The application or administration of an anti-(MHC-peptide complex) antibody can be in the form of a soluble compound, e.g., antibody alone or antibody conjugate, or on the surface of the cell, e.g., an effector cell. In some implementations, a nucleic acid encoding the anti-(MHC-peptide complex) antibody is administered.

As used herein, an amount of an anti-(MHC-peptide complex) ligand effective to treat a disorder, or a “therapeutically effective amount” refers to an amount of the ligand which is effective, upon single or multiple dose administration to a subject, in treating a cell, e.g., a cancer cell (e.g., a cell that presents a TAA in association with a MHC), or in prolonging curing, alleviating, relieving or improving a subject with a disorder as described herein beyond that expected in the absence of such treatment. As used herein, “inhibiting the growth” of the neoplasm refers to slowing, interrupting, arresting or stopping its growth and metastases and does not necessarily indicate a total elimination of the neoplastic growth.

As used herein, an amount of an anti-(MHC-peptide complex) ligand effective to prevent a disorder, or a “a prophylactically effective amount” of the ligand refers to an amount of an anti-(MHC-peptide complex) ligand, e.g., an anti-(MHC-peptide complex) antibody described herein, which is effective, upon single- or multiple-dose administration to the subject, in preventing or delaying the occurrence of the onset or recurrence of a disorder, e.g., a cancer.

The terms “induce,” “inhibit,” “potentiate,” “elevate,” “increase,” “decrease” or the like, e.g., which denote quantitative differences between two states, refer to a difference, e.g., a statistically significant difference, between the two states. For example, “an amount effective to inhibit the proliferation of the hyperproliferative cells that present a TAA” means that the rate of growth of the cells will be different, e.g., statistically significantly different, from the untreated cells. In a preferred embodiment, the TAA is hTERT, MUC1, TAX, or gr100.

As used herein, the term “subject” is intended to include human and non-human animals. Preferred human animals include a human patient having a disorder characterized by abnormal cell proliferation or cell differentiation. The term “non-human animals” of the invention includes all vertebrates, e.g., non-mammals (such as chickens, amphibians, reptiles) and mammals, such as non-human primates, sheep, dog, cow, pig, etc.

In one embodiment, the subject is a human subject. Alternatively, the subject can be a mammal that includes a cell that presents a TAA-like antigen on an MHC to form a complex with which a ligand of the invention cross-reacts. A protein ligand of the invention can be administered to a human subject for therapeutic purposes (discussed further below). Moreover, an anti-(MHC-peptide complex) ligand can be administered to a non-human mammal for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of the ligand (e.g., testing of dosages and time courses of administration).

In one embodiment, the invention provides a method of treating (e.g., ablating or killing) a cell (e.g., a
non-cancerous cell, e.g., a normal, benign or hyperplastic cell, or a cancerous cell, e.g., a malignant cell, e.g., cell found in a solid tumor, a soft tissue tumor, or a metastatic lesion (e.g., a cell found in renal, urothelial, colonic, rectal, pulmonary, breast or hepatic, cancers and/or metastasis). Methods of the invention include the steps of contacting the cell with an anti-(MHC-peptide complex) ligand, e.g., an anti-(MHC-peptide complex) antibody described herein, in an amount sufficient to treat, e.g., ablate or kill, the cell. [0348] The subject method can be used on cells in culture, e.g., in vitro or ex vivo. For example, cancerous or metastatic cells (e.g., renal, urothelial, colon, rectal, lung, breast, ovarian, prostatic, or liver cancerous or metastatic cells) can be cultured in vitro in culture medium and the contacting step can be effected by adding the anti-(MHC-peptide complex) ligand to the culture medium. The method can be performed on cells (e.g., cancerous or metastatic cells) present in a subject, as part of an in vivo (e.g., therapeutic or prophylactic) protocol. For in vivo embodiments, the contacting step is effected in a subject and includes administering the anti-(MHC-peptide complex) ligand to the subject under conditions effective to permit both binding of the ligand to the cell and the treating, e.g., the killing or ablating of the cell. [0349] The method can be used to treat a cancer. As used herein, the terms “cancer”, “hyperproliferative”, “malignant”, and “neoplastic” are used interchangeably, and refer to those cells an abnormal state or condition characterized by rapid proliferation or neoplasia. The terms include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. “Pathologic hyperproliferative” cells occur in disease states characterized by malignant tumor growth. [0350] The common medical meaning of the term “neoplasia” refers to “new cell growth” that results as a loss of responsiveness to normal growth controls, e.g., to neoplastic cell growth. A “hyperplasia” refers to cells undergoing an abnormally high rate of growth. However, as used herein, the terms neoplasia and hyperplasia can be used interchangeably, as their context will reveal, referring generally to cells experiencing abnormal cell growth rates. Neoplasias and hyperplasias include “tumors”, which may be benign, premalignant or malignant. [0351] Examples of cancerous disorders include, but are not limited to, solid tumors, soft tissue tumors, and metastatic lesions. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary tract (e.g., renal, urothelial cells), pharynx, prostate, ovary as well as adenocarcinomas which include malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and so forth. Metastatic lesions of the aforementioned cancers can also be treated or prevented using the methods and compositions of the invention. The subject method can be useful in treating malignancies of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary tract, prostate, ovary, pharynx, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Exemplary solid tumors that can be treated include: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing’s tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms’ tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, epedymoma, pheoaloma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma. [0352] The term “carcinoma” is recognized by those skilled in the art and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An “adenocarcinoma” refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures. [0353] The term “sarcoma” is recognized by those skilled in the art and refers to malignant tumors of mesenchymal derivation. [0354] The subject method can also be used to inhibit the proliferation of hyperplastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. For instance, the present invention contemplates the treatment of various myeloid disorders including, but not limited to, acute promyelocytic leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vainikka, L. (1991) Crit. Rev. in Oncol./Hematol. 11:267-97). Lymphoid malignancies which may be treated by the subject method include, but are not limited to acute lymphoblastic leukemia (ALL), which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom’s macroglobulinemia (WM). Additional forms of malignant lymphomas contemplated by the treatment method of the present invention include, but are not limited to, non-Hodgkin’s lymphoma and variants thereof, peripheral T-cell lymphomas, adult T-cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL) and Hodgkin’s disease. [0355] Methods of administering anti-(MHC-peptide complex) ligands are described in “Pharmaceutical Compositions”. Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used. The ligands can be used as competitive agents to inhibit, reduce an undesirable interaction, e.g., between a natural or pathological agent and a MHC-peptide complex, e.g., a
MHC-peptide complex wherein the peptide is a TAA such as hTERT, MUC1, TAX, or gp100.

In one embodiment, the anti-(MHC-peptide complex) ligands are used to kill or ablate cancerous cells and normal, benign hyperplastic, and cancerous cells in vivo. The ligands can be used by themselves or conjugated to an agent, e.g., a cytotoxic drug, radioisotope. This method includes: administering the ligand alone or attached to a cytotoxic drug, to a subject requiring such treatment.

The terms “cytotoxic agent” and “cytostatic agent” and “anti-tumor agent” are used interchangeably herein and refer to agents that have the property of inhibiting the growth or proliferation (e.g., a cytostatic agent), or inducing the killing, of hyperproliferative cells, e.g., an aberrant cancer cell. In cancer therapeutic embodiment, the term “cytotoxic agent” is used interchangeably with the terms “anti-cancer” or “anti-tumor” to mean an agent, which inhibits the development or progression of a neoplasm, particularly a solid tumor, a soft tissue tumor, or a metastatic lesion.

Nonlimiting examples of anti-cancer agents include, e.g., antitumoricidum agents, topoisomerase inhibitors, antimetabolites, mitotic inhibitors, alkylating agents, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis, radiation, and antibodies against other tumor-associated antigens (including naked antibodies, immunotoxins and radioconjugates). Examples of the particular classes of anti-cancer agents are provided in detail as follows: antitubulin/antitubulin, e.g., paclitaxel, vincristine, vinblastine, vindelesine, vinorelbine, taxotere; topoisomerase I inhibitors, e.g., topotecan, camptothecin, doxorubicin, etoposide, mitoxantrone, daunorubicin, idarubicin, teniposide, ammsacrine, epirubicin, melarbone, piroxantrone hydrochloride; antimetabolites, e.g., 5-fluourouracil (5-FU), methotrexate, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, cytarabine/Ara-C, trimetrexate, gemcitabine, acivicin, alanosine, pyrazofurin, N-Phosphoracetilyl-L-Asparagin-PALA, pentostatin, 5-azacitidine, 5-Aza 2’-deoxycytidine, ara-A, cladribine, 5-fluorouracil, FUDR, tazozofurin, N-[5-benzimidazole-2-ethyl-4-oxoquinazolin-6-ylmethyl]-N-methylamino]-2-henoyl]-L-glutamic acid; alkylating agents, e.g., cisplatin, carboplatin, mitomycin C, BCNU—Carmustine, melphalan, thiotepa, busulfan, chlorambucil, procaineycin, dacarbazine, ifosfamide phosphate, cyclophosphamide, nitrogen mustard, uracil mustard, pipobroman, 4-ipomeanol; agents acting via other mechanisms of action, e.g., dihydrodihydropyrene, spironustine, and desipemetabolite; biological response modifiers, e.g., to enhance anti-tumor responses, such as interferon; apoptotic agents, such as actinomycin D; and anti-hormones, for example anti-estrogens such as tamoxifen or, for example antiandrogens such as 4’-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3’-(trifluoromethyl)propionanilide.

Since the anti-(MHC-peptide complex) ligands that are specific for a TAA recognize cancerous cells that present the TAA, any such cells to which the ligands bind are destroyed. Alternatively, the ligands bind to cells in the vicinity of the cancerous cells and kill them, thus indirectly attacking the cancerous cells which may rely on surrounding cells for nutrients, growth signals and so forth. Thus, the anti-(MHC—peptide complex) ligands (e.g., modified with a cytotoxic) can selectively kill or ablate cells in cancerous tissue (including the cancerous cells themselves).

The ligands may be used to deliver a variety of cytotoxic drugs including therapeutic drugs, a compound emitting radiation, molecules of plants, fungal, or bacterial origin, biological proteins, and mixtures thereof. The cytotoxic drugs can be intracellularly acting cytotoxic drugs, such as short-range radiation emitters, including, for example, short-range, high-energy α- emitters, as described herein.

Enzymatically active toxins and fragments thereof are exemplified by diphteria toxin A fragment, nonbinding active fragments of diphteria toxin, exotoxin A (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, abrin B chain, modecellin A chain, α-sacrin, certain Azurites fordii proteins, certain Dianthin proteins, Phytolaccace americana proteins (PAP, PAPI and PAP-S), Morodica charantia inhibitor, curcin, erin, Saponaria officinalis inhibitor, gelfolin, mitogulin, restrictocin, phenomenic, and enomycin. Procedures for preparing enzymatically active polypeptides of the immunotoxins are described in WO84/03508 and WO85/03508, which are hereby incorporated by reference, and in the appended Examples below. Examples of cytotoxic moieties that can be conjugated to the antibodies include adriamycin, chlorambucil, daunomycin, methotrexate, neocarzinostatin, and platinum.

In the case of polypeptide toxins, recombinant nucleic acid techniques can be used to construct a nucleic acid that encodes the ligand (or a polypeptide component thereof) and the cytotoxin (or a polypeptide component thereof) as translational fusions. The recombinant nucleic acid is then expressed, e.g., in cells and the encoded fusion polypeptide isolated.


To kill or ablate normal, benign hyperplastic, or cancerous cells, a first protein ligand is conjugated with a prodrug which is activated only when in close proximity with a prodrug activator. The prodrug activator is conjugated with a second protein ligand, preferably one which binds to a non-competing site on the target molecule. Whether two protein ligands bind to competing or non-competing binding sites can be determined by conventional competitive binding assays. Drug-prodrug pairs suitable for use in the practice of the present invention are described in Blakely et al., (1996) Cancer Research, 56:3287-3292.

Alternatively, the anti-(MHC-peptide complex) ligand can be coupled to high energy radiation emitters, for example, a radioisotope, such as 311I, a γ-emitter, which, when localized at the tumor site, results in a killing of several cell diameters. See, e.g., S. E. Order, “Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy”, Monoclonal Antibodies for
Radioimmunotherapy (RTT) using antibodies labeled with $^{191}$Ir, $^{90}$Y and $^{177}$Lu is under intense clinical investigation. There are significant differences in the physical characteristics of these three nuclides and as a result, the choice of radioisotope is very critical in order to deliver maximum radiation dose to the tumor. The higher beta energy particles of $^{90}$Y may be good for bulky tumors. The relatively low beta energy particles of $^{191}$Ir are ideal, but in vivo delocalization of radiiodinated molecules is a major disadvantage for internalizing antibody. In contrast, $^{177}$Lu has low energy beta particle with only 0.2-0.3 mm range and delivers much lower radiation dose to bone marrow compared to $^{90}$Y. In addition, due to longer physical half-life (compared to $^{90}$Y), the tumor residence times are higher. As a result, higher activities (more mCi amounts) of $^{177}$Lu labeled agents can be administered with comparatively less radiation dose to marrow.


In one embodiment, the anti-(MHC-peptide complex) ligands can be used directly in vivo to eliminate antigen-expressing cells via natural complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC). The protein ligands of the invention, can include complement binding effector domain, such as the Fc portions from IgG, -2, or -3 or corresponding portions of IgM which bind complement. In one embodiment, a population of target cells is ex vivo treated with a binding agent of the invention and appropriate effector cells. The treatment can be supplemented by the addition of complement or serum containing complement. Further, phagocytosis of target cells coated with a protein ligand of the invention can be improved by binding of complement proteins. In another embodiment target, cells coated with the protein ligand which includes a complement binding effector domain are lysed by complement.

In another embodiment, the anti-(MHC-peptide complex) ligands are used to block recognition of the particular MHC-peptide complex by other effectors, e.g., the endogenous immune system. For this implementation, the “blocking” ligand may be an antibody that lacks an effector domain, e.g., a Fab. For example, the MHC-peptide complex may be on the surface of a glial cell or a Langerhans cell. Autoimmune diseases such as multiple sclerosis and diabetes have been implicated with endogenous immune system attacks on these cells. The anti-(MHC-peptide complex) ligands that block recognition of MHC-peptides specific for these cell types can be provided, e.g., systemically or locally. For example, the blocking ligands may be expressed by exogenous or endogenous cells that are in the same tissue, or are the very same cells.

In a related example, the blocking ligands include a signal sequence that causes retention of the blocking ligand in a cell, e.g., in the cell secretary pathway. For example, the KDEL sequence, which causes endoplasmic reticulum retention, can be appended to a polypeptide component of the blocking ligand (e.g., one of the chains, in the case of an antibody).

Also encompassed by the present invention is a method of killing or ablating which involves using the anti-(MHC-peptide complex) ligand for prophylaxis. For example, these materials can be used to prevent or delay development or progression of cancers.

Use of the therapeutic methods of the present invention to treat cancers has a number of benefits. In implementations where the protein ligands specifically recognize the varied protein component of the MHC-peptide complex, other tissue is spared and high levels of the agent are delivered directly to the site where therapy is required. Treatment in accordance with the present invention can be effectively monitored with clinical parameters. Alternatively, these parameters can be used to indicate when such treatment should be employed.

Anti-(MHC-peptide complex) ligands of the invention can be administered in combination with one or more of the existing modalities for treating cancers, including, but not limited to: surgery; radiation therapy, and chemotherapy.

T-Cell Reprogramming

T cells can be reprogrammed to target cells that display particular peptides on their MHC molecules. A protein that specifically recognizes the MHC-peptide complex can be isolated using a method described herein. Nucleic acid encoding the polypeptide chain or chains that form the protein is introduced into a T cell and then expressed.

In one embodiment, the nucleic acid encoding the anti-(MHC-peptide) ligand is functionally fused to a membrane anchor such that the ligand is expressed on the surface of the host cell. The anti-(MHC-peptide) ligand can be an antibody or fragment thereof. In one embodiment the nucleic acid encodes a Fab fragments, and one of the two chains of the fragment are membrane anchored. In another embodiment, both chains of the fragment are membrane anchored.

In another embodiment, if the isolated protein includes an antigen-binding domain, the nucleic acids encoding the variable domains of the antigen binding domain are joined in frame such that the fusion nucleic acid encodes a single-chain antibody domain. The use of a single chain construct insures that the two variable domains associate when expressed in a heterologous cell and that an excess of one or both domains is not produced.

In another embodiment, a nucleic acid is constructed that encodes both variable domains, but as separate polypeptides, e.g., by using a promoter for each coding nucleic acid, a divergent promoter, or a poly-cistronic cassette.

The nucleic acid is then introduced into the T cell, typically a human T lymphocyte, e.g., a self-A2.1 restricted T lymphocyte. For example, the nucleic acid can be introduced into the cells of a population of human T cells, e.g. from donors or patients with a proportion of T cells that express the allotype of interest. The nucleic acid can be introduced using a retroviral vector. For example, the nucleic acid can be cloned into a retroviral vector (e.g., as described in Willemsen et al. (2000) Gene Ther. 7:1369 and Stanislawski et al. (2001) Nature Immunol. 2:962).

The nucleic acid can be introduced into a retroviral packaging line, e.g., 293T cells by transfection, e.g., using calcium phosphate precipitation. In one embodiment, the nucleic acid is transferred to T lymphocytes in culture. For
example, the transfected 293T cells are cocultured with PBMCs activated with an antibody to CD3 and treated with IL-2. During the coculturing, retroviruses produced by the 293 cells infect the PBMC cells. The function of infected T cells can be tested, e.g., using the Cr-release assay in the presence of a target cell that presents the MHC-peptide to which the ligand is directed. The T cells can be also introduced into a subject.

[0380] In a related example, the recipient human T lymphocytes can be obtained from a subject, e.g., a patient, for which treatment is required (i.e., the T lymphocyte is an autologous cell). After introduction of the vector, the modified T lymphocyte can be reintroduced into the subject. Of course, T lymphocytes for such therapy can be obtained from other sources. For example, the recipient T lymphocyte can also be obtained from a relative of the subject or other individual with similar genetic composition, e.g., to minimize adverse immunological reactions.

[0381] Diagnostic Uses

[0382] Protein ligands that bind to a MHC-peptide complex and identified by the methods described herein have in vitro and in vivo diagnostic, therapeutic and prophylactic utilities.

[0383] In one aspect, the present invention provides a diagnostic method for detecting the presence of a MHC-peptide complex that presents a particular peptide, in vitro (e.g., a biological sample, such as tissue, biopsy, e.g., a cancerous tissue) or in vivo (e.g., in vivo imaging in a subject).

[0384] The method includes: (i) contacting a sample with anti-(MHC-peptide complex) ligand; and (ii) detecting formation of a complex between the anti-(MHC-peptide complex) ligand and the sample. The method can also include contacting a reference sample (e.g., a control sample) with the ligand, and determining the extent of formation of the complex between the ligand and the sample relative to the same for the reference sample. A change, e.g., a statistically significant change, in the formation of the complex in the sample or subject relative to the control sample or subject can be indicative of the presentation of a particular peptide (e.g., a TAA) on an MHC in the sample.

[0385] Another method includes: (i) administering the anti-(MHC-peptide complex) ligand to a subject; and (ii) detecting formation of a complex between the anti-(MHC-peptide complex) ligand, and the subject. The detecting can include determining location or time of formation of the complex.

[0386] The anti-(MHC-peptide complex) ligand can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes,prosthetic groups, fluorescent materials, luminescent materials and radioactive materials.

[0387] Complex formation between the anti-(MHC-peptide complex) ligand and a MHC-peptide complex can be detected by measuring or visualizing either the ligand bound to the MHC-peptide complex or unbound ligand. Conventional detection assays can be used, e.g., an enzyme-linked immunosorbent assays (ELISA), a radioimmunoassay (RIA) or tissue immunohistochemistry. Further to labeling the anti-(MHC-peptide complex) ligand, the presence of a MHC-peptide complex can be assayed in a sample by a competition immunoassay utilizing standards labeled with a detectable substance and an unlabeled anti-(MHC-peptide complex) ligand.

[0388] Fluorophore and chromophore labeled protein ligands can be prepared. Since antibodies and other proteins absorb light having wavelengths up to about 310 nm, the fluorescent moieties should be selected to have substantial absorption at wavelengths above 310 nm and preferably above 400 nm A variety of suitable fluorescers and chromophores are described by Stryer (1968) Science, 162:526 and Brand, L. et al. (1972) Annual Review of Biochemistry, 41:843-868. The protein ligands can be labeled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Pat. Nos. 3,940,475, 4,289,747, and 4,376,110. One group of fluorescers having a number of the desirable properties described above is the xantheine dyes, which include the fluorescins and rhodamines. Another group of fluorescent compounds are the naphthyamines. Once labeled with a fluorophore or chromophore, the protein ligand can be used to detect the presence or localization of the MHC-peptide complex in a sample, e.g., using fluorescent microscopy (such as confocal or deconvolution microscopy).

[0389] Histological Analysis. Immunohistochemistry can be performed using the protein ligands described herein. For example, in the case of an antibody, the antibody can be synthesized with a label (such as a purification or epitope tag), or can be detectably labeled, e.g., by conjugating a label or label-binding group. For example, a chelator can be attached to the antibody. The antibody is then contacted to a histological preparation, e.g., a fixed section of tissue that is on a microscope slide. After an incubation for binding, the preparation is washed to remove unbound antibody. The preparation is then analyzed, e.g., using microscopy, to identify if the antibody bound to the preparation.

[0390] Of course, the antibody (or other polypeptide or peptide) can be unlabeled at the time of binding. After binding and washing, the antibody is labeled in order to render it detectable.

[0391] Protein Arrays. The anti-(MHC-peptide complex) ligand can also be immobilized on a protein array. The protein array can be used as a diagnostic tool, e.g., to screen medical samples (such as isolated cells, blood, sera, biopsies, and the like). Of course, the protein array can also include other ligands, e.g., that bind to an MHC-peptide complex or to other target molecules, such as other cancer-specific antigens.


[0393] For example, the array can be an array of antibodies, e.g., as described in De Wildt, supra. Cells that produce the protein ligands can be grown on a filter in an arrayed format. Polypeptide production is induced, and the expressed polypeptides are immobilized to the filter at the location of the cell. At least some of the antibodies, for example, can recognize different MHC-peptide complexes.

[0394] A protein array can be contacted with a labeled target to determine the extent of binding of the target to each immobilized polypeptide ligand. If the target is unlabeled, a sandwich method can be used, e.g., using a labeled probe, to detect binding of the unlabeled target.
Information about the extent of binding at each address of the array can be stored as a profile, e.g., in a computer database. The protein array can be used to identify MHC–peptide complexes that are represented in the sample (e.g., presented on one or more cells in the sample).

FACS. (Fluorescent Activated Cell Sorting). The anti-(MHC–peptide complex) ligand can be used to label cells, e.g., cells in a sample (e.g., a patient sample). The ligand is also attached (or attachable) to a fluorescent compound. The cells can then be sorted using fluorescently activated cell sorted (e.g., using a sorter available from Becton Dickinson Immunocytometry Systems, San Jose Calif.), see also U.S. Pat. Nos. 5,627,037; 5,030,002; and 5,137,809. As cells pass through the sorter, a laser beam excites the fluorescent compound while a detector counts cells that pass through and determines whether a fluorescent compound is attached to the cell by detecting fluorescence. The amount of label bound to each cell can be quantified and analyzed to characterize the sample.

The sorter can also detect the cell and separate cells bound by the ligand from those cells not bound by the ligand. The separated cells can be collected and/or characterized.

In vivo Imaging. In still another embodiment, the invention provides a method for detecting the presence of cancerous tissues in vivo that are presenting TAs on MHC molecules. The method includes (i) administering to a subject (e.g., a patient having a cancer or neoplastic disorder) an anti-(MHC–peptide complex) ligand, conjugated to a detectable marker; (ii) exposing the subject to a means for detecting said detectable marker to the tissues or cells that are presenting the TAA. The protein ligand does not substantially bind the MHC in the absence of the peptide, and does not substantially bind the peptide in the absence of the MHC. For example, the subject is imaged, e.g., by NMR or other tomographic means.

Examples of labels useful for diagnostic imaging in accordance with the present invention include radioisotopes such as 51Cr, 111In, 125I, 99mTc, 32P, 125I, 1H, 13C, and 188Rh. Fluorescent labels such as fluorescein and rhodamine, nuclear magnetic resonance active labels, positron emission isotopes detectable by a positron emission tomography (“PET”) scanner, chemiluminescers such as luciferin, and enzymatic markers such as peroxidase or phosphatase. Short-range radiation emitters, such as isotopes detectable by short-range detector probes can also be employed. The protein ligand can be labeled with such reagents using known techniques. For example, see Wensel and Meares (1983) Radioimmunoimaging and Radioimmunotherapy, Elsevier, New York for techniques relating to the radioimaging of antibodies and D. Colcher et al. (1986) Meth. Enzymol. 121: 802-816.

A radiolabeled ligand of this invention can also be used for in vitro diagnostic tests. The specific activity of a isotopically-labeled ligand depends upon the half-life, the isotopic purity of the radioactive label, and how the label is incorporated into the antibody.


In the case of a radiolabeled ligand, the ligand is administered to the patient, is localized to the tumor bearing the antigen with which the ligand reacts, and is detected or “imaged” in vivo using known techniques such as radio-nuclear scanning using e.g., a gamma camera or emission tomography. See, e.g., A. R. Bradwell et al., “Developments in Antibody Imaging”, Monoclonal Antibodies for Cancer Detection and Therapy, R. W. Baldwin et al., (eds.), pp 65-85 (Academic Press 1985). Alternatively, a positron emission tomography scanner, such as designated PET V1 located at Brookhaven National Laboratory, can be used where the radioisotope emits positrons (e.g., 11C, 15O, 18F).

Magnetic Resonance Imaging (MRI) uses NMR to visualize internal features of living subject, and is useful for diagnosing, diagnosis, treatment, and surgery. MRI can be used without radioactive tracer compounds for obvious benefit. Some MRI techniques are summarized in EP-A-0 502 814. Generally, the differences related to relaxation time constants T1 and T2 of the water protons in different environments is used to generate an image. However, these differences can be insufficient to provide sharp resolution images.

The differences in these relaxation time constants can be enhanced by contrast agents. Examples of such contrast agents include a number of magnetic agents paramagnetic agents (which primarily alter T1) and ferromagnetic or superparamagnetic (which primarily alter T2 response). Chelates (e.g., EDTA, DTPA and NTA chelates) can be used to attach (and reduce toxicity) of some paramagnetic substances (e.g., Fe3+, Mn2+, Gd3+). Other agents can be in the form of particles, e.g., less than 10 μm to about 10 nm in diameter). Particles can have ferromagnetic, antiferromagnetic or superparamagnetic properties. Particles can include, e.g., magnetite (Fe3O4), γ-Fe2O3 ferrites, and other magnetic metallic compounds of transition elements. Magnetic particles may include: one or more magnetic crystals with and without nonmagnetic material. The nonmagnetic material can include synthetic or natural polymers (such as SEPHAROSE™, dextran, dextrin, starch and the like.)

The anti-(MHC–peptide complex) ligands can also be labeled with an indicating group containing of the NMR-active 15F atom, or a plurality of such atoms inasmuch as (i) substantially all of naturally abundant fluorine atoms are the 19F isotope and, thus, substantially all fluorine-containing compounds are NMR-active; (ii) many chemically active polyfluorinated compounds such as trifluoracetic anhydride.
are commercially available at relatively low cost, and (iii) many fluorinated compounds have been found medically acceptable for use in humans such as the perfluorinated polyethers utilized to carry oxygen as hemoglobin replacements. After permitting such time for incubation, a whole body MRI is carried out using an apparatus such as one of those described by Pykett (1982) Scientific American, 246:78-88 to locate and image cancerous tissues.

[0406] Also within the scope of the invention are kits comprising the protein ligand that binds to a MHC-peptide complex and instructions for diagnostic use, e.g., the use of the anti-(MHC-peptide complex) ligand, e.g., antibody or antigen-binding fragment thereof, or other polypeptide or peptide to detect MHC-peptide complex, in vitro, e.g., in a sample, e.g., a biopsy or cells from a patient having a cancer or neoplastic disorder, or in vivo, e.g., by imaging a subject. The kit can further contain a least one additional reagent, such as a label or additional diagnostic agent. For in vivo use the ligand can be formulated as a pharmaceutical composition.

[0407] Mass Spectroscopy

[0408] In another exemplary application, the protein ligands that specifically bind to an MHC-peptide complex are used to isolate cells that have such complexes on their surface or protein complexes released from cells. Peptides in the complexes are analyzed by mass spectroscopy.

[0409] The cells can be isolated by FACS or by binding to a support to which the protein ligand is attached (or becomes attached). After isolation, peptides can be eluted from the surface of the cells and analyzed by mass spectroscopy, e.g., MALDI mass spectroscopy. The molecular weight of the eluted peptides can be used to profile the cell, and e.g., to verify that identity of the peptides displayed by complex to which the ligand is directed, and to identify other peptides displayed by other MHC complexes on the surface.

[0410] Protein complexes can be purified by affinity chromatography using the peptide ligands and similarly analyzed. Flad et al. (1998) Cancer Res 58:5803-11 describe use of MALDI to identify peptides presented by HLA-Class I proteins.

[0411] Anti-(MHC-Peptide Complex) Ligands

[0412] Table 1 lists exemplary peptides that are displayed by cancer cells as an MHC complex. Protein ligands can be identified which specifically bind to these peptides when they are displayed on an MHC.

### TABLE 1

<table>
<thead>
<tr>
<th>Protein Fragment Name</th>
<th>Amino acid Sequence</th>
<th>SEQ ID NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp100 G9-209</td>
<td>(IMQVPFSV)</td>
<td>SEQ ID NO: 1</td>
</tr>
<tr>
<td>gp100 G9-280</td>
<td>(YLFQPPTTV)</td>
<td>SEQ ID NO: 2</td>
</tr>
<tr>
<td>gp100 G9-154</td>
<td>(KTVQFQKV)</td>
<td>SEQ ID NO: 3</td>
</tr>
<tr>
<td>MUC1 D6</td>
<td>(LLTTTLTV)</td>
<td>SEQ ID NO: 4</td>
</tr>
<tr>
<td>TAX</td>
<td>(LLFGPVTV)</td>
<td>SEQ ID NO: 121</td>
</tr>
<tr>
<td>hTERT T540</td>
<td>(ILAKFLHWL)</td>
<td>SEQ ID NO: 5</td>
</tr>
<tr>
<td>hTERT T685</td>
<td>(RVDIDPLUV)</td>
<td>SEQ ID NO: 6</td>
</tr>
</tbody>
</table>

[0413] See, also Renkvist et al. (2001) Cancer Immunol Immunother 50:3-15 for a list of additional peptide-MHC complexes for which protein ligands can be identified.

Table 2 lists antibodies that bind to an MHC-peptide complex wherein the peptide component is a peptide fragment of gp100.

### TABLE 2

<table>
<thead>
<tr>
<th>Antibody Name</th>
<th>Peptide Recognized</th>
<th>Nucleic acid Sequence SEQ ID NO:</th>
<th>Amino-acid sequence SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A11</td>
<td>G9-209</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>1A7</td>
<td>G9-209</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>1A9</td>
<td>G9-209</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>1C8</td>
<td>G9-209</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>1D7</td>
<td>G9-209</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>1G2</td>
<td>G9-209</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>2D2</td>
<td>G9-280</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>2C5</td>
<td>G9-280</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td>2D3</td>
<td>G9-280</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>2F1</td>
<td>G9-280</td>
<td>43</td>
<td>44</td>
</tr>
<tr>
<td>3G3F12</td>
<td>G9-154</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td>3G3F3</td>
<td>G9-154</td>
<td>51</td>
<td>52</td>
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<tr>
<td>3G3F4</td>
<td>G9-154</td>
<td>55</td>
<td>56</td>
</tr>
</tbody>
</table>

[0414] Table 2 lists antibodies that bind to an MHC-peptide complex wherein the peptide component is a peptide fragment of gp100.

[0415] Table 3 lists antibodies that bind to an MHC-peptide complex wherein the peptide component is a peptide fragment of hTERT.

### TABLE 3

<table>
<thead>
<tr>
<th>Antibody Name</th>
<th>Peptide Recognized</th>
<th>Nucleic acid Sequence SEQ ID NO:</th>
<th>Amino-acid sequence SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A12</td>
<td>TB65</td>
<td>99</td>
<td>102</td>
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<tr>
<td>3B1</td>
<td>TB65</td>
<td>103</td>
<td>104</td>
</tr>
<tr>
<td>3F5</td>
<td>TB65</td>
<td>107</td>
<td>108</td>
</tr>
<tr>
<td>3G3</td>
<td>TB65</td>
<td>111</td>
<td>112</td>
</tr>
<tr>
<td>3H2</td>
<td>TB65</td>
<td>115</td>
<td>116</td>
</tr>
</tbody>
</table>

[0416] Table 4 lists antibodies that bind to an MHC-peptide complex wherein the peptide component is a peptide fragment of MUC-1.

### TABLE 4

<table>
<thead>
<tr>
<th>Antibody Name</th>
<th>Peptide Recognized</th>
<th>Nucleic acid Sequence SEQ ID NO:</th>
<th>Amino-acid sequence SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>3M3A1</td>
<td>MUC-1 D6</td>
<td>63</td>
<td>64</td>
</tr>
<tr>
<td>3M3B8</td>
<td>MUC-1 D6</td>
<td>67</td>
<td>68</td>
</tr>
</tbody>
</table>

[0417] Table 5 lists antibodies that bind to an MHC-peptide complex wherein the peptide component is a peptide fragment of TAX.
TABLE 5

<table>
<thead>
<tr>
<th>Antibody Name</th>
<th>Peptide Sequence</th>
<th>Nucleic acid Sequence</th>
<th>Amino acid Sequence</th>
<th>MHC-Bound</th>
<th>SEQ ID NO:</th>
<th>Antibody Name</th>
<th>Peptide Sequence</th>
<th>Nucleic acid Sequence</th>
<th>Amino acid Sequence</th>
<th>MHC-Bound</th>
<th>SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3E3 TAX</td>
<td>71 73</td>
<td>light chain</td>
<td>heavy chain</td>
<td>72 74</td>
<td></td>
<td>T3F1 TAX</td>
<td>75 77</td>
<td>light chain</td>
<td>heavy chain</td>
<td>76 78</td>
<td></td>
</tr>
<tr>
<td>T3F2 TAX</td>
<td>79 81</td>
<td>light chain</td>
<td>heavy chain</td>
<td>80 82</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HLA Classes and Alleles

[0418] The following are exemplary HLA alleles: A; B; Cw; DMA; DMB; DOA; DPA1; DPB1; DQA1; DQB1; DRA; DRB1; DRB3; DRB4; DRB5; DRB6; DRB7; E; G; MICA; TAP1; TAP2. See also Human Mutation 11:1-3, 1998.

[0419] The following invention is further illustrated by the following examples, which should not be construed as further limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

Example 1

Methods for Selection and Screening

[0420] 1. Production of Biotinylated scMHC/peptide Complexes

[0421] scMHC/peptide complexes were produced by in vitro refolding of inclusion bodies produced in E. coli as described (Denkberg and Reiter (2000) Eur. J. Immunol. 30:3522-32). Briefly, a single chain 132 microglobulin -HLA/A2 (scMHC) construct, in which the β2m and HLA-A2 genes are connected to each other by a flexible peptide linker, was designed to contain the BiA recognition sequence for site-specific biotinylation at the C-terminus (scMHC-BiA). This construct is expressed in E. coli and upon induction with IPTG, intracellular inclusion bodies that contain large amounts of the recombinant protein accumulate. Inclusion bodies are purified, reduced and subsequently refolded in a redox-shuffling buffer system (0.1M Tris, 0.5M Arginine, 0.09 mM Oxidized Glutathione, pH 8.0) in the presence of a 5-10 molar excess of the antigenic peptides. Correctly folded MHC/peptide complexes were isolated and purified by anion exchange Q-SEPHAROSE™ chromatography (Pharmacia). Filtration using CENTRICON™ 30 units (CENTRICON™) was used to exchange the elution buffer with Tris-HCl (10 mM, pH 8.0) and concentrate the scMHC/peptide complex to 1 mg/ml for specific biotinylation using the BiA enzyme (Aviddy, Denver, Colo.) as previously described (Altmun et al. (1996) Science 274:94-96; Denkberg and Reiter (2000) Eur. J. Immunol. 30:3522-32). Excess biotin was removed from biotinylated complexes using a G-25 desalting column. The homogeneity and purity of the scMHC/peptide complexes were analyzed by various biochemical means including SDS-PAGE, Size exclusion chromatography, and ELISA assays as described previously (Denkberg and Reiter (2000) Eur. J. Immunol. 30:3522-32). The biological function of the scMHC/peptide complexes was determined by the ability of the scMHC/peptide complexes to stain CTL lines and clones in a peptide-specific manner. The generation of the scMHC/peptide tetramers and CTL staining procedures have been previously described in detail (Denkberg and Reiter (2000) Eur. J. Immunol. 30:3522-32; Denkberg and Reiter (2001) J Immunol 167, 270-6).

[0422] 1.2 Selection of Phage-Antibodies on Biotinylated Complexes

[0423] A large human Fab library containing 3.7 x 10^10 different Fab clones was used for the selection (de Haard et al. (1999) J Biol. Chem. 274:18218-30). Phages (10^5) were first preincubated for 1 hr at room temperature in PBS containing 2% nonfat dry milk with streptavidin-coated paramagnetic beads (200 μl; Dynal, Oslo) to deplete streptavidin binders. Streptavidin-coated paramagnetic beads (200 μl; Dynal, Oslo) were also incubated in PBS+2% milk for 1 hr at room temperature. The remaining phages were subsequently incubated for 1 hr with decreasing amounts of biotinylated scMHC/peptide complexes (500 nM for the first round and 100 nM for the following rounds). Streptavidin magnetic beads were added, and the mixture was incubated for 15 min with continuous rotation. A magnetic force was applied to pull down phages bound to biotinylated complexes. After 10 washes of the streptavidin-bound complexes with PBS (0.1% TWEEN and 2 washes with PBS, bound phages were eluted by incubation for min with 1 ml of Triethylenyl (TEA) (100 mM). The elution mixture was neutralized by the addition of 100 μl of Tris-HCl (1M, pH 7.4) and used to infect E. coli (TG1) cells (OD600=0.5) for 30 min at 37°C. Bacteria were grown overnight at 30°C on 2YT plates containing 100 μg/ml Ampicillin (2YT/A/G) and 2% glucose.

[0424] 1.3 Fab Selection and Purification of Soluble Recombinant Fab Antibodies

[0425] For Fab selection, colonies were collected from the plates in 2YT/A/G and diluted 1:100 in 50 ml of medium. Cells were grown to O.D600 nm = 0.5 and M13KO7 helper phage (5 x 10^12 cfu) was added to 5 ml of the culture. After incubation at 37°C for 30 min, the cells were centrifuged, resuspended in 25 ml of 2YT/Ampicillin (100 μg/ml) and grown overnight at 30°C. Phages were collected from culture supernatants and purified for the next round of panning by PEG precipitation. The diversity of the selected antibodies was determined by DNA fingerprinting. The Fab DNA of different clones was PCR-amplified using the primers pUC-reverse (5'-AGCGGATAACATTTCAACAGGG-3'; SEQ ID NO:119) and fl-tet-seq24 (5'-TTTTGGCCTTTTCCTACGCGTGAGT-3'; SEQ ID NO:120). The resulting PCR fragments were digested with BstNI (New England Biolabs, Mass. USA) (2 hr, 37°C) and analyzed by agarose gel electrophoresis.

[0426] 1.3 Expression and Purification of Soluble Recombinant Fab Antibodies

The Fab clones were purified from the periplasmic fraction of BL21 cells using the hexa-histidine tag fused to the CH1 domain of the Fabs. We have produced and analyzed 2-4 Fab clones for each complex, which were selected according to their specificity pattern assayed by ELISA with phage and soluble Fab fragments. An overnight starter culture of Fab specific clones was grown at 30°C. Cells were diluted 1:100 into 500 ml of 2YT/A/G, grown to OD600 nm = 0.8-1.0 and induced to express the recombinant Fab antibody by the addition of 1 mM IPTG for 4 hr at 30°C. The cells were centrifuged and the pellet was resuspended in 5 ml of a B-PER solution (Pierce) to release periplasmic contents. After 30 min of rotated incubation at RT, the solution was centrifuged (15000 rpm, 15 min) and the supernatant was incubated with 0.5 μl of pre-washed TALON beads suspension (Clontech) for 45 min at RT. The solution was applied onto a Biosan disposable column, and after sedimentation the beads were washed three times with 10 ml of PBS/0.1% Tween20 (pH
8.0). The bound Fabs were eluted using 0.5 ml of 100 mM Imidazole in PBS. The eluted Fabs were dialyzed twice against PBS (overnight; 4°C) to remove residual imidazole. The homogeneity and purity of the purified Fabs was determined by analysis on non-reduced and reduced SDS-PAGE.

**[0427]** 1.4 ELISA with Phage Clones and Purified Fab Antibodies

**[0428]** The binding specificity of individual phage clones and soluble Fab fragments was determined by ELISA using biotinylated scMHC-peptide complexes. ELISA plates (Falcon) were coated overnight with BSA-biotin (1 μg/well). After having been washed, the plates were incubated (1 hr, RT) with streptavidin (1 μg/well), washed extensively and further incubated (1 hr, RT) with 0.5 μg of MHC/peptide complex. Plates were blocked for 30 min at RT with PBS/2% and subsequently were incubated for 1 hr at RT with phage clones (~10⁶ phages/well) or various concentrations of soluble purified Fab, and after washing, with 1:1000 HRP-conjugated/anti-myc antibody. Detection was performed using TMB reagent (Sigma).

**[0429]** 1.5 Flow Cytometry

**[0430]** The B cell line RMAS-HHD, which is transfected with a single-chain 2M-HLA-A-2 gene (Pascolo et al. (1997) J Exp Med. 185, 2043-51), EBV-transformed B-lymphoblast JY cells or tumor cells as indicated were used to determine the reactivity of the recombinant Fab. The cells were incubated overnight at 26°C in medium containing 1000/1 of the peptide. JY cells were loaded with peptide (10004) at 37°C. The APCs were subsequently incubated at 37°C for 2-3 hours to stabilize cell surface expression of MHC-peptide complexes. The cells were incubated for 60-90 min at 4°C with recombinant Fab antibodies (10-100 μg/ml) in 100 μl. After three washes the cells were incubated with FITC-labeled anti-human Fab (Jackson). After a final wash, the cells were resuspended in ice-cold PBS.

**[0431]** Adherent tumor cells were harvested by trypsinization and resuspended in cold RPMI.

**[0432]** All subsequent washes and incubations were performed in ice-cold PBS as described above for RMAS-HHD peptide-loaded cells. Analysis of the cells was performed by FACStar flow cytometry (Becton Dickinson) and the results were analyzed with the WinMDI program (Trotter J. et al. online resource provided by the FACs facility at Scripps, La Jolla Calif.).

**[0433]** 1.6 Competition Binding Assays

**[0434]** Flexible ELISA plates were coated with BSA-biotin and scMHC-peptide complexes (10 μg in 100 μl) were immobilized as described. The binding of soluble purified Fabs was performed by competitive binding analysis examining the ability of purified Fab to inhibit the binding of [125I]-Fab to the specific immobilized scMHC-peptide complex. The recombinant Fab antibodies were labeled with [125I] using the Bolton-Hunter reagent. The labeled Fab was added to wells as a tracer (3×10⁵ CPM/well) in the presence of increasing concentrations of the cold Fab fragments as a competitor. Next, the binding assays were performed at RT for 1 hr in PBS. Finally, the plates were washed extensively (5 times) with PBS and the bound radioactivity was determined in a gamma counter. The apparent affinity of the Fabs was determined by extrapolating the concentration of competitor necessary to achieve 50% inhibition of [125I]-labeled Fab binding to the immobilized scMHC-peptide complex. Non-specific binding was determined by the addition of a 20-40-fold excess of unlabeled Fab.

**Example 2**

**GP100-HLA-A2 Antibodies**

**[0435]** Here, for the first time, we have isolated a panel of high affinity human recombinant Fab antibodies endowed with the antigen-specific, MHC-restricted specificity of T cells. These antibodies recognize three common HLA-A2-restricted epitopes of the human melanoma differentiation antigen gp100. HLA-A2 is the most frequent human MHC allel that displays many cancer-associated peptides. The antibodies were isolated from a large non-immune repertoire of phage antibody library selected on recombinant-engineered single-chain MHC-peptide complexes displaying a distinct gp100-derived epitope.

**[0436]** We show that this panel of antibodies recognizes HLA-A2 molecules only when displaying the specific peptide against which they were selected; they do not bind HLA-A2 molecules complexed with other gp100-derived epitopes or with other HLA-A2-restricted control peptides. Hence, they exhibit a TCR-like restriction. Moreover, these antibodies have been used to directly visualize the specific HLA-A2 gp100 epitopes on antigen-presenting cells as well as on the surface of melanoma tumor cells by flow cytometry.

**RESULTS:**

**[0437]** Recombinant Single-Chain MHC-Peptide Complexes with Three Melanoma-Derived gp100, HLA-A2-Restricted Peptides.

**[0438]** gp100 is a melanocyte lineage-specific membrane glycoprotein consisting of 661 amino acids that is expressed in most melanoma cells. This protein is recognized by many HLA-A2-restricted melanoma reactive tumor infiltrating lymphocytes (TILs) that have been isolated from melanoma patients (Kawakami et al. (1994) Proc. Natl. Acad. Sci. U.S.A 91:6458-62; Bakker et al. (1994) J. Exp. Med. 179:1005-09). Five T cell epitopes have been identified in gp100, three of them are common immunogenic epitopes recognized by CTLs derived from different patients (Kawakami et al. (1995) J. Immunol. 154:3961-68; Cox et al. (1994) Science 264:716-19); G9209 (IMDQVPFVS; SEQ ID NO:1), G9280 (YLEEPGVPVT; SEQ ID NO:2), and G9154 (KTWGQY-WQQ; SEQ ID NO:3). Recombinant MHC-peptide complexes that display the three

**[0439]** gp100-derived epitopes were generated by using a single-chain MHC (scMHC) construct that was previously described (Denkberg and Reiter (2000) Eur. J. Immunol. 30:3522-32; Denkberg and Reiter (2001) J Immunol 167, 270-6). In this construct, the extracellular domains of HLA-A2 are connected into a single-chain molecule with β2 microglobulin using a 15-amino-acid flexible linker. The scMHC-peptide complexes were produced by in vitro refolding of inclusion bodies, from bacterial cultures transformed with the scMHC construct, in the presence of each of the three gp100-derived peptides. Soluble recombinant scMHC-peptide complexes were obtained from refolding solutions using a purification protocol employing ion-exchange and size-exclusion chromatography. The refolded gp100-derived peptide-MHC complexes were very pure, homogeneous and in monomeric form as shown by analysis on SDS-PAGE and size-exclusion chromatography. Recombinant scMHC-pep-
tide complexes generated by this strategy have been previously characterized in detail for their biochemical, biophysical, and biological properties and were found to be functional (Denenberg and Reiter (2000) *Eur. J. Immunol.* 30:3522-32; Denenberg and Reiter (2001) *J. Immunol.* 167, 270-6). To demonstrate that the refolded gp100-derived MHC-peptide complexes are functional, we tested their ability to stain a gp100-derived G9209-specific CTL clone (Dudley and Rosenberg (2000) *Cancer Res.* 60:69-77). To this end, we generated scMHC-G9209 tetramers as described previously (Denenberg and Reiter (2000) *Eur. J. Immunol.* 30:3522-32). To date, this is a well-established strategy for overcoming the low affinity of the MHC-peptide-TCR interactions (Altman et al. (1996) *Science* 274:94-96; Lee et al. (1999) *Nat. Med.* 5:677-85; Ogg et al. (1998) *Science* 279:2103-06). The scMHC-G9209 tetramers could specifically stain the G9209-restricted CTL clone R6C12 (FIG. 29A). However, a G9280 epitope-containing tetramer did not bind to these cells (FIG. 29B) nor to tetramers could also activate the R6C12 CTLs, as demonstrated by secretion of interferon-γ. These results suggest that the recombinant scMHC complexes are functional and retain the conformation of the native MHC-peptide complex.

[0440] Selection of Recombinant Antibodies with TCR-Like Specificity to Three Common T Cell Epitopes of the Melanoma Antigen gp100

[0441] To enable efficient selection scMHC-peptide complexes were biotinylated using a BirA sequence tag that was engineered at the C-terminus of the HLA-A2 gene for site-specific biotinylation as previously described (Altman et al. (1996) *Science* 274:94-96; Denenberg and Reiter (2000) *Eur. J. Immunol.* 30:3522-32). A large naive repertoire of $3.7 \times 10^{10}$ human recombinant Fab fragments (de Haard et al. (1999) *J. Biol. Chem.* 274:18218-30) was incubated first with streptavidin-coated beads to avoid the selection of anti-streptavidin antibodies. A magnetic field was applied to precipitate the beads, and the supernatant containing the library depleted of streptavidin binders was used for the subsequent panning in solution on soluble recombinant scMHC-peptide complexes containing each of the three gp100-derived T cell epitopes. After incubation of the library with soluble complexes, binding phases were collected using streptavidin-coated magnetic beads followed by elution with triethylamine. A 1000 to-2500-fold enrichment in phage titer was observed after three rounds of panning using each of the three different gp100-derived peptide-MHC complexes (Table 1).

[0442] An ELISA with phage particles was performed to determine phage specificity on biotinylated recombinant scMHC-peptide complexes immobilized to BSA-biotin-streptavidin-coated immunoplates. The BSA-biotin-streptavidin spacer enables the correct folding of the complexes, which can be distorted by direct binding to plastic. About 70-90% of randomly selected phages from the third round of panning on each complex reacted with the corresponding MHC-peptide complex (Table 1). The precise specificity of the selected phage antibodies was determined by a differential ELISA on wells coated with scMHC HLA-A2 complexes containing either the specific gp100-derived peptide or control HLA-A2-restricted peptides including the two other gp100-derived epitopes (FIGS. 30A-30C). The diversity pattern of the selected specific clones was assessed by DNA fingerprinting analysis. Two types of Fab phage clones were observed following these specificity assays. One type bound to the HLA-A2/peptide complex without peptide specificity and the second bound to the HLA-A2 complex with a peptide specific manner (termed in Table 1 as TCR-like binders). We assayed these specific clones and revealed the following specificity results: for the G9154 epitope, 24 clones out of 90 analyzed (27%) reacted specifically with the HLA-A2-G9154 complex but not with complexes containing the gp100-derived peptides G9280, G9209, nor with HTLV-I TAX or melanoma MART1-containing scMHC complexes (Table 1 and FIG. 30A). As a representative analysis of 10 TCR-like Fab clones, diversity analysis of these clones identified 10 different patterns. Thus, several different antibodies with TCR-like specificity were selected. For the G9209 epitope, 20 clones out of the 94 analyzed (21%) reacted specifically with the HLA-A2-G9209 complex but not with control complexes (Table 1 and FIG. 30B analyzing 5 clones). These positive clones yielded 4 different fingerprint patterns. Finally, the panning on HLA-A2 complexes containing the G9280 epitope resulted in 15/94 specific peptide-restricted clones (16%) (Table 1 and FIG. 30C analyzing 5 clones), which exhibited 3 different fingerprint patterns. Most interesting is the unexpected high frequency of idiotropic TCR-like binders that represent 16-27% of the phage clones binding to the MHC-peptide complex (Table 1).

[0443] For all three HLA-A2-gp100 peptide complexes screened, we isolated several of such Fab antibodies displaying TCR-like binding pattern, and in all 3 cases, one particular clone dominated the population after 3 rounds of selection (at a frequency of 30-50%).

Characterization of Recombinant Soluble Fab Antibodies with TCR-Like Specificity

[0444] We have selected 2-4 Fab clones for each HLA-A2-gp100-derived complex that exhibited the most specific peptide-dependent and TCR-like binding pattern as analyzed by the phage ELISA assays presented above. These Fab fragments that bind specifically to each of the three gp100-derived HLA-A2-peptide complexes were produced in a soluble form in *E. coli* TG1 or BL21 cells and were purified by IMAC as described in materials and methods. Yields were approximately 0.5-2 mg of pure material from 1 liter of bacterial culture. SDS-PAGE analysis revealed a homogenous and pure population of Fabs with the predicted molecular size.

[0445] The binding specificity of these purified Fab fragments was determined by ELISA assays on biotinylated MHC-peptide complexes immobilized to wells through BSA-biotin-streptavidin. The correct folding of the bound complexes and their stability during the binding assays were determined by their ability to react with the conformational specific monoclonal antibody W6/32 which binds HLA complexes only when folded correctly and when it contains peptide. When we used soluble purified Fabs, these ELISA assays revealed a very specific recognition pattern (FIGS. 31A-31D). Two Fab clones, G2D12 and G3G4, selected to bind the G9154 HLA-A2 complex, bound only to the specific complex but not to complexes displaying the G9209 or G9280 peptides nor to HLA-A2 complexes containing a MUC1-derived peptide (Cannon et al. (2000) *Int J. Cancer*. 85:391-7) or the HTLV-I-derived TAX peptide (FIG. 31A).

[0446] Fab clones specific for the G9209 HLA-A2 complex recognized only this complex, but not the two other gp100-derived peptides in the same context, nor two telomerase-derived HLA-A2 complexes (FIG. 31D). Finally, the HLA-A2-G9280-specific Fab clones recognized only their G9280-derived complexes and no other MHC-peptide complexes
The Fab antibodies did not recognize any of 5-7 other HLA-A2-peptide complexes, the peptide alone, empty HLA-A2 molecules (which are difficult to produce because they are unstable in the absence of a peptide), neither streptavidin or other protein antigens. Thus, these antigen-specific Fab fragments exhibit binding characteristics and the fine specificity of a TCR-like molecule.

**[0447]** The ELISA binding specificity results were confirmed in competition experiments, in which excess specific and control soluble sMHC-peptide complexes were present in solution and competed for binding to the immobilized complex. Competition was observed with the specific soluble MHC-peptide complex but not with control complexes. An example for this type of assay is shown in FIG. 31D, in which soluble G9280-containing HLA-A2 but not G9154/HLA-A2 complexes in solution competed and inhibited the binding of Fab 2F1 to the immobilized G9280/HLA-A2 complexes.

**[0448]** Next, the affinity binding properties of the TCR-like soluble Fabs were determined using a saturation ELISA assay in which biotinylated complexes were bound to BSA-biotin-streptavidin-coated plates to which increasing amounts of Fab antibody were added. The binding of three specific Fabs to the corresponding gp100-derived HLA-A2-peptide complexes was dose-dependent and saturable (FIGS. 32A-32C). Extrapolating the 50% binding signal revealed that these antibodies possess high affinity with a binding affinity in the nanomolar range. To determine the apparent binding affinity of the TCR-like Fab fragments to their cognate MHC-peptide complex, we performed a competition binding assay in which binding of 125I-labeled Fab competed with increasing concentrations of unlabeled Fab fragment. The apparent binding affinity of three Fabs, each of them specific for one of the three gp100-derived T cell epitopes was measured to be 15 to 30 nM (FIGS. 32D-32F). These results underscore our success in isolating high affinity Fab antibodies with TCR-like specificity from a large non-immune phage-displayed repertoire of antibodies.

**[0449]** To demonstrate that the isolated soluble Fab antibodies can bind the specific MHC-peptide complex not only in its recombinant soluble form but also in the native form as expressed on the cell surface, we utilized two APC systems. The first consists of the murine TAP-deficient RMA-S cells transfected with the human HLA-A2 gene in a single-chain format (Figs. 33A and 33B) (HLA-A2,1;Cl-82m singl-chain) (RMA-S-HHD cells). gp100-derived or control peptides were loaded on the RMA-S-HHD cells and the ability of the selected Fab antibodies to bind to peptide-loaded cells monitored by FACS. Peptide-induced MHC stabilization of the TAP2 mutant RMA-S-HHD cells was determined by analyzing the reactivity of anti HLA-A2 MAb BB7.2 with peptide loaded and unloaded cells using FACS. Fab G2D12, which recognized the G9154-containing HLA-A2 complex, reacted only with RMA-S-HHD cells loaded with the G9154 peptide but not with cells loaded with the G9280 peptide or control cells not loaded with peptide. Similarly the G9209-HLA-A2-specific Fab antibody IA9 recognized RMA-S—HHD cells loaded with G9209 peptide but not at all loaded cells with G9154 peptide. Similar results were observed in FACs analysis of the G9280-specific Fab antibody 2F1. The Fab antibodies were analyzed on RMA-S-HHD cells loaded with 5 different control HLA-A2-restricted peptides including cross-reaction studies among the gp100-derived peptides and similar specificity results were observed. Moreover, RMAS-HHD cells loaded with the G9154 epitope reacted only with Fab G2D12 directed toward the G9154-containing complex but not with Fabs IA9 and 2F1 recognizing HLA-A2 in complex with the G9209 or G9280 epitopes respectively.

**[0450]** The second type of APCs tested were EBV-transformed B lymphoblast cell lines, which express HLA-A2, and were incubated with the gp100-derived or control peptides. These cells are TAP+, and consequently, displaying the exogenous peptide is facilitated by peptide exchange. Using this strategy, we obtained a mixture of exogenously and endogenously derived peptides presented on HLA-A2 that are displayed on the cell surface. In testing the HLA-A2/gp100-specific antibodies IA9, 2F1, and G2D12, we found intensive staining of JY cells loaded with the specific gp100-derived peptide to which they were selected but no binding was observed when other gp100 or control peptides were used. Control antibodies recognizing a telomerase-derived peptide in complex with selfHLA-A2 did not bind to the gp100-derived peptide-loaded JY cells. Furthermore, no binding was observed when these antibodies were incubated with an HLA-A2-EBV B cell line loaded with the gp100 or control peptides.

**[0451]** These results show that the Fab antibodies exhibit TCR-like fine specificity and can specifically recognize their corresponding native HLA-A2 complexes in situ on the surface of cells.

**[0452]** To explore whether these TCR-like Fab antibodies would bind endogenously derived MHC-peptide complexes and therefore may eventually be used to visualize these complexes on the surface of tumor cells, we performed flow cytometry analysis on HLA-A2+/melanoma tumor cell lines (FIGS. 33A-33H). These cells represent the normal situation in which MHC-peptide complexes are expected to be present at a much lower density on the cell surface compared with the peptide-loaded RMAS-HHD or JY cells used above. The G9154-specific Fab antibody G2D12 reacted very intensely in a dose dependent manner with the HLA-A2+ gp100+ melanoma FM3D (FIGS. 33A and 33B) and YU ZAZ6 cells (FIGS. 33C and 33D), but not with the HLA-A2-melanoma MZ2-MEL.3.0 cells (FIGS. 33E and 33F) or the HLA-A2+ breast carcinoma tumor cell line MCF7 (FIGS. 33G and 33H). Anti-HLA-A2 MAb BB7.2 was used to confirm HLA type expression (in addition to genomic PCR using HLA-A2-specific primers). A control Fab antibody specific for the HTLV-1-derived HLA-A2-TAX complex did not bind to either cell line (FIGS. 33A, 33C, 33E, and 33G). These results demonstrate that, although in a monovalent form, the high affinity of the Fab antibodies enables efficient detection and visualization of MHC-peptide complexes on the surface of tumor cells. Hence, these TCR-like antibodies can bind to cells that express the specific MHC-peptide complex at a density most likely to be found on gp100-expressing tumor cells, antigen-presenting cells, dendritic and other lymphoid cells involved in tumor antigen presentation to the immune system. Fab antibodies IA9 and 2F1 specific to the G9209 or G9280 gp100-derived epitopes, respectively, also reacted with FM3D cells but with a lower intensity. This may reflect differential expression of gp100-derived epitopes known as the antigenic variation phenomenon. Indeed, FM3D cells
were shown to express high levels of the G9154 epitope in comparison with the two other epitopes as revealed by their relative sensitivity to CTLs specific to the different gp100-derived epitopes in killing assays (Kirkpin et al. (1995) Cancer Immunol. Immunother. 41:71-81).

Discussion

[0453] In this study we have demonstrated the ability to select from a large non-immune repertoire of human Fab fragments a panel of antibodies directed to several T cell epitopes within a single cancer antigen, the melanoma associated antigen gp100.

[0454] These antibodies exhibit a very specific and special binding pattern, they can bind with a peptide-specific manner to HLA-A2 complexes. Hence, these are recombinant antibodies with T cell antigen receptor-like specificity. In contrast to the inherent low affinity of TCRs, these molecules display the high affinity binding characteristics of antibodies, while retaining TCR specificity. We have shown by direct ELISA assays and flow cytometry studies that the Fab antibodies selected against the three common immunogenic T cell epitopes of gp100 bind only to the specific HLA-A2 complex and not to control complexes generated with the other two gp100-derived epitopes nor to other HLA-A2-peptide complexes. Most importantly, these recombinant antibodies specifically recognize native gp100-derived MHC-peptide complexes on the surface of cells, including binding to melanoma tumor cells. In this way, they serve as an example of soluble high affinity recombinant TCR-like antibodies capable of binding and detecting specific MHC-peptide complexes on the surface of tumor cells. Interestingly, we were able to isolate a repertoire of several antibodies against each of the gp100-derived epitopes. They exhibit a very specific recognition pattern toward each of the three T cell epitopes even though they are encoded within a single cancer antigen. Until now antibodies with TCR-like specificity have been generated against murine MHC-peptide complexes employing various strategies of immunizations (Andersen et al. (1996) Proc. Natl. Acad. Sci. U.S.A 93:1820-24; Porgador (1997) Immunity 6:715-26; Dadaglio (1997) Immunity 6, 727-38; Murphy et al. (1989) Nature 338:765-8; Aharoni (1991) Nature 351:147-50). Recently the same Fab library was used to select for HLA-A1-MAGE-A1-specific binding antibodies (Chames and Hoogenboom (2000) Proc. Natl. Acad. Sci. U.S.A. 97:7969-74). One specific clone, G8, was selected which exhibited TCR-like specificity but revealed a relatively low affinity of 250 nM. Most strikingly, here we selected several different TCR-like antibodies against each MHC-peptide complex screened, whereas all previous successful experiments reported the ability to isolate only a single antibody clone (Andersen et al. (1996) Proc. Natl. Acad. Sci. U.S.A. 93:1820-24; Porgador (1997) Immunity 6:715-26; Chames and Hoogenboom (2000) Proc. Natl. Acad. Sci. U.S.A. 97:7969-74). We also selected an immune phage library constructed from HLA-A2 transgenic mice immunized with the gp100-derived G9209-containing HLA-A2 complex. In contrast to our ability to isolate several antibodies against each MHC-peptide complex using the large non-immune Fab library, we could only isolate a single antibody clone from the murine immunized library, which exhibited TCR-like fine specificity.

[0455] The fact that high-affinity antibodies with such unique fine specificity were readily obtained in this study, and that they were in some cases nanomolar affinity, underscores the power of the display technology for this application, as well as add proof to the quality of the human non-immune antibody library used in the selections. The observation that 20-30% of the MHC-peptide binding antibodies had the fine specificity of a TCR-like molecule is nevertheless surprising, especially since they were selected from a non-immune repertoire considered not to be biased towards such specificity. More recently we have been able to isolate from the same phage library recombinant Fab's against a large variety of MHC-peptide complexes containing other cancer-associated or viral HLA-A2-restricted peptides, indicating that this behavior is not gp100 or peptide related.


[0457] It would have been possible that one particular antibody family or antibody V-gene segment would have an intrinsic propensity to bind HLA-A2 molecules, and that the high frequency could be explained by a high abundance of such antibodies in the non-immune library. However, the sequences of the selected clones are derived from many different antibody families and germline segments, without any biases visible in the CDRs either. The high frequency and high affinities for some of the antibodies isolated here, suggest that these molecules may well be present at a high frequency in the antibody repertoires from the B-cell donors of the phage library, but an in vivo role for such antibodies remains unclear.

[0458] Whatever eventually the reason for this high frequency of antibodies to MHC-peptides may be, it appears that this phage-based approach can be successfully applied to isolate recombinant antibodies with TCR-like specificity to a large variety of MHC-peptide complexes. Thus, it may now become possible to dissect the role of antigens in various pathological conditions such as cancer, viral infections and autoimmune disease, not only at the level of the T-cell using MHC-tetramers, but also at the level of the APC and diseased cell, using antibodies of the type described in this paper.

[0459] Recombinant antibodies with TCR-like specificity, such as have been selected and characterized in this study, represent a valuable new tool in molecular immunology for several major fields of research: (1) for studying antigen presentation in cancer, (2) for developing new immunotherapy targeting molecules, and (3) for studying structure-function relationships in TCR-peptide-MHC interactions. We have shown that these antibodies can be used to detect and visualize the presence of specific T cell epitopes (MHC-peptide complexes) by standard methods of flow cytometry. With appropriate conservation of the MHC-peptide complexes during fixation, the antibodies can be used to detect such complexes by immuno-histochemistry opening the door for widespread use in pathology. Indeed, preliminary experiments demonstrate that these Fab's stain a fixed melanoma cell line by immuno-histochemistry. As such, they are useful for the study and analysis of antigen presentation on tumor cells by determining the expression of specific tumor-related MHC-peptide complexes on the surface of tumor cells, metastasis, antigen-presenting cells, and lymphoid cells. Such antibodies are also particularly useful for determining

[0460] The molecules described here are the first examples of high affinity human antibodies directed against the most frequent HLA haplotype, HLA-A2, complexed with cancer peptides. These very specific molecules, which recognize a very specific human tumor antigen, can be used as targeting moieties in various antibody-based immunotherapeutic approaches. This includes the use of these antibodies as recombinant immunotoxins (Pustan (1997) *Biochim Biophys Acta.* 1333, C1-6), fusions with cytokine molecules (Lode and Reisfeld (2000) *Immunol Res.* 21:279-88); bi-specific antibody therapy (Withoff (2001) *Curr Opin Mol. Ther.* 3:53-62) or immuno-gene therapy (Willemsen et al. (2000) *Gene Ther.* 7:1369).

[0461] Another interesting aspect for the use of these TCR-like Fab antibodies is for structure-function studies of MHC-peptide-TCR interactions. By mutating particular residues in the specific peptide and testing the influence of these mutations on the binding of the Fab antibodies and peptide-specific T cell clones it may be possible to obtain important data on structure-function relationship and the different nature of recognition between the TCR-like Fab and the native TCR. The fact that we have selected so many different antibody sequences binding the same fine-specificity is very interesting for structural studies. Structural models of these antibodies will enable identification common structural features or features also found in TCRs. Crystallization and structure determination of the TCR-like Fab’s in complex with the MHC-peptide ligand will be an important goal which would also enable to study the structural differences in molecular recognition by antibodies versus TCRs.

[0462] The most important question with respect to immunodiagnostic and -therapeutic applications of TCR-like Fabs relates to the low density and turnover of the specific epitope on the target cell surface. With regard to the density and targeted killing of cells we have previously shown in a murine model, that to achieve efficient killing with a TCR-like immunotoxin molecule a density of several thousand MHC-peptide complexes is required for selective elimination of APCs (Reiter and Pustan (1997) *Proc. Natl. Acad. Sci. USA* 94:4631-36).

[0463] It remains to be determined what the density of the 100-derived complexes on the cancer cells tested is. The fact that in FACS clear shifts can be seen, indicates that the level of display is detectable using the compositions and methods described herein. The other important issue to consider is the fine-specificity of the antibody. The antibodies characterized in this study were specific for their particular peptide in the HLA-A2 context, in two tests, ELISA and flow cytometry, with a panel of less than 10 other unrelated peptides tested as controls. It is clear from structural studies with MHC-peptide specific antibodies, that related peptides with one or a few mutations in the peptide may also be recognized. Additional methods, such as site-directed mutagenesis and re-selection techniques, can be used to fine tune the specificity of the antibodies, if this is deemed necessary. For example, specificity tuning may be required for certain applications, e.g., in the context of a true natural repertoire of peptides displayed in the MHC on the surface of cells. For other applications, fine tuning may not be necessary to determine the relative levels of the peptide-MHC complex investigated.

[0464] Further proof for the specificity of the TCR-like Fab antibodies isolated in this study was obtained in a T cell stimulation/inhibition assay, in which the G9209-specific Fab 1A9 was able to inhibit the release of cytokines (interferon γ and IL-2) from G9209-specific CTLs, R6C12, while a control G9280-specific Fab did not inhibit peptide-specific CTL stimulation.

[0465] To improve the sensitivity and targeting capabilities of these TCR-like antibody molecules, two antibody engineering approaches can be employed: one increases the affinity of the parental antibodies by affinity maturation strategies without altering their TCR-like fine specificity (Chowdhury and Pustan (1999) *Nat. Biotechnol.* 17:568-72) and the second increases the avidity of these recombinant monovalent molecules by making them multi-valent. Combining these strategies may well result in improved second-generation antibody molecules that will be sensitive enough and specific for immunotherapeutic approaches as well as for studying the interaction of tumor cells and the human immune system.

[0466] Our study strikingly shows the power of the phage display approach and its ability to select especially fine specificities from a repertoire containing a myriad of different antibodies.

[0467] The advent in recent years of the application of tetrameric arrays of class I MHC-peptide complexes now enables us to detect and study rare populations of antigen-specific T cells (Altman et al. (1996) *Science* 274:94-96). Our approach produces antibody molecules that enable phenotypic analysis of antigen (MHC-peptide) presentation, the other side of the coin to MHC-peptide-TCR interactions. Combining these two new approaches will significantly enhance our ability to understand immune responses in health as well as under various pathological conditions such as cancer, viral infections, and also when applied to class II MHC molecules, autoimmune diseases. The effectiveness and feasibility of this approach, as presented in this study, makes it realistic to generate in a generic form antibodies directed towards a large variety of specific MHC-peptide complexes.

**Example 3**

**Telomerase-HLA-A2 Antibodies**

[0468] The recent characterization of MHC-displayed tumor-associated antigens that recognize effector cells of the immune system has created new perspectives for cancer therapy. Antibodies that recognize these tumor associated MHC-peptide complexes with the same specificity as the T-cell antigen receptor will therefore be valuable tools for immunotherapy as well as for the studying antigen presentation in human cancers. Most tumor-associated antigens are expressed in only one or a few tumor types; however, recently specific T-cell epitopes derived from the telomerase catalytic subunit (hTER) that are widely expressed in many cancers were identified and shown to be recognized by CTLs derived from cancer patients. We selected a large non-immune repertoire of phage Fab antibodies on recombinant human class I HLA-A2 complexes displaying two distinct antigenic T-cell epitopes derived from hTER. We isolated a surprisingly large panel of high affinity human recombinant Fab antibodies that exhibited peptide-specific, MHC-restricted binding characteristics of T cells. The analyzed Fab’s not only recog-
ize the cognate MHC-peptide complex in a recombinant soluble form, but also the native complex as displayed on the surface of antigen-presenting cells and hTERT-expressing tumor cells. These findings demonstrate for the first time the ability to transform the unique fine specificity but low intrinsic affinity of TCRs on T cells into high affinity soluble antibody molecules endowed with a T-cell antigen receptor-like specificity. These molecules may prove to be very important and widely applicable for monitoring the expression of specific MHC-peptide complexes on the surface of tumor and immune cells, for structure-function studies of TCR-peptide-MHC interactions, as well as for developing new targeting agents for immunotherapy.

[0469] The design and development of strategies to augment active, specific immune-therapies in patients with a malignant disease has been greatly influenced by and benefitted from the progress made in better understanding the mechanisms that lead to an immune response. This is due mainly to the progress made in the availability of well-characterized tumor associated antigens (TAs) and to the advent of methodology developed to monitor immune responses (Boon and van der Bruggen (1996) J Exp Med 183:725-9; Rosenberg (2001) Nature 411:380-4; Renkvist and Parma (2001) Cancer Immunol Immunother 50:3-15; Altman et al. (1996) Science 274:94-96; Lee et al. (1999) Nat Med. 5:677-85). Consequently, anti-tumor immune responses can now be correlated with clinical responses in patients immunized with well-defined TAs. Especially with melanoma, it is now well established that human melanoma cells and other types of tumor cells express antigens that are recognized by cytotoxic T lymphocytes (CTLs) derived from cancer patients (Boon and van der Bruggen (1996) J Exp Med 183:725-9; Rosenberg (2001) Nature 411:380-4; Renkvist and Parma (2001) Cancer Immunol Immunother 50:3-15). Exciting clinical trials are therefore now in progress to target these TAs using various strategies such as vaccination with the cancer peptides or dendritic cells and adoptive cell therapy in order to generate more effective anti-tumor immune responses in cancer patients (Offera and Mielke (2000) Curr Opin Immunol 12:576-82; Esche (1999) Curr Opin Mol Ther 1:72-81; Kugler et al. (2000) Nat Med. 6:332-36). The presence of tumor-specific MHC-peptide complexes on the surface of tumor cells may also represent a unique and specific target for an antibody-based therapeutic approach. To develop such a strategy, new targeting moieties must be isolated such as recombinant antibodies that will recognize specific peptide-MHC complexes. In addition to being used as targeting agents, such antibodies would serve as a valuable tool for obtaining precise information about the presence, expression pattern, and distribution of the target tumor antigen, i.e., the MHC-peptide complex, on the tumor’s cell surface, on tumor metastases, in lymphoid organs, and on professional antigen-presenting cells. Such unique antibodies with T-cell receptor-like specificity will for the first time, enable measurement of the antigen presentation capabilities of tumor cells by direct visualization of the specific MHC-peptide complex on the tumor cell surface. Attempts to use soluble T-cell receptors for this purpose have proven difficult because of the inherent low affinity for their target and their instability as recombinant-engineered molecules (Wulff and Pluckthun (1994) J Mol Biol 242:655-69).


Results [0473] Recombinant Single-Chain MHC-Particle Complexes with Two hTERT-Derived HLA-A2-Restricted Peptides [0474] Two major T-cell epitopes were identified in hTERT that were recognized by HLA-A2-restricted CTLs derived from different patients (Vonderheide, et al. (1999) Immunity 10:673-9; Minev, et al. (2000) Proc Natl Acad Sci USA 97:4796-801.): peptide 540 (ILAKFLHVL; SEQ ID NO:5) (T540) and peptide 865 (RLVDFFLYV; SEQ ID NO:6) (T865). Recombinant MHC-peptide complexes that present the two hTERT-derived epitopes were generated by using a single-chain MHC (scMHC) construct that was described previously (Denkberg and Reiter (2000) Eur J. Immunol. 30:3522-32; Denkberg and Reiter (2001) J Immunol 167, 270-6). In this construct, the extracellular domains of HLA-A2 are connected into a single-chain molecule with β-2 microglobulin using a 15-amino acid flexible linker. The scMHC-peptide complexes were produced by in vitro refolding of inclusion bodies from bacterial cultures transformed
with the scMHC construct. Refolding was performed in the presence of the two hTERT-derived peptides followed by a purification protocol employing ion-exchange chromatography. The refolded hTERT-derived peptide-MHC complexes were very pure, homogenous and monomeric, as shown by analysis on SDS-PAGE and size-exclusion chromatography. Recombinant scMHC-peptide complexes generated by this strategy have been previously characterized in detail for their biochemical, biophysical, and biological properties and were found to be correctly folded and functional (Denkberg and Reiter, 2000) Eur. J. Immunol. 30:3522-32; Denkberg and Reiter (2001) J. Immunol. 167, 270-6).

[0475] To clearly demonstrate that the scMHC complex is folded correctly and contains peptide, we performed mass spectrometry analysis. The MHC-peptide complexes were deposited on a metal target as co-crystals with α-Xylene-4-hydroxycinamic acid (for the peptide identification) and separately as co-crystals with sinapinic acid (for the protein identification). The mass spectrometry analysis was done using Matrix-assisted laser-desorption time-of-flight (MALDI-TOF) in the positive ion mode. The peptide was easily detected, with the expected mass of 1140 dalton corresponding to the mass of the T540 peptide used for the refolding of the scMHC-peptide complex. This was the only peptide detected indicating that the refolded complex is a homogenous population of molecules containing a single specific peptide. The profile of the scMHC protein revealed a single peak with a mass of 44.5 kDa corresponding to the expected molecular weight of the scMHC protein. As shown above for the peptide, this was the only identified protein peak in the analyzed spectrum indicating that the protein consists of a very homogenous population of folded complexes.

[0476] Selection of Recombinant Antibodies with TCR-Like Specificity to HLA-A2-Restricted T-Cell Epitopes of hTERT

[0477] To enable efficient selection, scMHC-peptide complexes were biotinylated using a BirA sequence tag that was engineered at the C-terminus of the HLA-A2 gene for site-specific biotinylation as previously described (Altman et al. 1996) Science 274:94-96; Denkberg and Reiter (2000) Eur. J. Immunol. 30:3522-32). The phage display large repertoire of 3.7×10^16 human recombinant Fab fragments (de Haard et al. 1999) J. Biol. Chem. 274:18218-30), was incubated first with streptavidin-coated beads to avoid the selection of anti-streptavidin antibodies. A magnetic field was applied to precipitate the beads, and the supernatant containing the library depleted of streptavidin binders was used for the subsequent panning in solution on soluble recombinant MHC-peptide complexes containing the two hTERT-derived T cell epitopes. After incubation of the library with soluble complexes, binding phases were collected using streptavidin-coated magnetic beads followed by elution with triethylamine. A 600 to 1200-fold enrichment in phage titer was observed after three rounds of panning using the two different hTERT-derived peptide-MHC complexes (FIG. 34A). An ELISA with phage particles was performed on biotinylated recombinant scMHC-peptide complexes immobilized on streptavidin-coated immunoplates to determine antibody specificity. The fine specificity of the selected phage antibodies was determined by a differential ELISA on wells coated with scMHC HLA-A2 complexes containing either the specific hTERT-derived peptide, or control complexes containing other HLA-A2-restricted peptides. Phage clones analyzed after the third round of selection exhibited two types of binding pattern toward the MHC-peptide complex: one class of antibodies were pan-MHC binders which can not differentiate between the various MHC-peptide complexes; the second type were antibodies which bound the MHC-peptide complex in a peptide specific manner. The ELISA screen revealed that 62-64% of randomly selected clones from the third round of panning appeared to be binding to the HLA-A2/peptide complex. Twenty percent (for the T540 epitope) and 40% (for the T865) bound to 4-5 out of 5 different peptide/MHC complexes tested. However, a surprisingly high percentage of antibodies though were fully specific for the peptide/MHC used in selection when tested as phage antibodies in ELISA on different peptide/MHC complexes. As shown in FIG. 34A, 22% and 44% of the clones directed toward the T865 and T540 epitopes, respectively, exhibited antigen-specific, MHC-restricted binding characteristics of T cells. Thus, they bound only to the MHC peptide complex containing the specific T540 or T865 hTERT-derived peptides and did not bind to control complexes containing other HLA-A2-restricted peptides. These apparent MHC/peptide-specific positive clones remained specific in a secondary screening on more complexes (see materials and methods for list of HLA-A2 restricted peptides tested).

[0478] We examined the diversity pattern of these 21 respectively 41 peptide-specific clones by DNA fingerprint analysis and found 5-6 different restriction patterns (from round two of three) for each hTERT-derived complex, indicating the selection of several different antibodies with TCR-like specificity. DNA sequencing analysis confirmed these observations.

[0479] FIGS. 34A-34C shows a representative analysis of 5 TCR-like Fab clones of each of the two selections. The 5 different T540-specific clones tested reacted only with scMHC-T540 complexes and not with MHC-peptide complexes displaying the hTERT-derived T865 epitope or two melanoma gp100-derived epitopes, G9-209 and G9-280 (FIG. 34B) Similar results were observed in phage ELISA assays that determined the specificity of 6 phage clones isolated against the hTERT-derived T865 epitope (FIG. 34C).

[0480] Characterization of Recombinant Soluble Fab Antibodies with TCR-Like Specificity

[0481] We produced soluble Fab fragments from the phage clones (analyzed above, FIGS. 34B and 34C) that exhibited the specific binding pattern to the different hTERT-derived HLA-A2/peptide complexes in E. coli BL21 cells. These were purified by metal affinity chromatography from the periplasm by use of the hexahistidine tag fused to the CH1 domain of the Fabs. SDS-PAGE analysis of the affinity-purified material revealed homogenous, very pure Fab antibodies with the expected molecular weight. Approximately 0.5-2 mg of pure material could be obtained from 1 liter of bacterial culture.

[0482] We determined the fine specificity of the soluble molecules by ELISA on biotinylated MHC-peptide complexes that were immobilized to BSA-streptavidin-coated wells. The BSA-streptavidin-biotin spacer enables the correct folding of the complexes, which can be distorted by direct binding to plastic. To determine the correct folding of the bound complexes and their stability during the binding assays, we monitored their ability to react with the conformational specific monoclonal antibody W6/32, which recognizes HLA complexes only when folded correctly and when containing peptide. FIG. 35A shows a representative analysis of five soluble Fab antibodies directed to HLA-A2/T540 complexes. All five antibodies react specifically with the T540-
containing HLA-A2 complexes but not with control complexes containing the T865 hTERT-derived MHC-peptide complex, nor with HLA-A2 complexes containing the two melanoma gp100-derived epitopes, G9-209 and G9-280. We tested the fine specificity of these antibodies on five other MHC-peptide complexes displaying various HLA-A2-restricted peptides with similar results (see materials and methods for list of HLA-A2-restricted peptides tested). Similarly, soluble purified Fab fragment antibodies from the antibody clones isolated against the T865 epitope bound to the specific HLA-A2/T865 complexes, but not to control T540 hTERT-derived complexes nor to the melanoma gp100-derived HLA-A2/G9-209 and HLA-A2/G9-280 complexes (FIG. 35B). Thus, these peptide-specific and MHC-restricted Fab fragments exhibit the binding characteristics and fine specificity of a TCR-like molecule. The Fab antibodies did not recognize the peptide alone when immobilized on the plate neither streptavidin or other protein antigens (such as: BSA, IgG, RNase, Chymotrypsin).

[0483] Next, we tested the affinity binding properties of two of the TCR-like soluble Fabs, using a saturation ELISA assay in which biotinylated complexes were bound to streptavidin-coated plates and to which increasing amounts of Fab antibody were added. As shown in FIGS. 36A and 36B, the binding of two specific Fabs (4A9 and 3H2) was dose-dependent and saturable. Extrapolating the 50% binding signal of either antibody revealed that their affinity is in the nanomolar range.

[0484] Finally, we determined the apparent binding affinity of the TCR-like Fab fragments to their cognate MHC-peptide complex by a competition binding assay in which the binding of 125I-labeled Fab was competed with increasing concentrations of unlabeled Fab fragment. These binding studies (FIGS. 36C and 36D) revealed an apparent binding affinity of approximately 5 nM for the 4A9 antibody specific for the T540 hTERT epitope and 10-15 nM for the 3G3 antibody specific for the T865 epitope.

[0485] Binding of Fab Fragments to APCs Displaying the hTERT-Derived Epitopes

[0486] To demonstrate that the isolated Fab fragments can bind the specific MHC-peptide complex not only in the recombinant soluble form but also in the native form as expressed on the cell surface, we used murine TAP-deficient RMA-S cells transfected with the human HLA-A2 gene in a single-chain format38 (HLA-A2.1/D6-132m single chain) (RMA-S-HHD cells). The hTERT-derived and control peptides were loaded on RMA-S-HHD cells and the ability of the selected Fab antibodies to bind to peptide-loaded cells was monitored by FACS. Peptide-induced MHC stabilization of the TAP2 mutant RMA-S-HHD cells was demonstrated by the reactivity of MAbs w6/32 (HLA conformation-dependent) and BB7.2 (HLA-A2-specific) with peptide-loaded but not unloaded cells. Fabs 4A9 and 4G9, which recognize the T540-containing HLA-A2 complexes, reacted only with the T540-loaded RMA-S-HHD cells but not with cells loaded with the gp100-derived G9-209 peptide or the gp100-derived G9-280 peptide, respectively. Similarly the T865-HLA-A2-specific Fab antibodies 3G3 and 3H2 recognized only T865-loaded RMA-S-HHD cells and did not recognize cells loaded with the gp100-derived peptides at all. Similar results were observed in FACS analysis using 4 other HLA-A2 restricted peptides.

[0487] We have also used the TAP+EBV-transformed B-lymphoblast HLA-A2+JY cells as APCs. They have normal TAP and consequently peptide loading is facilitated by the exchange of endogenously derived peptides with HLA-A2-restricted peptides supplied externally by incubation of the cells with the desired peptides. We incubated these cells first with the T540, T865 telomerase-derived, and control HLA-A2-restricted peptides, then washed the cells, followed by incubation with Fab antibodies 4A9 and 3H2, respectively. These Fab fragments recognize only JY cells incubated with the specific telomerase peptide to which they were selected but not control HLA-A2-restricted peptides including the other telomerase epitope. We also tested the cross-reactivity of Fabs 4A9 and 3H2 on JY cells loaded with T540 and T865, respectively. JY cells loaded with T540 were only recognized by Fab 4A9 but not by Fab 3H2 nor by control Fabs recognizing a melanoma-derived gp100 epitope. Similarly, T865-loaded JY cells were recognized by Fab 3H2 specific for T865 in complex with HLA-A2 but not by Fab 4A9 nor by other gp100-specific Fabs. As control we used peptide-loaded HLA-A2/-HLA-A1+APD B cells. No binding of the Fab antibodies to these cells was detected. These results demonstrate that the Fab antibodies exhibit a TCR-like fine specificity and can recognize the corresponding native HLA-A2 complexes in situ on the surface of cells.

[0488] Binding of TCR-Like Fab Antibody to Telomerase-Expressing Tumor Cells

[0489] To confirm that the telomerase-specific TCR-like Fab antibodies can bind endogenously derived MHC-peptide complexes on the surface of tumor cells, we performed flow cytometry analysis on various tumor cells that express hTERT and HLA-A2. These cells represent the normal situation in which MHC-peptide complexes are expected to be present on tumor cells at a much lower density on the cell surface compared with the peptide-loaded APCs. The T540-specific Fab antibody 4A9 and T865-specific Fab 3H2 reacted with the HLA-A2+FM3D melanoma, LuCap prostate carcinoma, and HeLa epithelial carcinoma tumor cells (FIGS. 37A-37C) but not with the HLA-A2-prostate carcinoma PC3 cells that express hTERT (FIG. 37D). Telomerase activity in these cells was measured by a telomerase repeat amplification protocol (TRAP) using total cellular extracts, buffer control, and of telomerase-positive cells. The results were obtained using 100 or 500 ng of each extract with and without heat inactivation (15 min at 85°C). A 36-bp internal control for amplification efficiency and quantitative analysis was run for each reaction. The reaction products were separated on 10% nondenaturing polyacrylamide gel.

[0490] FM3D, LuCap, HeLa, and PC3 cells exhibit moderate to high telomerase activity. In these experiments we observed a moderate shift in fluorescence intensity in most of the cell population. However, a sub-population (20-30%) of the cells exhibited a substantial shift in staining intensity, indicating increased expression of telomerase T540 and T865-specific MHC-peptide complexes. These observations may reflect the antigenic variations in expression levels of MHC-peptide complexes expected to occur on the surface of tumor cells. Control HLA-A2+ cells that do not express hTERT were not stained by the antibodies. In addition, we tested the reactivity of Fabs 4A9 and 3H2 with HLA-A2 positive human foreskin fibroblasts that were transfected with hTERT and control non-transfected cells (FIGS. 37E and 37F). The telomerase-specific Fabs reacted only with the transfected cells but not with the control normal fibroblasts. TRAP activity assays revealed high telomerase activity in the transfected but not in control cells. These results therefore
demonstrate the ability of these high-affinity TCR-like antibodies to detect MHC-peptide complexes on the surface of tumor cells. This occurs despite the fact that the Fab antibodies are monovalent. Thus, these TCR-like antibodies can bind to cells that express the specific MHC-peptide complex at a density most likely to be found on tumor cells, antigen-presenting cells such as dendritic cells, and other cells involved in tumor-antigen presentation to the immune system.

Discussion

This study demonstrates our ability to select from a large non-immune repertoire of human Fab fragments displayed on phage a panel of antibodies directed against two HLA-A2-restricted T cell epitopes of the most widely expressed tumor-associated antigen identified so far, the human telomerase reverse transcriptase.

These antibodies can bind with high affinity in an antigen-specific, MHC-restricted manner, soluble HLA-A2 molecules complexed with the cognate peptides. Moreover, they can detect and visualize peptide/MHC complexes on the surface of cells. Hence, these are recombinant antibodies with the T-cell antigen receptor-like specificity of T cells. In contrast to the inherently low affinity of TCRs to MHC-peptide complexes, these molecules display the high affinity binding characteristics of antibodies, yet they retain TCR-like fine specificity.

Unlike recombinant TCRs, these recombinant antibodies recognize the corresponding native MHC-peptide complexes on cells.


The finding that CTLs specific for telomerase-derived epitopes isolated from a prostate cancer patient mediate efficient lysis of a variety of HLA-A2+ cancer cells such as prostate, breast, colon, lung, and melanoma is unprecedented (Vonderheide, et al. (1999) *Immunity* 10:673-9; Minev, et al. (2000) *Proc Natl Acad Sci U.S.A.* 97:4796-801.). Thus, we think that these cancer cells are equally effective in processing and presenting the same endogenous hTERT peptides. Therefore, similar hTERT peptides are expressed and complexed with MHC class I molecules on a variety of cancer cells of different histological origins and types. This suggests that hTERT represents the most widely expressed TAA described so far and renders telomerase-expressing tumor cells susceptible to destruction by CTL. Furthermore, this underscores the potential advantages that hTERT may have in controlling primary tumors and metastases in a large variety of cancer types in humans. Thus, hTERT-derived MHC-peptide complexes may turn out to be a very attractive target for cancer immunotherapy.

Our study demonstrates the power of the phage display approach for selecting antibodies with unusually and unique fine specificity. Until now antibodies with TCR-like specificity have been generated against murine MHC-peptide complexes employing various strategies of immunization (Andersen, et al. (1996) *Proc. Natl. Acad. Sci. U.S.A.* 93:1820-24; Porgador (1997) *Immunity* 6:715-26; Day (1997) *Proc Natl Acad Sci U.S.A.* 94:8064-9; Zhong (1997) *Proc Natl Acad Sci U.S.A.* 97:13856-61; Dadaglio (1997) *Immunity* 6, 727-38; Aharoni (1991) *Nature* 351:147-50; Krosgaard et al. (2000) *J Exp Med.* 191, 1395-412; Chames and Hoogenboom (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97:7969-74). Recently using the same phage-displayed Fab library, a recombinant Fab antibody was isolated that recognizes the melanoma antigen MAG-1 in complex with the human HLA-A1 MHC molecule. The affinity of this antibody was quite low (250 nM); therefore, it could be used to detect HLA-A1-MAG-1 complexes only when displayed in multiple copies on a phage (Chames and Hoogenboom (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97:7969-74). The fact that high-affinity antibodies with such unique, fine specificity targeting a rather difficult antigen were readily obtained in this study, and that they were in some cases with low nanomolar affinity, underscores the power of the display technology for this application, as well as add proof to the quality of the human non-immune antibody library used in the selections. The observation that 20-40% of the MHC-peptide binding antibodies had the fine specificity of a TCR-like molecule is nevertheless surprising, especially since they were selected from a non-immune repertoire considered not to be biased towards such specificity. More recently we have been able to isolate recombinant Fab antibodies against a large variety of MHC-peptide complexes containing other cancer-associated or viral HLA-A2-restricted peptides, indicating that this behavior is not telomerase peptides related. The unexpected high frequency of these antibodies and our ability to isolate several different antibodies directed to either complex is even more surprising in view of previous reports, in which the use of immunized or naive phage libraries resulted in only a single antibody clone (Andersen, et al. (1996) *Proc. Natl. Acad. Sci. U.S.A.* 93:1820-24; Porgador (1997) *Immunity* 6:715-26; Chames and Hoogenboom (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97:7969-74).

It would have been possible that one particular antibody family or antibody V-gene segment would have an intrinsic propensity to bind HLA-A2 molecules, and that the high frequency could be explained by a high abundance of such antibodies in the non-immune library. However, the sequences of the selected clones are derived from many different antibody families and germline segments, without any biases visible in the CDRs either. The high frequency and high affinities for some of the antibodies isolated here, suggest that these molecules may well be present at a high frequency in the antibody repertoires from the B-cell donors of the phage library, but a role for such antibodies remains unclear.

Whatever eventually the reason for this high frequency of antibodies to MHC-peptides may be, it appears that this phage-based approach can be successfully applied to
isolate recombinant antibodies with TCR-like specificity to a large variety of MHC-peptide complexes. Thus, it is possible to dissect the role of antigens in various pathological conditions such as cancer, viral infections and autoimmune disease, not only at the level of the T-cell using MHC-tetramers, but also at the level of the APC and diseased cell, using antibodies of the type described here. [0499]

The state and quality of the antigen used in the selection process was significant. In particular, with a trimolecular complex as an HLA-peptide complex, it is important to define those recombinant forms that do exhibit the "natural" conformation. We found that in vitro refolding from E coli inclusion bodies, of a single-chain MHC molecule complexed with various peptides yielded large quantities of correctly folded protein and that these refolded scMHC HLA-A2-peptide complexes are indeed functional, as demonstrated by their ability to stimulate T-cell activation, and can be used in the form of scMHC-peptide tetramers to phenotypically stain CTL clones specific for melanoma peptides (Denenberg and Reiter (2000) Eur. J. Immunol. 30:3522-32; Denenberg and Reiter (2001) J Immunol 167, 270-6).

[0500] Thus, these advantages may play a critical role in our ability to select these high-affinity TCR-like antibodies even though such peptide-specific binders are thought to be quite rare in even the most sizable library. [0501]

Recombinant antibodies with TCR-like specificity, such as we have selected and characterized herein, also represent an innovative and valuable tool in molecular immunology. These antibodies may now be used to detect and visualize the presence of specific MHC-restricted T-cell epitopes by standard methods of flow cytometry and immuno-histochemistry. As such, they are useful for the study and analysis of antigen presentation on tumor cells by determining the expression of specific tumor-related MHC-peptide complexes on the surface of tumor cells, metastases, antigen presenting cells, and lymphoid cells. These antibodies can be used to analyze immunotherapy-based approaches by determining the alterations in MHC-peptide complex expression on antigen-presenting cells before, during, and after vaccination protocols with peptides or with APCs loaded with tumor cell extracts or dendritic-tumor cell hybrid vaccinations (Offering a and Melief (2000) Curr Opin Immunol 12:576-82; Esche (1999) Curr Opin Mol Ther 1:72-81; Kjoller et al. (2000) Nat. Med. 6:332-36). For immunotherapeutic applications, this approach presents new opportunities for using these specific molecules, which recognize very specific and unique human tumor antigens as candidates to serve as targeting moieties for antibody-based immunotherapies. Such approaches could include recombinant immunotoxins (Pastan (1997) Biochim Biophys Acta. 1333, C1-6), fusions with cytokine molecules (Lode and Reisfeld (2000) Immunol Res. 21:279-88); bi-specific antibody therapy (Withoff (2001) Curr Opin Mol Ther. 3:53-62) or immuno-gene therapy (Willemsen et al. (2000) Gene Ther 7:1369).

[0502] These antibodies also represent a valuable tool for structural and functional studies of TCR-peptide-MHC interactions. As previously shown for a murine system, TCR-like antibodies were used to define fine specificities of TCR interactions (Stryhn et al. (1996) Proc. Natl. Acad. Sci. U.S.A 93:10336-42). A striking similarity between the specificity of the T-cells and that of the murine TCR-like antibody was found and most of the peptide residues, which could be recognized by the T-cells, could also be recognized by the antibody. [0503]

Here we have demonstrated binding of some of our antibodies to telomerase-expressing tumor cells, thus showing for the first time the feasibility of detecting and visualizing specific MHC-peptide complexes on the surface of tumor cells with a soluble pluge-library-derived antibody. The antibodies isolated in this study, which exhibit the specificity of hTERT-restricted T cells, can be used for the design of new antibody-based targeting molecules for immunotherapy because they have the unique antigen-specific, MHC-restricted specificity of T cells, combined with the high affinity characteristics of antibodies. This is in contrast to the inherently low affinity of TCR to MHC-peptide complexes.

[0504] The density (and turnover rate) of these specific epitopes on the target cell surface, and the specificity of the antibody may impact immunotherapy and research applications. With regard to surface density, we have previously shown in a murine model that, to achieve efficient killing with a TCR-like immunotoxin molecule, a density of several thousand specific MHC-peptide complexes is required for selective elimination of APCs (Reiter and Pastan (1997) Proc. Natl. Acad. Sci. U.S.A 94:4631-36).

[0505] It remains to be determined what the density of the telomerase complexes on the cancer cells tested is. Clear shifts in FACS analysis indicate that the density of TAA of the telomerase complex on cancer cells is higher than previously noted. The other important issue to consider is the fine-specificity of the antibody. The antibodies characterized in this study were specific for their particular peptide in the HLA-A2 context, in two tests, ELISA and flow cytometry, with a panel of less than 10 other unrelated peptides tested as controls. It is clear from structural studies with MHC-peptide specific antibodies, that related peptides with one or a few mutations in the peptide may also be recognized. I therefore remains to be seen that the specificity of the antibodies will be in the context of a true natural repertoire of peptides displayed in the MHC. New data on the use of such antibodies for retargeting T-cell to tumor cells are highly encouraging in this respect. A recent study with Fab G8, an antibody that targets the HLA-A1 complexed to MAGE-A1 (Chames and Hoogenboom (2000) Proc. Natl. Acad. Sci. U.S.A 97:7969-74), shows that expression of the Fab genes on the surface of transfected primary human T lymphocytes retargets these cells specifically to MAGE-A1 expressing tumor cells, and in a manner indistinguishable from a T-cell receptor with similar specificity (Willemsen et al. (2000) Gene Ther 7:1369).

[0506] To improve the targeting capabilities of these TCR-like antibody molecules two antibody engineering approaches can be employed: (1) increasing the affinity of the parental antibody by affinity maturation strategies without alteration of its TCR-like fine specificity (Chowdhary and Pastan (1999) Nat. Biotechnol. 17:568-72), and (2) increasing the avidity of these recombinant monovalent molecules by rendering them bi or multi-valent. The combination of these affinity maturation strategies and avidity engineering may well result in second-generation, improved antibodies that can recognize levels of MHC-peptide complexes with sufficient sensitivity for their eventual immunotherapeutic use.

Example 4

MUC1-HLA-A2 Antibodies

[0507] In this study we attempted to isolate human recombinant antibodies directed toward a T-cell epitope derived from the Mucin 1 antigen.
Mucin 1 (MUC1) is an epithelial cell-associated mucin that is developmentally regulated and aberrantly expressed by carcinomas, which makes it an important marker in malignancy (Mukherjee et al. (2000) J. Immunol. 165:3451-3460). This molecule exists as a large extended rod protruding from the apical cell membrane into the lumen of the ducts. MUC1 has an unusual structure, consisting mainly of a 20-amino acid sequence repeated in tandem on an average of 30-90 times. The tandem repeats (TRs) serve as the scaffold for O-linked oligosaccharides that cover the polypeptide core (Gendler et al. (1995) Annu. Rev. Physiol 57:607-634; Spicer et al. (1991) J. Biol. Chem. 266:15099-15109).

In cancer, there are differences in expression that distinguish this protein as tumor specific. There is a large increase in the amount of mucin expressed on cells and in the circulation. Its distribution is no longer restricted to the apical surface of ducts and glands, but it is found throughout the tumor mass and on the surface of tumor cells. Importantly, the glycosylation is altered; oligosaccharide structures are shorter and fewer in number, revealing immunodominant peptide sequences in every TR that on normal surfaces would be concealed by glycosylation. Underglycosylation of MUC1 reveals peptide epitopes presented in the context of MHC molecules and recognized by CTLs that can kill tumor cells expressing this form of MUC1 (Bald et al. (1989) Proc. Natl. Acad. Sci. 86:7159-7163).

The recent description of MUC1 as a target for CTLs has raised interest in using this protein as a target for immunotherapy. It is expressed by most adenocarcinomas of the breast, lung, stomach, pancreas, colon, prostate, ovary, endometrium, and cervix, which makes MUC1 an attractive therapeutic target. In 1999, cancers that expressed MUC1 accounted for about 72% of new cases and for 66% of the deaths (Greenlee et al. (2000) CA Cancer J. Clin. 50:7-33).

However, expression of the underglycosylated MUC1 is not sufficient to stimulate CTL killing, as 90% of existing carcinomas express MUC1 and these tumors progress.

Recently, Carmon et al. (Carmon et al. (2000) Int. J. Cancer 85:391-397) characterized three new HLA-A2.1-restricted MUC1-derived CTL epitopes. These peptides, which are not deduced from the extracellular Tandem Repeat Array (TRA), were shown to be processed and presented by a breast-tumor cell line. Moreover, CTL induced against these peptides lysed target cells pulsed with breast-carcinoma-derived peptide extract more efficiently than target cells pulsed with normal-breast-derived peptides. One of these MUC1 epitopes, was the D6 peptide (LLETVLTVV; SEQ ID NO:4), which exhibited high MHC-binding affinity, positively correlated with preferential immunogenic properties in CTL assays.

Therefore, there is a need to develop molecules that may specifically recognize tumor cells presenting MUC1 derived peptides; such molecules may serve as a targeting moiety to direct drugs or toxins to tumor cells. These molecules can also serve as a tool to study the presentation of MUC1 epitopes on the surface of tumor cells, antigen-presenting cells and lymphoid organs.

In the present work, we have isolated a panel of human recombinant antibodies with antigen-specific, MHC-restricted specificity of T cells binding with high affinity HLA-A2 complexes that display the specific Mucin-1D6 peptide. These antibodies have been used to directly visualize, by flow cytometry, the specific HLA-A2/MUC1-D6 epitope on antigen-presenting cells as well as on the surface of tumor cells. Recombinant Single-Chain MHC-Peptide Complexes with Mucin-Derived HLA-A2-Restricted Peptide

One of the potent T-cell epitope identified in the MUC1 antigen that was recognized by HLA-A2-restricted CTLs derived from HLA-A2 transgenic mice is the peptide D6 (LLETVLTVV; SEQ ID NO:4) (Carmon et al. (2000) Int. J. Cancer 85:391-397). Recombinant MHC-peptide complexes that present the MUC1-derived epitope were generated by using a single-chain MHC (scMHC) construct that was described previously (Denkberg et al. (2000) Eur. J. Immunol. 30:3522-3532). In this construct, the extracellular domains of HLA-A2 are connected into a single-chain molecule with β2-microglobulin using a 15-amino acid flexible linker. The scMHC-peptide complexes were produced by in vitro refolding of inclusion bodies from bacterial cultures transformed with the scMHC construct. Refolding was performed in the presence of the MUC1-derived peptide followed by a purification protocol employing ion-exchange chromatography. The refolded scHLA-A2/D6 complexes were very pure, homogenous and monomeric, as shown by analysis on SDS-PAGE and size-exclusion chromatography. Recombinant scMHC-peptide complexes generated by this strategy have been previously characterized in detail for their biochemical, biophysical, and biological properties and were found to be correctly folded and functional (Denkberg et al. (2000) Eur. J. Immunol. 30:3522-3532; Denkberg et al. (2001) J. Immunol. 167:270-276).

Selection of Recombinant Antibodies with TCR-Like Specificity to HLA-A2-Restricted T-Cell Epitope of MUC1

To enable efficient selection, scMHC-peptide complexes were biotinylated using a BirA sequence tag that was engineered at the C-terminus of the HLA-A2 gene for site-specific biotinylation as previously described (Altman et al. (1996) Science 274:94-96; Denkberg et al. (2000) Eur. J. Immunol. 30:3522-3532). The phage display large repertoire of 3.7×1012 human recombinant Fab fragments (de Haard et al. (1999) J. Biol. Chem. 274:18218-18230), was incubated first with streptavidin-coated beads to avoid the selection of anti-streptavidin antibodies. A magnetic field was applied to precipitate the beads, and the supernatant containing the library depleted of streptavidin binders was used for the subsequent panning in solution on soluble recombinant MHC-peptide complexes containing the MUC1-derived T-cell epitope. After incubation of the library with soluble complexes, binding phages were collected using streptavidin-coated magnetic beads followed by elution with triethylamine. A 580-fold enrichment in phage titer was observed after three rounds of panning using the MUC1-derived D6 peptide-MHC complexes (FIG. 38A). The fine specificity of the selected phage antibodies was determined by a differential ELISA on streptavidin-coated wells incubated with biotinylated scMHC HLA-A2 complexes containing either the specific MUC1-derived D6 peptide, or control complexes containing other HLA-A2-restricted peptides. Phage clones analyzed after the third round of selection exhibited two types of binding pattern toward the MHC-peptide complex: one class of antibodies were pan-MHC binders which cannot differentiate between the various MHC-peptide complexes; the second type were antibodies which bound the HMC-
peptide complex in a peptide specific manner. The ELISA screen revealed that 84% of randomly selected clones from the third round of panning appeared to be binding to the HLA-A2/peptide complex.

[0518] However, a surprisingly high percentage of antibodies though were fully specific for the peptide/MHC used in selection (i.e., the sHLA-A2/D6 complex) when tested as phage antibodies to ELISA on different peptide/MHC complexes. As shown in FIG. 43A, 80% of the clones exhibited antigen-specific, MHC-restricted binding characteristics of T cells. Thus, they bound only to the MHC peptide complex containing the specific D6 MUC1-derived peptide and did not bind to control complexes containing other HLA-A2-restricted peptides. These apparent MHC/peptide-specific positive clones remained specific in a secondary screening on more complexes. FIG. 38B shows a representative analysis of 5 TCR-like Fab clones. Clones M2B1 and M2F2 are from the second round of panning and clones M3A1 and M3B8 are from the third round. The different MUC1-D6 specific clones tested, reacted only with scMHC-MUC1-D6 complexes and not with MHC-peptide complexes displaying the MUC1-derived A7 epitope, the melanoma gp100-derived epitope, G9-154 and the viral TAX_{11-19} epitope (FIG. 38B).

[0519] We examined the diversity pattern of 26 peptide-specific clones (from round two or three) by DNA fingerprint analysis and found 16 different restriction patterns indicating the selection of several different antibodies with TCR-like specificity. DNA sequencing analysis confirmed these observations.

Characterization of Recombinant Soluble Fab Antibodies with TCR-Like Specificity

[0520] We produced, in E. coli BL21 cells, soluble Fab fragments from the phage clones (analyzed above) that exhibited the specific binding pattern to the MUC1-derived HLA-A2/peptide complexes.

[0521] These were purified by metal affinity chromatography from the periplasm by use of the hexahistidine tag fused to the CH1 domain of the Fabs. SDS-PAGE analysis of the affinity-purified material revealed homogenous, very pure Fabs antibodies with the expected molecular weight. Approximately 0.5-2 mg of pure material could be obtained from 1 liter of bacterial culture.

[0522] We determined the fine specificity of the soluble molecules by ELISA on biotinylated MHC-peptide complexes that were immobilized to BSA-streptavidin-coated wells. The HSA-streptavidin-biotin spacer enables the correct folding of the complexes, which can be distorted by direct binding to plastic. To determine the correct folding of the bound complexes and their stability during the binding assays, we monitored their ability to react with the conformational specific monoclonal antibody w6/32, which recognizes HLA complexes only when folded correctly and when containing peptide. FIG. 44B shows a representative analysis of five soluble Fab antibodies directed to HLA-A2/MUC1-D6 complexes. All five antibodies react specifically with the D6-containing HLA-A2 complexes but not with control complexes containing the A7 MUC1-derived MHC-peptide complex, nor with HLA-A2 complexes containing the melanoma gp100-derived epitope, G9-154 or the viral TAX_{11-19} epitope. We tested the fine specificity of these antibodies on five other MHC-peptide complexes displaying various HLA-A2-restricted peptides with similar results. Thus, these peptide-specific and MHC-restricted Fab fragments exhibit the binding characteristics and fine specificity of a TCR-like molecule. The Fab antibodies did not recognize the peptide alone when immobilized on the plate neither streptavidin or other protein antigens (such as: BSA, IgG, RNAse, Chymotrypsin).

[0523] Next, we tested the affinity binding properties of two of the TCR-like soluble Fabs, using a saturation ELISA assay in which biotinylated complexes were bound to streptavidin-coated plates and to which increasing amounts of Fab antibody were added. As shown in FIGS. 40A and 40B, the binding of two specific Fabs (M3A1 and M3B8) was dose-dependent and saturable. Extrapolating the 50% binding signal of either antibody revealed that their affinity is in the nanomolar range.

[0524] Finally, we determined the apparent binding affinity of the TCR-like Fab fragments to their cognate MHC-peptide complex by a competition binding assay in which the binding of [125]labeled Fab was competed with increasing concentrations of unlabeled Fab fragment. These binding studies revealed an apparent binding affinity of approximately 10-15 nM for the M3A1 antibody and the M3B8 antibody specific for the MUC1-D6 epitope.

Binding of Fab Fragments to APCs Displaying the MUC1-Derived Epitope

[0525] To demonstrate that the isolated Fab fragments can bind the specific MHC-peptide complex not only in the recombinant soluble form but also in the native form as expressed on the cell surface, we used murine TAP2-deficient RMA-S cells transfected with the human HLA-A2 gene in a single-chain format (HLA-A2.1/D8-132g single chain) (RMA-S-HHD cells). The MUC1-derived D6 and control peptides were loaded on RMA-S-HHD cells and the ability of the selected Fab antibodies to bind to peptide-loaded cells was monitored by FACS. Peptide-induced MHC stabilization of the TAP2 mutant RMA-S-HHD cells was demonstrated by the reactivity of MAb's w6/32 (HLA-conformation-dependent) and BB7.2 (HLA-A2-specific) with peptide-loaded but not unloaded cells. Fab's M3A1 and M3B8, reacted only with D6-loaded RMA-S-HHD cells but not with cells loaded with the 100-derived G9-154 peptide. Similar results were observed in FACS analysis using 4 other HLA-A2 restricted peptides.

[0526] We have also used the TAP+EBV-transformed B-lymphoblast HLA-A2+JY cells as APCs. They have normal TAP and consequently peptide loading is facilitated by the exchange of endogenously derived peptides with HLA-A2-restricted peptides supplied externally by incubation of the cells with the desired peptides. We incubated these cells first with the D6 MUC1-derived, and control HLA-A2-restricted peptides, then washed the cells, followed by incubation with Fab antibodies M3A1 and M3B8. These Fab fragments recognize only JY cells incubated with the specific Mucin1 peptide to which they were selected but not control HLA-A2-restricted peptides including the other MUC1 epitope. As control we used peptide-loaded HLA-A2+HLA-A1+APD B cells. No binding of the Fab antibodies to these cells was observed.

[0527] Binding of TCR-Like Fab Antibody to MUC1-Expressing Tumor Cells

[0528] To confirm that the MUC1-specific TCR-like Fab antibodies can bind endogenously derived MHC-peptide complexes on the surface of tumor cells, we performed flow cytometry analysis on various tumor cells that express MUC1 and HLA-A2.
Since the density of a particular peptide-HLA complex on these tumor cells is expected to be lower compared to peptide-pulsed APCs we increased the avidity of Fab M3A1 by making Fab tetramers which are directly tagged with a fluorescent probe. This approach was used previously to increase the binding avidity of peptide-MHC complexes to the TCR or to increase sensitivity of recombinant antibody molecules (Cloutier et al (2000) Mol. Immunol. 37:1067-1077). Another advantage in using fluorescent labeled tetramers lies in the fact that only a single staining step is required while monomeric unlabeled Fab's require a fluorescent labeled secondary antibody. We thus used our Fab tetramers, which were generated with fluorescent-labelled streptavidin, to measure the expression of MUC1-derived D6 peptide-MHC complexes on the surface of MUC1 expressing tumor cells. The intensity of binding measured by flow cytometry with peptide loaded JY cells was dramatically increased by two logs compared to the staining intensity with the M3A1 Fab monomer. Next, we tested the ability of the Fab M3A1 tetramer to stain breast cancer HLA-A2+ tumor cells pulsed with the Muc1-derived D6 peptide. As shown in FIG. 41A, significant staining of peptide-pulsed MDA-MB-231 cells was observed with the tetramer while a lower degree of staining was observed when cells were stained using the Fab monomer. Titration of peptide-pulsed MDA-MB-231 cells with different concentrations of the MUC1-derived D6 peptide demonstrated that staining intensity was dependent on the concentration of peptide used for pulsing and that pulsing with a concentration as low as 10-15 nM was sufficient to detect binding using the Fab M3A1 tetramer (FIG. 41B). Similar experiments were performed on MUC1-expressing MCF7 breast carcinoma cells, however the staining intensity with these cells was lower compared to MDA-MB-231 cells. This may be explained by the expression level of HLA-A2 molecules on the surface of these cells. MDA-MB-231 cells express significantly higher levels of HLA-A2 compared to MCF7 cells as monitored by the anti-HLA-A2 antibody BB7.2.

We also detected the natural occurrence of HLA-A2/Mucin1-D6 complexes on MCF7 cells without prior peptide pulsing, using the Fab M3A1 tetramer. These cells represent the normal situation in which MHC-peptide complexes are expected to be present on tumor cells at a much lower density on the cell surface compared with the peptide-loaded APCs or peptide-pulsed tumor cells. As control, we used MCF7 cells pulsed with the MUC1/D6 peptide (positive control) and other HLA-A2 restricted peptides (negative control) at a concentration of 10 μM. The MUC1/D6-specific Fab M3A1 tetramer reacted specifically and yielded a significant intensity of staining compared to controls with the D6-pulsed and native MCF7 cells (FIG. 42), but not with the cells pulsed with non-specific peptide. MUC1 expression in these cells was visualized by staining with an anti-Mucin1 antibody. These results demonstrate the ability of these high-affinity TCR-like antibodies to detect MHC-peptide complexes on the surface of tumor cells.

Thus, these TCR-like antibodies can bind to cells that express the specific MHC-peptide complex at a density most likely to be found on tumor cells, antigen-presenting cells such as dendritic cells, and other cells involved in tumor-antigen presentation to the immune system.

TAX

Using the methods described above, antibodies against the TAX-MHC complex were isolated. Three exemplary antibodies are T3F3, T3F1, and T3F2.

Other embodiments are within the claims and in the summary.
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<400> SEQUENCE: 18

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1  5  10  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20  25  30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35  40  45
Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50  55  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65  70  75  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85  90  95
Ala Arg Asp Tyr Tyr Gly Asp Tyr Ala Leu Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 19
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19
gacatccagt tgacccagtc ttctctcctctctgttgtcat cttgttgaga cagagtcacc 60
tctactgccc ggctgcaagcc gaggtattgac accttatctaaa tttggtgatca acacagacca 120
gggatccgcc tcaacgtccct cttttatatcccggtcagtgc ggtgccggcc 180
gaggtctggcc ggccgacagct gccaccttctct cccatccagct cttccaaacct 240
gagacatc ctcacataa tgcagcagc cctctctctact tccctgag 300
gggacagcagct cctctgccag 324

ggtgcgagt ggtgcgagt gttgtaatg atgtaatgaa taatgtat 180

<210> SEQ ID NO 20
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Amp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1  5  10  15
Amp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Thr Tyr
20  25  30
gaaagcacc tccagcagtc tcctaggacc ctgcttttgt ctccaggaga gagagcacc 60
cctctcgcc ggcagcagtc gctattac acgccatctct tcgccttgtag ccagcagaa 120
cctggcaggt cttcagcagtc ctcctctcct catgtgatcac ccctggccac acctggatcctca 180
gacagctgca tcgtggcagcc tcctggggcaca gctcctactct tcctcactcag cagctctgacagacacacccctcagcagac 240
cctgaaggctgctcagtcgagacagtcctga ggttcacgcagatctctgtgctgcttgacgac 300
caggggccac aggggaaat cagacacg 327

<210> SEQ ID NO 24
<211> LENGTH: 109
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24
Glu Thr Thr Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Arg Tyr Ile Arg Ala Arg
20 25 30
Phe Leu Ala Trp Tyr Gln Glu Gly Ala Pro Arg Leu Leu
35 40 45
Ile Tyr Asp Ala Ser Thr Arg Ala Thr Gln Gly Ile Pro Asp Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
85 90 95
Arg Thr Phe Gly Gln Gly Thr Leu Val Glu Ile Lys Arg
100 105

<210> SEQ ID NO 25
<211> LENGTH: 366
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25
ccgctcagc ctggtcgagtct tggggtctag gtcgaagaac ctgggtcctct ctggtaaggtc 60
tctgcaagct ctctgcctg gcccctcag cagctacgca ttcgctgtgggt ggcacagggg 120
cctggcaac ggtctgtcag gatggaggag atccatccta tgtctgtgctac agcaacacac 180
gcagcaggt ctcagcagtc ctcagcagtc aatctcagag cagctcagctcc 240
atggcagtg gcagcagctg atctgagtcag acctccttgt ctatctgtgc gaagatttgc 300
agccagtgat ggtctctagtct tgtttttgtat actctcgggtg cagggactaag ggtccagcagtc 360
tcagc 366

<210> SEQ ID NO 26
<211> LENGTH: 122
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr

A continued...
caggtgccag tcgtacagtc tggggagggc gttgtccagc tgtggaggtc cctgagactc 60
tctgtgcag cctctggtatt cactttcag tacctagcct tgcacttggt cgcocaggt 120
cacggcaag ggtggtggagt ggtggcattt atactaatag atggaagtaa taaatactac 180
gcaggtccgg tgtgagggcag attcaccctc tccagagac aatcccaagaa cacgtgttat 240
ctgtcaattga aggctctgag acogcagggc acggcctgtat attactgtag gagactgtg 300
ggtgtcagct tgtctcggga tgtctttgtat atatgagggc aaggacactat ggtccaggc 360
tcagc 366

<210> SEQ ID NO 30
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 30

Gln Val Glu Leu Val Gln Ser Gly Gly Val Val Gln Pro Gly Arg 1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr 20 25 30
Gly Leu His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45
Ala Phe Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Ala Asp Ser Val 50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr 65 70 75 80
Leu Gln Met Asn Gly Leu Arg Ala Glu Asp Thr Ala Val Tyr Cys 85 90 95
Ala Lys Thr Val Gln Val Thr Val Ser Asp Ala Phe Asp Ile Trp 100 105 110
Gly Lys Gly Thr Met Val Thr Val Ser Ser 115 120

<210> SEQ ID NO 31
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 31

gatgttgtga tgtctcagct tccaggccac cttgctctgtg ctcgagggga tagcgcacc 60
cctctcaggt gggcaggtta gacttctagt gccagctcag tgtcctctgta ccaacagag 120
cctggtccagg cttccagcagt cctaatctat acogcaggtca tccagccccc tgtccacccog 180
gcaggtcga ggtggacagtgt gtctggcagcg gttcaccctc tggcatccag cggacctggag 240
cctgaagatt tgtgaggtga ttctgtcgcag cagatggttt tctactcttg gacctggcgc 300
caggggacca aggtggagat cagacga 327

<210> SEQ ID NO 32
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 32

Asp Val Val Met Thr Gln Ser Pro Gly Thr Leu Ser Val Ser Pro Gly
Asp Ser Ala Thr Leu Ser Cys Trp Ala Ser Gln Ser Leu Ser Aep Ser
20 25 30
Tyr Val Ser Trp Tyr Gln Gln Lys Pro Gly Gin Ala Pro Arg Leu Leu
35 40 45
Ile His Ser Ala Ser Ile Arg Ala Pro Gly Ile Pro Asp Arg Phe Ser
50 55 60
Gly Ser Val Ser Gly Thr Glu Thr Leu Thr Ile Ser Gly Leu Glu
65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Ser Cys His Gin Tyr Gly Phe Leu Pro
85 90 95
Trp Thr Phe Gly Gin Gly Thr Lys Val Glu Ile Arg Arg
100 105

<210> SEQ ID NO 33
<211> LENGTH: 360
<212> ORGANISM: Homo sapiens
<400> SEQUENCE: 33
caggtccagc tggtagagtg gtagaagaagc ctagggcctc agtgaaggtc 60
tctgcaaggg tggacgtgta cacccttacc aggtaggta tcagctgggt cgacagggcc 120
cctggcagag ggttaggtgg gatgggaggg atcaagcttt ccaatggotta cacaagat 180
gcacagata tccgggacag acctaccttg accagagaca acatccaggg gcacagcctac 240
atggggtctg agaaggtgc atgggagac acggcccttt attacgtgct ggatgatgtat 300
atagtggcc tagatgtggt tggatatggt ggccaaaggg caatggtcag cgtttcaagc 360

<210> SEQ ID NO 34
<211> LENGTH: 120
<212> ORGANISM: Homo sapiens
<400> SEQUENCE: 34
Gln Val Gln Leu Val Gin Ser Gly Ala Gin Leu Val Lys pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Gly Val Leu Val Gin Ser Gly Ala Ser Lys Tyr Thr Phe Thr Arg Tyr
20 25 30
Gly Ile Ser Trp Val Arg Gin Ala Pro Gly Gin Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Ser Ser Ser Asn Gly Tyr Thr Lys Tyr Ala Gin Asn Leu
50 55 60
Gln Gly Arg Leu Thr Leu Thr Thr Ser Thr Thr Gly Thr Ala Tyr
65 70 75 80
Met Glu Leu Arg Ser Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95
Ala Arg Tyr Asp Ile Ser Gly Leu Asp Gly Phe Asp Ile Trp Gly Gln
100 105 110
Gly Thr Met Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 35
<211> LENGTH: 324
<212> ORGANISM: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

gaaagcacac tcaccaagtc tccagtgacc ctgtctcgtgt ctccagggga aagagcacc 60
tccctcagc gggcagagtc gacacactcc atagctgtgc cagcggacaa 120
tctggccaggg tctccagtgct cctccatctat gctgtctgca gacagcgcac tcgcctccca 180
gcgagttga gcctgcatgag gcctgggaca gctctcactc tcacactcag cagactgtag 240
cctgaagagt ttgcaagtgta ttactgtcag cagatggtgt ctcacgcag tttgggccag 300
gggaccaagc tcggagatcc aaca 324

<210> SEQ ID NO 36
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Glu Thr Thr Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly 1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Thr 20 25 30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu 35 40 45
Ile Tyr Ala Ser Ser Arg Ala Thr Gln Pro Asp Arg Phe Ser 50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu 65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Arg 85 90 95
Ser Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg 100 105

<210> SEQ ID NO 37
<211> LENGTH: 363
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37

caggtgcaagc tcagggagtg tggggagggc ctggtcaagc ctgggggggtg cctggagcct 60
tctgtgcaagc tcctgtgatt cacctcagtt acataagca tgaactggt cggacagctgt 120
cggagggag gcgtggaggtc gttgtcata ccattaagta gttggtagac cattataactc 180
gcagactcgag tggaggggccag tattcaccct tcagagacac acggccagag cagctgttat 240
tcggactgtg acagctgagc acagctggttt attacaagtt aagaggtgtat 300
cctactctt actaactcg ttagacatg tggggccag ggacacaggt caccgtctca 360
agc 363

<210> SEQ ID NO 38
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20                      25                      30
Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35                      40                      45
Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50                      55                      60
Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                      70                      75                      80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85                      90                      95
Val Arg Gly Asp Pro Tyr Phe Tyr Tyr Gly Met Asp Ile Trp Gly
100                     105                     110
Gln Gly Thr Val Thr Val Thr Val Ser Ser
115                     120

<210> SEQ ID NO 39
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 39

gacatcgcagt gcacccagt tcctacctcc ctgtctgctt ctgtaggaga cagagtcac<br><br>60
atcgcac gccgctcagc gacattagc accacttaa attgtatca gcagacgca<br><br>120
gggaaagccgcc ctgctgcttt ggtatgtattg ccgtcaggtgtcagcttgt<br><br>180
agagactgc ggcagcagtgcttgagcag ttcattctcattcactcagc<br><br>240
gcgccgct gctgccctt cactgtctct cactgtctt cactgctct<br><br>300
cagacgccac gcacattcag taaacga<br><br>327

<210> SEQ ID NO 40
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Ile Ile Thr Cys Arg Ala Thr Gln Ser Ile Ser Thr His
20                      25                      30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35                      40                      45
Tyr Ser Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50                      55                      60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                      70                      75                      80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Ser Pro Pro
85                      90                      95
Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg
100                     105

<210> SEQ ID NO 41
<211> LENGTH: 360
<212> TYPE: DNA
-continued
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 41

```
caggtgcagc tgccaggtgc cggcggcagg ctcgtaagct cctcggagac cctgctccct 60
acgtcagtc ttctctgctg ctctacagct gtaaaccagc actactgggg ctggctccgc 120
cagcccccccag ggaaggggct ggagctggat attataagtgg ggaaggctac 180
tacaatcgtg ccccaggtgg tggagcgacc agtcgctag acacgtccaa gagaacttcc 240
tccctgagaa tgacccctgt gacccgtgac ggaggggctg tggctttctg tgggagagaa 300
tcccctgctc ccatactacttg aagcttctcg ccagttaagac ccctggctac cgcttcaagc 360
```
-continued

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1  5  10  15
Thr Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Arg Asn
20  25  30
Tyr Val Ser Trp Phe Gln Gln Val Pro Gly Arg Ala Pro Lys Leu Leu
35  40  45
Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Ile Pro Gly Arg Phe Ser
50  55  60
 Ala Ser Lys Ser Asp Thr Ser Ala Thr Leu Asp Ile Thr Gly Leu Gln
65  70  75  80
Ser Gly Asp Glu Ala Val Tyr Tyr Cyc Gly Thr Trp Asp Ser Thr Leu
85  90
Asp Leu Tyr Val Phe Gly Gly Gly Thr His Val Pro Val Leu
100 105 110

<210> SEQ ID NO 45
<211> LENGTH: 384
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 45

gaggtccacg tgggtcgagtc tggggctgag gttgaaagaac ctggggcctc aagtgaagtt  60
tccctgcaag ctcttggtata caccttcacc agctactata tccacttggt ggcacagggc 120
cctggacaag gttcttgagtc gatgggagca atcaacccga gtgggtctag cacaacctac 180
goacagaagt tcccggggag agtcaccagc ccacagacg cagctctcag 240
atgtacgtga ccagctgctag atctggaagac agggcggtgt atactgtgca ggagatgggg 300
acccatgttc ccgggagttta tcctactac tactactac gatggcaagt ctggggccaa 360
gggacacgg tccacgtctc aagc 384

<210> SEQ ID NO 46
<211> LENGTH: 128
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 46

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ala
1  5  10  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20  25  30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Gin Gly Leu Glu Trp Met
35  40  45
Gly Ala Ile Asn Pro Ser Gly Gin Ser Thr Pro Tyr Ala Gin Lys Phe
50  55  60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65  70  75  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Cyc
85  90  95
Ala Arg Asp Gly Thr Tyr Gly Ser Gly Ser Tyr Pro Tyr Tyr Tyr
100 105 110
Tyr Gly Met Asp Val Trp Gly Gin Gly Thr Thr Val Thr Val Ser Ser
115 120 125
<210> SEQ ID NO 47
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

aatttatgct gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
tcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctc
cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc
gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
cgagtcagcttc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
tcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctc
cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc
gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
cgagtcagcttc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
tcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctc
cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc
gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
cgagtcagcttc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
tcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctc
cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatg cc

<210> SEQ ID NO 48
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

Anp Phe Met Leu Thr Gin Pro His Ser Val Ser Glu Ser Pro Gly Lys    1     5     10     15
Thr Val Thr Ile Ser Cys Thr Gly Ser Gly Ser Ile Ser Amh Asn    20     25     30
Tyr Val His Trp Tyr Gin Gin Gin Asp Pro Ile Gin Pro Thr Gin    35     40     45
Met Phe Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin    50    55     60
Gly Ser Ile Gin Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser    65     70     75     80
Leu Lys Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin    85    90    95
Ser Lys Val Val Phe Gin Gin Gin Thr Gin Gin Gin Gin Gin Gin Gin Gin    100   105    110

<210> SEQ ID NO 49
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

gaggtcagcc tgtgtcagtc gccgccggc ggtgtcagcc gccgccggc ggtgtcagcc
tcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctc
cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatg cc
gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
cgagtcagcttc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
tcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctc
cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatg cc

<210> SEQ ID NO 50
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 50

aatttatgct gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc gcagcagcccc
tcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctcgctc
cagaagatgcc cagaagatgcc cagaagatgcc cagaagatgcc cagaagatg cc

372
-continued

<400> SEQUENCE: 50

Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1  5  10  15

Ser Leu Thr Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20  25  30  

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35  40  45  

Ser Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50  55  60  

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65  70  75  80  

Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val Tyr Tyr Cys
85  90  95  

Ala Lys Thr Leu Ser Ala Gly Trp Ile Gly Gly Gly Ala Phe Asp
100  105  110  

Ile Trp Gly His Gly Thr Met Val Thr Val Ser Ser
115  120

<210> SEQ ID NO: 51
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

gaaacgcacc tccgcagtc tccagcacc ctgcttttgt ctccagggga aagagccacc 60
cctcttgca gggccagtcg gaggtttgag acgagctgc tagcttggta ccagcagaa 120
cctggcagg cttcaggccc cttcatcttt gttcactcgc caggcacc ccagcttccc 180
gacagttca gtggcagtgg gcctgggaca gacttcactc tccacactcag cagactggag 240
cctgaagtt ttgacgtgta ttacgctcag cagatgata gttcaccagc gagttcggc 300
cagggcacc aggtggaaat caaagca 327

<210> SEQ ID NO: 52
<211> LENGTH: 199
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 52

Glu Thr Thr Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Leu Ser Pro Gly
1  5  10  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20  25  30  

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35  40  45  

Ile Tyr Gly Ala Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50  55  60  

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65  70  75  80  

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln His Asp Ser Ser Pro
85  90  95  

Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105
<210> SEQ ID NO: 53
<211> LENGTH: 363
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 53

caggctccag tcgtgcagtc tgggggaggg gttggtccagc ctggagagct ccggagactc 60
tcctgtgcag cctctggatt cacctcaggt agttatgcca tgcacttgggt cggcaggt 120
cagcagcaggg ggtctggaggt ggtggtcttt atcctatagtt atggagaattga taagaacattt 180
gcagctcgtc tgaaggggctgc attcacactca tccagagacca attcacaagaa cactcctat 240
cctgcaataaga cagcgctgctg agctgctgagc acggctgtggt attaagctctgca gaaagatcc 300
tacttgata atagtgtcttt tccggcagac tggggcagcg gcaccccttggt caccgtctca 360
agc 363

<210> SEQ ID NO: 54
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 54

Gln Val Gln Leu Val Gln Ser Gly Gly Val Val Gln Pro Gly Arg 1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr 20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45
Ala Phe Ile Ser Tyr Asp Gly Ser Asp Lys Asn Phe Ala Asp Ser Val 50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr 65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95
Ala Lys Asp Ser Tyr Tyr Asp Asn Ser Ala Phe Gln Ala Asp Trp Gly 100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser 115 120

<210> SEQ ID NO: 55
<211> LENGTH: 330
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 55

aattttatgc tgaactcagc ccactctgtg tggagtcttc ctgggaagac ggtacaacctc 60
tccgctccag gcagctggtgc gggacttagc aacaattatgc tccactgta ccaacgcgcg 120
cggggagctgc ccaccaacac tgctattgtt gaagataacc aagaccctc tgggttccct 180
gatcggtttct cttgctcctat gcaacagctcc tccaacctctg ctctcctctg ctcttctgga 240
cctagagctg aggacaggg tgaagtactac tggатаcttt cttgatggaag taagaatggtc 300
tctgggagc ggaccaagct gaccgtccta 330
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 56

Asp Phe Met Leu Thr Gln Pro His Val Ser Glu Ser Pro Gly Lys
1  5    10   15

Thr Val Thr Ile Ser Cys Thr Gly Ser Gly Ser Ile Asp Asn Asn
20 25   30

Tyr Val His Trp Tyr Gln Glu Arg Pro Gly Ser Ala Pro Thr Thr Val
35 40   45

Met Phe Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Arg Phe Ser
50 55   60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Val Ile Ser Gly
65 70   75   80

Leu Lys Thr Glu Asp Gly Gly Tyr Tyr Cys Gln Ser Ser Asp Gly
85 90   95

Ser Lys Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
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<210> SEQ ID NO: 57
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 57

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tcggcagctg ggtgctagtg ggtgtccagtt atacatagtt atggagcat ttattactat 180
cggcactctg tgaaggccgct attcaccatc tccagagaca tttcaggaag cagctgtat 240
tcgacaattg acacgtcagtc aagtctgagac acgcatgtgt atacactgct caaaaaacctg 300
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acgcctctca gc 372
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<210> SEQ ID NO: 58
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 58

Glu Val Gln Leu Val Val Ser Gly Gly Val Val Gln Pro Gly Arg
1  5    10   15

Ser Leu Thr Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25   30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40   45

Ser Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55   60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70   75   80

Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val Tyr Cys
85 90   95

Ala Lys Thr Leu Ser Ala Gly Glu Trp Ile Gly Gly Gly Ala Phe Asp
100 105 110
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Ile Trp Gly His Gly Thr Met Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 59
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 59

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120
cctggccagg ctccagcaag ccctcctctat ggctcaatcca gcagggccac tggcatccca
180
gacaggtca gtggcagttg gttgggaca gagctcctc tcaccctcag cagacttgag
240
cctgaagatt ttgcaagtga ttatctcag cagcatgata gctcaaccag gcagctcggc
300
caggggcaag aggtgggaat ccaacga
327

<210> SEQ ID NO 60
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 60

Glu Thr Thr Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1  5  10  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gin Ala Pro Arg Leu Leu
35 40 45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gin Gin His Asp Ser Ser Pro
85 90 95
Arg Thr Phe Gly Gin Gly Thr Val Gin Ile Lys Arg
100 105

<210> SEQ ID NO 61
<211> LENGTH: 363
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 61

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cgacggaccc gggcgatgtt atacatatat gttgggattc taaagggatatctctttc
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gcagcctcgc tgaagggcccg attccaccata tcacattaga attccagaga cacttctat
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tctgacaaat gccacagctctcagctcagctcatgccagcctgaccctggtcctggtgaagagaatccc
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360
agc
363
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<210> SEQ ID NO 62
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Ser Tyr Asp Gly Ser Asp Lys Asn Phe Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys Asp Ser Tyr Tyr Asp Asn Ser Ala Phe Glu Ala Asp Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 63
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

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30 45
ccctggggcc agtctctat acttgctatca gccggtgacc cgggtatcag
50 65
agcagcttc aaagctggtgc gtttgtagcag ttcagcagtcagacagctact
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ttgctgagg ccaagctgtg gtttcagcag ttcagcagtcagacagctact
110 125
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140

<210> SEQ ID NO 64
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 64

Glu Thr Thr Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Leu Ser Leu Ser Leu Ser
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Ser Val Ser Ser Ser
20 25 30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Arg Leu Leu
35 40 45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gin Gin Tyr Gly Ser Ser Pro
85 90 95
Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> SEQ ID NO 65
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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tctgtcagc ccttgcagc cgtcttgatatg tctgtgcttc ctgtgacttc ctgtgacttc
tctgtcagc ccttgcagc cgtcttgatatg tctgtgcttc ctgtgacttc ctgtgacttc
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<210> SEQ ID NO 66
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 66
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ser Tyr
20 25 30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Glu Leu Glu Trp Met
35 40 45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Ala Asp Gly Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Pro Glu Tyr Cys Ile Asn Gly Val Cys Ser Leu Asp Val
100 105 110
Trp Gly Glu Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 67
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 67
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<210> SEQ ID NO 68
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 68
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tctggtcagttgcc tggcagttgcc tggtcagttgcc ctgtgacttc ctgtgacttc ctgtgacttc
tctggtcagttgcc tggcagttgcc tggtcagttgcc ctgtgacttc ctgtgacttc ctgtgacttc
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ggsaccaagg tggsaatcga acga

<210> SEQ ID NO 68
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 68

Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1   5   10   15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20  25   30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35  40   45
Tyr Asp Ala Ser Asn Arg Ala Thr G1y Ile Pro Ala Arg Phe Ser Gly
50  55   60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65  70   75   80
Glu Asp Phe Ala Val Tyr Tyr Cys His Gln Tyr Gly Ser Ser Pro Gln
85  90   95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> SEQ ID NO 69
<211> LENGTH: 363
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 69

gagtcagcg tgggtcagc tggggctgag gttgagsagc tgggtcctc ggtgaaggtc
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tcggacaggg ctccgaggg cgccttcgga cgtgctggtt gggcgaggc
120
cctgacagc ggtggtctgg gatggggagc atcagctcct gttggtctac gccaagactc
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gacacactt tccggcagcg acgacagt tccgcgagc ggtggtctac gcagcctctc
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gccgcaagcg ccagccttc aacccgactc acgactgtc
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gccgcaagcg

<210> SEQ ID NO 70
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 70

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ser
1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Ser Tyr
20  25   30
Ala Ile Ser Trp Val Arg Glu Ala Pro Gly Gln Gly Leu Glu Trp Met
35  40   45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50  55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65  70   75   80
-continued

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85  90  96
Ala Val His Tyr Gly Asp Tyr Val Ser Ser Met Asp Val Trp Gly
100 105 110
Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 71
<211> LENGTH: 330
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 71

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cctctctgca ggacgtgtca gatgtgggcc agctatctag ctggctacca acagaagcct
120
ggcaggtc ccagactctc cattctgat gctccgccca gggccagtcc gccccacggc 180
aggtctcagc gcagttggtgc ttggagccac ttcactctca ccctccacag cctagagcct
240
gaaggtttgt cagtttata ctgctcagcag cgtagcaact ggcttcgcata gttacattttt
300
ggccagggga cccagctgga gatcaacaga 330

<210> SEQ ID NO 72
<211> LENGTH: 109
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 72

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1  5  10  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Gly Ser Tyr
20  25  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Ala Pro Arg Leu Ile Tyr
35  40  45
Asp Ala Ser His Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser
50  55  60
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gly Pro Glu
65  70  75  80
Asp Phe Ala Val Tyr Tyr Gln Gln Arg Ser Asn Trp Pro Pro Met
85  90  95
Tyr Thr Phe Gly Gln Gly Thr Leu Gly Ile Lys Arg
100 105

<210> SEQ ID NO 73
<211> LENGTH: 366
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 73

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tcggtcagc tttctgtgag caccctcagc agctataca tcagctgggt ggcacagggc 120
cctggcagc ggcttgtggt gattggaagg acctacccct tccttggtac agcgaactac 180
gcagagaagt tccagggcag agoaacagtt acgccgacat aatccgcagc cacagctac
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tagagctga gccagctgag atctgagagc acgcctgtgt attacaagtc ggccctgacg 300
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gatagtgtc gttattacgg gcgcgggtgac tacaaggtggct tgtcacaggct ggtcaccgtc

<210> SEQ ID NO 74
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 74

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ser
1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Thr Phe Ser Ser Tyr
20  25   30
Thr Ile Ser Trp Val Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Met
35   40   45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Aem Tyr Ala Gln Lys Phe
50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lye Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85   90   95
Ala Gly Asp Thr Asp Ser Ser Gly Tyr Tyr Gly Ala Val Asp Tyr Trp
100  105  110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115  120

<210> SEQ ID NO 75
<211> LENGTH: 330
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 75

gaaatgtgct tgtacgtgc tccagccaco ctgtttttgt ctcaggga aagagccacc
cctctctgca ggacgacctc gacttctggcc acgtacttag cctgtaccca acagaagcct
ggaggacttc cccagactctt cactcatgtg gcaaccccac gggccagtgg catccccagc
aggtacggct gcagctggtgc tgtgaggccct ctcacagcgc ccaacgagc ccatcgtacc
gamagACATTAGGATCTGACGACGCTGACCTACGAGC

ggcccaggcc ccaagotgga gatcagcag

<210> SEQ ID NO 76
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 76

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1   5   10   15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Gly Ser Tyr
20  25   30
Leu Ala Thr Tyr Gln Gly Leu Pro Gly Ala Pro Arg Leu Leu Ile Tyr
35   40   45
Asp Ala Ser His Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser
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<211> LENGTH: 366
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 77

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60

tctgtgcaag
cacagtgcag
tctgctgtg
gagacggcc  
120

cctggtacga
gagttgctgg
gtagggaggg
tcatcctctt
tctgtgagca
gcactgcc  
180

gocagagag
tccagctgag
ttgccagc
taccccaacag
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<210> SEQ ID NO 78
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 78

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<210> SEQ ID NO 79
<211> LENGTH: 339
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 79

gaaattgtgc
tgacctagtc
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tgccccgtca
cccctgsgaga
gccgcttcc  
60

attcctggca
ggctctagca
gagctctgtg
catagtaatg
gatacaacat
tttggttgg  
120

tacgccagc
gacagcggca
gtcctccagc
tccgatct
tttgggttcc
taaggggcc  
180
<210> SEQ ID NO: 80
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 80

Glu Ile Val Leu Thr Gin Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1     5     10    15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gin Ser Leu His Ser
20    25    30

Aam Gly Tyr Aam Tyr Leu Aep Trp Tyr Leu Gin Lys Pro Gly Gin Ser
35    40    45

Pro Gin Leu Leu Ile Tyr Leu Gly Ser Aam Arg Ala Ser Gly Val Pro
50    55    60

Amp Arg Phe Ser Gly Ser Gly Ser Gly Thr Aep Phe Thr Leu Lys Ile
65    70    75    80

Ser Arg Val Glu Ala Glu Arg Val Gly Val Tyr Tyr Cys Met Gin Ala
85    90    95

Leu Gln Thr Pro Arg Thr Phe Gly Gin Gly Thr Lys Val Glu Ile Lys
100   105   110

Arg

<210> SEQ ID NO: 81
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 81

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tctgtgcccgc tccctgggatt caccctcagc agctatggca tgcactgggt ccgccaggtc 120
cagccgaggg ggtgctggtg gttgctgcct atataataat agggactatt taataactat 180
gcagactgct cgaaggccgct attcaccatt tccagagcata attccagcaag cagctgtat 240
cagctcattg acagctgcag acgctggagc acggotgtgt atactgtgct gagaagcctt 300
gatcagctg atctactaata ctaactatgt atggagctcttgt ggggcaaggg gaccaggtgc 360
gacgctccaa gc 372

<210> SEQ ID NO: 82
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 82

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gin Pro Gly Arg
1     5     10    15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20    25    30

Gly Met His Trp Val Arg Glu Ala Pro Gly Lys Gly Leu Glu Trp Val
35    40    45
Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
 Ala Arg Asp Phe Asp Tyr Gly Asp Ser Tyr Tyr Tyr Gly Met Asp
100 105 110
Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> SEQ ID NO: 83
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 83
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atccctggcc gggcagctca gagaaggttg gattacctgg ccctggtatca gcagaagcga 120
ggacacgcct ctaagctctc gatctagtgg gatccaccttc gcagagtgg ggtccatca 180
aggttcagcg ccagttggct tggcagacag ttcacctccta ccacagcggg cctgcagcct 240
gagaagtttg caacacctta ttgcacgcag gcataagttt tccocacatc ottogccaa 300
ggacagcgcc tgacatcag aca 324

<210> SEQ ID NO: 84
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 84
Asp Ile Gln Met Thr Glu Ser Pro Ser Ile Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Arg Phe Gly Asp Tyr
20 25 30
Leu Ala Trp Tyr Gln Glu Pro Gly Glu Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Gly Ala Ser Thr Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Gln Phe Thr Leu Thr Ile Ser Gly Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Ile
85 90 95
Thr Phe Gly Lys Gly Thr Arg Leu Asp Ile Arg Arg
100 105

<210> SEQ ID NO: 85
<211> LENGTH: 357
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 85
cagttgccagc tggcaccagt tccttccatc ctgtctgcat ctgtggsaga cagagtcacc 60
tcctgcaagg ctcctgctgc acccctcagc agctatgcct tcagctggtt ggacacggc 120
-continued

cctggacaaag ggttgagttg gatggatgg atcaagtcttg gcgtgggcct caaatatatc 180
tccaagagt tctcgagcag acctcttgg caacggagac cactcttgc cacaagcctc 240
atggagctg gacagcagtt atctgatagc accgtgtgta ataccagctg gagaagcccc 300
gagagagcct gggacccctt tataaacgggcc cgggggcccc tgggatcagg ctcaagc 357

<210> SEQ ID NO 86
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 86

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ser 1
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr 20
Ser Ile Ser Trp Val Arg Gin Ala Pro Gly Gin Gly Leu Glu Trp Met 30
Gly Trp Ile Asn Val Gly Asn Gly Asn Ala Ile Tyr Ser Gin Lys Phe 40
Gly Gin Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Thr Thr Ala Tyr 50
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys 60
Val Ala Arg Asp Gly Glu Arg Ala Trp Asp Leu Asp Tyr Trp Gly Gin Gly 70
Thr Leu Val Thr Val Ser Ser 80

<210> SEQ ID NO 97
<211> LENGTH: 330
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 97

aattttatgc tgaacctcgc ccaaccttggt tcggagtctc cggggagac ccgaacccatc 60
ttcgaaccg cgaccagttgc cagcaattgc caaccatag ttcagttgta ccaacagcgc 120
cggggacgt ccaccgcagc tgggttctat gaggtgcc caagacctct tcgggtctct 180
gatcggctct cttgctccat cggacagctc tcaacattg tcctccatat cactcttgga 240
cgtgagacgt agagacgacg tggcaactac tgcagttcct atgatagcag caatcaggtc 300
ttcgagcag aggaccaagct gacgcctcct 330

<210> SEQ ID NO 88
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 88

Aas Phe Met Leu Thr Glu Pro His Ser Val Ser Glu Ser Pro Gly Lys 1
Thr Val Thr Ile Ser Cys Thr Gly Ser Gly Gly Ser Ile Ala Thr Asn 20
Tyr Val Glu Trp Tyr Gin Gin Arg Pro Gly Ser Ala Pro Ala Thr Val
-continued

35 40 45
Ile Tyr Glu Asp A Asp Gln Arg Pro Ser Gly Val Pro A Asp Arg Phe Ser
50 55 60
Gly Ser Ile Asp Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 60
Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser
95 100 105 95
Ser Asn Gln Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
110

<210> SEQ ID NO 89
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 89

cagggcagc tacgcatgt ggcgcagga ctgtagcagc cttcgagac cttgtccctc 60
acctgctcg tctatgtgtgg gtcctcagc ggtagctagt ggacgctagc ccgcagc 120
ccaggggag ggctgctagg gattgataa atcaatata tggcagccag caactacaac 180
cggcctcga aagtggtcagt cccgatatca gtgtaggctgg ccagaaaca cttctctcta 240
aacgtcagct cttgacagcc ccgggcagc ccagcgttga actgtgcagc gatgtgcagt 300
tactactcag gtatggaagct cttgggccaag gggcagccag tcagctctct aacc 354

<210> SEQ ID NO 90
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 90

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Leu Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Glu Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Asp Thr Ala Val Tyr Cys Ala
85 90 95
Arg Met Val Arg Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr
100 105 110
Thr Val Thr Val Ser Ser
115

<210> SEQ ID NO 91
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 91
tctatgtgc tgactcagcc accctcaagt tcagagcgcc caggaagac gggcagatt 60
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acctgtgagg gcatcaagat tggaggagag agtgtgcaat ggtaccagca gaagccaggg 120
caggccctcg tgtgtgctcg ctattagcat actgtccagg ctctcaggggt ccttgagcga 180
tccctctggt ccaacctctgg gaacacggcc accctgatca tcagcggagt cgaagccggg 240
gatgagggg actattacct ccaagcttgtg gataagctgca tctatcccaaca agtgccttc 300
ggagcggaga ccagagtgac cgtcctg 327

<210> SEQ ID NO 92
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 92

Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Glu Ala Pro Gly Lys
1     5     10     15
Thr Ala Arg Ile Thr Cys Glu Gly Ile Thr Ile Gly Arg Lys Ser Val
20    25    30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
35    40    45
Amp Asp Thr Val Arg Pro Ser Gly Val Pro Glu Arg Phe Ser Gly Ser
50    55    60
Ann Ser Gly Ann Thr Ala Thr Leu Ile Ile Ser Gly Val Glu Ala Gly
65    70    75    80
Amp Gly Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Thr Asp Pro
85    90    95
Gln Val Val Phe Gly Gly Gly Gly Thr Lys Val Thr Val Leu
100   105

<210> SEQ ID NO 93
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 93

cagstacagc tggcagcagtc aggtccagga cttgtgaagc ctctgcagac ccctccactc 60
acctgtgcac tctcgggaga cagtgcctct acgaagcatt ctctcggag cttgatcagg 120
cattctcccagacagcttgagtcgagagcatactacagtc caagtgttat 180
tatgatgctcgcctgaagagcgat ctcaccacac cccccacac attcaagagac 240
caggctctcc tgtcagctgac cgctgtgact cccagagac ccgctatgta ttctgtgta 300
agggggagcact aatattggtg grgggcccc aagcactgccg ctcggtcctc aagc 364

<210> SEQ ID NO 94
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 94

Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1     5     10     15
Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Ser Val Ser Ser Lys
20    25    30
Ann Ser Ser Trp Ann Trp Ile Arg Gln Ser Pro Ser Arg Gly Leu Glu
35    40    45
Trp  Leu  Gly  Arg  Thr  Tyr  Tyr  Arg  Ser  Lys  Trp  Tyr  Tyr  Asp  Tyr  Ala
45       65       60
Val  Ser  Val  Lys  Gly  Arg  Ile  Thr  Phe  Thr  Pro  Asp  Thr  Ser  Lys  Aen
65       70       75       80
Gln  Val  Ser  Leu  His  Leu  Aen  Ala  Val  Thr  Pro  Glu  Asp  Thr  Ala  Met
85       90       95
Tyr  Tyr  Cys  Val  Arg  Gly  Ser  Ile  Phe  Asp  Val  Val  Trp  Gly  Gln  Gly  Thr
100      105      110
Met  Val  Thr  Val  Ser  Ser  
115

<210> SEQ ID NO 95
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 95

(gacatcacaagtgacaccagtctcttcatactgtctgctagtggagagcagagtcacc
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atcacgtgaccgggcaacgtgtagttggtgactttggtcgttagatgaccacgcaac
120
gggcaagctctagctctgtctgattatagtgtggtcgtcacttcttgacaggtggcgtcac
180
aggtcagctgcaaacgcagttcacttcctaaccagcaggggcaagcgcagcttcagcagc
240
gaaagtatttgaaccactcaattgctcagcagcaaaactttactactacttcagcttggcagaa
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gggacagggttgacataccagaca
324)

<210> SEQ ID NO 96
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 96

Asp  Ile  Gln  Met  Thr  Gln  Ser  Pro  Ser  Ile  Leu  Ser  Ala  Ser  Val  Gly
1       5       10      15
Asp  Arg  Val  Thr  Ile  Thr  Cys  Arg  Ala  Ser  Gln  Arg  Phe  Gly  Asp  Tyr
20      25      30
Leu  Ala  Trp  Tyr  Gln  Gln  Lys  Pro  Gln  Ala  Pro  Lys  Leu  Leu  Ile
35      40      45
Tyr  Gly  Ala  Ser  Thr  Leu  Gln  Ser  Gly  Val  Pro  Ser  Arg  Phe  Ser  Gly
50      55      60
Ser  Gly  Ser  Gly  Thr  Glu  Phe  Thr  Leu  Thr  Ile  Ser  Gly  Leu  Gln  Pro
65      70      75      80
Glu  Asp  Phe  Ala  Thr  Tyr  Tyr  Cys  Gln  Gln  Ala  Aen  Ser  Phe  Pro  Ile
85      90      95
Thr  Phe  Gly  Lys  Gly  Thr  Arg  Leu  Asp  Ile  Arg  Arg
100     105

<210> SEQ ID NO 97
<211> LENGTH: 357
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 97

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tctggcaaggctctggagagctccactgtgactcgcttgaggctggagggcagagcgcggc
120
-continued

cctggaacag ggccttgctg gatgggatgg atcaaccgttg gcaatgttac gcgaatatat 160
taccagatg ttgaggagct agttaccatt accagggcac ccaggggac cacagctac 240
atggaactga gcagcccttgac tctcggagac aagccgttgtg atatcgtggtc gagagacggg 300
gagagagcct gggagctgag tcaatggggc caggggaccc tggccacgct ctcagc 357

<210> SEQ ID NO 98
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 98

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ser 1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Lys Thr Phe Ser Ser Tyr 20 25 30
Ala Ile Ser Thr Val Arg Gln Ala Pro Gly Glu Leu Glu Trp Met 35 40 45
Gly Thr Ile Asn Val Gly Asn Gly Asn Ala Ile Tyr Ser Glu Lys Phe 50 55 60
Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Thr Thr Ala Tyr 65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95
Ala Arg Asp Gly Glu Arg Ala Thr Asp Leu Asp Tyr Trp Gly Glu Gly 100 105 110
Thr Leu Val Thr Val Ser Ser 115

<210> SEQ ID NO 99
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 99
gaaaacgcac tcacgcagtt ctcagggccc cttgttcctgct ctcaggggga aagagccacc 60
tcttcctcga ggcacagctca gaacggggtgctgctcgtgta cagcagaaa 120
ctctggcagct cctcaggtct cctcagttct cgcgtcggct ggcacagctca cggcagccac 180
gacaggcct gcagacggtgg ggttcagggcag cattcagtc accagcagct gacagcagct 240
tcttacaagtt tcacagtctga ccagttcgct gtcgcatctt gagcttgccagct 300
ggacacgcc ctggacgacct accga 324

<210> SEQ ID NO 100
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 100

Glu Thr Thr Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly 1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Arg 20 25 30
Tyr Leu Ala Trp Tyr Gln Glu Lys Pro Gly Gln Ala Pro Arg Leu Leu 35 40 45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser
50  55  60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65  70  75  80
Pro Glu Asp Phe Ala Val Tyr Cys Glu Glu Tyr Gly Ser Ser Ser
85  90  95
Thr Phe Gly Gln Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> SEQ ID NO 101
<211> LENGTH: 369
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 101
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acagtgactg tctctgtgag ctcctagcgc agtagtgatt actactgggg ctggtacccg 120
cagcccggcg ggaagggcgt ggaaggtgatt ggagatctct atatatgtgg gacaacctgc 180
tacaacccgt ccctcaagag tcaggtcacc atatccgtag acagcccacc gaacaggacc 240
tccctgaagc tgagccgttg gacagccgca gcaaggtgct tgagttacctc tcgagatcc 300
agagctggga gtacctccaa tgatcttttt gatatctggg gcacaggggac aatggtcacc 360
gctccagc  369

<210> SEQ ID NO 102
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 102
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1   5   10  15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
20  25  30
Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35  40  45
Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Tyr Ser Ser
50  55  60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Arg Glu Phe
65  70  75  80
Ser Leu Lys Leu Ser Ser Val Ala Ala Asp Thr Ala Val Tyr Tyr
85  90  95
Cys Ala Arg Ser Arg Ser Gly Ser Tyr Leu Arg Asp Ala Phe Asp Ile
100 105 110
Trp Gly Glu Gly Thr Met Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 103
<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 103
gaacgacac tcaocacgtc tccaggccac ctgcttttgt ctccaggagga aagagccacc  60
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cctctctgca gggcagcgtca gagtgttgcag acagactact tagcttggtga cccagcagaa
120
cctgycacag ctctcaagct cctctcatat gggaatccca gcggggtcag tgggatcca
180
gacaggtccca gttgcaaggt gttgsgsca gctccacttc ctaacactcag cagaogtggag
240
cctgcaagat ttaactggcag cgaatgtggt gttctagcgg gaaggtcggc
300
cagggggcca aggtggaat csaacga
327

<210> SEQ ID NO 104
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 104
Glu Thr Thr Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Ser Pro Gly
1  5   10  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gin Ser Val Ser Ser Ser
20  25   30
Tyr Leu Ala Trp Tyr Gln Gin Leu Pro Gly Gin Ala Pro Arg Leu Leu
35  40   45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50  55   60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65  70   75   80
Pro Glu Asp Phe Ala Val Tyr Tyr Gln Gin Gin Tyr Gly Ser Ser Ser
85  90   95
Gly Thr Phe Gly Gin Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> SEQ ID NO 105
<211> LENGTH: 363
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 105
caggtacagc tgtccgaagtc agggggcttag gttgaagaagc ctggggtgcc gcgggtgc
60
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180
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240
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gac
363

<210> SEQ ID NO 106
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 106
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Pro Gly Ser
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Ser
20  25   30
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 107

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ggccagcgc ccaagctctc catatatgtg gcatccagaca ggccacactgg cgctccagac 180
aggtcagtg gcaagctgtc tgggagacag ttcaactctca ccaatcagcag acctgagct 240
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ggcagggga ccagacctgaga gataaaacga 330

<210> SEQ ID NO 108
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 108

Glu Thr Thr Leu Thr Gln Ser Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly 1      5      10      15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gin Ser Val Gly Ser Asn 20     25     30
Leu Ala Trp Tyr Gin Gin Gin Arg Pro Gly Gin Ala Pro Ser Leu Ile 35     40     45
Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Asp Arg Phe Ser Gly 50     55     60
Ser Gin Ser Gin Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro 65     70     75     80
Glu Asp Phe Ala Val Tyr Tyr Cys Gin Gin Tyr Gly Asp Ser Pro Arg 85     90     95
Leu Tyr Thr Phe Gly Gin Gly Thr Lys Leu Glu Ile Lys Arg 100    105    110

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<213> ORGANISM: Homo sapiens

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cgctgctcta aggaagcagct ccaactatca gtagaacag tccaagaaaca gttctccttg 240
aagctgtacgt ctgtgacgacg gctggacagc ggtctgctatt aagtgcgcag aggagctcaac 300
tatgatgta ggtgattat ccccttattt gctttttgata tctgggcccag aggggaatgt 360
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<210> SEQ ID NO 110
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 110

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20    25    30
Tyr Trp Ser Trp Ile Arg Glu Pro Pro Gly Lys Gly Leu Glu Trp Ile
35    40    45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50    55    60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Glu Phe Ser Leu
65    70    75    80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Cys Ala
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Arg Val Ala Tyr Tyr Asp Ser Ser Gly Tyr Tyr Pro Tyr Asp Ala Phe
100   105   110
Asp Ile Thr Gly Glu Gly Thr Met Val Thr Val Ser Ser
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<211> LENGTH: 327
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 111

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cctggggcgct ctcacgacct ctgtcatctat ggtcactcga cccagagcacc ttgcatctca 180
gacgagttca gttgacgctg gtctggagca gacttacact tcacactcag cagacgtagg 240
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 112

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<210> SEQ ID NO: 113  
<211> LENGTH: 369  
<212> ORGANISM: Homo sapiens  
<400> SEQUENCE: 113  

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cacggcagag ggttgccagtt ggtgccagtt atacatagat tggagaat caaat 180
gcagacttgc tgaaggctctt atcaccctc tcagagaac attaaaga aacagctgtg 240
cctcgcaacta aacagctcag gcggagagcc acggcgctgt tgttctagtttagaagtttaga 300
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<210> SEQ ID NO: 114  
<211> LENGTH: 123  
<212> ORGANISM: Homo sapiens  
<400> SEQUENCE: 114  

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Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  35  40  45
Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val  50  55  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  65  70  75  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  85  90  95
Ala Arg Glu Leu Arg Phe Leu Glu Trp Ser Ser Asp Ala Phe Asp Ile 100 105 110
Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser  115 120
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<210> SEQ ID NO: 115  
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 115

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cctgagcagg ctccacagct cttcacttat ggtgcaatcc caagggccac tcgcttccca 180
gacagtgtc ctgagcgttg gctctgggaca gcctcacttc tcaccatcag cagactggag 240
cctggagatt ttgcaagtta ttactgtcma cagtcaggtta cctcactttac gtggacgttc 300
ggccacagga ccaaggtgta aatccaaacga 330

<210> SEQ ID NO: 116
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 116

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Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu 35 40 45
Ile Tyr Gly Ala Ser Thr Arg Ala Thr Gln Val Pro Asp Arg Phe Ser 50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu 65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Thr Ser Leu 85 90 95
Thr Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg 100 105 110

<210> SEQ ID NO: 117
<211> LENGTH: 375
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 117

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cagccccccag gggaaggtggt gactgttagt ggggaatcag aatacatgag aagccacaac 180
ccacacacag gcctcaagag cgagagctag acatagagc aacgtcacaac gcacagcttc 240
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gctcagact cagacgc 375

<210> SEQ ID NO: 118
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 118

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<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 119
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<210> SEQ ID NO 120
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: primer

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<210> SEQ ID NO 121
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 121
Leu Leu Phe Gly Tyr Pro Val Tyr Val 1 5

1-3. (canceled)

4. A protein comprising an immunoglobulin heavy chain variable (VH) domain and an immunoglobulin light chain variable (VL) domain, wherein the protein binds a complex comprising an MHC and a peptide, does not substantially bind said MHC in an absence of said peptide, and does not substantially bind said peptide in an absence of said MHC, wherein said peptide is a peptide fragment of gp100, MUC1 or TAX.

5. The protein of claim 4, wherein said MHC is MHC class I.

6. The protein of claim 5, wherein said MHC class I comprises HLA-A*0201.

7. The protein of claim 4, wherein said peptide fragment of said gp100 is G9-209 (SEQ ID NO:1).

8. The protein of claim 4, wherein said peptide fragment of said gp100 is G9-280 (SEQ ID NO:2).

9. The protein of claim 4, wherein said peptide fragment of said gp100 is G9-154 (SEQ ID NO:3).

10. The protein of claim 4, wherein said peptide fragment of said MUC1 is D6 (SEQ ID NO:4).

11. The protein of claim 4, wherein said peptide fragment of said TAX is set forth by SEQ ID NO:121.

12. The protein of claim 7, wherein said protein comprises all six CDRs of 1A11 (SEQ ID NOs: 8 and 10), 1A7 (SEQ ID
13. The protein of claim 8, wherein said protein comprises all six CDRs of 2B2 (SEQ ID NOs:32 and 34), 2C5 (SEQ ID NOs:36 and 38), 2D1 (SEQ ID NOs:40 and 42) or 2F1 (SEQ ID NOs:44 and 46).

14. The protein of claim 9, wherein said protein comprises all six CDRs of G2D12 (SEQ ID NOs:48 and 50), G3F12 (SEQ ID NOs:52 and 54), G3F3 (SEQ ID NOs:56 and 58) or G3G4 (SEQ ID NOs:60 and 62).

15. The protein of claim 10, wherein said protein comprises all six CDRs of M3A1 (SEQ ID NOs:64 and 66) or M3B8 (SEQ ID NOs:68 and 70).

16. The protein of claim 10, wherein said protein comprises all six CDRs of T3E3 (SEQ ID NOs:72 and 74), T3F1 (SEQ ID NOs:76 and 78) or T3F2 (SEQ ID NOs:80 and 82).