US 20040001828A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0001828 A1**

Tuscano et al. (43) **Pub. Date: Jan. 1, 2004**

(54) **TREATMENT METHODS USING ANTI-CD22 ANTIBODIES**

(76) Inventors: **Joseph Tuscano**, Folsomo, CA (US); **Thomas F. Tedder**, Durham, NC (US)

Correspondence Address:
HELLER EHRMAN WHITE & MCAULIFFE LLP
275 MIDDLEFIELD ROAD
MENLO PARK, CA 94025-3506 (US)

(21) Appl. No.: **10/371,797**

(22) Filed: **Feb. 21, 2003**

Related U.S. Application Data

(60) Provisional application No. 60/359,419, filed on Feb. 21, 2002. Provisional application No. 60/420,472, filed on Oct. 21, 2002.

Publication Classification

(51) **Int. Cl.**[7] **A61K 39/395**

(52) **U.S. Cl.** **424/144.1**

(57) **ABSTRACT**

The invention concerns treatment methods using anti-CD22 monoclonal antibodies with unique physiologic properties. In particular, the invention concerns methods for the treatment of B-cell malignancies by administering an effective amount of a blocking anti-CD22 monoclonal antibody specifically binding to the first two Ig-like domains, or to an epitope within the first two Ig-like domains of native human CD22 (hCD22).

FIGURE 1

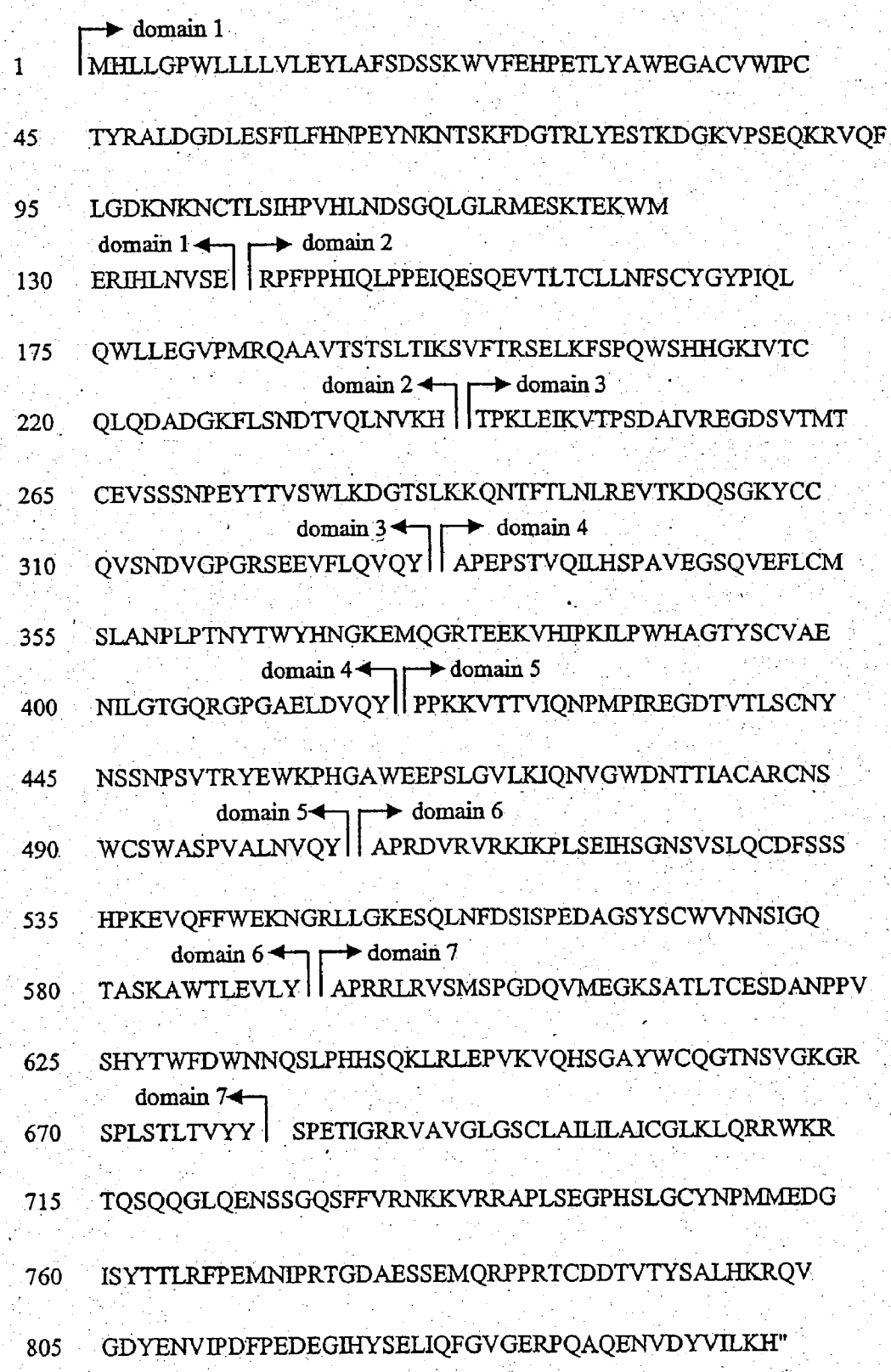

domain 1
1 MHLLGPWLLLLVLEYLAFSDSSKWVFEHPETLYAWEGACVWIPC
45 TYRALDGDLESFILFHNPEYNKNTSKFDGTRLYESTKDGKVPSEQKRVQF
95 LGDKNKNCTLSIHPVHLNDSGQLGLRMESKTEKWM
domain 1
domain 2
130 ERIHLNVSE RPFPPHIQLPPEIQESQEVTLTCLLNFSCYGYPIQL
175 QWLLEGVPMRQAAVTSTSLTIKSVFTRSELKFSPQWSHHGKIVTC
domain 2
domain 3
220 QLQDADGKFLSNDTVQLNVKH TPKLEIKVTPSDAIVREGDSVTMT
265 CEVSSSNPEYTTVSWLKDGTSLKKQNTFTLNLREVTKDQSGKYCC
domain 3
domain 4
310 QVSNDVGPGRSEEVFLQVQY APEPSTVQILHSPAVEGSQVEFLCM
355 SLANPLPTNYTWYHNGKEMQGRTEEKVHIPKILPWHAGTYSCVAE
domain 4
domain 5
400 NILGTGQRGPGAELDVQY PPKKVTTVIQNPMPIREGDTVTLSCNY
445 NSSNPSVTRYEWKPHGAWEEPSLGVLKIQNVGWDNTTIACARCNS
domain 5
domain 6
490 WCSWASPVALNVQY APRDVRVRKIKPLSEIHSGNSVSLQCDFSSS
535 HPKEVQFFWEKNGRLLGKESQLNFDSISPEDAGSYSCWVNNSIGQ
domain 6
domain 7
580 TASKAWTLEVLY APRRLRVSMSPGDQVMEGKSATLTCESDANPPV
625 SHYTWFDWNNQSLPHHSQKLRLEPVKVQHSGAYWCQGTNSVGKGR
domain 7
670 SPLSTLTVYY SPETIGRRVAVGLGSCLAILILAICGLKLQRRWKR
715 TQSQQGLQENSSGQSFFVRNKKVRRAPLSEGPHSLGCYNPMMEDG
760 ISYTTLRFPEMNIPRTGDAESSEMQRPPRTCDDTVTYSALHKRQV
805 GDYENVIPDFPEDEGIHYSELIQFGVGERPQAQENVDYVILKH"

Figure 2

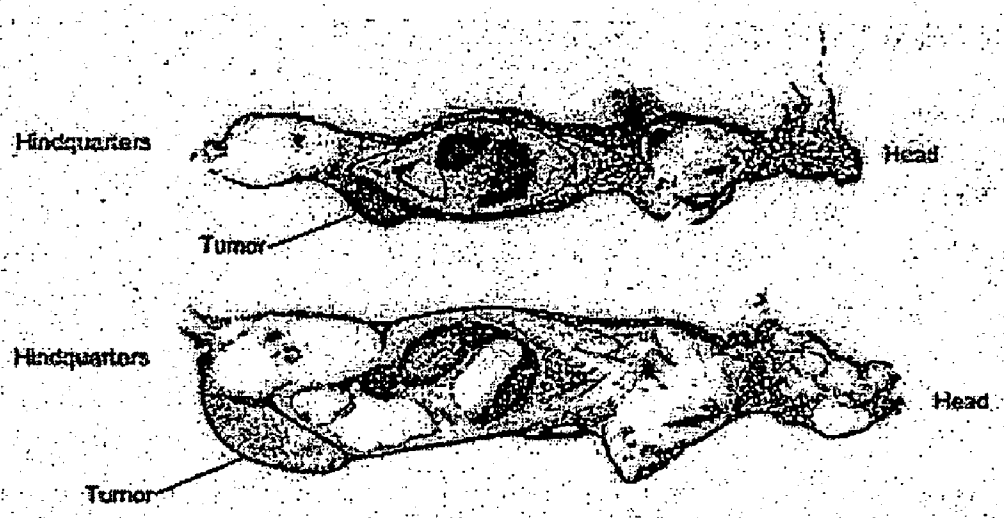


Figure 3

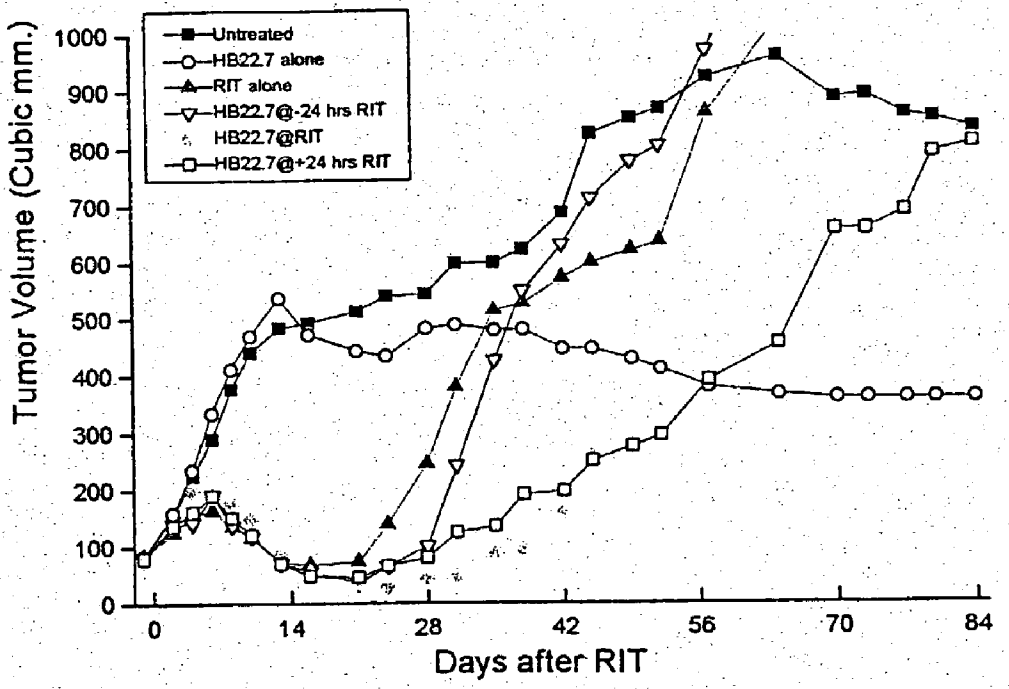
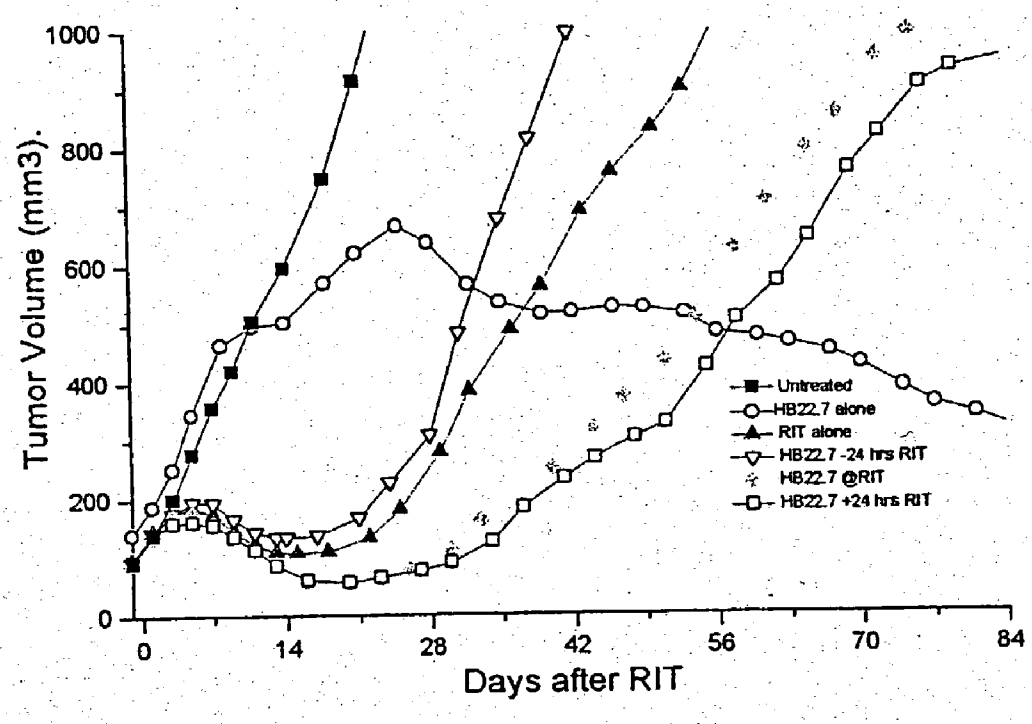

Untreated
HB22.7 alone
RIT alone
HB22.7@-24 hrs RIT
HB22.7@RIT
HB22.7@+24 hrs RIT
Tumor Volume (Cubic mm.)
1000
900
800
700
600
500
400
300
200
100
0
0
14
28
42
56
70
84
Days after RIT

Figure 4


Tumor Volume (mm3).
1000
800
600
400
200
0
0
14
28
42
56
70
84
Days after RIT
Untreated
HB22.7 alone
RIT alone
HB22.7 -24 hrs RIT
HB22.7 @RIT
HB22.7 +24 hrs RIT

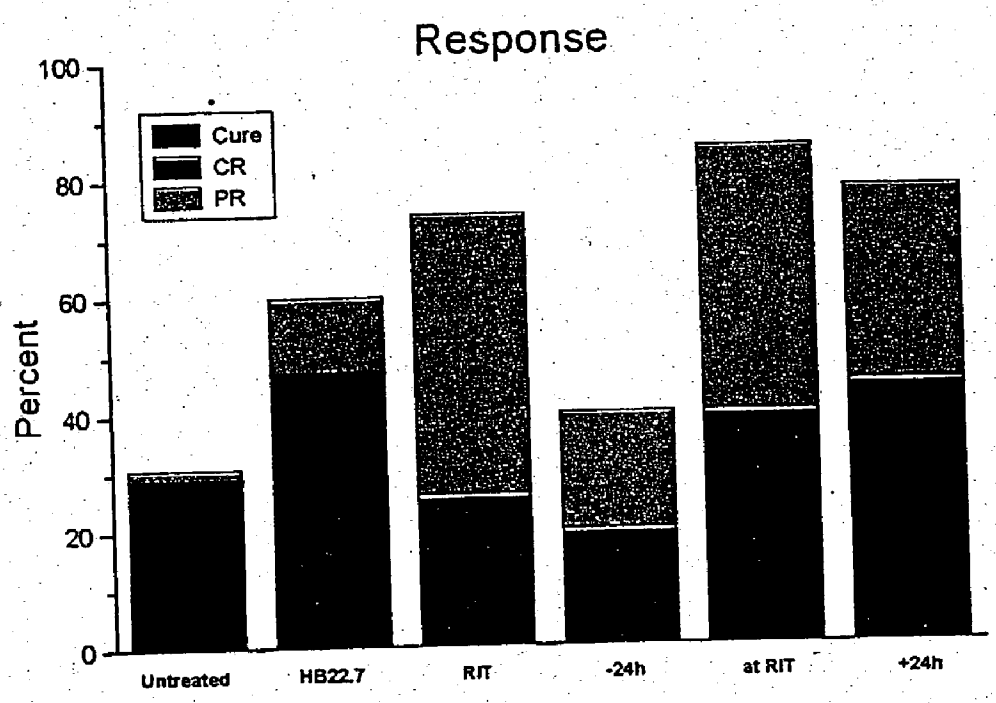

Response
Cure
CR
PR
Percent
100
80
60
40
20
0
Untreated
HB22.7
RIT
-24h
at RIT
+24h

Figure 5

Figure 6

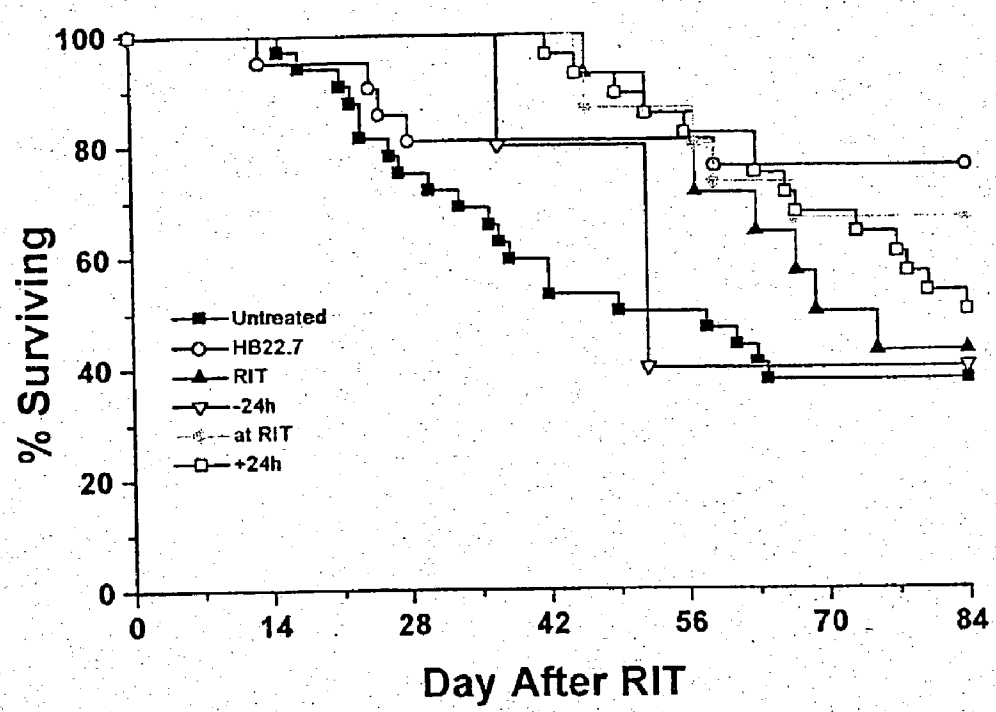
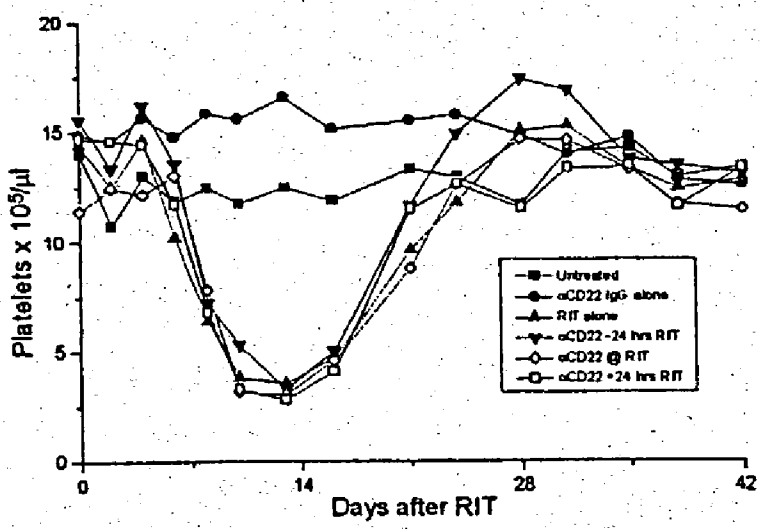

100
80
60
40
20
0
% Surviving
Untreated
HB22.7
RIT
-24h
at RIT
+24h
0
14
28
42
56
70
84
Day After RIT

Figure 7


Platelets x 10^5/µl
20
15
10
5
0
0
14
28
42
Days after RIT
Untreated
αCD22 IgG alone
RIT alone
αCD22 -24 hrs RIT
αCD22 @ RIT
αCD22 +24 hrs RIT

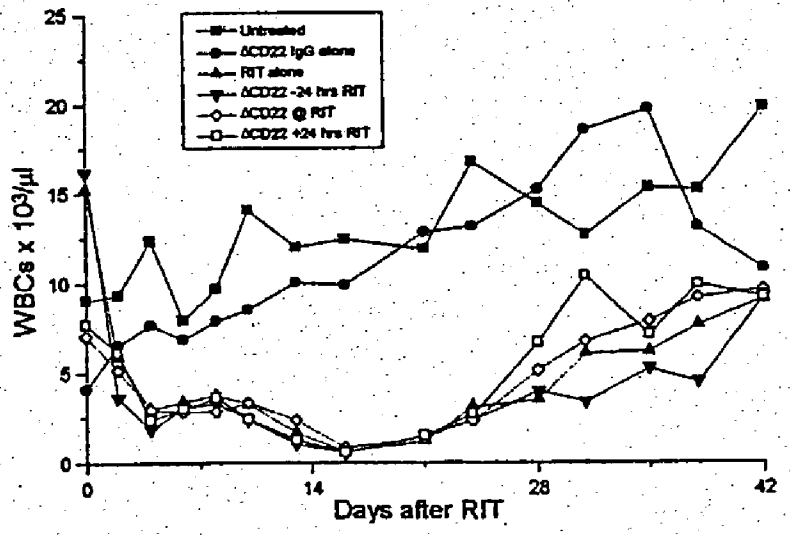

WBCs x 10^3/µl
25
20
15
10
5
0
0
14
28
42
Days after RIT
Untreated
ΔCD22 IgG alone
RIT alone
ΔCD22 -24 hrs RIT
ΔCD22 @ RIT
ΔCD22 +24 hrs RIT

Figure 8

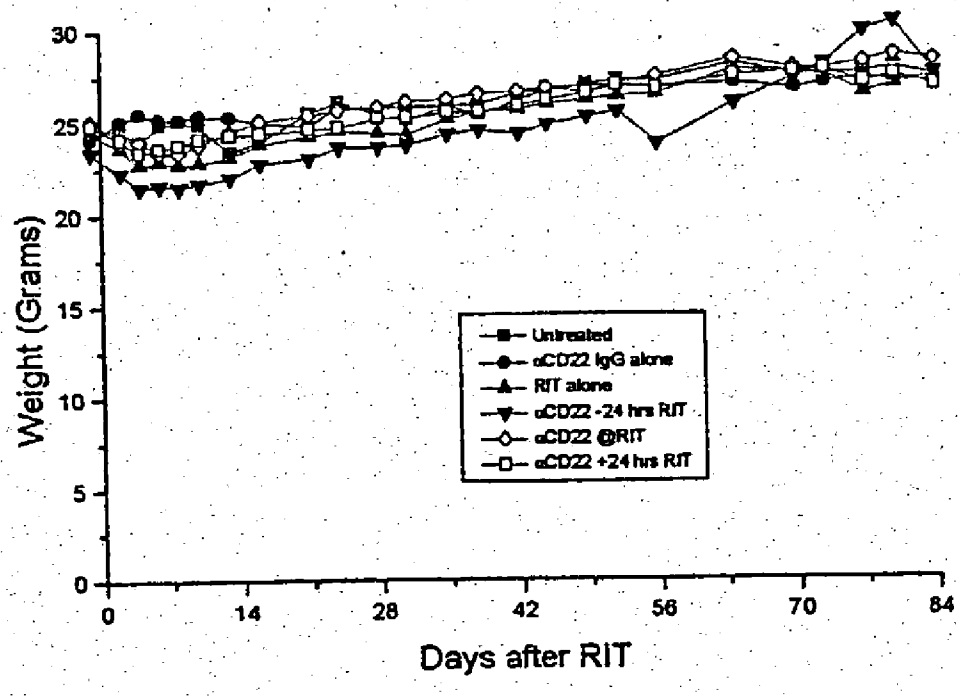

Weight (Grams)
30
25
20
15
10
5
0
0
14
28
42
56
70
84
Days after RIT
Untreated
αCD22 IgG alone
RIT alone
αCD22 -24 hrs RIT
αCD22 @RIT
αCD22 +24 hrs RIT

Figure 9

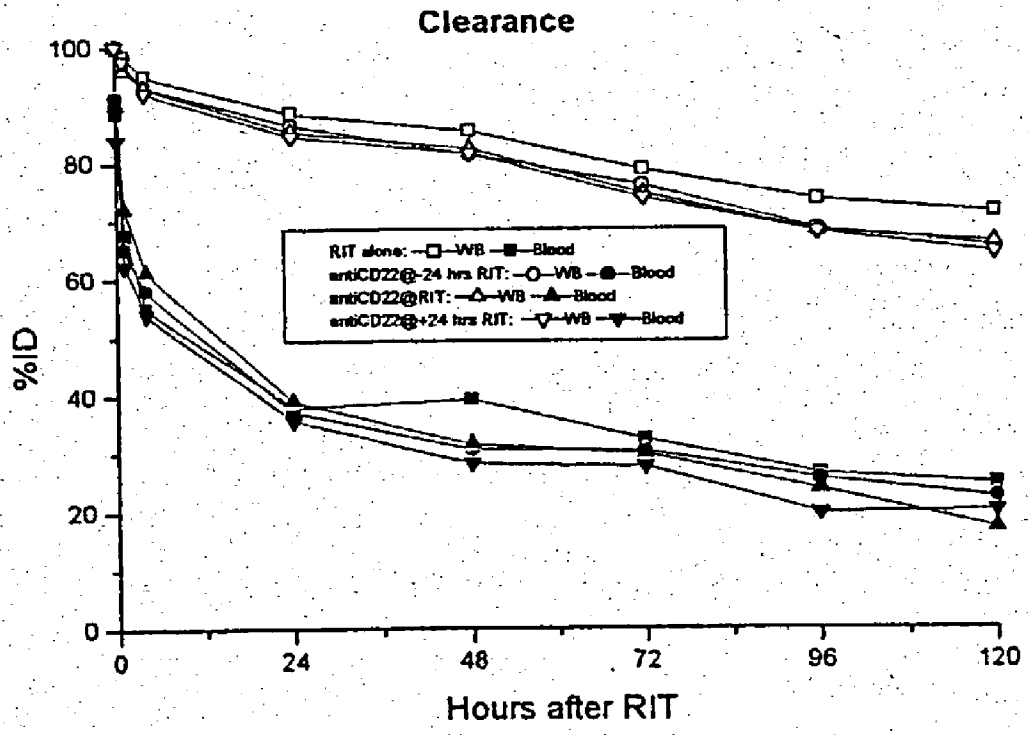

Clearance
100
80
60
40
20
0
%ID
0
24
48
72
96
120
Hours after RIT
RIT alone: —□—WB —■—Blood
antiCD22@-24 hrs RIT: —○—WB —●—Blood
antiCD22@RIT: —△—WB —▲—Blood
antiCD22@+24 hrs RIT: —▽—WB —▼—Blood

Figure 10

HB22 Hybridoma Antibody Heavy Chair Sequences

Residue	10	20	30 CDR1	40	50 CDR2	60	70	80	90	94	CDR3
HB22-5	GPELVKPGASMKISCKASGYSF.		TDYTMNWVKQSHGKNLEWIGLLH.		PFNG.	GTSYNQKFKGKATLSVDKSSSTAFMELLSLTSEDSAVYFCAR					GTGRN YAMDYWGQGTSVTVSS
HB22-196	GPDLVKPGASVKISCKASGYSF.		IGYYMHWLKQSHGKSLEWIGRVN.		PNTA.	GLTYNQRFKDKAILTVDKSSNTAYMELRSLTSEDSAVYYCSR					VDYDDYG YWFFDVWGAGTTVTVSS
HB22-7	GPGLVAPSQSLSITCTVSGFSL.		SDYGVNWVRQIPGKGLEWLGIIW.		.GDG.	RTDYNSALKSRLNISKDNSKSQVFLKMNSLKADDTARYYCAR					APGNR AMEYWGQGTSVTVSS
HB22-33	GPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYIR.				.YDG.	SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAR					GGITV AMDYWGQGTSVTVSS
HB22-13	GGGLVQPGGSLRLSCATSGFTF.		IDYYMNWVRQPPGKALEWLGFIKNKFNGYTTEYNTSVKGRFTISRDNSQSILYLQMNTLRAEDSATYYCAR								GLGRS YAMDYWGQGTSVTVSS
HB22-23	GGGLGATWRSMKLSCVASGFTF.		SYYWMNWVRQSPEKGLEWIAEIRLKSNNYATHYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYCTR								YDGSSR DYWGQGTTLTVSS
						V_H Sequences					D Region J Sequences

Figure 11

HB22-5 VH Sequence

```
1                                   10                                  20
E   V   Q   L   Q   E   S   G   P   E   L   V   K   P   G   A   S   M   K   I
GAG GTG CAG CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGA GCT TCA ATG AAG ATA   60

21                                  30                                  40
S   C   K   A   S   G   Y   S   F   T   D   Y   T   M   N   W   V   K   Q   S
TCC TGC AAG GCT TCT GGT TAC TCA TTC ACT GAC TAC ACC ATG AAC TGG GTG AAG CAG AGC  120

41                                  50                                  60
H   G   K   N   L   E   W   I   G   L   L   H   P   F   N   G   G   T   S   Y
CAT GGA AAG AAC CTT GAG TGG ATT GGA CTT CTT CAT CCT TTC AAT GGT GGT ACT AGC TAC  180

61                                  70                                  80
N   Q   K   F   K   G   K   A   T   L   S   V   D   K   S   S   S   T   A   F
AAC CAG AAG TTC AAG GGC AAG GCC ACA TTA TCT GTA GAC AAG TCA TCC AGC ACA GCC TTC  240

81                                  90                                  100
M   E   L   L   S   L   T   S   E   D   S   A   V   Y   F   C   A   R   G   T
ATG GAG CTC CTC AGT CTG ACA TCT GAG GAC TCT GCA GTC TAT TTC TGT GCA AGA GGG ACA  300

101                                 110                                 120
G   R   N   Y   A   M   D   Y   W   G   Q   G   T   S   V   T   V   S   S
GGT CGG AAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA      357
```

Figure 12

HB22-7 VH Sequence

```
1                                  10                                  20
 E   V   Q   L   Q   E   S   G   P   G   L   V   A   P   S   Q   S   L   S   I
GAG GTG CAG CTG CAG GAG TCT GGA CCT GGC CTG GTG GCG CCC TCA CAG AGC CTG TCC ATC   60

21                                 30                                  40
 T   C   T   V   S   G   F   S   L   S   D   Y   G   V   N   W   V   R   Q   I
ACA TGC ACC GTC TCA GGG TTC TCA TTA AGC GAC TAT GGT GTA AAC TGG GTT CGC CAG ATT  120

41                                 50                                  60
 P   G   K   G   L   E   W   L   G   I   I   W   G   D   G   R   T   D   Y   N
CCA GGA AAG GGT CTG GAG TGG CTG GGA ATA ATA TGG GGT GAT GGA AGG ACA GAC TAT AAT  180

61                                 70                                  80
 S   A   L   K   S   R   L   N   I   S   K   D   N   S   K   S   Q   V   F   L
TCA GCT CTC AAA TCC AGA CTG AAC ATC AGC AAG GAC AAC TCC AAG AGC CAA GTT TTC TTG  240

81                                 90                                 100
 K   M   N   S   L   K   A   D   D   T   A   R   Y   Y   C   A   R   A   P   G
AAA ATG AAC AGT CTG AAA GCT GAT GAC ACA GCC AGG TAC TAC TGT GCC AGA GCC CCC GGT  300

101                               110                            117
 N   R   A   M   E   Y   W   G   Q   G   T   S   V   T   V   S   S
AAT AGG GCT ATG GAG TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA              351
```

Figure 13

HB22-13 VH Sequence

```
1                                                  10                                                  20
 E   V   Q   L   Q   E   S   G   G   G   L   V   Q   P   G   G   S   L   R   L
GAG GTG CAG CTG CAG GAG TCT GGA GGA GGC TTG GTA CAG CCT GGG GGT TCT CTG AGA CTC   60

21                                                 30                                                  40
 S   C   A   T   S   G   F   T   F   I   D   Y   Y   M   N   W   V   R   Q   P
TCC TGT GCA ACT TCT GGG TTC ACC TTC ATT GAT TAC TAC ATG AAC TGG GTC CGC CAG CCT  120

41                                                 50                                                  60
 P   G   K   A   L   E   W   L   G   F   I   K   N   K   F   N   G   Y   T   T
CCA GGA AAG GCA CTT GAG TGG TTG GGT TTT ATT AAA AAC AAA TTT AAT GGT TAC ACA ACA  180

61                                                 70                                                  80
 E   Y   N   T   S   V   K   G   R   F   T   I   S   R   D   N   S   Q   S   I
GAA TAC AAT ACA TCT GTG AAG GGT CGG TTC ACC ATC TCC AGA GAT AAT TCC CAA AGC ATC  240

81                                                 90                                                  100
 L   Y   L   Q   M   N   T   L   R   A   E   D   S   A   T   Y   Y   C   A   R
CTC TAT CTT CAA ATG AAC ACC CTG AGA GCT GAG GAC AGT GCC ACT TAT TAC TGT GCA AGA  300

101                                                110                                                 120
 G   L   G   R   S   Y   A   M   D   Y   W   G   Q   G   T   S   V   T   V   S
GGG CTG GGA CGT AGC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC  360

121
 S
TCA                                                                                                 363
```

Figure 14

HB22-23 VH Sequence

```
1                                   10                                  20
 E   V   Q   L   Q   E   S   G   G   G   L   G   A   T   W   R   S   M   K   L
GAG GTG CAG CTG CAG GAG TCT GGA GGA GGG CTT GGT GCA ACC TGG AGA TCC ATG AAA CTC   60

21                                  30                                  40
 S   C   V   A   S   G   F   T   F   S   Y   Y   W   M   N   W   V   R   Q   S
TCC TGT GTT GCC TCT GGA TTC ACT TTC AGT TAC TAC TGG ATG AAC TGG GTC CGC CAG TCT  120

41                                  50                                  60
 P   E   K   G   L   E   W   I   A   E   I   R   L   K   S   N   N   Y   A   T
CCA GAG AAG GGG CTT GAG TGG ATT GCT GAA ATT AGA TTG AAA TCT AAT AAT TAT GCA ACA  180

61                                  70                                  80
 H   Y   A   E   S   V   K   G   R   F   T   I   S   R   D   D   S   K   S   S
CAT TAT GCG GAG TCT GTG AAA GGG AGG TTC ACC ATC TCA AGA GAT GAT TCC AAA AGT AGT  240

81                                  90                                 100
 V   Y   L   Q   M   N   N   L   R   A   E   D   T   G   I   Y   Y   C   T   R
GTC TAC CTG CAA ATG AAC AAC TTA AGA GCT GAA GAC ACT GGC ATT TAT TAC TGT ACC AGG  300

101                                 110                                 120
 Y   D   G   S   S   R   D   Y   W   G   Q   G   T   T   L   T   V   S   S
TAT GAT GGT TCC TCC CGG GAC TAC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA      357
```

Figure 15

HB22-33 VH Sequence

```
1                                                  10                                                  20
 E   V   Q   L   Q   E   S   G   P   G   L   V   K   P   S   Q   S   L   S   L
GAG GTG CAG CTG CAG GAG TCT GGA CCT GGC CTC GTG AAA CCT TCT CAG TCT CTG TCT CTC   60

21                                                 30                                                  40
 T   C   S   V   T   G   Y   S   I   T   S   G   Y   Y   W   N   W   I   R   Q
ACC TGC TCT GTC ACT GGC TAC TCC ATC ACC AGT GGT TAT TAC TGG AAC TGG ATC CGG CAG  120

41                                                 50                                                  60
 F   P   G   N   K   L   E   W   M   G   Y   I   R   Y   D   G   S   N   N   Y
TTT CCA GGA AAC AAA CTG GAA TGG ATG GGC TAC ATT AGG TAC GAC GGT AGC AAT AAC TAC  180

61                                                 70                                                  80
 N   P   S   L   K   N   R   I   S   I   T   R   D   T   S   K   N   Q   F   F
AAC CCA TCT CTC AAA AAT CGA ATC TCC ATC ACT CGT GAC ACA TCT AAG AAC CAG TTT TTC  240

81                                                 90                                                  100
 L   K   L   N   S   V   T   T   E   D   T   A   T   Y   Y   C   A   R   G   G
CTG AAG TTG AAT TCT GTG ACT ACT GAG GAC ACA GCT ACA TAT TAC TGT GCA AGA GGG GGG  300

101                                                110                                                 118
 I   T   V   A   M   D   Y   W   G   Q   G   T   S   V   T   V   S   S
ATT ACG GTT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA          360
```

Figure 16

HB22-196 VH Sequence

```
1                                                  10                                                  20
 E   V   Q   L   Q   E   S   G   P   D   L   V   K   P   G   A   S   V   K   I
GAG GTG CAG CTG CAG GAG TCT GGA CCT GAC CTG GTG AAG CCT GGG GCT TCA GTG AAG ATA   60

21                                                 30                                                  40
 S   C   K   A   S   G   Y   S   F   I   G   Y   Y   M   H   W   L   K   Q   S
TCC TGT AAG GCT TCT GGT TAC TCA TTC ATT GGC TAT TAC ATG CAC TGG CTG AAG CAG AGC  120

41                                                 50                                                  60
 H   G   K   S   L   E   W   I   G   R   V   N   P   N   T   A   G   L   T   Y
CAT GGA AAG AGC CTT GAG TGG ATT GGA GCT GTT AAT CCT AAC ACT GCT GGT CTT ACC TAC  180

61                                                 70                                                  80
 N   Q   R   F   K   D   K   A   I   L   T   V   D   K   S   S   N   T   A   Y
AAC CAG AGG TTC AAG GAC AAG GCC ATA TTA ACT GTA GAC AAG TCA TCC AAC ACA GCC TAT  240

81                                                 90                                                  100
 M   E   L   R   S   L   T   S   E   D   S   A   V   Y   Y   C   S   R   V   D
ATG GAG CTC CGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT TCA AGA GTG GAC  300

101                                                110                                                 120
 Y   D   D   Y   G   Y   W   F   F   D   V   W   G   A   G   T   T   V   T   V
TAT GAT GAC TAC GGG TAC TGG TTC TTC GAT GTC TGG GGC GCA GGG ACC ACG GTC ACC GTC  360

121
 S   S
TCC TCA                                                                          366
```

Figure 17

HB22 Hybridoma Antibody V kappa light chain sequences

```
Antibody -20      -10       1        10        20 CDR1 ABCDEFG    35        40        50 CDR2   60        70        80        90 CDR3  J Segment  Constant
HB22-5    MESQTQVFVFLLLCVSGAHGSIVMTQTPKFLLVSTGDRVTITCKASQTVT    NDLAWYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFTINTVQAEDLAVYFCQQDHSSP LTFGAGTKLELK RADAAPTV
HB22-7    MESQTQVFVFLLLCVSGAHGSIVMTQTPKFLLVSAGDRITLTCKASQSVT    NDVAWTQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYRSP WTFGGGTKLEIK RADAAPTV
HB22-13   MESQTQVFVFLLLCVSGAHGSIVMTQTPKFLLVSAGDRVSITCKASQSVT    NDVTWYQQKPGQSPKLLIYFASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYSSP LTFGAGTKLELK RADAAPTV
HB22-23   MESQTQVFVFLLCVSGAHGSIVMTQTPKFLLVSAGDRVSITCKASQSVT    NDVTWYQQKPGQSPKLLIYFASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYSSP LTFGAGTKLELK RADAAPTV
HB22-33    MKLPVRLLVLMFWIPASSSDVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVP YTFGGGTKLEIK RADAAPTV
HB22-196  MESQTQVFISILLWLYGADGNIVMTQSPKSMSMSVGERVTLTCKASENVV    TYVSWYQQKPEQSPKLLIYGASNRYTGVPDRFTGSGSATDFTLTISSVQAEDLADYHCGQGYSYP YTFGGGTKLEIK RADAAPTV
```

Figure 18

HB22-5 Vk Sequence

```
    M  E  S  Q  T  Q  V  F  V  F  L  L  L  C  V  S  G  A  H
AAG ATG GAG TCA CAG ACC CAG GTC TTC GTA TTT CTA CTG CTC TGT GTG TCT GGT GCT CAT     60

 G  S  I  V  M  T  Q  T  P  K  F  L  L  V  S  T  G  D  R  V
GGG AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA ACA GGA GAC AGG GTT    120

 T  I  T  C  K  A  S  Q  T  V  T  N  D  L  A  W  Y  Q  Q  K
ACC ATT ACC TGC AAG GCC AGT CAG ACT GTG ACT AAT GAT TTA GCT TGG TAC CAA CAG AAG    180

 P  G  Q  S  P  K  L  L  I  Y  Y  A  S  N  R  Y  T  G  V  P
CCA GGG CAG TCT CCT AAA CTG CTG ATA TAC TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT    240

 D  R  F  T  G  S  G  Y  G  T  D  F  T  F  T  I  N  T  V  Q
GAT CGC TTC ACT GGC AGT GGA TAT GGG ACG GAC TTC ACT TTC ACC ATC AAC ACT GTG CAG    300

 A  E  D  L  A  V  Y  F  C  Q  Q  D  Y  S  S  P  L  T  F  G
GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGC TCT CCT CTC ACG TTC GGT    360

 A  G  T  K  L  E  L  K  R  A  D  A  A  P  T  V
GCT GGG ACC AAG CTG GAA CTG AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                 410
```

Figure 19

HB22-7 Vk Sequence

```
    M  E  S  Q  T  Q  V  F  V  F  L  L  L  C  V  S  G  A  H
AAG ATG GAG TCA CAG ACC CAG GTC TTC GTA TTT CTA CTG CTC TGT GTG TCT GGT GCT CAT      60

 G  S  I  V  M  T  Q  T  P  K  F  L  L  V  S  A  G  D  R  I
GGG AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA GCA GGA GAC AGG ATT     120

 T  L  T  C  K  A  S  Q  S  V  T  N  D  V  A  W  Y  Q  Q  K
ACC TTA ACC TGC AAG GCC AGT CAG AGT GTG ACT AAT GAT GTA GCT TGG TAC CAA CAG AAG     180

 P  G  Q  S  P  K  L  L  I  Y  Y  A  S  N  R  Y  T  G  V  P
CCA GGG CAG TCT CCT AAA CTG CTG ATA TAC TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT     240

 D  R  F  T  G  S  G  Y  G  T  D  F  T  F  T  I  S  T  V  Q
GAT CGC TTC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG     300

 A  E  D  L  A  V  Y  F  C  Q  Q  D  Y  R  S  P  W  T  F  G
GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGG TCT CCG TGG ACG TTC GGT     360

 G  G  T  K  L  E  I  K  R  A  D  A  A  P  T  V
GGA GGC ACC AAG CTG GAA ATC AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                  410
```

Figure 20

HB22-13 Vk Sequence

```
     M  E  S  Q  T  Q  V  F  V  F  L  L  L  C  V  S  G  A  H
AAG ATG GAG TCA CAG ACC CAG GTC TTC GTA TTT CTA CTG CTC TGT GTG TCT GGT GCT CAT     60

 G  S  I  V  M  T  Q  T  P  K  F  L  L  V  S  A  G  D  R  V
GGG AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA GCA GGA GAC AGG GTT    120

 S  I  T  C  K  A  S  Q  S  V  T  N  D  V  T  W  Y  Q  Q  K
TCC ATA ACC TGC AAG GCC AGT CAG AGT GTG ACT AAT GAT GTA ACT TGG TAC CAA CAG AAG    180

 P  G  Q  S  P  K  L  L  I  Y  F  A  S  N  R  Y  T  G  V  P
CCA GGG CAG TCT CCT AAA TTG CTG ATA TAC TTT GCA TCC AAT CGC TAC ACT GGA GTC CCT    240

 D  R  F  T  G  S  G  Y  G  T  D  F  T  F  T  I  S  T  V  Q
GAT CGC TTC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG    300

 A  E  D  L  A  V  Y  F  C  Q  Q  D  Y  S  S  P  L  T  F  G
GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGC TCT CCG CTC ACG TTC GGT    360

 A  G  T  K  L  E  L  K  R  A  D  A  A  P  T  V
GCT GGG ACC AAG CTG GAG CTG AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                 410
```

Figure 21

HB22-23 Vk Sequence

```
     M   E   S   Q   T   Q   V   F   V   F   L   L   L   C   V   S   G   A   H
AAG ATG GAG TCA CAG ACC CAG GTC TTC GTA TTT CTA CTG CTC TGT GTG TCT GGT GCT CAT      50

 G   S   I   V   M   T   Q   T   P   K   F   L   L   V   S   A   G   D   R   V
GGG AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA GCA GGA GAC AGG GTC     100

 T   I   S   C   K   A   S   Q   S   V   S   N   D   V   A   W   Y   Q   Q   K
ACC ATA AGC TGC AAG GCC AGT CAG AGT GTG AGT AAT GAT GTA GCT TGG TAC CAA CAG AAG     150

 P   G   Q   S   P   K   L   L   I   Y   Y   A   S   K   R   Y   T   G   V   P
CCA GGG CAG TCT CCT AAA CTG CTG ATA TAC TAT GCA TCC AAG CGC TAT ACT GGA GTC CCT     200

 D   R   L   T   G   S   G   Y   G   T   D   F   T   F   T   I   S   T   V   Q
GAT CGC CTC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG     250

 A   E   D   L   A   V   Y   F   C   Q   Q   D   H   S   Y   P   W   T   F   G
GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT CAT AGC TAT CCG TGG ACG TTC GGT     300

 G   G   T   K   L   E   I   K   R   A   D   A   A   P   T   V
GGA GGC ACC AAG CTG GAG ATC AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                  350
```

Figure 22

HB22-33 Vk Sequence

```
 M  K  L  P  V  R  L  L  V  L  M  F  W  I  P  A  S  S  S  D
ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG ATG TTC TGG ATT CCT GCT TCC AGC AGT GAT      60

 V  V  M  T  Q  T  P  L  S  L  P  V  S  L  G  D  Q  A  S  I
GTT GTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA GAT CAA GCC TCC ATC     120

 S  C  R  S  S  Q  S  L  V  H  S  N  G  N  T  Y  L  H  W  Y
TCT TGC AGA TCT AGT CAG AGC CTT GTA CAC AGT AAT GGA AAC ACC TAT TTA CAT TGG TAC     180

 L  Q  K  P  G  Q  S  P  K  L  L  I  Y  K  V  S  N  R  F  S
CTG CAG AAG CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT TCT     240

 G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  L  K  I  S
GGG GTC CCA GAT AGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACA CTC AAG ATC AGC     300

 R  V  E  A  E  D  L  G  V  Y  F  C  S  Q  S  T  H  V  P  Y
AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TTC TGC TCT CAA AGT ACA CAT GTT CCG TAC     360

 T  F  G  G  G  T  K  L  E  I  K  R  A  D  A  A  P  T  V
ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA CGG GCT GAT GCT GCA CCA ACT GTA TC      419
```

Figure 23

HB22-196 Vk Sequence

```
    M  E  S  Q  T  Q  V  F  I  S  I  L  L  W  L  Y  G  A  D
AAG ATG GAG TCA CAG ACC CAG GTC TTC ATA TCC ATA CTG CTC TGG TTA TAT GGA GCT GAT      60

 G  N  I  V  M  T  Q  S  P  K  S  M  S  M  S  V  G  E  R  V
GGG AAC ATT GTA ATG ACC CAA TCT CCC AAA TCC ATG TCC ATG TCA GTA GGA GAG AGG GTC     120

 T  L  T  C  K  A  S  E  N  V  V  T  Y  V  S  W  Y  Q  Q  K
ACC TTG ACC TGC AAG GCC AGT GAG AAT GTG GTT ACT TAT GTT TCC TGG TAT CAA CAG AAA     180

 P  E  Q  S  P  K  L  L  I  Y  G  A  S  N  R  Y  T  G  V  P
CCA GAG CAG TCT CCT AAA CTG CTG ATA TAC GGG GCA TCC AAC CGG TAC ACT GGG GTC CCC     240

 D  R  F  T  G  S  G  S  A  T  D  F  T  L  T  I  S  S  V  Q
GAT CGC TTC ACA GGC AGT GGA TCT GCA ACA GAT TTC ACT CTG ACC ATC AGC AGT GTG CAG     300

 A  E  D  L  A  D  Y  H  C  G  Q  G  Y  S  Y  P  Y  T  F  G
GCT GAA GAC CTT GCA GAT TAT CAC TGT GGA CAG GGT TAC AGC TAT CCG TAC ACG TTC GGA     360

 G  G  T  K  L  E  I  K  R  A  D  A  A  P  T  V
GGG GGG ACC AAG CTG GAA ATA AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                  410
```

TREATMENT METHODS USING ANTI-CD22 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application Serial No. 60/359,419, filed Feb. 21, 2002 and to U.S. Provisional Application Serial No. 60/420,472, filed Oct. 21, 2002, both of which applications are hereby incorporated by reference in their entireties and from each of which priority is claimed under 35 U.S.C. §119(e).

[0002] This invention was made with Government support by Grant Nos. CA 47829, CA 54464, and CA 81776, awarded by the National Institutes of Health. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] 1. Field of the Invention

[0004] The present invention concerns the therapeutic use of certain anti-CD22 monoclonal antibodies with unique physiologic properties. More specifically, the invention concerns methods of treating B-cell malignancies, such as lymphomas and leukemias, and autoimmune diseases with blocking anti-CD22 antibodies having unique pro-apoptotic properties.

[0005] 2. Description of the Related Art

[0006] CD22 is a membrane glycophosphoprotein found on nearly all B lymphocytes and most B-cell lymphomas. Cross-linking CD22 triggers CD22 tyrosine phosphorylation and assembles a complex of effector proteins that activate the stress-activated protein kinase (SAPK) pathway. CD22 cross-linking provides a potent costimulatory signal in primary B-cells and pro-apoptotic signal in neoplastic B-cells. Structurally, CD22 is a member of the "sialoadhesin" subclass of the immunoglobulin (Ig) gene superfamily, having seven extracellular Ig domains with a single amino-terminal V-set Ig domain and six C-2 set Ig domains. Wilson et al., *J. Exp. Med.* 173:137-146 (1991); Engel et al., *J. Exp. Med.* 181:1581-1586 (1995); and Torres et al., *J. Immunol.* 149:2641-2649 (i992). It has been shown that CD22 is a critical lymphocyte-specific signal transduction molecule which negatively and positively regulates B lymphocyte antigen receptor (BCR) signaling by recruiting signaling effector molecules to physiologically pertinent sites. Tedder et al., *Annu. Rev. Immunol* 15:481-504 (1997); Sato et al., *Immunology* 10:287-297 (1998).

[0007] Anti-CD22 antibodies have been described, for example in U.S. Pat. Nos. 5,484,892; 6,183,744; 6,187,287; 6,254,868, and in Tuscano et al., *Blood* 94(4):1382-92 (1999). The use of monoclonal antibodies, including anti-CD22 antibodies, in the treatment of non-Hodgkin's lymphoma is reviewed, for example, by Renner et al., *Leukemia* 11(Suppl. 2):S55-9 (1997). A humanized anti-CD22 antibody, LymphoCide™ (empatuzumab, Immunomedics, Inc.) is in Phase III clinical trials for the treatment of indolent and aggressive forms of non-Hodgkin's lymphomas. An yttrium-90-labeled version of this antibody is currently in Phase I clinical trials for the same indication.

[0008] Despite recent advances in cancer therapy, B-cell malignancies, such as the B-cell subtype of non-Hodgkin's lymphoma, and chronic lymphocytic leukemia, are major contributors of cancer-related deaths. Accordingly, there is a great need for further, improved therapeutic regimens for the treatment of B-cell malignancies.

SUMMARY OF THE INVENTION

[0009] The present invention concerns an improved clinical approach for the treatment of B-cell malignancies in human patients, taking advantage of the unique properties of certain blocking anti-CD22 monoclonal antibodies.

[0010] In one aspect, the invention concerns a method for treating a human patient diagnosed with a B-cell malignancy, comprising (1) administering to the patient an effective amount of a blocking anti-CD22 monoclonal antibody specifically binding to the first two Ig-like domains or to an epitope associated with the first two Ig-like domains of native human CD22 (hCD22) of SEQ ID NO: 1, and (2) monitoring the response of the malignancy to the treatment.

[0011] In a particular embodiment, the antibody used binds to essentially the same epitope as an antibody selected from the group consisting of HB22-7 (HB11347), HB22-23 (HB11349), HB22-33, HB22-5, HB22-13, and HB22-196, preferably HB22-7, HB22-23, or HB22-33, more preferably HB22-7 or HB22-33.

[0012] In a further embodiment, the antibody blocks CD22 binding to its ligand by at least 70%, preferably by at least 80%.

[0013] In another embodiment, the antibody comprises a heavy chain comprising a V_H sequence having at least about 95% sequence identity with the sequence of amino acids 1 to 100 of SEQ ID NO: 9 (HB22-5 V_H sequence); or amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 13 (HB22-13 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); or amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 19 (HB22-196 V_H sequence).

[0014] In yet another embodiment, the antibody comprises a heavy chain comprising a V_H sequence having at least about 95% sequence identity with the sequence of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); or amino acids 1 to 160 of SEQ ID NO: 15 (HB22-23 V_H sequence); or amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence).

[0015] In a still further embodiment, the antibody comprises a V_H sequence selected from the group consisting of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 VH sequence); amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); and amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence).

[0016] In a different embodiment, the antibody comprises a light chain comprising a V_κ sequence having at least about 95% sequence identity with the amino acid sequence of SEQ ID NO: 21 (HB22-5 V_κ sequence); or SEQ ID NO: 23 (HB22-7 V_κ sequence); or SEQ ID NO: 25 (HB22-13 V_κ sequence); or SEQ ID NO: 27 (HB22-23 VK_κ sequence); or SEQ ID NO: 29 (HB22-33 V_κ sequence); or SEQ ID NO: 31 (HB22-196 V_κ sequence).

[0017] In a particular embodiment, the antibody comprises a light chain comprising a V_κ sequence having at least about

95% sequence identity with the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); or SEQ ID NO: 27 (HB22-23 V_κ sequence); or SEQ ID NO: 29 (HB22-33 V_κ sequence).

[0018] In a further embodiment, the antibody comprises a V_κ sequence selected from the group consisting of the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); SEQ ID NO: 27 (HB22-23 V_κ sequence); and SEQ ID NO: 29 (HB22-33 V_κ sequence).

[0019] In a preferred embodiment, the antibody comprises V_H and V_κ sequences selected from the group consisting of amino acids 1 to 97. of SEQ ID NO: 11 (HB22-7 V_H sequence) and the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence) and the amino acid sequence of SEQ ID NO: 27 (HB22-23 V_κ sequence); and amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence) and the amino acid sequence of SEQ ID NO: 29 (HB22-33 V_κ sequence).

[0020] In a different aspect, the invention concerns nucleic acid encoding any of the antibody heavy or light chain variable regions discussed above, or any portion thereof.

[0021] The targeted condition can be any type of B-cell malignancy, including but not limited to localized B-cell malignancies. Typical representatives of B-cell malignancies are B-cell subtype of non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, chronic lymphocytic leukemia, hairy cell leukemia, and prolymphocytic leukemia.

[0022] The treatment method of the present invention may be performed without any further treatment of malignant B cells, including radiation therapy, chemotherapy, combined modality radioimmunotherapy (CMRIT), and the like. The treatment method of the present invention typically provides improved cure rate and/or increased survival and/or superior tumor volume reduction when compared to no treatment, combination treatment with the same antibody and radioimmunotherapy, or with radioimmunotherapy alone.

[0023] The antibody can be a complete antibody, or an antibody fragment, including, for example, Fab, Fab', $F(ab')_2$, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. Thus, the antibody may have an additional antigen specificity, e.g. may be a bispecific antibody. The bispecific antibody may, for example, additionally bind to another epitope to CD22. In addition, the bispecific antibody may have binding specificity for other antigens, such as, CD19, CD20, CD52, CD3, CD28, or HLA-DR10(Lym-1); or for Fc receptors, e.g. CD16, CD64 and CD89.

[0024] The antibody may be chimeric, humanized, primatized, or human.

[0025] The administration of the antibody may be performed by any conventional route, such as intravenous (i.v.) administration by repeated intravenous infusions.

[0026] The response to the treatment may be monitored by methods well known for a skilled practitioner, including monitoring shrinkage of a solid tumor, e.g. by magnetic resonance imaging (MRI)

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] **FIG. 1** shows the amino acid sequence of human CD22 (hCD22), where the boundaries of the Ig-like domains (domains 1-7) are indicated

[0028] **FIG. 2**. Whole body autoradiography of Raji and Ramos tumor-bearing nude mice injected with ^{111}In-21T-BAD-antiCD22 (HB22-7). Mice were sacrificed and autoradiographed 48 hours after injection. Upper image is Raji-tumored mouse, lower image is Ramos-tumored mouse.

[0029] **FIG. 3**. The temporal assessment of tumor volume in Raji-xenografted mice that were untreated or treated with 125 uCi ^{90}Y-DOTA-peptide-Lym-1 (RIT) alone, anti-CD22 alone (HB22-7), or three different sequences of RIT and HB22-7 (CMRIT) in trial 081500. Tumor volume was assessed three times per week. Mouse numbers for each treatment group are tabulated

[0030] **FIG. 4**. Summary analysis of tumor volume observed in all independent xenograft trials. The trials were conducted as described in **FIG. 2**. Mouse numbers for each trail are tabulated (Table 2).

[0031] **FIG. 5**. The response and cure rate for Raji-xenografted mice that were treated as described in **FIG. 2**. The tumor responses were categorized as follows: C, cure (tumor disappeared and did not regrow by the end of the 84-day study); CR, complete regression (tumor at least 7 days but later regrew); PR, partial regression (tumor volume decreased by 50% or more for at least 7 days, then regrew). The data represents results of all independent trials.

[0032] **FIG. 6**. Overall survival was assessed for Raji xenografted mice that were treated as described in **FIG. 2**. Mice were euthanized when the tumor burden exceeded 2000 mg or at the end of the 84 day trial. The data represents results of all independent trials.

[0033] **FIGS. 7*a***, **7*b*** and **7*c***. Hematologic toxicity was assessed by measuring white blood cell (WBC) (**FIG. 7*b***), red blood cell (RBC) (**FIG. 7*c***) and platelet counts (**FIG. 7*a***) twice weekly in the Raji-xenografted mice that were treated as described in **FIG. 2**. When compared to RIT alone there was no difference in hematologic toxicity in the CMRIT groups. In addition, there was no hematologic toxicity observed in the mice treated with HB22-7 alone.

[0034] **FIG. 8**. Non-hematologic toxicity was assessed by measuring body weights twice weekly in Raji xenografted mice that were treated as described in **FIG. 2**. There were no significant differences in body weights in any of the treatment groups in all five xenograft trials.

[0035] **FIG. 9**. RIT clearance was assessed by measuring radioactivity in whole body (WB) and blood daily for 5 days after initiation of treatment with RIT. The results were reported after adjusting for decay based on the $T_{1/2}$ of ^{90}Y. There were no significant differences in RIT clearance in any of the CMRIT treatment groups.

[0036] **FIG. 10**. V_H amino acid sequence analysis of anti-CD22 antibodies (Abs) that block ligand binding. Amino acid numbering and designations of the origins of the coding sequence for each Ab is according to the convention of Kabat et al. (*Sequences of Proteins of Immunological Interest*, U.S. Government Printing Office, Bethesda, Md., 1991), where amino acid positions 1-94, CDR1 and 2, and

FR1, 2, and 3 are encoded by a V_H gene. Sequences that overlap with the 5' PCR primers are not shown. A dot indicates a gap inserted in the sequence to maximize alignment of similar amino acid sequences. Gaps in the sequences were introduced between V_H, D and J segments for clarity. The rank order of sequences shown was based on relatedness to the HB22-5 sequence.

[0037] FIGS. **11-16**. Nucleotide and encoded amino acid sequences for heavy chain V_H-D-J_H junctional sequences for anti-CD22 Abs from hybridomas HB22-5 (SEQ ID NOS: 8 and 9), HB22-7 (SEQ ID NOS: 10 and 11); HB22-13 (SEQ ID NOS: 12 and 13); HB22-23 (SEQ ID NOS: 14 and 15); HB22-33 (SEQ ID NOS: 16 and 17); and HB22-196 (SEQ ID NOS: 18 and 19). Sequences that overlap with the 5' PCR primers are indicated by double underlining. D region sequences are underlined.

[0038] **FIG. 17**. Light chain V_κ amino acid sequence analysis of anti-CD22 Abs that block ligand binding. Amino acid numbering and designation of origins of the coding sequence for each Ab is according to the convention of Kabat et al, supra. The amino acid following the predicted signal sequence cleavage site is numbered 1. A dot indicates a gap inserted in the sequence to maximize alignment of similar amino acid sequences. Gaps in the sequences were introduced between V_κ, J segments and κ constant region (double underlined) sequences for clarity.

[0039] FIGS. **18-23**. Nucleotide and deduced amino acid sequences for kappa light chain V-J-constant region junctional sequences for anti-CD22 Abs from hybridomas HB22-5 (SEQ ID NOS: 20 and 21); HB22-7 (SEQ ID NOS: 22 and 23): HB22-13 (SEQ ID NOS: 24 and 25): HB22-23 (SEQ ID NOS: 26 and 27); HB22-33 (SEQ ID NOS: 28 and 29); and HB22-196 (SEQ ID NOS: 30 and 31). Sequences that overlap with the 5' PCR primers are indicated by double underlining.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0040] A. Definitions

[0041] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0042] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

[0043] The term "immunoglobulin" (Ig) is used to refer to the immunity-conferring portion of the globulin proteins of serum, and to other glycoproteins, which may not occur in nature but have the same functional characteristics. The term "immunoglobulin" or "Ig" specifically includes "antibodies" (Abs). While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Native immunoglobulins are secreted by differentiated B cells termed plasma cells, and immunoglobulins without any antigen specificity are produced at low levels by the lymph system and at increased levels by myelomas. As used herein, the terms "immunoglobulin,""Ig," and grammatical variants thereof are used to include antibodies (as hereinabove defined), and Ig molecules without antigen specificity.

[0044] Native immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

[0045] The main Ig isotypes (classes) found in serum, and the corresponding Ig heavy chains, shown in parentheses, are listed below:

[0046] IgG (γ chain): the principal Ig in serum, the main antibody raised in response to an antigen, this antibody crosses the placenta;

[0047] IgE (ϵ chain): this Ig binds tightly to mast cells and basophils, and when additionally bound to antigen, causes release of histamine and other mediators of immediate hypersensitivity; plays a primary role in allergic reactions, including hay fever, asthma and anaphylaxis; and may serve a protective role against parasites;

[0048] IgA (α chain): this Ig is present in external secretions, such as saliva, tears, mucous, and colostrum;

[0049] IgM (μ chain): the Ig first induced in response to an antigen; it typically has lower affinity than other antibody isotypes produced later and is typically pentameric.

[0050] IgD (δ chain): this Ig is found in relatively high concentrations in umbilical cord blood, may be an early cell receptor for antigen, and is the main lymphocyte cell surface molecule.

[0051] The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies (including, but not limited to, full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

[0052] "Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable (V) domain. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0053] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific,

being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

[0054] The monoclonal antibodies herein specifically include "chireric" antibodies (immunoglobulins), as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Oi et al., *Biotechologies* 4(3):214-221 (1986); and Liu et al., *Proc. Natl. AcadSci. USA* 84:3439-43 (1987)).

[0055] "Humanized" or "CDR grafted" forms of non-human (e.g., murine) antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are also replaced by corresponding non-human residues (so called "back mutations"). Furthermore, humanized antibodies may be modified to comprise residues which are not found in the recipient antibody or in the donor antibody, in order to further improve antibody properties, such as affinity. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature* 321:522-525 (1986); and Reichmann et al., *Nature* 332:323-329 (1988).

[0056] "Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds. Springer-Verlag, N.Y., pp. 269-315 (1994).

[0057] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993).

[0058] The expression "linear antibodies" when used throughout this application refers to the antibodies described in Zapata et al. *Protein Eng.* 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0059] Antibodies of the IgG, IgE, IgA, IgM, and IgD isotypes may have the same variable regions, i.e. the same antigen binding cavities, even though they differ in the constant region of their heavy chains. The constant regions of an immunoglobulin, e.g. antibody are not involved directly in binding the antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity (ADCC).

[0060] Some of the main antibody isotypes (classes) are divided into further sub-classes. IgG has four known sub-classes: IgG1 (γ1), IgG2 (γ2), IgG3 (γ3), and IgG4 (γ4), while IgA has two known sub-classes: IgA1 (α1) and IgA2 (α2).

[0061] The term "epitope" is used to refer to binding sites for (monoclonal or polyclonal) antibodies on protein antigens.

[0062] Antibodies which bind to domain 1 and/or 2 within the amino acid sequence of native sequence human CD22, or to essentially the same epitope(s) bound by any of monoclonal antibodies specifically disclosed herein, such as HB22-7, HB22-23, and HB22-33, can be identified by "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999. According to the gene fragment expression assays, the open reading frame encoding the protein is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of the protein with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein in vitro, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled protein fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. The latter approach is suitable to define linear epitopes of about 5 to 15 amino acids.

[0063] An antibody binds "essentially the same epitope" as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two epitopes bind to identical or sterically overlapping epitopes are competition assays (e.g. competition ELISA assays), which can be configured in all number of different formats, using either labeled antigen or labeled antibody. Usually, the antigen is immobilized on a 96-well plate, and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive or enzyme labels.

[0064] The term amino acid or amino acid residue, as used herein, refers to naturally occurring L amino acids or to D

amino acids as described further below with respect to variants. The commonly used one- and three-letter abbreviations for amino acids are used herein (Bruce Alberts et al., Molecular Biology of the Cell, Garland Publishing, Inc., New York (3d ed. 1994)).

[0065] "Sequence identity" is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a native polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. The % sequence identity values are generated by the NCBI BLAST2.0 software as defined by Altschul et al., (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.,* 25:3389-3402. The parameters are set to default values, with the exception of the Penalty for mismatch, which is set to −1.

[0066] As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. In the context of B cell malignancies, the treatment may reduce the number of malignant cells; reduce the tumor size; inhibit (slow down or stop) the spread of malignant cells, including infiltration into peripheral organs, e.g. soft tissue or bone; inhibit (slow down or stop) metastasis; inhibit tumor growth; provide relief from symptoms associated with a B cell malignancy; reduce mortality; improve quality of life, etc. Treatment with the antibodies herein may result in cytostatic and/or cytotoxic effects.

[0067] The term "B cell malignancy," and grammatical variants thereof, are used in the broadest sense to refer to malignancies or neoplasms of B cells that typically arise in lymphoid tissues, such as bone marrow or lymph nodes, but may also arise in non-lymphoid tissues, such as thyroid, gastrointestinal tract, salivary gland and conjunctiva. The treatment methods of the present invention specifically concern CD22-positive B cell malignancies including, without limitation, B-cell subtype of non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, chronic lymphocytic leukemia, hairy cell leukemia, and prolymphocytic leukemia.

[0068] B. Detailed Description

[0069] 1. Antibodies

[0070] Blocking anti-CD22 monoclonal antibodies designated HB22-7, HB22-23, HB22-33, HB22-5, HB22-13, and HB22-196 are known, and have been disclosed in U.S. Pat. No. 5,484,892, Tuscano et al., *Eur. J Immunol.* 26:1246 (1996), and Tuscano et al, Blood 94(4), 1382-1392 (1999). HB22-7 and HB22-23 are available from the American Type Culture Collection (ATCC), 12302 Parklawn Drive, Rockville, Md. 20852, under Accession Nos. HB22347 and HB 11349, respectively. The preparation of these antibodies is also described in Example 1 below. Epitope mapping of CD22 has shown that these blocking monoclonal antibodies bind to the first two Ig-like domain or to epitopes which are associated with the first two Ig-like domain of human CD22 (U.S. Pat. No. 5,484,892 and Tedder et al., *Annu. Rev. Immunol.* 15:481-504 (1997)). The heavy and light chain variable region sequences of the antibodies are also disclosed in the present application.

[0071] The present invention is based on the unexpectedly superior properties of blocking anti-CD22 antibodies having the overall characteristics of HB22-7, HB22-23, HB22-33, HB22-5, HB22-13, and HB22-196 in the treatment of B-cell malignancies, based on results obtained in a xenograft model of B-cell type non-Hodgkin's lymphoma (NHL).

[0072] The anti-CD22 monoclonal antibodies can be made by any standard method known in the art, such as, for example, by the hybridoma method (Koehler and Milstein, *Nature* 256:495-497 (1975); and Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103, (Academic Press, 1986)), or by recombinant techniques, disclosed, for example, in U.S. Pat. No. 4,816,567, and by Wood et al., *Nature* 314:446-9 (1985).

[0073] It is now also possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g. Jakobovits et al., *Proc. Natl. Acad. Sci. USA* 90, 2551-255 (1993); Jakobovits et al., *Nature* 362, 255-258 (1993). Mendez et al. (*Nature Genetics* 15: 146-156 (1997)) have further improved the technology and have generated a line of transgenic mice designated as "Xenomouse II" that, when challenged with an antigen, generates high affinity fully human antibodies. This was achieved by germ-line integration of megabase human heavy chain and light chain loci into mice with deletion into endogenous J_H segment as described above. The Xenomouse II harbors 1,020 kb of human heavy chain locus containing approximately 66 V_H genes, complete D_H and J_H regions and three different constant regions (μ, δ and χ), and also harbors 800 kb of human κ locus containing 32 V_κ genes, J_κ segments and CK genes. The antibodies produced in these mice closely resemble that seen in humans in all respects, including gene rearrangement, assembly, and repertoire. The human antibodies are preferentially expressed over endogenous antibodies due to deletion in endogenous J_H segment that prevents gene rearrangement in the murine locus.

[0074] Alternatively, the phage display technology (McCafferty et al., *Nature* 348, 552-553 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene rep-

ertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g. Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3, 564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., *Nature* 352, 624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V-genes derived from the spleens of immunized mice. A repertoire of V-genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al, *J. Mol. Biol.* 222, 581-597 (1991), or Griffith et al., *EMBO J.* 12, 725-734 (1993). In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks et al., *Bio/Technol.* 10, 779-783 [1992]). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V-region genes with repertoires of naturally occurring variants (repertoires) of V-domain genes obtained from unimmunized donors. This techniques allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires has been described by Waterhouse et al., *Nucl. Acids Res.* 21, 2265-2266 (1993).

[**0075**] For further information concerning the production of monoclonal antibodies see also Goding, J. W., *Monoclonal Antibodies: Principles and Practice,* 3rd Edition, Academic Press, Inc., London, San Diego, 1996; Liddell and Weeks: *Antibody Technology: A Comprehensive Overview*, Bios Scientific Publishers: Oxford, UK, 1995; Breitling and Dubel: *Recombinant Antibodies*, John Wiley & Sons, New York, 1999; and Phage Display: A Laboratory Manual, Barbas et al, editors, Cold Springs Harbor Laboratory, Cold Spring Harbor, 2001.

[**0076**] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *J. Biochem. Biophys. Methods* 24:107-117 (1992) and Brennan et al., *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al, *Bio/Technology* 10:163-167 (1992)). In another embodiment, the $F(ab')_2$ is formed using the leucine zipper GCN4 to promote assembly of the $F(ab')_2$ molecule. According to another approach, Fv, Fab or $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

[**0077**] Heteroconjugate antibodies, composed of two covalently joined antibodies, are also within the scope of the present invention. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (PCT application publication Nos. WO 91/00360 and WO 92/200373). Heteroconjugate antibodies may be made using any convenient cross-linking methods, using well known, commercially available cross-linking agents.

[**0078**] The antibodies of the present invention, whether rodent, human, or humanized may also have a further antigen-specificity, to form bispecific antibodies. The second binding specificity may be directed, for example, against a further B cell antigen, such as CD19, CD20, CD52, and other CD antigens expressed on B cells, especially antigens associated with the targeted B cell malignancy. For example, CD20 is known to be expressed in more than 90% of non-Hodgkin's lymphomas. An anti-CD20 antibody (Rituxan®, IDEC Pharmaceuticals) is in clinical use for the treatment of non-Hodgkin's lymphoma. CAMPATH-1H (anti-CD52w) is another antibody developed for treating B cell malignancies. Bispecific antibodies including a binding specificity to the CD20 or CD52 antigen are specifically included within the scope herein. Another B cell antigen to which the bispecific antibodies of the present invention can bind is HLA-DR10 (Lym-1), a known marker of non-Hodgkin's lymphoma. Bispecific antibodies can be generated to enhance tumor localization as well as to recruit and/or augment the tumor-specific immune response. Examples of other antigen targets include, CD3, CD28, and the Fc receptors (CD16, CD64 and CD89). Bispecific antibodies are expected to have enhanced cytotoxicity and, as a result, improved remission rate and survival.

[**0079**] Antibodies binding to essentially the same epitope as HB22-7, HB22-23, HB22-33, HB22-5, HB22-13, and/or HB22-196 can be identified by epitope mapping. The simplest way to determine whether two different antibodies recognize the same epitope is a competition binding assay. This method determines if the antibodies are able to block each other's binding to the antigen, and works for both conformational and linear epitopes. The competition binding assay can be configured in a large number of different formats using either labeled antigen or labeled antibody. In the most common version of this assay, the antigen is immobilized on a 96-well plate. The ability of unlabeled antibodies to block the binding of labeled antibodies to the antigen is then measured using radioactive or enzyme labels. For further details see, for example, Wagener et al., *J. Immunol.,* 130:2308-2315 (1983); Wagener et al., *J. Immunol. Methods,* 68:269-274 (1984); Kuroki et al., *Cancer Res.* 50:4872-4879 (1990); Kuroki et al., *Immunol. Invest.* 21:523-538 (1992); Kuroki et al., *Hybridoma* 11:391-407 (1992), and Using Antibodies: A Laboratory Manual, Ed Harlow and David Lane editors, Cold Springs Harbor Laboratory Press, Cold Springs Harbor, N.Y., 1999, pp. 386-389.

[**0080**] Alternatively, or in addition, epitope mapping can be preformed by using a technique based on fragmentation of the antigen to which the antibody binds, either randomly or by specific genetic construction, and determining the

reactivity of the fragments obtained with the antibody. Fragmentation can also be performed on the nucleic acid level, for example by PCR technique, followed by transcription and translation into protein in vitro in the presence of radioactive amino acids. For further details see, for example, Harlow and Lane, supra, pp. 390-392.

[0081] According to a further method of epitope mapping, a set of overlapping peptides is synthesized, each corresponding to a small linear segment of the protein antigen, and arrayed on a solid phase. The panel of peptides is then probed with the test antibody, and bound antibody is detected using an enzyme-labeled secondary antibody. (Harlow and Lane, supra, pp. 393-396.)

[0082] An additional method well known in the art for epitope mapping is antibody selection from random synthetic or phage display peptide library. Phage display libraries are constructed by cloning complex mixtures of peptide-encoding oligonucleotides into the amino terminus of the minor coat protein gene of the fl-type ssDNA phage. Such phage display libraries are commercially available, for example, from New England Biolabs. The libraries are amplified as stocks, and then an aliquot sufficient to represent multiple copies of each independent clone is mixed with the antibody of interest. Antibody-bound phage are collected by a procedure called "biopanning," and unbound phage are removed. The bound phage are eluted and used to infect bacteria, and the selected stock is amplified. Individual plaques of the final selected stock are growth and checked for specific antibody reactivity, e.g. by ELISA, and the DNA around the insert site is sequenced. Analysis of the sequence encoding the peptide to which the antibody binds defined the specificity of the antibody. For further details see, e.g. Smith and Scott, *Methods Enzymol.* 217:228-257 (1993), and Harlow and Lane, supra, pp. 397-398.

[0083] Non-human (rodent) antibodies can be further modified, to make them more suitable for human clinical application. Chimeric antibodies are produced with mouse variable region gene segments of desired specificity spliced into human constant domain gene segments (see, e.g. U.S. Pat. No. 4,816,567).

[0084] Non-human (rodent) antibodies can also be humanized, in order to avoid issues of antigenicity when using the antibodies in human therapy. Generally, a humanized antibody has one or more amino acid residues introduced into it from a non-human source. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature,* 321:522-525 (1986); Riechmann et al., *Nature,* 332:323-327 (1988); Verhoeyen et al., *Science,* 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Despite the relatively straightforward nature of antibody humanization, simple grafting of the rodent CDR's into human frameworks (FR) does not always reconstitute the binding affinity and specificity of the original rodent monoclonal antibody. Properties of a humanized antibody can be improved by suitable design, including, for example, substitution of residues from the rodent antibody into the human framework (backmutations). The positions for such backmutations can be determined by sequence and structural analysis, or by analysis of the variable regions' three-dimensional model. In addition, phage display libraries can be used to vary amino acids at chosen positions within the antibody sequence. The properties of a humanized antibody are also affected by the choice of the human framework. Early experiments used a limited subset of well-characterized human monoclonal antibodies, irrespective of the sequence identity to the rodent monoclonal antibody (the so-called fixed frameworks approach). More recently, some groups use variable regions with high amino acid sequence identity to the rodent variable regions (homology matching or best-fit method). According to another approach, consensus or germline sequences are used, or fragments of the framework sequences within each light or heavy chain variable region are selected from several different human monoclonal antibodies.

[0085] Amino acid variants of antibodies prepared by any technique discussed above or otherwise available can be prepared by introducing appropriate nucleotide changes into the anti-CD22 DNA, or, for example, by peptide synthesis. The amino acid changes also may alter post-translational processes of the humanized or variant anti-CD22 antibody, such as changing the number or position of glycosylation sites.

[0086] Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, *Chem. Immunol.* 65:111-128 (1997); Wright and Morrison, *TibTECH* 15:26-32 (1997)). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., *Mol Immunol.* 32:1311-1318 (1996); Wittwe and Howard, *Biochem.* 29:4175-4180 (1990)), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Jefferis and Lund, supra; Wyss and Wagner, *Current Opin. Biotech.* 7:409-416 (1996)). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. For example, it has been reported that in agalactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-CH2 space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (Malhotra et al., *Nature Med.* 1:237-243 (1995)). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL) (Boyd et al., *Mol. Immunol.* 32:1311-1318 (1996)), while selective removal of sialic acid residues using neuraminidase resulted in no loss of CMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting G1cNAc, was reported to have improved ADCC activity (Umana et al., *Mature Biotech.* 17:176-180 (1999)).

[0087] Glycosylation variants of antibodies can be prepared by modifying the glycosylation sites in the underlying nucleotide sequence. In addition, the glycosylation of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type

used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse et al., *J. Biol. Chem.* 272:9062-9070 (1997)). In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U.S. Pat. Nos. 5,047,335; 5,510,261 and 5,278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g. make defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

[0088] The antibodies of the present invention may also be used by the antibody-directed enzyme prodrug therapy (ADEPT). ADEPT is a technology that utilizes the specificity of monoclonal antibodies targeting tumor antigens to target catalytic enzymes to the surface of cancer cells. There, the enzymes are in position to activate prodrug forms (e.g., a peptidyl chemotherapeutic agent, see WO81/01145) of anti-cancer drugs to their fully active form. See, for example, WO 88/07378 and U.S. Pat. No. 4,975,278.

[0089] Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, e.g., Massey, *Nature* 328:457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

[0090] Immunoconjugates of the antibodies herein are also specifically encompassed by this invention. Immunoconjugates comprise an antibody conjugated to a cytotoxic agent, such as chemotherapeutic agent, a toxin, or a radioisotope.

[0091] Specifically, the efficacy of the anti-CD22 antibodies herein can be further enhanced by conjugation to a cytotoxic radioisotope, to allow targeting a radiotherapy specifically to target sites (radioimmunotherapy). Suitable radioisotopes include, for example, I^{131} and Y^{90}, both used in clinical practice. Other suitable radioisotopes include, without limitation, In^{111}, Cu^{67}, I^{131}, As^{211}, Bi^{212}, Bi^{213}, and Re^{186}.

[0092] Chemotherapeutic agents useful in the generation of immunoconjugates include, for example, include adriamycin, doxorubicin, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxin, taxoids, e.g., paclitaxel (Taxol, Bristol-Myers Squibb Oncology, Princeton, N.J.), and doxetaxel (Taxotere, Rhône-Poulenc Rorer, Antony, Rnace), toxotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), 5-FU, 6-thioguanine, 6-mercaptopurine, actinomycin D, VP-16, chlorambucil, melphalan, and other related nitrogen mustards.

[0093] Toxins to be used in the immunoconjugates herein include, for example, diptheria A chain, exotoxin A chain, ricin A chain, enomycin, and tricothecenes. Specifically included are antibody-maytansinoid and antibody-calicheamicin conjugates. Immunoconjugates contaning maytansinoids are disclosed, for example, in U.S. Pat. Nos. 5,208,020, 5,416,020 and European Patent EP 0 425 235. See also Liu et al., *Proc. Natl. Acad. Sci. USA* 93:8618-8623 (1996). Antibody-calicheamicin conjugates are disclosed, e.g. in U.S. Pat. Nos. 5,712,374; 5,714,586; 5,739,116; 5,767,285; 5,770,701; 5,770,710; 5,773,001; and 5,877,296.

[0094] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science,* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, WO94/11026.

[0095] Covalent modifications of the anti-CD22 antibodies are also included within the scope of this invention. They may be made by chemical synthesis or by enzymatic or chemical cleavage of the antibody, if applicable. Other types of covalent modifications of the antibody are introduced into the molecule by reacting targeted amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues. A preferred type of covalent modification of the antibodies comprises linking the antibodies to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner well known in the art.

[0096] 2. Pharmaceutical Formulations and Treatment Methods

[0097] B-cell type Non-Hodgkin's Lymphoma is a term that is used to encompass a large group (over 29 types) of

lymphomas caused by malignant (cancerous) B cell lymphocytes, and represents a large subset of the known types of lymphoma. B-cells are known to undergo many changes in their life cycle dependent on complex intracellular signaling processes, and apparently different types of B-cell malignancies can occur at different stages of the life cycle of B-cells. At the stem cell stage, acute lymphocytic leukemia (ALL) or lymphoblastic lymphoma/leukemia can typically develop. Precursor B-cells can develop precursor B lymphoblastic lymphoma/leukemia. Typical malignancies of immature B-cells include small non-cleaved cell lymphoma and possibly Burkitt's/non-Burkitt's lymphoma. B cells before antigen exposure typically develop chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma, while after antigen exposure typically follicular lymphomas, large cell lymphoma and immunoblastic lymphoma are observed. There are also classification systems that characterize B-cell lymphomas by the rate of growth distinguishing aggressive (fast growing) and indolent (slow growing) lymphomas. For example, Burkitt's/non-Burkitt's lymphoma and LCL lymphoma belong in the aggressive group, while indolent lymphomas include follicular center cell lymphomas (FCCL), follicular large cell lymphomas, and follicular small cleaved cell lymphomas.

[0098] Non-Hodgkin's Lymphomas are also characterized by the stage of development. Stage I: cancer is found in only one lymph node area, or in only one area or organ outside the lymph nodes. Stage II: (1) Cancer is found in two or more lymph node areas on the same side of the diaphragm (the thin muscle under the lungs that helps breathing), or, (2) cancer is found in only one area or organ outside the lymph nodes and in the lymph nodes around it, or (3) other lymph node areas on the same side of the diaphragm may also have cancer. Stage III: Cancer is found in lymph node areas on both sides of the diaphragm. The cancer may also have spread to an area or organ near the lymph node areas and/or to the spleen. Stage IV: (1) Cancer has spread to more than one organ or organs outside the lymph system; cancer cells may or may not be found in the lymph nodes near these organs, or (2) cancer has spread to only one organ outside the lymph system, but lymph nodes far away from that organ are involved.

[0099] Current treatment options of B-cell malignancies, including non-Hodgkin's lymphomas depend on the type and stage of malignancy. Typical treatment regimens include radiation therapy, also referred to as external beam therapy, chemotherapy, immunotherapy, and combinations of these approaches. One promising approach is radioimmunotherapy (RIT). With external beam therapy, a limited area of the body is irradiated. With chemotherapy, the treatment is systemic, and often adversely affects normal cells, causing severe toxic side-effects. Targeted RIT is an approach in which a B-cell specific antibody delivers a toxic substance to the site of tumor. The therapeutic potential of RIT in patients with B-cell NHL has been shown using different targets, including CD20, CD19, CD22, and HLA-DR10 (Lym-1). More recently, combined modality therapy (CMT) has become an increasingly frequent maneuver for the treatment of solid tumors, and includes radiosensitization of cancer cells by drugs, and the direct cytotoxic effect of chemotherapy. The most common chemotherapy regiment for treating NHL is Cyclophosphamide-Hydroxydoxorubicin-Oncovin (vincristine)-Prednisone (CHOP) combination therapy. A randomized study of aggressive, but early stage NHL showed superior results with CHOP plus involved field radiation over treatment with CHOP alone. Despite its promise, the disadvantage of treatments involving external beam radiation is that external beam radiation can only be delivered in high doses to a limited region of the body, while NHL is mostly widespread. Accordingly, CMT has proven clinically useful for locally advanced malignancies.

[0100] Another current approach is combined modality radioimmunotherapy (CMRIT), which pairs the specific delivery of systemic radiation (e.g. ^{90}Y-DOTA-peptide-Lym-1) to NHL with the systemic radiation sensitizing effects of an additional chemotherapeutic agent. Because in CMRIT radiation is delivered continuously, cancer cells that are hypoxic may re-oxygenate, or pass through the radiosensitive G_2/M phase of the cell cycle during the course of treatment, making cure more likely. In addition, CMRIT provides specificity first, by the specific targeting of NHL by Lym-1, and second by timing. This allows the radiation sensitizer to potentially synergize only at the sites targeted by RIT, thus maximizing efficacy and minimizing toxicity. Several previous xenograft studies have demonstrated improved synergy when the radiation synthesizer (Taxol) was given 24-48 hours after RIT.

[0101] Although CMRIT is currently viewed as the most advanced therapeutic approach for the treatment of NHL, the antibodies of the present invention alone have been demonstrated to provide superior results both in terms of tumor volume reduction, cure rate and overall survival, when tested in the well accepted Raji and Ramos lymphoma xenograft models.

[0102] The anti-CD22 antibodies herein are typically administered in the form of pharmaceutical formulations well known to all pharmaceutical chemists. See, e.g. *Remington's Pharmaceutical Sciences,* (15th Edition, Mack Publishing Company, Easton, Pa. (1975)), particularly Chapter 87, by Blaug, Seymour. These formulations include for example, powders, pastes, ointments, jelly, waxes, oils, lipids, anhydrous absorption bases, oil-in-water or water-in-oil emulsions, emulsions carbowax (polyethylene glycols of a variety of molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. A typical dosage form is a sterile, isotonic, water-based solution suitable for administration by the intravenous (i.v.) route. The concentration of the antibodies of the invention in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

[0103] The compositions of the invention may also be administered via liposomes. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the composition of the invention to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a desired target, such as antibody, or with other therapeutic or immunogenic compositions. Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size, acid

lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al. *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

[0104] The antibodies of the present invention can be administered alone or in combination with other therapeutic regimens, including chemotherapy, radioimmunotherapy. (RIT), chemotherapy and external beam radiation (combined modality therapy, CMT), combined modality radioimmunotherapy (CMRIT), or cytokines alone or in combination, etc. Thus, the anti-CD22 antibodies of the present invention can be combined with CHOP (Cyclophosphamide-Hydroxydoxorubicin-Oncovin (vincristine)-Prednisolone), the most common chemotherapy regimen for treating non-Hodgkin's lymphoma. In addition, the anti-CD22 antibodies herein may be administered in combination with other antibodies, including anti-CD19, anti-CD20 and other anti-CD22 antibodies, such as LymphoCide™ (Immunomedics, Inc.) or LymphoCide Y-90. See, for example, Stein et al, *Drugs of the Future* 18:997-1004 (1993); Behr et al., *Clinical Cancer Research* 5:3304s-33314s, 1999 (suppl.); Juweid et al., *Cancer Res.* 55:5899s-5907s, 1995; Behr et al., *Tumor Targeting* 3:32-40 (1998), and U.S. Pat. Nos. 6,183,744, 6,187,287, and 6,254,868.

[0105] The patients to be treated in accordance with the present invention will have CD22 expressed on their malignant B cells. The presence of the CD22 antigen can be confirmed by standard techniques, such as immunohistochemistry, FACS, binding assay with labeled (e.g. radiolabeled) anti-CD22 antibody.

[0106] The preferred route of administration is via bolus or continuous infusion over a period of time, such as continuous or bolus infusion, once or twice a week. Another preferred route is subcutaneous injection. The dosage depends on the nature, form, and stage of the targeted B cell malignancy, the patients sex, age, condition, prior treatment history, other anti-cancer treatments used (including, e.g. radiation, chemotherapy, immunotherapy, etc.) and other factors typically considered by a skilled physician. For example, non-Hodgkin's lymphoma patients may receive from about 50 to about 1500 mg/m^2/week, specifically from about 100 to about 1000 mg/m^2/week, more specifically from about 150 to about 500 mg/m^2/week of an anti-CD22 antibody herein.

[0107] The patients will be monitored by standard techniques, such as by monitoring tumor regression, e.g. tumor size in the case of solid tumors, the phenotype of circulating B-cells or of biopsied tissues using anti-CD22 antibodies.

[0108] While the invention has been discussed with reference to human therapy, it will be understood that the antibodies of the present invention also find use in veterinary medicine. For example, feline malignant lymphoma occurs frequently in domestic cats, and shows similar characteristics to human non-Hodgkin's lymphoma (Bertone et al., *Am. J. Epidemiol.* 156:268-73 (2002)). Similarly, dogs are known to develop a variety of lymphomas. Accordingly, the antibodies herein can be used to treat feline and canine malignant lymphoma. Dosages, and routes of administration depend on the animal species to be treated, and their determination is well within the skill of a veterinary of ordinary skill.

[0109] Further details of the invention are provided in the following non-limiting examples.

EXAMPLES

[0110] Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. In addition to production as disclosed in the following examples, hybridoma producing monoclonal antibody HB22-7 (ATCC Accession No. HB 11349) may be obtained from the American Type Culture Collection, Rockville, Md.

Example 1

Production of Anti-CD22 Monoclonal Antibodies

[0111] Monoclonal antibodies (mAbs) HB22-7 (IgG2b), HB22-23 (IgG2a) HB22-33 (IgM), HB22-5 (IgG2a), HB22-13 (IgG2a), HB22-22 (IgA), and HB22-196 were produced according to the method of Engel et al., *J Immunol* 15:4710 (1993) and U.S. Pat. No. 5,484,892. See, also Tuscano et al, *Blood* 94:1382-1392 (1999). However, other methods may be used. Briefly, the HB22 mAbs were produced via hybridoma techniques using a mouse pre-B cell line 300.19, stably transfected with full length CD22 cDNA, as the immunogen. More specifically, thirty-three mAbs reactive with CD22 were generated by the fusion of NS-1 myeloma cell with spleen cells from Balb/c mice immunized three times with a mouse pre-B cell line, 300.19, stably transfected with a full-length CD22 cDNA. Hybridomas producing mAb reactive with mouse L cells transfected with CD22 cDNA, but not with untransfected cells, were cloned twice and used to generate supernatant or ascites fluid. mAb isotypes were determined using the Mouse Monoclonal Antibody Isotyping Kit (Amersham, Arlington Heights, Ill.). IgGmAb were purified using the Affi-Gel Protein A MAPS II Kit (Bio-Rad, Richmond, Calif.). The HB22-33 mAb (IgM) containing euglobulin fraction of ascites fluid was precipitated by extensive dialysis against distilled water and was shown to be essentially pure mAb by SDS-PAGE analysis. As disclosed in Table II of U.S. Pat. No. 5,484,892, mAbs HB22-7, HB22-22, HB22-23, and HB22-33 completely blocked (80-100%) the binding of Daudi, Raji and Jurkat cells to CD22 transfected COS cells. mAbs HB22-5, HB22-13, HB22-24, and HB22-28 partially blocked adhesion (20-80%).

[0112] The region(s) on CD22 that mediates ligand binding was characterized by mAb cross-inhibition studies using the "Workshop" CD22-blocking mAb and a panel of mAb that identify five different epitopes on CD22 (epitopes A, B, C, D, and E (Schwartz-Albiez et al., "The carbohydrate moiety of the CD22 antigen can be modulated by inhibitors of the glycosylation pathway." The binding specificities of the Workshop mAb are depicted pictorially in **FIG. 3**. In Leukocyte Typing IV. White Cell Differentiation Antigens, Knapp et al., eds., Oxford University Press, Oxford, p. 65 (1989)). It has been found that three of the monoclonal antibodies herein, HB22-7, HB22-22, and HB22-23, bind to very close or the same epitopes on CD22. Results of the epitope-mapping of these and other blocking antibodies are disclosed in Tedder et al., *Annu. Rev. Immunol* 15:481-504 (1997). Unlike other anti-CD22 antibodies proposed for therapy, the blocking antibodies of the present invention bind to an epitope within the first two Ig-like domains of the hCD22 amino acid sequence.

Example 2

Raji and Ramos Lymphoma Xenograft Trials

[0113] This example describes the results from our independent Raji and Ramos lymphoma xenograft trials. Nude mice xenografts are important tools for preclinical evaluations. Nude mice bearing human non-Hodgkin's lymphoma (NHL) xenografts utilizing the lymphoma cell lines Raji and Ramos have proven utility for evaluating efficacy for treatment of NHL (Buchsbaum et al., *Cancer Res.* 52(23):6476-6481 (1992) and Flavell et al., *Cancer Res.* 57:4824-4829 (1997)).

[0114] Materials and Methods

[0115] Reagents. Carrier-free ^{90}Y (Pacific Northwest National Laboratory, Richland, WA) and ^{111}In (Nordion, Kanata, Ontario, Canada) were purchased as chlorides in dilute HCl. Lym-1 (Techniclone, Inc Tustin, Calif.) is an IgG_{2a} mAb generated in mice immunized with human Burkitt's lymphoma cell nuclei. Lym-1 recognizes a cell surface 31-35 kD antigen on malignant B cells, and reacts with greater than 80% of human B cell NHL. Lym-1 purity was assessed according to the specifications that required greater than 95% pure monomeric IgG by polyacrylamide gel electrophoresis. ^{90}Y-DOTA-peptide-Lym-1 was prepared as previously described (O'Donnell et al., *Cancer. Biother. Radiopharm.* 13:251-361 (1998)). Assessment by HPLC, TLC, and cellulose acetate electrophore.sis revealed that ^{90}Y-DOTA-peptide-Lym-1 was prepared to 98% radiochemical purity with less than 5% aggregate content.

[0116] The anti-CD22 mAb, HB22-7, was prepared as previously described (Tuscano et al., *Blood* 94:1382-1392 (1999)), using a Protein A Sepharose Fast Flow column (Pharmacia). HB22-7 purity was determined by HPLC and flow cytometry, and found to be >95% pure. Physiologic properties were determined by flow cytometric-based analysis of apoptotic induction (Apo-Tag, Pharmacia) and found to be consistent with previous published results (Tuscano et al, supra). Endotoxin removal was achieved using an Acti-Clean ETOX column (Sterogene), with final endotoxin levels determined to be <0.15 Endotoxin Units (EU)/mg mAb (Bio Whitaker). The Lym-1 and HB22-7 mAbs met MAP (mouse antibody production) guidelines for murine, viral, mycoplasma, fungal, and bacterial contamination, as well as endotoxin, pyrogen and DNA content and general safety testing in animals.

[0117] Cell lines and Scatchard Analysis. Raji and Ramos Burkitt lymphoma cell lines were purchased from American Type Culture Collection (ATCC, Gathersberg, Md.). Both cell lines stained for CD22 expression by flow cytometric methods utilizing the HB22-7 mAb, as described previously (Tuscano et al., supra). The cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum at 0.5×10^6 cells/ml. A Scatchard analysis using Raji and Ramos cells was performed as described previously (Scatchard, G., *Ann. of NY Acad Sci.* 51:660 (1947)). Briefly, HB22-7 was labeled with ^{125}I by the chloramine T method (specific activity of 1.1 μCi/μg). A competitive binding assay was performed utilizing serially diluted, unlabeled HB22-7.

[0118] Mouse studies. Female athymic BALB/c nu/nu mice (Harlan Sprague-Dawley), 7-9 weeks of age were maintained according to University of California, Davis animal care guidelines on a normal diet ad libitum and under pathogen-free conditions. Five mice were housed per cage. Raji or Ramos cells were harvested in logarithmic growth phase; $2.5\text{-}5.0\times10^6$ cells were injected subcutaneously into both sides of the abdomen of each mouse. Studies were initiated 3 weeks after implantation, when tumors were 28-328 mm^3. Groups consisted of untreated, 125 μCi of RIT alone, 1.4 mg of HB22-7 alone, or the combination of RIT and HB22-7, with HB22-7 being administered 24 hours prior, simultaneously, or 24 hours after RIT. To minimize ambient radiation, bedding was changed daily for 1 week after treatment with ^{90}Y-DOTA-peptide-Lym-1, and twice weekly thereafter.

[0119] Tumoricidal Effect. Tumor volume was calculated as described by the formula for hemiellipsoids (DeNardo et al., *Clin. Cancer Res.* 3:71-79 (1997)). Initial tumor volume was defined as the volume on the day prior to treatment. Mean tumor volume was calculated for each group on each day of measurement; tumors that had completely regressed were considered to have a volume of zero. Tumor responses were categorized as follows: C, cure (tumor disappeared and did not regrow by the end of the 84 day study); CR, complete regression (tumor disappeared for at least 7 days, but later regrew); PR, partial regression (tumor volume decreased by 50% or more for at least 7 days, then regrew).

[0120] Statistical Analysis. Differences in response among treatment groups were evaluated using the Kruskall Walis rank sum test with the response ordered as none, PR, CR, and Cure. Survival time was also evaluated using the Kruskall Walis test. Tumor volume was compared at 3 time points: month 1 (day 26-29), month 2 (day 54-57), and at the end of the study (day 84). If an animal was sacrificed due to tumor-related causes, the last volume was carried forward and used in the analysis of later time points. Analysis of variance was used to test for differences among treatment groups. P values are two-tailed and represent the nominal p-values. Protection for multiple comparisons is provided by testing only within subsets of groups found to be statistically significantly different.

[0121] Results

[0122] Scatchard Analysis

[0123] Scatchard analysis was utilized to assess the binding affinity of HB22-7 and the number of CD22 receptors on Ramos and Raji cells. The cells were assayed for maximum binding percentage (Bmax), disassociation constant (Ka) and number of antibodies bound per cell. The results shown in Table 1 are the average of two experiments.

TABLE 1

I. PARAMETER	Cell Lines	
Cell line	Raji	Ramos
Bmax	53.5 ± 0.9%	21.0 ± 1.3%
R^2	0.954	0.926
Ka	$1.3 \pm 0.08 \times 10^9$	$5.95 \pm 1.0 \times 10^8$
Antibody/cell	118,000	43,000

[0124] The Scatchard analysis (Table 1) revealed a nearly 2.5 fold increase in the number of HB22-7 antibodies bound per cell, and Bmax, and a 2 fold increase in Ka for Raji cells versus Ramos cells, respectively.

[0125] Whole Body Autoradiography

[0126] In order to assess HB22-7-specific tumor targeting, whole body autoradiography of tumor-bearing nude mice injected with ^{111}In-2IT-BAD-anti-CD22 (HB22-7) was performed. Forty eight hours after injection mice were sacrificed, sectioned and autoradiographed (**FIG. 2**), as previously described (DeNardo et al., *Cancer* 3:71-79 (1997)). Autoradiography revealed intense tumor localization in the Raji-tumored mice and moderate localization in the Ramos-tumored mice. This targeting study is consistent with the Scatchard analysis that revealed less HB22-7 bound per Ramos cells as compared to Raji. However the rapid growth of Ramos tumors, and likely central necrosis, may also contribute to the apparent inferior targeting of Ramos.

[0127] Efficacy of RIT and CMRIT

[0128] The initial trial (081500) utilized 125 uCi of ^{90}Y-DOTA-peptide-Lym-1 alone or in combination with HB22-7 (1.4 mg) given either 24 hours prior, simultaneously, or 24 hours after RIT, (**FIG. 3**). In this trial there were 5 mice per group with the exception of the group treated with RIT alone, which had 9 mice and 5 untreated controls (mouse numbers are tabulated in Table 2).

TABLE 2

	Treatment Groups					
Trial	No Tx	HB22-7	RIT	−24	@RIT	+24
081500	5	4	9	5	5	5
101600	5	6	5	5	3	5
011601	—	5	4	—	9	7
032701	—	5	2	—	3	12
052401	3	—	3	—	—	—
060401	5	5	—	—	—	—
071701	7	5	—	—	—	4
092101	4	—	—	—	—	—
102401	13	—	—	—	—	—
Total	42	30	23	10	20	33

[0129] As predicted from similar Raji xenograft studies with ^{90}Y-2IT-BAD-Lym-1, RIT alone resulted in maximal mean tumor volume reduction by day 21, with increasing tumor volume thereafter. Xenografts treated with ^{90}Y-2IT-BAD-Lym-1 (RIT) and HB22-7 (CMRIT) demonstrated greater and more sustained mean tumor volume reduction, which was greatest when HB22-7 was administered simultaneously, and 24 hours after RIT. Surprisingly, HB22-7 administered alone resulted in stabilization of mean tumor volume by 2-3 weeks, then a gradual and sustained tumor volume reduction.

[0130] Several additional replicate trials were conducted with highly reproducible results (Table 2). The data from all trials were compiled and, when compared graphically, revealed results highly consistent with the initial study, (**FIG. 4**). The initial tumor volume reductions were again greatest at approximately day 21 when HB22-7 was administered simultaneously and 24 hours after RIT. In mice treated with HB22-7 alone, the stabilization in tumor growth that began 2 weeks after treatment followed by gradual sustained tumor volume reduction was also replicated in all subsequent trials. Using analysis of variance, when examining all treatment groups at day 30 the differences were highly significant ($p<0.001$). While analysis of volume reduction in all treatment groups at day 60 did not demonstrate significant differences ($p=0.39$), the differences at day 84 again were significant ($p=0.003$). The results observed graphically revealed that the difference in volume reduction in the RIT/CMRIT groups was highly reproducible and different from HB22-7 alone and untreated control, however, comparison of volume reduction only in only RIT treatment groups (including CMRIT) at all time points assessed (day 30, 60, and 84) did not reveal significant differences ($p\geqq0.5$). Additional CMRIT trials were done with HB22-7 being administered 48 and 72 hours after RIT. The extended interval between the administration of RIT and HB22-7 did not result in improved tumor volume reduction when compared to trials in which HB22-7 was given simultaneously and 24 hours after RIT (data not shown).

[0131] Response and cure rates were consistent with the effects of treatment on tumor volume, (**FIG. 5**). Treatment with ^{90}Y-DOTA-peptide-Lym-1 alone produced 48% PR, 13% CR, and a 13% cure rate. In the CMRIT groups, the overall response rate was maximized when HB22-7 and RIT were administered simultaneously generating 45% PR, 15% CR and 25% cure. However in the CMRIT groups the cure rate was the greatest (39%) when HB22-7 was administered 24 hours after RIT, which compared favorably to the cure rates observed in the untreated (29%), RIT alone (13%), 24 hours prior (10%) and simultaneous (25%) treatment groups. When examining the degree of response (ranking cure better than CR, better than PR) in all treatment groups using the Kruskal Walis test, the differences were statistically significant ($p=0.01$). Individual comparisons against untreated controls were all statistically significant ($p<0.05$), with the exception of RIT alone ($p=0.06$) and HB22-7 given 24 hours prior to RIT ($p=0.16$). While comparison of only active treatment groups (RIT alone, CMRIT, and HB22-7) was not significantly different ($p=0.18$), the CMRIT groups treated with HB22-7 simultaneously and after 24 hours had the best observed pattern of response. Interestingly the group treated with HB22-7 alone had the highest cure rate (47%) which was a significant improvement when compared to the untreated controls ($p<0.05$).

[0132] Tumor volume regression and cure rates translated into a similar pattern of survival. At the end of the 84 day study period 38 and 42% of the untreated and RIT alone groups were alive respectively, (**FIG. 6**). In the CMRIT treatment groups, survival increased to 67 and 50% when HB22-7 was administered simultaneously and 24 hours after RIT, respectively. Analysis of survival using Kruskal Walis was significant ($p<0.05$) for comparison of all groups. Similar to the response rate analysis, comparison of survival in the RIT groups only did not reveal significant differences ($p=0.41$), however the best survival in these groups was consistently observed when HB22-7 was administered either simultaneous or 24 hours after RIT.

[0133] The best overall survival, 76%, was observed in the group treated with HB22-7 alone, a significant difference when compared to untreated control ($p=0.02$).

[0134] Toxicity

[0135] Hematologic and non-hematologic toxicities were assessed by blood counts and mouse weights, respectively (**FIG. 7*a-c***). WBC and platelet nadirs in the RIT treatment

groups were at 14-20, and 10-14 days respectively. WBC and platelet recovery was approximately 28 and 21 days after treatment, respectively. The WBC and platelet nadirs were consistent with observations in previous studies that utilized 150 uCi of ^{90}Y-2IT-BAD-Lym-1. The hematologic toxicity of RIT was not altered by co-administration of HB22-7. No hematologic toxicity was detected in mice treated with HB22-7 alone. Analysis of mononuclear cell counts in all treatment groups revealed that HB22-7 had no effect on RIT-mediated mononuclear cell nadirs (data not shown). Non-hematologic toxicity as assessed by changes in mouse weight, and was found to be equivalent in all treatment groups (**FIG. 8**). There were no deaths due to toxicity in any treatment groups.

[**0136**] ^{90}Y-DOTA-peptide-Lym-1 Pharmacokinetics

[**0137**] Blood and whole body clearances of ^{90}Y-DOTA-peptide-Lym-1 in Raji-tumored mice with or without HB22-7 were similar (**FIG. 9**). The blood biological $T_{1/2}\alpha$ was 1.4 hours for RIT alone, and 2.2, 2.4, and 2.0 hours for the 24 hour prior, simultaneous and 24 hour after groups respectively. The blood biological $T_{1/2}\beta$ was 127 hours for the RIT alone group and 133, 87, and 103 hours for the 24 hours prior, simultaneous and 24 hours after groups respectively. The whole body $T_{1/2}$ was 246 hours for RIT alone and 207, 207, and 196 hours for the 24 hours prior, simultaneous and 24 hours after groups respectively. The addition of HB22-7 to RIT did not change the pharmacokinetics of ^{90}Y-DOTA-peptide-Lym-1.

[**0138**] Discussion

[**0139**] Raji xenograft studies were designed to determine if the anti-CD22 mAb (HB22-7) would generate additive or synergistic effects when combined with RIT to enhance apoptosis and/or DNA damage induced by low dose-rate radiation. The Raji xenograft nude mouse model has proven useful when used to assess toxicity and efficacy of RIT using ^{90}Y-2IT-BAD-Lym-1 RIT alone (O'donnell et al., *Cancer Biotherapy and Radiopharmaceuticals* 13:351-361 (1998)). Responses in this pre-clinical model translated into significant efficacy in human clinical trials (O'Donnell et al., *Anticancer Res.* 20:3647-55 (2000); O'Donnell et al., *J. Nucl. Med.* 40:216 (1999) (Abstract)).

[**0140**] In the studies described in this Example, the addition of the anti-CD22 mAb HB22-7 to ^{90}Y-DOTA-peptide-Lym-1(125 uCi) enhanced the efficacy of RIT without any change in toxicity. Previous Raji xenograft studies with 150 and 200 μCi of ^{90}Y-2IT-BAD-Lym-1 generated response and cure rates that were comparable to those observed in the present study (O'Donnel et al., (1998), supra). The 125 μCi dose of ^{90}Y-DOTA-peptide-Lym-1 was chosen based on these previous studies with the 2IT-BAD linker. While the previous studies with 2IT-BAD demonstrated greatest efficacy with the 200 μCi dose, the choice of 125 μCi was based on the hypothesis that HB22-7 would be synergistic or additive with RIT and the lower dose would allow for better assessment of these effects. The studies of this Example utilized a novel linker (DOTA-peptide) that has not been previously examined in lymphoma xenograft models. The DOTA-peptide linker was designed for enhanced hepatic degradation of unbound radiopharmaceutical thereby leading to a more favorable biodistribution. While tumor-specific uptake was not assessed in detail in this study, the toxicity profile observed with 125 uCi of ^{90}Y-DOTA-peptide-Lym-1 alone was acceptable with no treatment-related mortality and predictable leukocyte and platelet nadirs.

[**0141**] HB22-7 was chosen based on in vitro studies demonstrating pro-apoptotic and signaling effects (Tuscano et al,. *Blood* 94:1382-1392 (1999)). The treatment dose of HB22-7 utilized was empiric, however, it was based on the amount that was shown to be effective at inducing apoptosis in vitro and extrapolating this to the mouse model. In addition, when formulating the dose of HB22-7 consideration was given to the equivalent (when adjusted for body surface area differences in humans versus mice) dose of Rituximab® used in human clinical trials. The approximation to the Rituximab® dose was utilized based on the fact that this is the only naked mAb available that has demonstrated efficacy for the treatment of lymphoma, granted the optimal dose of Rituximab® is currently undefined.

[**0142**] The study was designed to assess the efficacy of HB22-7 alone, the combination of RIT and HB22-7 as well as the effect of three different sequence combinations. The tumor volume reduction observed with ^{90}Y-DOTA-peptide-Lym-1 alone was consistent with previous studies with ^{90}Y-21T-BAD-Lym-1 in terms of timing, magnitude, and duration of response (O'Donnel et al., 1998, supra). RIT alone resulted in approximately 50% reduction in tumor volume 14 days after therapy. When assessing at the approximate point of maximal volume reduction (day 21-30) the addition of HB22-7 to RIT significantly enhanced the magnitude of response in a sequence specific manner. It appears that the addition of HB22-7 was most effective when administered simultaneously or 24 hours after RIT. The distinctive pattern of volume reduction was highly reproducible. Independent replicate trials demonstrated similar patterns and magnitude of tumor volume reduction. The improved reductions in tumor volume translated into superior response rates and survival. RIT alone generated 13% CR and 13% cures, the addition of HB22-7 increased the cure rate to 25% when administered simultaneously with RIT, and to 39% when HB22-7 was administered 24 hours after RIT.

[**0143**] This is the first time that a second monoclonal antibody has been combined with RIT, and demonstrates the potential of utilizing monoclonal antibodies or other agents with well defined physiologic properties that may augment efficacy without increasing toxicity.

[**0144**] Surprisingly the mice treated with HB22-7 alone had impressive tumor volume reduction and superior cure and survival rates when compared to all other treatment groups. Again, several independent trials generated highly consistent results with a delayed initial tumor volume stabilization, and then tumor volume reduction beginning approximately 14 days after treatment. This translated into the best cure and overall survival rates observed in any of the treatment groups.

[**0145**] In conclusion, the antibodies of the present invention, when administered alone, have been demonstrated to provide superior results in terms of tumor volume reduction, cure rate and overall survival when compared to other treatment regimens, including CMRIT, which is currently viewed as the most advanced therapeutic approach for the treatment of NHL.

Example 3

Sequence Analysis of anti-CD22 Antibodies

[0146] V_H and Light Chain Gene Utilization

[0147] Cytoplasmic RNA was extracted from 1-10×10^5 hybridoma cells using the RNeasy Mini Kit (Qiagen Chatsworth, Calif.). First strand cDNA was synthesized from cytoplasmic RNA using oligo-dT primers (dT_{18}) and a Superscript Kit (Gibco BRL, Gaithersburg, Md.). One μl of cDNA solution was used as template for PCR amplification of V_H genes. PCR reactions were carried out in a 100-μl volume of a reaction mixture composed of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTP (Perkin Elmer, Foster City, Calif.), 50 pmol of each primer, and 5 U of Taq polymerase (ISC Bioexpress, Kaysville, Utah). Amplification was for 30 cycles (94° C. for min, 58° for 1 min, 72° C. for 1 min; Thermocycler, Perkin Elmer). V_H genes were amplified using a promiscuous sense 5' V_H primer (Ms V_HE: 5' GGG AAT TCG AGG TGC AGC TGC AGG AGT CTG G 3'; SEQ ID NO: 2) as previously described (Kantor et al., *J. Immunol.* 158:1175-86 (1996)), and antisense primers complementary to the Cμ coding region (primer Cμ-in: 5' GAG GGG GAC ATT TGG GAA GGA CTG 3'; SEQ ID NO: 3) or the Cγ region (Primer Cγ1: 5' GAG TTC CAG GTC ACT GTC ACT GGC 3'; SEQ ID NO: 4).

[0148] Light chain cDNA was amplified using a sense V_κ primer [5' ATG GGC (AT)TC AAG ATG GAG TCA CA(GT) (AT)(CT)(CT) C(AT)G G 3'; SEQ ID NO: 5] and a Cγ antisense primer (5' ACT GGA TGG TGG GAA GAT G 3'; SEQ ID NO: 6).

[0149] HB22-33 light chain sequences were amplified using a different sense $V_\kappa$primer (5' ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG 3'; SEQ ID NO: 7).

[0150] Amplified PCR products were purified from agarose gels using the QIAquick gel purification kit (Qiagen) and were sequenced directly in both directions using an ABI 377 PRISM DNA sequencer after amplification using the Perkin Elmer Dye Terminator Sequencing system with AmpliTaq DNA polymerase and the same primers for initial PCR amplification. All V_H and light chain regions were sequenced completely on both the sense and anti-sense DNA strands.

[0151] The alignment of the V_H and V_κ amino amino acid sequences for anti-CD22 monoclonal antibodies HB22-5, HB22-7, HB22-13, HB22-23, HB22-33, and HB22-196 are shown in **FIGS. 10 and 17**, respectively. FIGS. **11-16** show the nucleotide and amino acid sequences for heavy chain V_H-D-J_H junctions of anti-CD22 Abs from hybridomas HB22-5 (SEQ ID NOS: 8 and 9), HB22-7 (SEQ ID NOS: 10 and 11); HB-22-13 (SEQ ID NOS: 12 and 13); HB-22-23 (SEQ ID NOS: 14 and 15); HB-22-33 (SEQ ID NOS: 16 and 17); and HB-22-196 (SEQ ID NOS: 18 and 19). FIGS. **18-23** show the nucleotide and deduced amino acid sequences for kappa light chain V-J-constant region junctions of anti-CD22 Abs from hybridomas HB22-5 (SEQ ID NOS: 20 and 21); HB22-7 (SEQ ID NOS: 22 and 23); HB22-13 (SEQ ID NOS: 24 and 25) HB22-23 (SEQ ID NOS: 26 and 27); HB22-33 (SEQ. ID NOS: 28 and 29); and HB22-196 (SEQ ID NOS: 30 and 31).

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 31
<210> SEQ ID NO 1
<211> LENGTH: 847
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 1

```
Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Val Leu Glu Tyr Leu
 1               5                  10                  15

Ala Phe Ser Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu Thr Leu
            20                  25                  30

Tyr Ala Trp Glu Gly Ala Cys Val Trp Ile Pro Cys Thr Tyr Arg Ala
        35                  40                  45

Leu Asp Gly Asp Leu Glu Ser Phe Ile Leu Phe His Asn Pro Glu Tyr
    50                  55                  60

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

Lys Asp Gly Lys Val Pro Ser Glu Gln Lys Arg Val Gln Phe Leu Gly
                85                  90                  95

Asp Lys Asn Lys Asn Cys Thr Leu Ser Ile His Pro Val His Leu Asn
            100                 105                 110

Asp Ser Gly Gln Leu Gly Leu Arg Met Glu Ser Lys Thr Glu Lys Trp
        115                 120                 125
```

-continued

```
Met Glu Arg Ile His Leu Asn Val Ser Glu Arg Pro Phe Pro Pro His
    130                 135                 140

Ile Gln Leu Pro Pro Glu Ile Gln Glu Ser Gln Glu Val Thr Leu Thr
145                 150                 155                 160

Cys Leu Leu Asn Phe Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp
                165                 170                 175

Leu Leu Glu Gly Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser
            180                 185                 190

Leu Thr Ile Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro
        195                 200                 205

Gln Trp Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala
    210                 215                 220

Asp Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His
225                 230                 235                 240

Thr Pro Lys Leu Glu Ile Lys Val Thr Pro Ser Asp Ala Ile Val Arg
                245                 250                 255

Glu Gly Asp Ser Val Thr Met Thr Cys Glu Val Ser Ser Ser Asn Pro
            260                 265                 270

Glu Tyr Thr Thr Val Ser Trp Leu Lys Asp Gly Thr Ser Leu Lys Lys
        275                 280                 285

Gln Asn Thr Phe Thr Leu Asn Leu Arg Glu Val Thr Lys Asp Gln Ser
    290                 295                 300

Gly Lys Tyr Cys Cys Gln Val Ser Asn Asp Val Gly Pro Gly Arg Ser
305                 310                 315                 320

Glu Glu Val Phe Leu Gln Val Gln Tyr Ala Pro Glu Pro Ser Thr Val
                325                 330                 335

Gln Ile Leu His Ser Pro Ala Val Glu Gly Ser Gln Val Glu Phe Leu
            340                 345                 350

Cys Met Ser Leu Ala Asn Pro Leu Pro Thr Asn Tyr Thr Trp Tyr His
        355                 360                 365

Asn Gly Lys Glu Met Gln Gly Arg Thr Glu Glu Lys Val His Ile Pro
    370                 375                 380

Lys Ile Leu Pro Trp His Ala Gly Thr Tyr Ser Cys Val Ala Glu Asn
385                 390                 395                 400

Ile Leu Gly Thr Gly Gln Arg Gly Pro Gly Ala Glu Leu Asp Val Gln
                405                 410                 415

Tyr Pro Pro Lys Lys Val Thr Thr Val Ile Gln Asn Pro Met Pro Ile
            420                 425                 430

Arg Glu Gly Asp Thr Val Thr Leu Ser Cys Asn Tyr Asn Ser Ser Asn
        435                 440                 445

Pro Ser Val Thr Arg Tyr Glu Trp Lys Pro His Gly Ala Trp Glu Glu
    450                 455                 460

Pro Ser Leu Gly Val Leu Lys Ile Gln Asn Val Gly Trp Asp Asn Thr
465                 470                 475                 480

Thr Ile Ala Cys Ala Arg Cys Asn Ser Trp Cys Ser Trp Ala Ser Pro
                485                 490                 495

Val Ala Leu Asn Val Gln Tyr Ala Pro Arg Asp Val Arg Val Arg Lys
            500                 505                 510

Ile Lys Pro Leu Ser Glu Ile His Ser Gly Asn Ser Val Ser Leu Gln
        515                 520                 525
```

-continued

```
Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln Phe Phe Trp Glu
    530                 535                 540

Lys Asn Gly Arg Leu Leu Gly Lys Glu Ser Gln Leu Asn Phe Asp Ser
545                 550                 555                 560

Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser Cys Trp Val Asn Asn Ser
                565                 570                 575

Ile Gly Gln Thr Ala Ser Lys Ala Trp Thr Leu Glu Val Leu Tyr Ala
            580                 585                 590

Pro Arg Arg Leu Arg Val Ser Met Ser Pro Gly Asp Gln Val Met Glu
        595                 600                 605

Gly Lys Ser Ala Thr Leu Thr Cys Glu Ser Asp Ala Asn Pro Pro Val
    610                 615                 620

Ser His Tyr Thr Trp Phe Asp Trp Asn Asn Gln Ser Leu Pro His His
625                 630                 635                 640

Ser Gln Lys Leu Arg Leu Glu Pro Val Lys Val Gln His Ser Gly Ala
                645                 650                 655

Tyr Trp Cys Gln Gly Thr Asn Ser Val Gly Lys Gly Arg Ser Pro Leu
            660                 665                 670

Ser Thr Leu Thr Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg Val
        675                 680                 685

Ala Val Gly Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala Ile Cys
    690                 695                 700

Gly Leu Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly
705                 710                 715                 720

Leu Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys Lys
                725                 730                 735

Val Arg Arg Ala Pro Leu Ser Glu Gly Pro His Ser Leu Gly Cys Tyr
            740                 745                 750

Asn Pro Met Met Glu Asp Gly Ile Ser Tyr Thr Thr Leu Arg Phe Pro
        755                 760                 765

Glu Met Asn Ile Pro Arg Thr Gly Asp Ala Glu Ser Ser Glu Met Gln
    770                 775                 780

Arg Pro Pro Arg Thr Cys Asp Asp Thr Val Thr Tyr Ser Ala Leu His
785                 790                 795                 800

Lys Arg Gln Val Gly Asp Tyr Glu Asn Val Ile Pro Asp Phe Pro Glu
                805                 810                 815

Asp Glu Gly Ile His Tyr Ser Glu Leu Ile Gln Phe Gly Val Gly Glu
            820                 825                 830

Arg Pro Gln Ala Gln Glu Asn Val Asp Tyr Val Ile Leu Lys His
        835                 840                 845


<210> SEQ ID NO 2
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 2

gggaattcga ggtgcagctg caggagtctg g                                  31


<210> SEQ ID NO 3
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: homo sapiens
```

-continued

<400> SEQUENCE: 3

gaggggaca tttgggaagg actg 24

<210> SEQ ID NO 4
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 4

gagttccagg tcactgtcac tggc 24

<210> SEQ ID NO 5
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 5

atgggcwtca agatggagtc acakwyycwg g 31

<210> SEQ ID NO 6
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 6

actggatggt gggaagatg 19

<210> SEQ ID NO 7
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 7

atgaagttgc ctgttaggct gttggtgctg 30

<210> SEQ ID NO 8
<211> LENGTH: 357
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 8

gaggtgcagc tgcaggagtc tggacctgag ctggtgaagc ctggagcttc aatgaagata 60

tcctgcaagg cttctggtta ctcattcact gactacacca tgaactgggt gaagcagagc 120

catggaaaga accttgagtg gattggactt cttcatcctt tcaatggtgg tactagctac 180

aaccagaagt tcaagggcaa ggccacatta tctgtagaca agtcatccag cacagccttc 240

atggagctcc tcagtctgac atctgaggac tctgcagtct atttctgtgc aagagggaca 300

ggtcggaact atgctatgga ctactggggt caaggaacct cagtcaccgt ctcctca 357

<210> SEQ ID NO 9
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 9

Glu Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr

-continued

```
            20                  25                  30

Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp Ile
        35                  40                  45

Gly Leu Leu His Pro Phe Asn Gly Gly Thr Ser Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Ser Val Asp Lys Ser Ser Ser Thr Ala Phe
65                  70                  75                  80

Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
                85                  90                  95

Ala Arg Gly Thr Gly Arg Asn Tyr Ala Met Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Ser Val Thr Val Ser Ser
        115


<210> SEQ ID NO 10
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 10

gaggtgcagc tgcaggagtc tggacctggc ctggtggcgc cctcacagag cctgtccatc     60

acatgcaccg tctcagggtt ctcattaagc gactatggtg taaactgggt tcgccagatt    120

ccaggaaagg gtctggagtg gctgggaata atatggggtg atggaaggac agactataat    180

tcagctctca aatccagact gaacatcagc aaggacaact ccaagagcca agttttcttg    240

aaaatgaaca gtctgaaagc tgatgacaca gccaggtact actgtgccag agcccccggt    300

aatagggcta tggagtactg gggtcaagga acctcagtca ccgtctcctc a             351


<210> SEQ ID NO 11
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 11

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

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Asp Tyr
            20                  25                  30

Gly Val Asn Trp Val Arg Gln Ile Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45

Gly Ile Ile Trp Gly Asp Gly Arg Thr Asp Tyr Asn Ser Ala Leu Lys
    50                  55                  60

Ser Arg Leu Asn Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65                  70                  75                  80

Lys Met Asn Ser Leu Lys Ala Asp Asp Thr Ala Arg Tyr Tyr Cys Ala
                85                  90                  95

Arg Ala Pro Gly Asn Arg Ala Met Glu Tyr Trp Gly Gln Gly Thr Ser
            100                 105                 110

Val Thr Val Ser Ser
        115


<210> SEQ ID NO 12
<211> LENGTH: 363
<212> TYPE: DNA
```

-continued

<213> ORGANISM: homo sapiens

<400> SEQUENCE: 12

gaggtgcagc tgcaggagtc tggaggaggc ttggtacagc ctgggggttc tctgagactc 60

tcctgtgcaa cttctgggtt caccttcatt gattactaca tgaactgggt ccgccagcct 120

ccaggaaagg cacttgagtg gttgggtttt attaaaaaca aatttaatgg ttacacaaca 180

gaatacaata catctgtgaa gggtcggttc accatctcca gagataattc ccaaagcatc 240

ctctatcttc aaatgaacac cctgagagct gaggacagtg ccacttatta ctgtgcaaga 300

gggctgggac gtagctatgc tatggactac tggggtcaag gaacctcagt caccgtctcc 360

tca 363

<210> SEQ ID NO 13
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 13

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

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

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

Gly Phe Ile Lys Asn Lys Phe Asn Gly Tyr Thr Thr Glu Tyr Asn Thr
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Ser Ile
65 70 75 80

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

Tyr Cys Ala Arg Gly Leu Gly Arg Ser Tyr Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 14
<211> LENGTH: 357
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 14

gaggtgcagc tgcaggagtc tggaggaggg cttggtgcaa cctggagatc catgaaactc 60

tcctgtgttg cctctggatt cactttcagt tactactgga tgaactgggt ccgccagtct 120

ccagagaagg ggcttgagtg gattgctgaa attagattga aatctaataa ttatgcaaca 180

cattatgcgg agtctgtgaa agggaggttc accatctcaa gagatgattc caaaagtagt 240

gtctacctgc aaatgaacaa cttaagagct gaagacactg gcatttatta ctgtaccagg 300

tatgatggtt cctcccggga ctactggggc caaggcacca ctctcacagt ctcctca 357

<210> SEQ ID NO 15
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

-continued

<400> SEQUENCE: 15

Glu Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Gly Ala Thr Trp Arg
1 5 10 15

Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Tyr Tyr
20 25 30

Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Ile
35 40 45

Ala Glu Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80

Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Arg Tyr Asp Gly Ser Ser Arg Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

<210> SEQ ID NO 16
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 16

gaggtgcagc tgcaggagtc tggacctggc ctcgtgaaac cttctcagtc tctgtctctc 60

acctgctctg tcactggcta ctccatcacc agtggttatt actggaactg gatccggcag 120

ccaggaaaca aactggaatg gatgggctac attaggtacg acggtagcaa taactaccca 180

tctctcaaaa atcgaatctc catcactcgt gacacatcta agaaccagtt tttcaagttg 240

ctgaagttga attctgtgac tactgaggac acagctacat attactgtgc aagagggggg 300

attacggttg ctatggacta ctggggtcaa ggaacctcag tcaccgtctc ctca 354

<210> SEQ ID NO 17
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 17

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

Ser Leu Ser Leu Thr Cys Ser Val Thr Gly Tyr Ser Ile Thr Ser Gly
20 25 30

Tyr Tyr Trp Asn Trp Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp
35 40 45

Met Gly Tyr Ile Arg Tyr Asp Gly Ser Asn Asn Tyr Asn Pro Ser Leu
50 55 60

Lys Asn Arg Ile Ser Ile Thr Arg Asp Thr Ser Lys Asn Gln Phe Phe
65 70 75 80

Leu Lys Leu Asn Ser Val Thr Thr Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Ile Thr Val Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Ser Val Thr Val Ser Ser

-continued

115

<210> SEQ ID NO 18
<211> LENGTH: 366
<212> TYPE: DNA
<213> ORGANISM: homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 173
<223> OTHER INFORMATION: n=I

<400> SEQUENCE: 18

gaggtgcagc tgcaggagtc tggacctgac ctggtgaagc ctggggcttc agtgaagata 60

tcctgtaagg cttctggtta ctcattcatt ggctattaca tgcactggct gaagcagagc 120

catggaaaga gccttgagtg gattggagct gttaatccta acactgctgg tcntacctac 180

aaccagaggt tcaaggacaa ggccatatta actgtagaca agtcatccaa cacagcctat 240

atggagctcc gcagcctgac atctgaggac tctgcggtct attactgttc aagagtggac 300

tatgatgact acgggtactg gttcttcgat gtctggggcg cagggaccac ggtcaccgtc 360

tcctca 366

<210> SEQ ID NO 19
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 19

Glu Val Gln Leu Gln Glu Ser Gly Pro Asp Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Ile Gly Tyr
20 25 30

Tyr Met His Trp Leu Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Arg Val Asn Pro Asn Thr Ala Gly Leu Thr Tyr His Gly Lys Ser
50 55 60

Leu Glu Trp Ile Gly Arg Val Asn Pro Asn Thr Ala Gly Leu Thr Tyr
65 70 75 80

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

Ser Arg Val Asp Tyr Asp Asp Tyr Gly Tyr Trp Phe Phe Asp Val Trp
100 105 110

Gly Ala Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 20
<211> LENGTH: 410
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 20

aagatggagt cacagaccca ggtcttcgta tttctactgc tctgtgtgtc tggtgctcat 60

gggagtattg tgatgaccca gactcccaaa ttcctgcttg tatcaacagg agacagggtt 120

accattacct gcaaggccag tcagactgtg actaatgatt tagcttggta ccaacagaag 180

ccagggcagt ctcctaaact gctgatatac tatgcatcca atcgctacac tggagtccct 240

-continued

gatcgcttca ctggcagtgg atatgggacg gacttcactt tcaccatcaa cactgtgcag 300

gctgaagacc tggcagttta tttctgtcag caggattata gctctcctct cacgttcggt 360

gctgggacca agctggaact gaaacgggct gatgctgcac caactgtatc 410

<210> SEQ ID NO 21
<211> LENGTH: 135
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 21

Met Glu Ser Gln Thr Gln Val Phe Val Phe Leu Leu Leu Cys Val Ser
1 5 10 15

Gly Ala His Gly Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu
20 25 30

Val Ser Thr Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Thr
35 40 45

Val Thr Asn Asp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro
50 55 60

Lys Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp
65 70 75 80

Arg Phe Thr Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Asn
85 90 95

Thr Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr
100 105 110

Ser Ser Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
115 120 125

Ala Asp Ala Ala Pro Thr Val
130 135

<210> SEQ ID NO 22
<211> LENGTH: 410
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 22

aagatggagt cacagaccca ggtcttcgta tttctactgc tctgtgtgtc tggtgctcat 60

gggagtattg tgatgaccca gactcccaaa ttcctgcttg tatcagcagg agacaggatt 120

accttaacct gcaaggccag tcagagtgtg actaatgatg tagcttggta ccaacagaag 180

ccagggcagt ctcctaaact gctgatatac tatgcatcca atcgctacac tggagtccct 240

gatcgcttca ctggcagtgg atatgggacg gatttcactt tcaccatcag cactgtgcag 300

gctgaagacc tggcagttta tttctgtcag caggattata ggtctccgtg gacgttcggt 360

ggaggcacca agctggaaat caaacgggct gatgctgcac caactgtatc 410

<210> SEQ ID NO 23
<211> LENGTH: 135
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 23

Met Glu Ser Gln Thr Gln Val Phe Val Phe Leu Leu Leu Cys Val Ser
1 5 10 15

Gly Ala His Gly Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu
20 25 30

-continued

```
Val Ser Ala Gly Asp Arg Ile Thr Leu Thr Cys Lys Ala Ser Gln Ser
        35                  40                  45

Val Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro
    50                  55                  60

Lys Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp
65                  70                  75                  80

Arg Phe Thr Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser
                85                  90                  95

Thr Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr
            100                 105                 110

Arg Ser Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
        115                 120                 125

Ala Asp Ala Ala Pro Thr Val
    130                 135


<210> SEQ ID NO 24
<211> LENGTH: 410
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 24

aagatggagt cacagaccca ggtcttcgta tttctactgc tctgtgtgtc tggtgctcat     60

gggagtattg tgatgaccca gactcccaaa ttcctgcttg tatcagcagg agacagggtt    120

tccataacct gcaaggccag tcagagtgtg actaatgatg taacttggta ccaacagaag    180

ccagggcagt ctcctaaatt gctgatatac tttgcatcca atcgctacac tggagtccct    240

gatcgcttca ctggcagtgg atatgggacg gatttcactt tcaccatcag cactgtgcag    300

gctgaagacc tggcagttta tttctgtcag caggattata gctctccgct cacgttcggt    360

gctgggacca agctggagct gaaacgggct gatgctgcac caactgtatc               410


<210> SEQ ID NO 25
<211> LENGTH: 135
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 25

Met Glu Ser Gln Thr Gln Val Phe Val Phe Leu Leu Leu Cys Val Ser
 1               5                  10                  15

Gly Ala His Gly Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu
            20                  25                  30

Val Ser Ala Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Ser
        35                  40                  45

Val Thr Asn Asp Val Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro
    50                  55                  60

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

Arg Phe Thr Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser
                85                  90                  95

Thr Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr
            100                 105                 110

Ser Ser Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
        115                 120                 125
```

-continued

Ala Asp Ala Ala Pro Thr Val
130 135

<210> SEQ ID NO 26
<211> LENGTH: 410
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 26

aagatggagt cacagaccca ggtcttcgta tttctactgc tctgtgtgtc tggtgctcat 60

gggagtattg tgatgaccca gactcccaaa ttcctgcttg tatcagcagg agacagggtc 120

accataagct gcaaggccag tcagagtgtg agtaatgatg tagcttggta ccaacagaag 180

ccagggcagt ctcctaaact gctgatatac tatgcatcca agcgctatac tggagtccct 240

gatcgcctca ctggcagtgg atatgggacg gatttcactt tcaccatcag cactgtgcag 300

gctgaagacc tggcagttta tttctgtcag caggatcata gctatccgtg gacgttcggt 360

ggaggcacca agctggagat caaacgggct gatgctgcac caactgtatc 410

<210> SEQ ID NO 27
<211> LENGTH: 135
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 27

Met Glu Ser Gln Thr Gln Val Phe Val Phe Leu Leu Leu Cys Val Ser
1 5 10 15

Gly Ala His Gly Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu
20 25 30

Val Ser Ala Gly Asp Arg Val Thr Ile Ser Cys Lys Ala Ser Gln Ser
35 40 45

Val Ser Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro
50 55 60

Lys Leu Leu Ile Tyr Tyr Ala Ser Lys Arg Tyr Thr Gly Val Pro Asp
65 70 75 80

Arg Leu Thr Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser
85 90 95

Thr Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp His
100 105 110

Ser Tyr Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
115 120 125

Ala Asp Ala Ala Pro Thr Val
130 135

<210> SEQ ID NO 28
<211> LENGTH: 419
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 28

atgaagttgc ctgttaggct gttggtgctg atgttctgga ttcctgcttc cagcagtgat 60

gttgtgatga cccaaactcc actctccctg cctgtcagtc ttggagatca agcctccatc 120

tcttgcagat ctagtcagag ccttgtacac agtaatggaa acacctattt acattggtac 180

ctgcagaagc caggccagtc tccaaagctc ctgatctaca aagtttccaa ccgattttct 240

-continued

ggggtcccag ataggttcag tggcagtgga tcagggacag atttcacact caagatcagc 300

agagtggagg ctgaggatct gggagtttat ttctgctctc aaagtacaca tgttccgtac 360

acgttcggag gggggaccaa gctggaaata aaacgggctg atgctgcacc aactgtatc 419

<210> SEQ ID NO 29
<211> LENGTH: 139
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 29

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1 5 10 15

Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
20 25 30

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
35 40 45

Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
50 55 60

Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
100 105 110

Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu
115 120 125

Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Val
130 135

<210> SEQ ID NO 30
<211> LENGTH: 410
<212> TYPE: DNA
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 30

aagatggagt cacagaccca ggtcttcata tccatactgc tctggttata tggagctgat 60

gggaacattg taatgaccca atctcccaaa tccatgtcca tgtcagtagg agagagggtc 120

accttgacct gcaaggccag tgagaatgtg gttacttatg tttcctggta tcaacagaaa 180

ccagagcagt ctcctaaact gctgatatac ggggcatcca accggtacac tggggtcccc 240

gatcgcttca caggcagtgg atctgcaaca gatttcactc tgaccatcag cagtgtgcag 300

gctgaagacc ttgcagatta tcactgtgga cagggttaca gctatccgta cacgttcgga 360

gggggggacca agctggaaat aaaacgggct gatgctgcac caactgtatc 410

<210> SEQ ID NO 31
<211> LENGTH: 135
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 31

Met Glu Ser Gln Thr Gln Val Phe Ile Ser Ile Leu Leu Trp Leu Tyr
1 5 10 15

Gly Ala Asp Gly Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser
20 25 30

-continued

```
Met Ser Val Gly Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn
        35                  40                  45

Val Val Thr Tyr Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro
    50                  55                  60

Lys Leu Leu Ile Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp
65                  70                  75                  80

Arg Phe Thr Gly Ser Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95

Ser Val Gln Ala Glu Asp Leu Ala Asp Tyr His Cys Gly Gln Gly Tyr
            100                 105                 110

Ser Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
        115                 120                 125

Ala Asp Ala Ala Pro Thr Val
    130                 135
```

What is claimed is:

1. A method for treating a human patient diagnosed with a B-cell malignancy, comprising (1) administering to said human patient an effective amount of a blocking anti-CD22 monoclonal antibody binding to the first two Ig-like domains, or to an epitope within the first two Ig-like domains of native human CD22 (hCD22) of SEQ ID NO: 1, and (2) monitoring the response of said malignancy to said treatment.

2. The method of claim 1 wherein said antibody binds to essentially the same epitope of an antibody selected from the group consisting of HB22-7 (HB 11347), HB22-23 (HB11349), HB22-33, HB22-5, HB22-13, and HB22-196.

3. The method of claim 2 wherein said antibody binds to essentially the same epitope as an antibody selected from the group consisting of HB22-7 (HB 11347), HB22-23 (HB 11349), and HB22-33.

4. The method of claim 3 wherein said antibody binds to essentially the same epitope as HB22-7.

5. The method of claim 3 wherein said antibody binds to essentially the same epitope as HB22-33.

6. The method of claim 1 wherein said antibody blocks CD22 binding to its ligand by at least about 70%.

7. The method of claim 1 wherein said antibody blocks CD22 binding to its ligand by at least about 80%.

8. The method of claim 1 wherein said B-cell malignancy is localized.

9. The method of claim 1 wherein said B-cell malignancy is selected from the group consisting of B-cell subtype of non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, chronic lymphocytic leukemia, hairy cell leukemia, and prolymphocytic leukemia.

10. The method of claim 1 wherein said treatment is unaccompanied by any other treatment of malignant B cells.

11. The method of claim 1 wherein said treatment is unaccompanied by radiation therapy.

12. The method of claim 1 wherein said treatment is unaccompanied by chemotherapy.

13. The method of claim 1 wherein said treatment is unaccompanied by radioimmunotherapy (RIT) or combined modality radioimmunotherapy (CMRIT).

14. The method of claim 10 wherein treatment with said antibody alone provides improved cure rate in a Raji lymphoma xenograft model when compared to combination treatment with said antibody and radioimmunotherapy.

15. The method of claim 10 wherein treatment with said antibody alone provides increased survival in a Raji lymphoma xenograft model when compared to combination treatment with said antibody and radioimmunotherapy.

16. The method of claim 10 wherein treatment with said antibody alone provides superior tumor volume reduction in a Raji lymphoma xenograft model when compared to combination treatment with said antibody and radioimmunotherapy.

17. The method of claim 1 wherein said antibody is a fragment of a complete antibody.

18. The method of claim 17 wherein said antibody is selected from the group consisting of Fab, Fab', $F(ab')_2$, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments.

19. The method of claim 1 wherein said antibody has an additional antigen-specificity.

20. The method of claim 19 wherein said antibody is a bispecific antibody.

21. The method of claim 20 wherein said antibody additionally binds to another epitope of CD22.

22. The method of claim 1 wherein said antibody is chimeric.

23. The method of claim 1 wherein said antibody is humanized.

24. The method of claim 1 wherein said antibody is human.

25. The method of claim 1 wherein said antibody is administered intravenously.

26. The method of claim 25 wherein said antibody is administered by weekly intravenous infusions.

27. The method of claim 6 wherein the response to said treatment is monitored by following shrinkage of a solid B-cell tumor.

28. The method of claim 27 wherein shrinkage is monitored by magnetic resonance imaging (MRI).

29. The method of claim 1 wherein said antibody comprises a heavy chain comprising a V_H sequence having at least about 95% sequence identity with the sequence of amino acids 1 to 100 of SEQ ID NO: 9 (HB22-5 V_H sequence); or amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 13 (HB22-3 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); or amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 19 (HB22-196 V_H sequence).

30. The method of claim 29 wherein said antibody comprises a heavy chain comprising a V_H sequence having at least about 95% sequence identity with the sequence of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); or amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence).

31. The method of claim 30 wherein said antibody comprises a V_H sequence selected from the group consisting of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); and amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence).

32. The method of claim 1 wherein said antibody comprises a light chain comprising a V_κ sequence having at least about 95% sequence identity with the amino acid sequence of SEQ ID NO: 21 (HB22-5 V_κ sequence); or SEQ ID NO: 23 (HB22-7 V_κ sequence); or SEQ ID NO: 25 (HB22-13 V_κ sequence); or SEQ ID NO: 27 (HB22-23 V_κ sequence); or SEQ ID NO: 29 (HB22-33 V_κ sequence); or SEQ ID NO: 31 (HB22-196 V_κ sequence).

33. The method of claim 32 wherein said antibody comprises a light chain comprising a V_κ sequence having at least about 95% sequence identity with the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); or SEQ ID NO: 27 (HB22-23 V_κ sequence); or SEQ ID NO: 29 (HB22-33 V_κ sequence).

34. The method of claim 33 wherein said antibody comprises a V_κ sequence selected from the group consisting of the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); SEQ ID NO: 27 (HB22-23 V_κ sequence); and SEQ ID NO: 29 (HB22-33 V_κ sequence).

35. The method of claim 1 wherein said antibody comprises V_H and V_κ sequences selected from the group consisting of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence) and the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence) and the amino acid sequence of SEQ ID NO: 27 (HB22-23 V_κ sequence); and amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence) and the amino acid sequence of SEQ ID NO: 29 (HB22-33 V_κ sequence).

36. The method of claim 35 wherein said antibody is chimeric.

37. The method of claim 35 wherein said antibody is humanized.

38. The method of claim 35 wherein said antibody is human.

* * * * *