CLAIMS:

1. A monomeric calicheamicin derivative/anti-CD22 antibody conjugate having the formula, ~~Pr(-X-S-S-W)m~~Pr(-X-W)m wherein:

Pr is an anti-CD22 antibody;

X is a hydrolyzable linker ~~that comprises a product of any reactive group that can react with an antibody~~ having the formula (CO - Alk^1 - Sp^1 - Ar - Sp^2 - Alk^2 - $C(Z^1)$ = Q - Sp), wherein

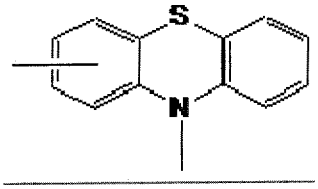
Alk^1 and Alk^2 are independently a bond or branched or unbranched (C_1-C_{10}) alkylene chain;

Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$, or -X-Ar'-Y-$(CH_2)_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, with the proviso that when n = 0, then at least one of Y and Z must be a bond and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, -COOR', -CONHR', $-(CH_2)_nCOOR'$, $-S(CH_2)_nCOOR'$, $-O(CH_2)_nCONHR'$, or $-S(CH_2)_nCONHR'$, with the proviso that when Alk^1 is a bond, Sp^1 is a bond;

n is an integer from 0 to 5;

R' is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of -OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium $-A^-$ where A^- is a pharmaceutically acceptable anion completing a salt;

Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, -COOR', -CONHR', $-O(CH_2)_nCOOR'$, $-S(CH_2)_nCOOR'$, $-O(CH_2)_nCONHR'$, or $-S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined or a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene or



with each naphthylidene or phenothiazine optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, -COOR', -CONHR', $-O(CH_2)_nCOOR'$, $-S(CH_2)_nCOOR'$, or $-S(CH_2)_nCONHR'$ wherein n and R' are as defined above, with the proviso that when Ar is phenothiazine, Sp^1 is a bond only connected to nitrogen;

Sp^2 is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is a bond;

Z^1 is H, (C_1-C_5) alkyl, or phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, -COOR', -ONHR', -$O(CH_2)_nCOOR'$, -$S(CH_2)_nCOOR'$, -$O(CH_2)_nCONHR'$, or -$S(CH_2)_nCONHR'$ wherein n and R' are as defined above;

Sp is a straight or branched-chain divalent or trivalent (C_1-C_{18}) radical, divalent or trivalent aryl or heteroaryl radical, divalent or trivalent (C_3-C_{18}) cycloalkyl or heterocycloalkyl radical, divalent or trivalent aryl- or heteroaryl-aryl (C_1-C_{18}) radical, divalent or trivalent cycloalkyl- or heterocycloalkyl-alkyl (C_1-C_{18}) radical or divalent or trivalent (C_2-C_{18}) unsaturated alkyl radical, wherein heteroaryl is preferably furyl, thienyl, N-methylpyrrolyl, pyridinyl, N-methylimidazolyl, oxazolyl, pyrimidinyl, quinolyl, isoquinolyl, N-methylcarbazoyl, aminocourmarinyl, or phenazinyl and wherein if Sp is a trivalent radical, Sp can be additionally substituted by lower (C_1-C_5) dialkylamino, lower (C_1-C_5) alkoxy, hydroxy, or lower (C_1-C_5) alkylthio groups; and

Q is =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH-, or =NHO-;

W is a calicheamicin ~~radical~~;

m is the average loading for a purified conjugation product such that the calicheamicin constitutes 4 – 10% of the conjugate by weight; and

~~(-X-S-S-W)m~~(-X-W)m is a calicheamicin derivative, wherein the antibody molecule comprises a heavy chain variable domain comprising

(a) SEQ ID NO: 1 for CDR-H1,

(b) ~~the sequence GINPGNNYATYRRKFQG of gH7 in Figure 6 or~~ SEQ ID NO: 2 or SEQ ID NO: 13 or SEQ ID NO: 15 or SEQ ID NO: 16 or residues 50-66 of SEQ ID NO: 23 or residues 50-66 of SEQ ID NO: 27 for CDR-H2, and

(c) SEQ ID NO: 3 for CDR-H3;

and a light chain variable domain comprising

(d) SEQ ID NO: 4 for CDR-L1,

(e) SEQ ID NO: 5 for CDR-L2, and

(f) SEQ ID NO: 6 for CDR-L3.

2. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 1, wherein the antibody is a CDR-grafted anti-CD22 antibody.

3. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 2, wherein the antibody comprises a variable domain comprising human acceptor framework regions and non-human donor CDRs.

4. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 3, wherein the human acceptor framework regions of the variable domain of the heavy chain of the antibody are based on SEQ ID NOs: 21 and 22 and comprise donor residues at positions 1, 28, 48, 72 and 97 in SEQ ID NO: 8.

5. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 4, wherein the antibody further comprises non-human donor residues at positions 68 and 70 in SEQ ID NO: 8.

6. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 2, wherein the CDR-grafted antibody comprises a variable domain of the light chain comprising a human acceptor framework region based on SEQ ID NOs: 17 and 18 and further comprising donor residues at positions 2, 4, 42, 43, 50 and 65 in SEQ ID NO: 7.

7. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 6, wherein the CDR-grafted antibody further comprises a donor residue at position 3 in SEQ ID NO: 7.

8. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 2, wherein the CDR-grafted antibody comprises a light chain variable region comprising SEQ ID NO: 19 and a heavy chain variable region comprising SEQ ID NO: 27.

9. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 2, wherein the CDR-grafted antibody comprises a light chain having the sequence as

set forth in SEQ ID NO: 28 and a heavy chain having the sequence as set forth in SEQ ID NO: 30.

10. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 2, wherein the CDR-grafted antibody is a variant antibody obtained by an affinity maturation protocol ~~and has increased affinity for human CD22~~.

11. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 2, wherein the antibody comprises a light chain consisting of an amino acid sequence resulting from the expression of SEQ ID NO: 29 in a mammalian cell and a heavy chain consisting of an amino acid sequence resulting from the expression of SEQ ID NO: 31 in a mammalian cell.

12. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of any one of the preceding claims, wherein the calicheamicin ~~derivative~~ is a gamma calicheamicin or a N-acetyl gamma calicheamicin ~~derivative~~.

13. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim 12, wherein the calicheamicin ~~derivative~~ is functionalized with 3-mercapto-3-methyl butanoyl hydrazide.

~~14. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of any one of the preceding claims, wherein the hydrolyzable linker is a bifunctional linker that is capable of releasing the calicheamicin derivative from the conjugate after binding and entry into target cells.~~

~~15~~14. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim ~~14~~13, wherein the ~~bifunctional~~ linker is 4-(4-acetylphenoxy) butanoic acid (AcBut).

~~16~~15. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate according to any one of claims 1 to ~~15~~14 for use in the treatment of a proliferative disorder.

~~17~~16. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~16~~15, wherein the proliferative disorder is a B-cell malignancy.

~~18~~17. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~17~~16, wherein the B-cell malignancy is leukemia.

~~19~~18. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~20,~~17 wherein the leukemia expresses cell surface antigen CD22.

19. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim 17 or 18, wherein the leukemia is acute lymphocytic leukemia.

~~22~~20. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~19~~16, wherein the B-cell malignancy is lymphoma.

~~23~~21. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~22~~20, wherein the lymphoma expresses cell surface antigen CD22.

~~24~~22. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~22 or 23~~20 or 21, wherein the lymphoma is a Non-Hodgkin's lymphoma.

~~25~~23. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to ~~claim 24~~ any one of claims 15-22, wherein the conjugate ~~or the composition~~ is ~~administered~~ administrable with one or more bioactive agents.

~~26~~24. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~25~~23, wherein the bioactive agents are selected from a group consisting of antibodies, growth factors, hormones, cytokines, anti-hormones, xanthines, interleukins, interferons, and cytotoxic ~~drugs~~agents.

~~27~~25. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~26~~24, wherein the bioactive agent is an antibody.

~~28~~26. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~27~~25, wherein the antibody is directed against a cell surface antigen expressed on B-cell malignancies.

~~29~~27. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~28~~26, wherein the antibody directed against cell surface antigens expressed on B-cell malignancies is selected from a group consisting of anti-CD19, anti-CD20 and anti-CD33 antibodies.

~~30~~28. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~29~~27, wherein the anti-CD20 antibody is rituximab.

~~31~~29. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~26~~24, wherein the cytotoxic agent is selected from the group consisting of doxorubicin, daunorubicin, idarubicin, aclarubicin, zorubicin, mitoxantrone, epirubicin, carubicin, nogalamycin, menogaril, pitarubicin, valrubicin, cytarabine, gemcitabine, trifluridine, ancitabine, enocitabine, azacitidine, doxifluridine, pentostatin, broxuridine, capecitabine, cladribine, decitabine, floxuridine, fludarabine, gougerotin, puromycin, tegafur, tiazofurin, adriamycin, cisplatin, carboplatin, cyclophosphamide, dacarbazine, vinblastine, vincristine, mitoxantrone, bleomycin, mechlorethamine, prednisone, procarbazine methotrexate, flurouracils, etoposide, taxol, taxol analogs, and mitomycin.

~~32~~30. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~26~~24, wherein the conjugate is ~~administered~~ administrable together with, prior to, or subsequent to the administration of one or more combinations of cytotoxic agents as part of a treatment regimen, wherein the combination of cytotoxic agents is selected from:

A. CHOPP (cyclophosphamide, doxorubicin, vincristine, prednisone, and procarbazine);

B. CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone);

C. COP (cyclophosphamide, vincristine, and prednisone);

D. CAP–BOP (cyclophosphamide, doxorubicin, procarbazine, bleomycin, vincristine, and prednisone);

E. m–BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone, and leucovorin);

F. ProMACE–MOPP (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, leucovorin, mechloethamine, vincristine, prednisone, and procarbazine);

G. ProMACE–CytaBOM (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, leucovorin, cytarabine, bleomycin, and vincristine);

H. MACOP–B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin, and leucovorin);

I. MOPP (mechloethamine, vincristine, prednisone, and procarbazine);

J. ABVD (adriamycin/doxorubicin, bleomycin, vinblastine, and dacarbazine);

K. MOPP (mechloethamine, vincristine, prednisone, and procarbazine) alternating with ABV (adriamycin/doxorubicin, bleomycin, and vinblastine);

L. MOPP (mechloethamine, vincristine, prednisone, and procarbazine) alternating with ABVD (adriamycin/doxorubicin, bleomycin, vinblastine, and dacarbazine);

M. ChlVPP (chlorambucil, vinblastine, procarbazine, and prednisone);

N. IMVP–16 (ifosfamide, methotrexate, and etoposide);

O. MIME (methyl–gag, ifosfamide, methotrexate, and etoposide);

P. DHAP (dexamethasone, high–dose cytarabine, and cisplatin);

Q. ESHAP (etoposide, methylpredisolone, high–dose cytarabine, and cisplatin);

R. CEPP(B) (cyclophosphamide, etoposide, procarbazine, prednisone, and bleomycin);

S. CAMP (lomustine, mitoxantrone, cytarabine, and prednisone);

T. CVP–1 (cyclophosphamide, vincristine, and prednisone);

U. ESHOP (etoposide, methylpredisolone, high–dose cytarabine, vincristine and cisplatin);

V. EPOCH (etoposide, vincristine, and doxorubicin for 96 hours with bolus doses of cyclophosphamide and oral prednisone);

W. ICE (ifosfamide, cyclophosphamide, and etoposide);

~~X. CEPP(B) (cyclophosphamide, etoposide, procarbazine, prednisone, and bleomycin);~~

~~Y~~X. CHOP–B (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin); and

~~Z~~Y. P/DOCE (epirubicin or doxorubicin, vincristine, cyclophosphamide, and prednisone).

~~33~~31. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~26~~24, wherein the conjugate is ~~administered~~ administrable together with an antibody directed against a cell surface antigen on B-cell malignancies, and optionally comprising one or more combinations of cytotoxic agents as part of a treatment regimen, wherein the combination of cytotoxic agents is selected from:

A. CHOPP (cyclophosphamide, doxorubicin, vincristine, prednisone, and procarbazine);

B. CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone);

C. COP (cyclophosphamide, vincristine, and prednisone);

D. CAP–BOP (cyclophosphamide, doxorubicin, procarbazine, bleomycin, vincristine, and prednisone);

E. m–BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone, and leucovorin);

F. ProMACE–MOPP (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, leucovorin, mechloethamine, vincristine, prednisone, and procarbazine);

G. ProMACE–CytaBOM (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, leucovorin, cytarabine, bleomycin, and vincristine);

H. MACOP–B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin, and leucovorin);

I. MOPP (mechloethamine, vincristine, prednisone, and procarbazine);

J. ABVD (adriamycin/doxorubicin, bleomycin, vinblastine, and dacarbazine);

K. MOPP (mechloethamine, vincristine, prednisone, and procarbazine) alternating with ABV (adriamycin/doxorubicin, bleomycin, and vinblastine);

L. MOPP (mechloethamine, vincristine, prednisone, and procarbazine) alternating with ABVD (adriamycin/doxorubicin, bleomycin, vinblastine, and dacarbazine);

M. ChlVPP (chlorambucil, vinblastine, procarbazine, and prednisone);

N. IMVP–16 (ifosfamide, methotrexate, and etoposide);

O. MIME (methyl–gag, ifosfamide, methotrexate, and etoposide);

P. DHAP (dexamethasone, high–dose cytarabine, and cisplatin);

Q. ESHAP (etoposide, methylpredisolone, high–dose cytarabine, and cisplatin);

R. CEPP(B) (cyclophosphamide, etoposide, procarbazine, prednisone, and bleomycin);

S. CAMP (lomustine, mitoxantrone, cytarabine, and prednisone);

T. CVP–1 (cyclophosphamide, vincristine, and prednisone);

U. ESHOP (etoposide, methylpredisolone, high–dose cytarabine, vincristine and cisplatin);

V. EPOCH (etoposide, vincristine, and doxorubicin for 96 hours with bolus doses of cyclophosphamide and oral prednisone);

W. ICE (ifosfamide, cyclophosphamide, and etoposide);

~~X. CEPP(B) (cyclophosphamide, etoposide, procarbazine, prednisone, and bleomycin);~~

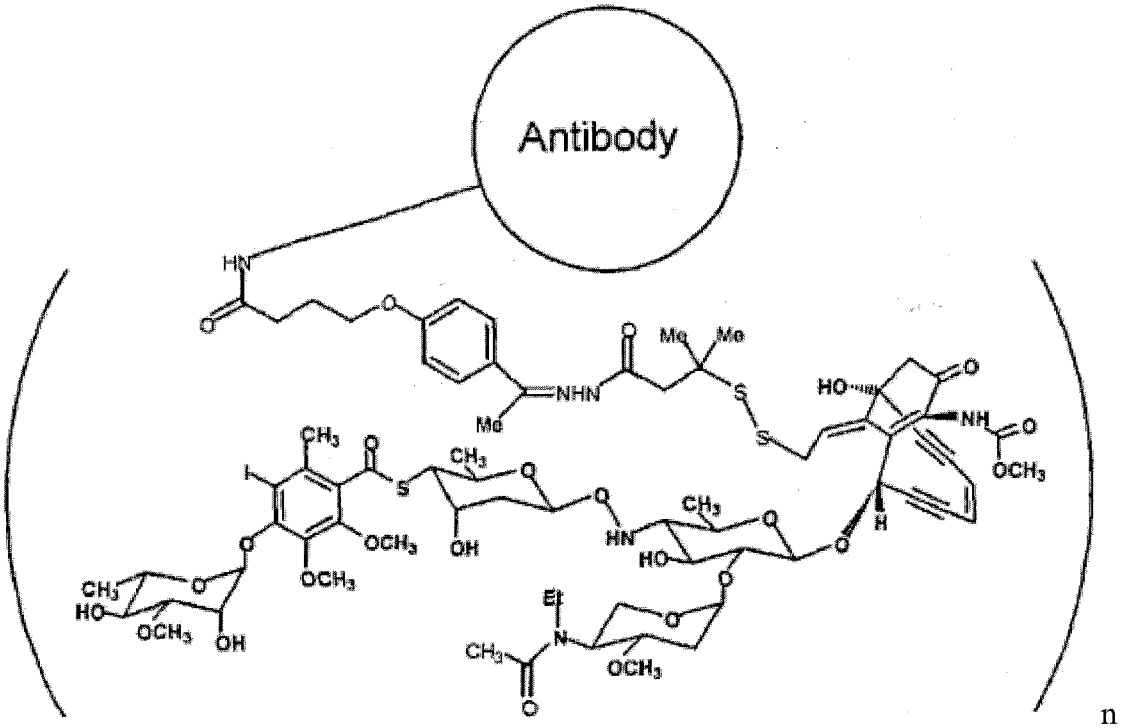
~~Y~~X. CHOP–B (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin); and

~~Z~~Y. P/DOCE (epirubicin or doxorubicin, vincristine, cyclophosphamide, and prednisone).

~~34~~32. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to any one of claims ~~16 to 33~~15-31, wherein the conjugate is ~~administered~~ administrable at an effective dose of from 0.1 mg/m^{2} to 50 mg/m^{2}.

~~35~~33. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate for use according to claim ~~34~~32, wherein the conjugate is ~~administered~~ administrable at an effective dose of from 0.4 mg/m^{2} to 30 mg/m^{2}.

~~36~~34. A monomeric calicheamicin derivative/anti-CD22 antibody conjugate having the formula:



wherein the antibody comprises SEQ ID NO: 1 for CDR-H1, residues 50-66 of SEQ ID NO: 27 for CDR-H2, SEQ ID NO: 3 for CDR-H3, SEQ ID NO: 4 for CDR-L1, SEQ ID NO: 5 for CDR-L2 and SEQ ID NO: 6 for CDR-L3; and

wherein n is 3 to 9.

~~37~~35. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim ~~36~~34, wherein the antibody comprises a light chain variable region as set forth in SEQ ID NO: 19 and a heavy chain variable region as set forth in SEQ ID NO: 27.

~~38~~36. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim ~~36~~34, wherein the antibody is expressed in a mammalian cell and comprises a light chain consisting of residues 21 to 239 of SEQ ID NO: 28 and a heavy chain consisting of residues 20 to 466 of SEQ ID NO: 30.

~~39~~37. The monomeric calicheamicin derivative/anti-CD22 antibody conjugate of claim ~~36~~34, wherein the antibody comprises a light chain consisting of an amino acid sequence resulting from the expression of SEQ ID NO: 29 in a mammalian cell and a heavy chain consisting of an amino acid sequence resulting from the expression of SEQ ID NO: 31 in a mammalian cell.

~~40~~38. A pharmaceutical composition comprising the ~~composition~~ monomeric calicheamicin derivative/anti-CD22 antibody conjugate of any one of claims ~~36~~34 to 37 and a pharmaceutically acceptable excipient, diluent or carrier.