CLAIMS

1. A humanized antibody specifically recognizing human CA IX, comprising:

a) a heavy chain variable region sequence comprising CDR sequences identical to or differing in 1 or 2 amino acids from the following sequences:

GFTFTDYYMH (SEQ ID NO. 1), and

FIRNKASGYTPQYSASVKG (SEQ ID NO. 2), and

VRGGHAGSNYWYFDV (SEQ ID NO. 3); or

a heavy chain variable region sequence comprising CDR sequences identical to or differing in 1 or 2 amino acids from the following sequences:

GFTFNTNAMH (SEQ ID NO. 17), and

RIRSKSNNYTTYYADSVKD (SEQ ID NO. 18), and

VCGSWFAY (SEQ ID NO. 19);

and

b) a light chain variable region sequence comprising the CDR sequences identical to or differing in 1 or 2 amino acids from the following sequences:

HVSQNINVWLS (SEQ ID NO. 9), and

QASNLHT (SEQ ID NO. 10), and

QQGQSYPFT (SEQ ID NO. 11); or

a light chain variable region sequence comprising the CDR sequences identical to or differing in 1 or 2 amino acids from the following sequences:

KSSQSLLNSSNQKNYLA (SEQ ID NO. 25), and

FTSTRQS (SEQ ID NO. 26), and

QQHYSIPLT (SEQ ID NO. 27).

2. The humanized antibody according to claim 1, containing a heavy chain variable region sequence comprising CDR sequences identical to or differing in 1 or 2 amino acids from the sequences GFTFTDYYMH (SEQ ID NO. 1), and FIRNKASGYTPQYSASVKG (SEQ ID NO. 2), and VRGGHAGSNYWYFDV (SEQ ID NO. 3); and a light chain variable region sequence comprising the CDR sequences identical to or differing in 1 or 2 amino acids from the following sequences: HVSQNINVWLS (SEQ ID NO. 9), and QASNLHT (SEQ ID NO. 10), and QQGQSYPFT (SEQ ID NO. 11).

3. The humanized antibody according to claim 1, containing a heavy chain variable region sequence comprising CDR sequences identical to or differing in 1 or 2 amino acids from the sequences GFTFNTNAMH (SEQ ID NO. 17), and RIRSKSNNYTTYYADSVKD (SEQ ID NO. 18), and

VCGSWFAY (SEQ ID NO. 19); and a light chain variable region sequence comprising the CDR sequences identical to or differing in 1 or 2 amino acids from the following sequences: KSSQSLLNSSNQKNYLA (SEQ ID NO. 25), and FTSTRQS (SEQ ID NO. 26), and QQHYSIPLT (SEQ ID NO. 27).

4. The humanized antibody according to any one of the preceding claims, comprising at least one variable region selected from the group consisting of:

- a heavy chain variable region comprising or having the sequence: $X^1VX^2LVX^3SGGGLVQPGX^4SLRLSCX^5ASGFTFTDYYMHWVRQAPGX^6GLEWX^7X^8FIRNKASGYTPQYSASVKGRFTISRDX^9X^{10}X^{11}X^{12}X^{13}X^{14}YLQMNSLX^{15}X^{16}EDTAX^{17}YYVRGGHAGSNYWYFDVWGX^{18}GTX^{19}VTVSS$ (SEQ ID NO. 33)

  wherein

  X^1 = E or Q

  X^2 = Q or R

  X^3 = E or Q

  X^4 = G or R

  X^5 = A or T

  X^6 = K or R

  X^7 = V or L

  X^8 = A or G

  X^9 = D or N

  X^{10} = S or A

  X^{11} = K or T or E

  X^{12} = N or T

  X^{13} = S or I

  X^{14} = L or A

  X^{15} = K or R

  X^{16} = T or A or I

  X^{17} = V or I

  X^{18} = Q or K

  X^{19} = T or L or M; and

- a light chain variable region comprising or having the sequence: $DIQMTQSPSX^{20}LSASVGDRVTIX^{21}CHVSQNINVWLSWYQQKPGX^{22}APX^{23}LLIYQASNLHTGVPSRFSGSGSGTX^{24}FTX^{25}TIX^{26}SLQPX^{27}DX^{28}X^{29}TYYCQQGQSYPFTFGX^{30}GTKX^{31}EIK$ (SEQ ID NO. 34)

  wherein

X^{20} = S or T

X^{21} = T or N

X^{22} = K or N or E

X^{23} = N or K

X^{24} = H or E or D or G

X^{25} = L or F

X^{26} = S or G or R

X^{27} = E or D

X^{28} = I or F or Y

X^{29} = A or V

X^{30} = Q or G

X^{31} = V or L; and

- a heavy chain variable region comprising or having the sequence:

X^{32}VQLVESGGGX^{33}VQPGX^{34}SLX^{35}LSCAASGFTFNTNAMHWVRQAX^{36}GX^{37}GLEWVX^{38}RIRSKSNNYTTYYADSVKDRFTISRDX^{39}SKX^{40}TX^{41}YLQX^{42}NSLX^{43}X^{44}EDTAVYYCVCGSWFAYWGQGTX^{45}VTVSS (SEQ ID NO. 35)

wherein

X^{32} = E or Q

X^{33} = L or V

X^{34} = G or R

X^{35} = K or R

X^{36} = S or P

X^{37} = K or R

X^{38} = A or G

X^{39} = D or N

X^{40} = N or S

X^{41} = A or L

X^{42} = M or V

X^{43} = K or R

X^{44} = T or A

X^{45} = L or T; and

- a light chain variable region comprising or having the sequence:

DX^{46}X^{47}MTQSPDSLAVSLGERX^{48}TINCKSSQSLLNSSNQKNYLAWX^{49}QQKPGQX^{50}PX^{51}X^{52}X^{53}IYFTSTRQSGVPDRFX^{54}GSGSGTDFTLTIX^{55}SLQAEDVAVYX^{56}CQQHYSIPLTFGQGTX^{57}X^{58}EIK (SEQ ID NO. 36)

X^{46} = V or I

X^{47} = V or Q
X^{48} = V or A
X^{49} = Y or F
X^{50} = S or P
X^{51} = K or N
X^{52} = L or V
X^{53} = L or V
X^{54} = S or T
X^{55} = S or N
X^{56} = Y or F
X^{57} = K or Q
X^{58} = L or V.

5. The humanized antibody according to claim 4, containing the heavy chain regions having SEQ ID NO. 33 and the light chain regions having SEQ ID NO. 34.

6. The humanized antibody according to claim 4, containing the heavy chain regions of SEQ ID NO. 35 and the light chain regions of SEQ ID NO. 36.

7. The humanized antibody according to claim 4, comprising at least one variable region selected from the group consisting of:

a) a heavy chain variable region amino acid sequence comprising or having the sequence selected from the group consisting of
EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYYMHWVRQAPGKGLEWVAFIRNKASGYTPQYSASVKGRFTISRDDSKNSLYLQMNSLKIEDTAVYYCVRGGHAGSNYWYFDVWGQGTTVTVSS (SEQ ID NO. 4),
EVRLVESGGGLVQPGGSLRLSCAASGFTFTDYYMHWVRQAPGKGLEWLGFIRNKASGYTPQYSASVKGRFTISRDDSTNSLYLQMNSLKTEDTAIYYCVRGGHAGSNYWYFDVWGQGTLVTVSS (SEQ ID NO. 5),
QVQLVQSGGGLVQPGRSLRLSCTASGFTFTDYYMHWVRQAPGKGLEWVGFIRNKASGYTPQYSASVKGRFTISRDDSKTIAYLQMNSLKTEDTAVYYCVRGGHAGSNYWYFDVWGQGTLVTVSS (SEQ ID NO. 6),
EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYYMHWVRQAPGRGLEWVAFIRNKASGYTPQYSASVKGRFTISRDNAENSLYLQMNSLRAEDTAVYYCVRGGHAGSNYWYFDVWGKGTTVTVSS (SEQ ID NO. 7), and

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYYMHWVRQAPGKGLEWVGFIRNKASGYTPQYSASVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCVRGGHAGSNYWYFDVWGQGTMVTVSS (SEQ ID NO. 8);

b) a light chain variable region amino acid sequence comprising or having the sequences selected from the group consisting of
DIQMTQSPSSLSASVGDRVTITCHVSQNINVWLSWYQQKPGKAPNLLIYQASNLHTGVPSRFSGSGSGTHFTLTISSLQPEDIATYYCQQGQSYPFTFGGGTKVEIK (SEQ ID NO. 12),
DIQMTQSPSTLSASVGDRVTITCHVSQNINVWLSWYQQKPGNAPKLLIYQASNLHTGVPSRFSGSGSGTEFTLTIGSLQPDDFVTYYCQQGQSYPFTFGQGTKVEIK (SEQ ID NO. 13),
DIQMTQSPSSLSASVGDRVTITCHVSQNINVWLSWYQQKPGEAPKLLIYQASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGQSYPFTFGQGTKLEIK (SEQ ID NO. 14),
DIQMTQSPSSLSASVGDRVTINCHVSQNINVWLSWYQQKPGEAPKLLIYQASNLHTGVPSRFSGSGSGTGFTLTIRSLQPEDYATYYCQQGQSYPFTFGQGTKLEIK (SEQ ID NO. 15), and
DIQMTQSPSSLSASVGDRVTITCHVSQNINVWLSWYQQKPGKAPKLLIYQASNLHTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQGQSYPFTFGQGTKLEIK (SEQ ID NO. 16);

c) a heavy chain variable region amino acid sequence comprising or having the sequences selected from the group consisting of
EVQLVESGGGLVQPGGSLKLSCAASGFTFNTNAMHWVRQASGKGLEWVGRIRSKSNNYTTYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVCGSWFAYWGQGTLVTVSS (SEQ ID NO. 20),
EVQLVESGGGLVQPGGSLKLSCAASGFTFNTNAMHWVRQASGKGLEWVGRIRSKSNNYTTYYADSVKDRFTISRDDSKSTAYLQMNSLKTEDTAVYYCVCGSWFAYWGQGTLVTVSS (SEQ ID NO. 21),
QVQLVESGGGVVQPGGSLRLSCAASGFTFNTNAMHWVRQAPGRGLEWVARIRSKSNNYTTYYADSVKDRFTISRDNSKNTLYLQVNSLRAEDTAVYYCVCGSWFAYWGQGTLVTVSS (SEQ ID NO. 22),
EVQLVESGGGVVQPGRSLRLSCAASGFTFNTNAMHWVRQAPGKGLEWVARIRSKSNNYTTYYADSVKDRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVCGSWFAYWGQGTLVTVSS (SEQ ID NO. 23), and
EVQLVESGGGLVQPGGSLKLSCAASGFTFNTNAMHWVRQASGKGLEWVGRIRSKSNNYTTYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVCGSWFAYWGQGTTVTVSS (SEQ ID NO. 24); and

d) a light chain variable region amino acid sequence comprising or having the sequences selected from

the group consisting of
DVVMTQSPDSLAVSLGERVTINCKSSQSLLNSSNQKNYLAWYQQKPGQSPKLLIYFTSTRQSG
VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO. 28),
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSSNQKNYLAWFQQKPGQPPNLVIYFTSTRQSG
VPDRFSGSGSGTDFTLTINSLQAEDVAVYFCQQHYSIPLTFGQGTQVEIK (SEQ ID NO. 29),
DIQMTQSPDSLAVSLGERATINCKSSQSLLNSSNQKNYLAWYQQKPGQPPKLLIYFTSTRQSG
VPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQHYSIPLTFGQGTKVEIK (SEQ ID NO. 30),
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSSNQKNYLAWFQQKPGQPPKVLIYFTSTRQSG
VPDRFTGSGSGTDFTLTISSLQAEDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO. 31), and
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSSNQKNYLAWYQQKPGQPPKLLIYFTSTRQSG
VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO. 32).

8. The humanized antibody according to claim 7, containing at least one of the heavy chain variable regions listed in a) and at least one of the light chain variable regions listed in b) in one antibody.

9. The humanized antibody according to claim 7, containing at least one of the heavy chain variable regions listed in c) and at least one of the light chain variable regions listed in d) in one antibody.

10. The humanized antibody according to claim 7, wherein the said humanized antibody contains:
- a heavy chain variable region amino acid sequence comprising or having the sequence of SEQ ID NO. 7; and a light chain variable region amino acid sequence comprising or having the sequence selected from the group consisting of SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15 and SEQ ID NO. 16;

or
- a heavy chain variable region amino acid sequence comprising or having the sequence selected from a group consisting of SEQ ID NO. 22 and SEQ ID NO. 23; and a light chain variable region amino acid sequence comprising or having the sequence selected from a group consisting of SEQ ID NO. 28, SEQ ID NO. 29 and SEQ ID NO. 32.

11. The humanized antibody according to claim 7, wherein the said humanized antibody contains:
- a heavy chain variable region amino acid sequence comprising or having the sequence of SEQ ID NO. 7 and a light chain variable region amino acid sequence comprising or having the sequence of SEQ ID NO. 15; or
- a heavy chain variable region amino acid sequence comprising or having the sequence of SEQ ID NO. 23 and a light chain variable region amino acid sequence comprising or having the sequence of SEQ ID NO. 32.

12. The humanized antibody according to any one of the preceding claims which has human IgG constant regions (allotype G1m17,1) of the heavy chains and human kappa constant regions (allotype Km3) of the light chains.

13. A pharmaceutical composition comprising a therapeutically effective amount of a humanized antibody of any one of claims 1-12, which specifically recognizes human CA IX, and a pharmaceutically acceptable carrier, diluent or excipient.

14. The humanized antibody or the pharmaceutical composition of claim 13, for use in the treatment of a disease or disorder associated with expression, activation or function of a CA IX protein, preferably selected from cell proliferative disease or disorder, more preferably said disease or disorder is cancer selected from the group consisting of: squamous cell carcinoma, myeloma, small-cell lung cancer, non-small cell lung cancer, glioma, hodgkin's lymphoma, non-hodgkin's lymphoma, acute myeloid leukemia, multiple myeloma, gastrointestinal (tract) cancer, renal cancer, ovarian cancer, liver cancer, lymphoblastic leukemia, lymphocytic leukemia, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, melanoma, chondrosarcoma, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, brain cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, mesothelioma, and head and neck cancer.

15. The humanized antibody or the pharmaceutical composition of claim 14, for use in the treatment of breast cancer, mesothelioma, or glioblastoma expressing CA IX.

16. The pharmaceutical composition comprising a combination of therapeutically effective amounts of humanized antibodies of any one of claims 1-12, which specifically recognize human CA IX, wherein one of the humanized antibodies is administered prior to or subsequently to the administration of the second humanized antibody.

17. Use of a therapeutically effective amount of the humanized antibody or of the pharmaceutical composition of claim 13 for the manufacture of a medicament for treating a disease or disorder associated with expression, activation or function of a CA IX protein, preferably selected from cell proliferative disease or disorder, more preferably said disease or disorder is cancer selected from the group consisting of: squamous cell carcinoma, myeloma, small-cell lung cancer, non-small cell lung cancer, glioma, hodgkin's lymphoma, non-hodgkin's lymphoma, acute myeloid leukemia, multiple myeloma, gastrointestinal (tract) cancer, renal cancer, ovarian cancer, liver cancer, lymphoblastic leukemia, lymphocytic leukemia, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer,

thyroid cancer, melanoma, chondrosarcoma, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, brain cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, mesothelioma, and head and neck cancer.

18. Use of a therapeutically effective amount of the humanized antibody or the pharmaceutical composition of claim 13 for the manufacture of a medicament for treating of breast cancer, mesothelioma, or glioblastoma expressing CA IX.

19. Use of a therapeutically effective amount of the humanized antibody or the pharmaceutical composition of claim 13 for the manufacture of a medicament for reducing or inhibiting invasiveness of a tumor in a subject.

20. The use of any one of claims 17-19 comprising administering of the appropriate daily or weekly dose of the humanized antibody to CA IX to a patient is preferably ranging from 0.001 mg/kg to 15 mg/kg body weight.

21. The use of claim 20 comprising administering
i) multiple, identical or different doses of the humanized antibody;
ii) multiple escalating doses of the humanized antibody; or
iii) a dose of the humanized antibody once every week, once every 2 weeks, once every 3 weeks, once every 4 weeks, or once every 5 weeks.

22. The use of claim 17, comprising 1-10 administration cycles, each cycle comprising 2-5 infusions/doses every 1-4 weeks, with a humanized antibody, followed by a break 1-8 weeks between each two cycles.

23. A diagnostic composition comprising at least one humanized antibody of any one of claims 1-12, and at least one carrier, diluent, or excipient.

24. A method for diagnosing a cancer in a subject in need thereof, the method comprising contacting a biological sample derived or obtained from said subject with the diagnostic composition of claim 23, wherein a complex formation beyond a predetermined threshold is indicative of the cancer in said subject.

25. The method for diagnosing a cancer in a subject according to claim 24, wherein the humanized antibody, according to any one of the claims 1-12, is linked, bound or conjugated to a paramagnetic,

radioactive or fluorogenic moiety that is detectable upon imaging.