

CLAIMS

1. A humanized antibody specifically recognizing the proteoglycan domain of human CA IX 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. 1), and RIRSKSNNTTYADSVKD (SEQ ID NO. 2), and VCGSWFAY (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: KSSQSLNSSNQKNYLA (SEQ ID NO. 4), and FTSTRQS (SEQ ID NO. 5), and QQHYIPLT (SEQ ID NO. 6).
2. The humanized antibody according to claim 1, comprising at least one variable region selected from the group consisting of:
- a heavy chain variable region comprising or having the sequence:
 X^{32} VQLVESGGGX ^{33} VQPGX ^{34} SLX ^{35} LSCAASGFTFNTNAMHWVRQAX ^{36} GX ^{37} GLEWVX ^{38} RI
RSKSNNTTYADSVKDRFTISRDX ^{39} SKX ^{40} TX ^{41} YLQX ^{42} NSLX ^{43} X ^{44} EDTAVYYCVCGSWF
AYWGQGTGX ^{45} VTVSS (SEQ ID NO. 7)
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} TINCKSSQSLNSSNQKNYLAWX ^{49} QQKPGQX ^{50} PX ^{51} X ^{52} X
 ^{53} IYFTSTRQSGVPDRFX ^{54} GSGSGTDFLTIX ^{55} SLQAEDVAVYX ^{56} CQQHYIPLTFGQGTGX ^{57} X
 ^{58} EIK (SEQ ID NO. 8)
 X^{46} = V or I

- $X^{47} = V \text{ or } Q$
 $X^{48} = V \text{ or } A$
 $X^{49} = Y \text{ or } F$
 $X^{50} = S \text{ or } P$
5 $X^{51} = K \text{ or } N$
 $X^{52} = L \text{ or } V$
 $X^{53} = L \text{ or } V$
 $X^{54} = S \text{ or } T$
 $X^{55} = S \text{ or } N$
10 $X^{56} = Y \text{ or } F$
 $X^{57} = K \text{ or } Q$
 $X^{58} = L \text{ or } V.$

3. The humanized antibody according to claim 2, 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 sequences selected from the group consisting of

20 EVQLVESGGGLVQPGGSLKLSCAASGFTFNTNAMHWVRQASGKGLEWVGRIRSKSNYYTTY
YADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVCGSWFAYWGQGTLLTVSS (SEQ
ID NO. 9),

EVQLVESGGGLVQPGGSLKLSCAASGFTFNTNAMHWVRQASGKGLEWVGRIRSKSNYYTTY
YADSVKDRFTISRDDSKSTAYLQMNSLKTEDTAVYYCVCGSWFAYWGQGTLLTVSS (SEQ
ID NO. 10),

25 QVQLVESGGGVVQPGGSLRLSCAASGFTFNTNAMHWVRQAPGRGLEWVARIRSKSNYYTTY
YADSVKDRFTISRDN SKNTLYLQVNSLRAEDTAVYYCVCGSWFAYWGQGTLLTVSS (SEQ
ID NO. 11),

EVQLVESGGGVVQGRSLRLSCAASGFTFNTNAMHWVRQAPGKGLEWVARIRSKSNYYTTY
YADSVKDRFTISRDN SKNTLYLQMNSLRAEDTAVYYCVCGSWFAYWGQGTLLTVSS (SEQ
30 ID NO. 12), and

EVQLVESGGGLVQPGGSLKLSCAASGFTFNTNAMHWVRQASGKGLEWVGRIRSKSNYYTTY
YADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVCGSWFAYWGQGTLLTVSS (SEQ
ID NO. 13); and

- 35 b) a light chain variable region amino acid sequence comprising or having the sequences selected from the group consisting of

DVVMTQSPDSLAVSLGERVTINCKSSQSLLNSSNQKNYLAWYQQKPGQSPKLLIYFTSTRQSG
 VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO. 14),
 DIVMTQSPDSLAVSLGERATINCKSSQSLLNSSNQKNYLAWFQQKPGQPPNLVIYFTSTRQSG
 VPDRFSGSGSGTDFTLTINSLQAEDVAVYFCQQHYSIPLTFGQGTQVEIK (SEQ ID NO. 15),
 5 DIQMTQSPDSLAVSLGERATINCKSSQSLLNSSNQKNYLAWYQQKPGQPPKLLIYFTSTRQSG
 VPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQHYSIPLTFGQGTKVEIK (SEQ ID NO. 16),
 DIVMTQSPDSLAVSLGERATINCKSSQSLLNSSNQKNYLAWFQQKPGQPPKVLIIYFTSTRQSG
 VPDRFTGSGSGTDFTLTISSLQAEDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO. 17), and
 DIVMTQSPDSLAVSLGERATINCKSSQSLLNSSNQKNYLAWYQQKPGQPPKLLIYFTSTRQSG
 10 VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO. 18).

4. The humanized antibody according to claim 3, wherein the said humanized antibody contains a heavy chain variable region amino acid sequence comprising or having the sequence selected from a group consisting of SEQ ID NO. 11 and SEQ ID NO. 12; and a light chain variable region amino acid sequence
 15 comprising or having the sequence selected from a group consisting of SEQ ID NO. 14, SEQ ID NO. 15 and SEQ ID NO. 18.

5. The humanized antibody according to claim 4, wherein the said humanized antibody contains a heavy chain variable region amino acid sequence comprising or having the sequence of SEQ ID NO. 12 and a
 20 light chain variable region amino acid sequence comprising or having the sequence of SEQ ID NO. 18.

6. The humanized antibody according to any one of the preceding claims which has human IgG constant regions of allotype G1m17,1 of the heavy chains and human kappa constant regions of allotype Km3 of the light chains.
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7. A pharmaceutical composition comprising a therapeutically effective amount of a humanized antibody of any one of claims 1-6, which specifically recognizes human CA IX, and a pharmaceutically acceptable carrier, diluent or excipient.

8. The humanized antibody according to any one of claims 1-6 or the pharmaceutical composition of claim 7, for use in the treatment of a disease or disorder selected from cell proliferative disease or disorder, wherein 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,
 30 gastrointestinal (tract) cancer, renal cancer, ovarian cancer, liver cancer, lymphoblastic leukemia,
 35 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.

5 9. The humanized antibody or the pharmaceutical composition for use according to claim 8 in the treatment of breast cancer, mesothelioma, or glioblastoma expressing CA IX.

10 10. A pharmaceutical composition comprising a combination of therapeutically effective amounts of humanized antibodies of any one of claims 1-6, which specifically recognize human CA IX, for use in the treatment of cell proliferative disease or disorder wherein one of the humanized antibodies is administered prior to or subsequently to the administration of a second humanized antibody.

15 11. The humanized antibody or the pharmaceutical composition for use according to any one of claims 8-10, wherein the daily or weekly dose of the humanized antibody to CA IX ranges from 0.001 mg/kg to 15 mg/kg body weight.

12. The humanized antibody or the pharmaceutical composition for use according to any one of claims 8-11, wherein dosage regimen includes:
i) multiple, identical or different doses of the humanized antibody;
20 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.

25 13. The humanized antibody or the pharmaceutical composition for use according to any one of claims 8-12, wherein dosage regimen comprises 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.

30 14. A diagnostic composition comprising at least one humanized antibody of any one of claims 1-6, and at least one carrier, diluent, or excipient.

35 15. 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 14, wherein complex formation beyond a predetermined threshold is indicative of the cancer in said subject.

16. The method for diagnosing a cancer in a subject according to claim 15, wherein the humanized

antibody, according to any one of the claims 1-6, is linked, bound or conjugated to a paramagnetic, radioactive or fluorogenic moiety that is detectable upon imaging.