

Title: Therapeutic binding compounds

The invention relates to the fields of biology, immunology and medicine.

5 Melanoma is caused by malignant melanocytes. It is primarily caused by ultraviolet light exposure. Out of all different types of skin cancer malignant melanoma has the highest rate of mortality. It is estimated that world wide around 55,000 people have died from metastatic melanoma in 2012, a number which is steadily increasing every year. If spread has not yet occurred, most patients are cured by removing the melanoma. Patients with spread melanoma are treated with chemotherapy, radiation therapy and/or recently developed immunotherapies such as adoptive T-cell therapy or so called checkpoint inhibitor antibodies. These new immunotherapies clearly show that the immune system is able to recognize and attack the melanoma tumor cells. However, the response rate to such therapies is less than 50% and 5-years survival rates are around 30%. Therefore, additional treatment options are highly needed. The present invention provides means and methods for counteracting, preventing and/or detecting melanoma and other diseases.

SUMMARY

20 In one aspect, the present invention provides an isolated, synthetic or recombinant antibody, or functional part or functional equivalent thereof, that is specific for an epitope of CD9 comprising at least one amino acid selected from the group consisting of K169, D171, V172 and L173 of the CD9 sequence as depicted in Figure 2.

25 In another aspect, the present invention provides an isolated, synthetic or recombinant antibody or functional part or functional equivalent thereof that competes with antibody AT14-012 for binding to amino acids K169, D171, V172, L173 and F176 of the CD9 sequence as depicted in Figure 2.

30 In yet another aspect, the present invention provides an isolated, recombinant or purified CD9 peptide with a length of at most 60 amino acid residues, wherein said peptide comprises at least 6 amino acid residues that are identical to at least 6 amino acid residues located within CD9 amino acid positions 169-176 as depicted in Figure 2.

35 In yet another aspect, the present invention provides an isolated, synthetic or recombinant nucleic acid molecule with a length of at least 15 nucleotides, or a functional equivalent thereof, encoding at least one CDR sequence of an antibody or functional part or functional equivalent disclosed herein.

In yet another aspect, the present invention provides a nucleic acid molecule or functional equivalent thereof, encoding an antibody or functional part or functional equivalent disclosed herein.

5 In yet another aspect, the present invention provides a vector comprising a nucleic acid molecule or functional equivalent disclosed herein.

In yet another aspect, the present invention provides an isolated or recombinant cell, or a non-human animal, comprising a nucleic acid molecule or functional equivalent disclosed herein or a vector disclosed herein.

10 In yet another aspect, the present invention provides a composition comprising an antibody or functional part or functional equivalent disclosed herein, or a nucleic acid molecule or functional equivalent disclosed herein, or a vector disclosed herein, or a cell disclosed herein.

In yet another aspect, the present invention provides a kit of parts comprising an antibody or functional part or functional equivalent disclosed herein, a nucleic acid
15 molecule or functional equivalent disclosed herein, a vector disclosed herein or a cell disclosed herein and a therapeutic agent useful in the treatment and/or prevention of a disorder associated with CD9-expressing cells, preferably a CD9 positive cancer.

20 In yet another aspect, the present invention provides an antibody or functional part or functional equivalent disclosed herein, or a nucleic acid molecule or functional equivalent disclosed herein, or a vector disclosed herein, or a cell disclosed herein, for use as a medicament or prophylactic agent.

In yet another aspect, the present invention provides an antibody or functional part or functional equivalent disclosed herein, or a nucleic acid molecule or functional equivalent disclosed herein, or a vector disclosed herein, or a cell disclosed herein, for
25 use in a method for at least in part treating or preventing a disorder associated with CD9-expressing cells.

In yet another aspect, the present invention provides an antibody or functional part or functional equivalent disclosed herein for use in diagnosis of a disorder associated with CD9-expressing cells.

30 In yet another aspect, the present invention provides use of an antibody or functional part or functional equivalent disclosed herein for determining whether a sample comprises CD9-expressing cells.

In yet another aspect, the present invention provides a method for determining whether CD9-expressing cells are present in a sample comprising:

35 - contacting said sample with an antibody or functional part or functional equivalent

disclosed herein, and

- allowing said antibody or functional part or functional equivalent to bind CD9-expressing cells, if present, and
- determining whether or not CD9-expressing cells are bound to said antibody or functional part or functional equivalent, thereby determining whether or not CD9-expressing cells are present in said sample.

In yet another aspect, the present invention provides a method for producing an antibody or functional part or functional equivalent disclosed herein, the method comprising providing a cell with a nucleic acid molecule or functional equivalent or a vector disclosed herein, and allowing said cell to translate said nucleic acid molecule or functional equivalent or vector, thereby producing said antibody or functional part or functional equivalent disclosed herein, the method preferably further comprising harvesting, purifying and/or isolating said antibody or functional part or functional equivalent disclosed herein.

In yet another aspect, the present invention provides use of a therapeutically effective amount of an antibody or functional part or functional equivalent disclosed herein, or a nucleic acid molecule or functional equivalent disclosed herein, or a vector disclosed herein, or a cell disclosed herein, or a composition disclosed herein in the manufacture of a medicament for at least in part treating and/or preventing a disorder associated with CD9-expressing cells.

In yet another aspect, the present invention provides an *ex vivo* method for determining whether an individual is suffering from a CD9-positive cancer, the method comprising:

- contacting tumor cells from said individual with an antibody or functional part or functional equivalent disclosed herein,
- allowing said antibody or functional part or functional equivalent to bind CD9-expressing cells, if present, and
- determining whether or not CD9-expressing cells are bound to said antibody or functional part or functional equivalent, thereby determining whether or not said individual is suffering from a CD9-positive cancer.

In yet another aspect, the present invention provides an antibody disclosed herein, or an antibody for use disclosed herein, or a use disclosed herein, or a method disclosed herein, wherein said antibody is antibody AT14-012 or a functional part or functional equivalent thereof.

In yet another aspect, the present invention provides an isolated, synthetic or recombinant nucleic acid molecule, or a functional equivalent thereof, encoding a CD9 peptide disclosed herein.

5 In yet another aspect, the present invention provides a vector comprising a nucleic acid molecule or functional equivalent disclosed herein.

In yet another aspect, the present invention provides a host cell comprising a nucleic acid molecule or functional equivalent disclosed herein, and/or a vector disclosed herein.

10 In yet another aspect, the present invention provides use of a CD9 peptide disclosed herein, or a nucleic acid molecule or functional equivalent disclosed herein, or a vector disclosed herein, for producing, binding, detecting and/or obtaining an immune cell and/or an antibody, or a functional part or functional equivalent thereof, that is specific for CD9.

15 In yet another aspect, the present invention provides a method for producing a CD9-specific immune cell or a CD9-specific antibody, wherein a non-human animal is to be immunized with a CD9 peptide disclosed herein or with a nucleic acid molecule or functional equivalent disclosed herein or with a vector disclosed herein.

In yet another aspect, the present invention provides an antibody or immune cell obtainable by a method disclosed herein.

20 In yet another aspect, the present invention provides an immunogenic composition comprising a CD9 peptide disclosed herein, or a nucleic acid molecule or functional equivalent disclosed herein or a vector disclosed herein.

In yet another aspect, the present invention provides a diagnostic kit comprising a CD9 peptide disclosed herein and means for detecting antibody-bound or immune cell-
25 bound CD9 peptides.

In yet another aspect, the present invention provides a CD9 peptide disclosed herein or a nucleic acid molecule or functional equivalent disclosed herein or a vector disclosed herein for use as a medicament or prophylactic agent.

30 In yet another aspect, the present invention provides a CD9 peptide disclosed herein or a nucleic acid molecule or functional equivalent disclosed herein or a vector disclosed herein for use in immunotherapy.

In yet another aspect, the present invention provides a CD9 peptide disclosed herein for use as a diagnostic agent.

DETAILED DESCRIPTION

Some embodiments of the present invention provide a patient-derived, human antibody that is specific for CD9. Importantly, this antibody is derived from a late stage IV melanoma patient who was in complete remission after immunotherapy and is still
5 alive and well 10 years after treatment. The human antibody, designated AT14-012, is able to bind CD9-containing cells like melanoma, pancreas carcinoma, esophagus carcinoma and colon carcinoma cells.

The transmembrane protein CD9, also referred to as, amongst other things, MRP-1, MIC3, DRAP-27 and TSPAN-29, is a tetraspanin with a molecular weight of
10 about 23-27 kDa. It is ubiquitously present on the surface of many kinds of cells, including melanocytes, endothelial cells, certain types of nervous cells, musculoskeletal cells and certain types of immune cells. CD9 is also present on platelets. CD9 has four transmembrane domains, a small intracellular loop and two extracellular loops, which are referred to as the EC1 domain and the EC2 domain (Figure 1). CD9 interacts with
15 numerous other proteins, such as the most important integrins (Beta1 integrin), EWI proteins (EWI-2 and EWI-F), CD81, CD63 and EGFR. CD9 plays amongst other things a role in cell adhesion, proliferation and migration, including tumor proliferation and metastasis. In view of the presence of CD9 on the cell surface of many kinds of cells,

48. A method for determining whether CD9-expressing cells are present in a sample comprising:
- contacting said sample with an antibody or functional part or functional equivalent according to any one of claims 1-15, and
 - 5 - allowing said antibody or functional part or functional equivalent to bind CD9-expressing cells, if present, and
 - determining whether or not CD9-expressing cells are bound to said antibody or functional part or functional equivalent, thereby determining whether or not CD9-expressing cells are present in said sample.
- 10 49. A method according to claim 48, wherein said CD9 expressing cells are CD9 positive tumor cells.
50. A method for producing an antibody or functional part or functional equivalent according to any one of claims 1-15, the method comprising providing a cell with a nucleic acid molecule or functional equivalent or a vector according to any one of
- 15 claims 19-27, and allowing said cell to translate said nucleic acid molecule or functional equivalent or vector, thereby producing said antibody or functional part or functional equivalent according to any one of claims 1-15, the method preferably further comprising harvesting, purifying and/or isolating said antibody or functional part or functional equivalent according to any one of claims 1-15.
- 20 51. Use of a therapeutically effective amount of an antibody or functional part or functional equivalent according to any one of claims 1-15, or a nucleic acid molecule or functional equivalent according to any one of claims 19-26, or a vector according to claim 27, or a cell according to claim 28, or a composition according to claim 29 or 30 in the manufacture of a medicament for at least in part treating and/or
- 25 preventing a disorder associated with CD9-expressing cells.
52. A use according to claim 51 wherein said disorder is selected from the group consisting of CD9 positive cancer, osteoporosis, arthritis, lung inflammation, COPD, colitis, and a disorder associated with innate lymphoid cells.
53. A use according to claim 52, wherein said CD9 positive cancer is selected from the
- 30 group consisting of melanoma, colorectal cancer, pancreatic cancer, esophageal cancer, lung cancer, breast cancer, ovarian cancer, stomach cancer, squamous cell carcinoma, AML, multiple myeloma, gastric cancer, liver cancer, brain cancer, Kaposi sarcoma, carcinoma mucoepidermoid, choriocarcinoma, fibrosarcoma, cervical carcinoma, glioma, adenocarcinoma, lung adenocarcinoma, non-small-cell
- 35 lung carcinoma, bladder cancer and small cell lung cancer.

54. A use according to any one of claims 51-53 whereby said antibody or functional part or functional equivalent or nucleic acid molecule or functional equivalent or vector or cell is combined with a therapeutic agent useful in the treatment and/or prevention of a disorder associated with CD9-expressing cells, preferably a CD9 positive cancer.
- 5 55. A use according to claim 54 wherein said agent is an agent capable of stimulating C3 convertase formation or capable of counteracting inhibition of C3 convertase formation.
56. A use according to claim 55 wherein said agent is a CD55 blocking antibody.
57. A use according to claim 54 wherein said agent is a blocking antibody specific for a
10 co-inhibitory T cell molecule.
58. A use according to claim 57 wherein said antibody is selected from the group consisting of an anti-CTLA4 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-SIRPα antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-CD276 antibody, an anti-CD272 antibody, an anti-
15 KIR antibody, an anti-A2AR antibody, an anti-VISTA antibody and an anti-IDO antibody, and preferably is a PD1 blocking antibody or a PDL1 blocking antibody.
59. An *ex vivo* method for determining whether an individual is suffering from a CD9-positive cancer, the method comprising:
- contacting tumor cells from said individual with an antibody or functional part or
20 functional equivalent according to any one of claims 1-15,
 - allowing said antibody or functional part or functional equivalent to bind CD9-expressing cells, if present, and
 - determining whether or not CD9-expressing cells are bound to said antibody or functional part or functional equivalent, thereby determining whether or nor said
25 individual is suffering from a CD9-positive cancer.
60. An antibody according to any one of claims 1-15, or an antibody for use according to any one of claims 36-45, or a use according to any one claims 46, 47, and 51-58, or a method according to any one of claims 48-50 and 59, wherein said antibody is antibody AT14-012 or a functional part or functional equivalent thereof.
- 30 61. An isolated, synthetic or recombinant nucleic acid molecule, or a functional equivalent thereof, encoding a CD9 peptide according to any one of claims 16-18.
62. A vector comprising a nucleic acid molecule or functional equivalent according to claim 61.
63. A host cell comprising a nucleic acid molecule or functional equivalent according to
35 claim 61, and/or a vector according to claim 62.

64. Use of a CD9 peptide according to any one of claims 16-18, or a nucleic acid molecule or functional equivalent according to claim 61, or a vector according to claim 62, for producing, binding, detecting and/or obtaining an immune cell and/or an antibody, or a functional part or functional equivalent thereof, that is specific for CD9.
- 5 65. A method for producing a CD9-specific immune cell or a CD9-specific antibody, wherein a non-human animal is to be immunized with a CD9 peptide according to any one of claims 16-18 or with a nucleic acid molecule or functional equivalent according to claim 61 or with a vector according to claim 62.
66. A method according to claim 65, wherein a CD9-specific immune cell or antibody is
10 to be further harvested from said non-human animal.
67. An antibody or immune cell obtainable by a method according to claim 65 or 66.
68. An immunogenic composition comprising a CD9 peptide according to any one of claims 16-18, or a nucleic acid molecule or functional equivalent according to claim 61 or a vector according to claim 62.
- 15 69. A diagnostic kit comprising a CD9 peptide according to any one of claims 16-18 and means for detecting antibody-bound or immune cell-bound CD9 peptides.
70. A CD9 peptide according to any one of claims 16-18 or a nucleic acid molecule or functional equivalent according to claim 61 or a vector according to claim 62 for use as a medicament or prophylactic agent.
- 20 71. A CD9 peptide according to any one of claims 16-18 or a nucleic acid molecule or functional equivalent according to claim 61 or a vector according to claim 62 for use in immunotherapy.
72. A CD9 peptide according to any one of claims 16-18 for use as a diagnostic agent.