

Title: Therapeutic binding compounds

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

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

In one aspect, the present disclosure refers to an isolated, synthetic or recombinant antibody, ~~or functional part 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 and that comprises:

- a heavy chain CDR1 sequence DYAMH or DYAMY; and
- a heavy chain CDR2 sequence GISWNSGSIVYADSVKG; and
- a heavy chain CDR3 sequence AVSGYYPYFDY or AVSGYFPYFDY or AVSGYYPYFHY or AVSGYFPYFHY; and
- a light chain CDR1 sequence KSSQSVLYSSNNKNYLG; and
- a light chain CDR2 sequence WASTRES or WASIRES; and
- a light chain CDR3 sequence QQYYTTP; ~~or a functional part of said antibody.~~

In another ~~aspect~~example, the present disclosure refers to an isolated, synthetic or recombinant antibody or functional part thereof that competes with an antibody as disclosed herein for binding to amino acids K169, D171, V172, L173 and F176 of the CD9 sequence as depicted in Figure 2.

In yet another example, the present disclosure refers to an isolated, recombinant or purified CD9 peptide with a length of at most 35 amino acid residues, wherein said peptide comprises amino acids corresponding to K169, D171, V172, L173 and F176 of the CD9 sequence as depicted in Figure 2.

In yet another aspect, the present disclosure refers to an isolated, synthetic or recombinant nucleic acid molecule with a length of at least 15 nucleotides, encoding at least the heavy chain CDR 1-3 and the light chain CDR 1-3 sequences of an antibody or functional part disclosed herein.

Claims

1. An isolated, synthetic or recombinant antibody ~~_, or functional part 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 and that comprises:
- a heavy chain CDR1 sequence DYAMH or DYAMY; and
 - a heavy chain CDR2 sequence GISWNSGSIVYADSVKG; and
 - a heavy chain CDR3 sequence AVSGYYPYFDY or AVSGYFPYFDY or AVSGYYPYFHY or AVSGYFPYFHY; and
 - a light chain CDR1 sequence KSSQSVLYSSNNKNYLG; and
 - a light chain CDR2 sequence WASTRES or WASIRES; and
 - a light chain CDR3 sequence QQYYTTP; ~~or a functional part of said antibody.~~
2. An antibody or functional part according to claim 1, comprising a heavy chain variable region sequence having at least 80% sequence identity with the sequence EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSIVYADSVKGRFTISRDNANKNSLYLQLNSLRAEDTAFYYCAKAVSGYYPYFDYWGQGILVTVSS and a light chain variable region sequence having at least 80% sequence identity with the sequence DIVMTQSPDLSVSLGERATINCKSSQSVLYSSNNKNYLGWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYTTPSTFGQGTRL EIK.
3. An antibody or functional part according to claim 1 or 2 comprising:
- a heavy chain variable region sequence EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSIVYADSVKGRFTISRDNANKNSLYLQLNSLRAEDTAFYYCAKAVSGYYPYFDYWGQGILVTVSS, or EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMYWVRQAPGKGLEWVSGISWNSGSIVYADSVKGRFTISRDNANKNSLYLQLNSLRAEDTAFYYCAKAVSGYFPYFDYWGQGILVTVSS or EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSIVYADSVKGRFTISRDNANKNSLYLQLNSLRAEDTAFYYCAKAVSGYFPYFDYWGQGILVTVSS or EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGIS

WNSGSIVYADSVKGRFTISRDNNAKNSLYLQLNSLRAEDTAFYYCAKAVSGYFP
YFHYWGQGILVTVSS or

EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMYWVRQAPGKGLEWVSGIS
WNSGSIVYADSVKGRFTISRDNNAKNSLYLQLNSLRAEDTAFYYCAKAVSGYFP
YFDYWGQGILVTVSS or

EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMYWVRQAPGKGLEWVSGIS
WNSGSIVYADSVKGRFTISRDNNAKNSLYLQLNSLRAEDTAFYYCAKAVSGYFP
YFDYWGQGILVTVSS or

EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMYWVRQAPGKGLEWVSGIS
WNSGSIVYADSVKGRFTISRDNNAKNSLYLQLNSLRAEDTAFYYCAKAVSGYFP
YFHYWGQGILVTVSS or and

EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMYWVRQAPGKGLEWVSGIS
WNSGSIVYADSVKGRFTISRDNNAKNSLYLQLNSLRAEDTAFYYCAKAVSGYFP
YFDYWGQGILVTVSS or

EVQVVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGIS
WNSGSIVYADSVKGRFTISRDNNAKNSLYLQLNSLRAEDTAFYYCAKAVSGYFP
YFHYWGQGILVTVSS or

EVQVVESGGGLVQPGRSLRLSCAASGFNFDDYAMHWVRQAPGKGLEWVSGIS
WNSGSIVYADSVKGRFTISRDNNAKNSLYLQLNSLRAEDTAFYYCAKAVSGYFP
YFDYWGQGILVTVSS or

EVQVVESGGGLVQPGRSLRLSCAASGFNFDDYAMHWVRQAPGKGLEWVSGIS
WNSGSIVYADSVKGRFTISRDNNAKNSLYLQLNSLRAEDTAFYYCAKAVSGYFP
YFHYWGQGILVTVSS or

EVQVVESGGGLVQPGRSLRLSCAASGFNFDDYAMYWVRQAPGKGLEWVSGIS
WNSGSIVYADSVKGRFTISRDNNAKNSLYLQLNSLRAEDTAFYYCAKAVSGYFP
YFHYWGQGILVTVSS; and

- a light chain variable region sequence

DIVMTQSPDLSVSLGERATINCKSSQSVLYSSNNKNYLGWYQQKPGQPPKLL
IYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYTTPSTFGQG
TRLEIK or

DIVMTQSPDLSVSLGERATINCKSSQSVLYSSNNKNYLGWYQQKPGQPPKLL
IYWASIRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYTTPSTFGQG
TRLEIK.

4. An antibody or functional part according to any one of claims 1-3, that is a human antibody or functional part thereof.

5. An antibody or functional part according to any one of claims 1-4, that is able to bind melanoma cells, colon carcinoma cells, pancreas carcinoma cells and esophagus carcinoma cells.

6. An antibody or functional part according to any one of claims 1-5, wherein said antibody is of the IgG isotype, preferably IgG1 or IgG3.

7. An antibody or functional part according to claim 6 comprising an arginine at amino acid position 345.

~~8. An isolated, synthetic or recombinant antibody or functional part thereof that competes with an antibody according to claim 1 for binding to amino acids K169, D171, V172, L173 and F176 of the CD9 sequence as depicted in Figure 2.~~

~~9.8.~~ An antibody or functional part according to any one of claims 1-~~78~~, that is coupled to another compound.

~~10.9.~~ An antibody or functional part according to claim ~~89~~, wherein said other compound is a detectable label, a chemotherapeutic drug, a toxic moiety, an immunomodulatory molecule, another CD9-specific binding compound, or a radioactive compound.

~~11.10.~~ An isolated, synthetic or recombinant nucleic acid molecule with a length of at least 15 nucleotides, encoding at least the heavy chain CDR 1-3 and the light chain CDR 1-3 sequences of an antibody or functional part according to any one of claims 1-7.

~~12.11.~~ A nucleic acid molecule according to claim ~~101~~, that encodes at least the heavy chain variable region sequence and the light chain variable region sequence of an antibody or functional part according to any one of claims 1-7.

~~13.12.~~ A nucleic acid molecule according to claim ~~101~~ or ~~112~~, comprising the following sequences:

- gat tat gcc atg cac; and
- ggt att agt tgg aat agt ggt agc ata gtc tat gcg gac tct gtg aag ggc; and
- gcc gtg agt ggt tat tat ccc tac ttt gac tac; and
- aag tcc agc cag agt gtt tta tac agc tcc aac aat aag aac tac tta ggt; and
- tgg gca tct acc cgg gaa tcc; and
- . cag caa tat tat act act cct.

~~14.13.~~ A nucleic acid molecule according to any one of claims ~~101-123~~, comprising a sequence that has at least 80% sequence identity with the sequence gaa gtg cag gtg gtg gag tct ggg gga ggc ttg gta cag cct ggc agg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttt gat gat tat gcc atg cac tgg gtc cgg caa gct cca ggg aag ggc ctg gag tgg gtc tca

ggt att agt tgg aat agt ggt agc ata gtc tat gcg gac tct gtg aag ggc cga ttc acc atc tcc
aga gac aac gcc aag aac tcc ctg tat ctg caa ctg aac agt ctg aga gct gag gac acg gcc ttc
tat tac tgt gca aaa gcc gtg agt ggt tat tat ccc tac ttt gac tac tgg ggc cag gga att ttg gtc
acc gtc tcc tca, and comprising a sequence that has at least 80% sequence identity
5 with the sequence gac atc gtg atg acc cag tct cca gac tcc ctg tct gtg tct ctg ggc gag agg
gcc acc atc aac tgc aag tcc agc cag agt gtt tta tac agc tcc aac aat aag aac tac tta ggt
tgg tac cag cag aaa cca gga cag cct cct aag ctg ctc att tac tgg gca tct acc cgg gaa tcc
ggg gtc cct gac cga ttc agt ggc agc ggg tct ggg aca gat ttc act ctc acc atc agc agc ctg
cag gct gaa gat gtg gca gtt tat tac tgt cag caa tat tat act act cct tcc acc ttc ggc caa ggg
10 aca cga ctg gag att aaa.

~~15.14.~~ A nucleic acid molecule encoding an antibody or functional part according to any one of claims 1-7.

~~16.15.~~ A nucleic acid molecule according to any one of claims ~~101-145~~, that comprises cDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), or a DNA/RNA helix.

15 ~~17.16.~~ A nucleic acid molecule according to any one of claims ~~101-156~~, that is codon optimized for expression in a non-human host cell.

~~18.17.~~ A vector comprising a nucleic acid molecule according to any one of claims ~~101-167~~.

20 ~~19.18.~~ An isolated or recombinant cell, or a non-human animal, comprising a nucleic acid molecule according to any one of claims ~~101-167~~ or a vector according to claim ~~178~~.

~~20.19.~~ A composition comprising an antibody or functional part according to any one of claims 1-~~910~~, or a nucleic acid molecule according to any one of claims ~~101-167~~, or a vector according to claim ~~178~~, or a cell according to claim ~~189~~.

25 ~~21.20.~~ A composition according to claim ~~1920~~, wherein said composition is a pharmaceutical composition that also comprises a pharmaceutically acceptable carrier, diluent or excipient.

30 ~~22.21.~~ A kit of parts comprising an antibody or functional part according to any one of claims 1-~~910~~, a nucleic acid molecule according to any one of claims ~~101-167~~, a vector according to claim ~~178~~ or a cell according to claim ~~189~~ and a therapeutic agent useful in the treatment and/or prevention of a CD9 positive cancer, osteoporosis, arthritis, lung inflammation, COPD, colitis, a virus infection, a bacterial infection, CMV retinitis, oral candidiasis, Glanzmann thrombasthenia, or diphtheria.

~~23.22.~~ The kit of parts according to claim ~~212~~, wherein said agent is a complement regulatory protein or an agent capable of stimulating C3 convertase formation or capable of counteracting inhibition of C3 convertase formation.

~~24.23.~~ The kit of parts according to claim ~~223~~, wherein said agent is a CD55 blocking antibody or a CD46 blocking antibody or a CD59 blocking antibody or an antibody against another complement regulatory protein, preferably a CD55 blocking antibody.

~~25.24.~~ The kit of parts according to claim ~~212~~, wherein said agent is a blocking antibody specific for a co-inhibitory T cell molecule, or a small molecule against mutated BRAF, or another chemotherapy agent.

~~26.25.~~ The kit of parts according to claim ~~245~~, 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-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.

~~27.26.~~ An antibody or functional part according to any one of claims 1-~~910~~, or a nucleic acid molecule according to claim ~~145~~, or a vector according to claim ~~178~~, or a cell according to claim ~~189~~, for use as a medicament.

~~28.27.~~ An antibody or functional part according to any one of claims 1-~~910~~, or a nucleic acid molecule according to claim ~~145~~, or a vector according to claim ~~178~~, or a cell according to claim ~~189~~, for use in a method for at least in part treating a CD9 positive cancer, osteoporosis, arthritis, lung inflammation, COPD, colitis, a virus infection, a bacterial infection, CMV retinitis, oral candidiasis, Glanzmann thrombasthenia, or diphtheria.

~~29.28.~~ An antibody or functional part according to any one of claims 1-~~910~~ for *in vivo* use in diagnosis of a CD9 positive cancer, osteoporosis, arthritis, lung inflammation, COPD, colitis, a virus infection, a bacterial infection, CMV retinitis, oral candidiasis, Glanzmann thrombasthenia, or diphtheria.

~~30.29.~~ *In vitro* use of an antibody or functional part according to any one of claims 1-~~910~~ for diagnosing a CD9 positive cancer, osteoporosis, arthritis, lung inflammation, COPD, colitis, a virus infection, a bacterial infection, CMV retinitis, oral candidiasis, Glanzmann thrombasthenia, or diphtheria.

~~31.30.~~ An antibody or functional part or nucleic acid molecule or vector or cell for use according to claim 27 or 28, or use according to claim ~~2930~~, wherein said CD9

positive cancer is selected from the 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 lung carcinoma, bladder cancer and small cell lung cancer.

~~32~~31. An antibody or functional part or nucleic acid molecule or vector or cell for use according to ~~any one of claims 267~~ or ~~278~~ whereby said antibody or functional part or nucleic acid molecule or vector or cell is to be combined with a therapeutic agent useful in the treatment and/or prevention of a CD9 positive cancer, osteoporosis, arthritis, lung inflammation, COPD, colitis, a virus infection, a bacterial infection, CMV retinitis, oral candidiasis, Glanzmann thrombasthenia, or diphtheria.

~~33~~32. An antibody or functional part or nucleic acid molecule or vector or cell for use according to claim ~~312~~ wherein said agent is a complement regulatory protein or an agent capable of stimulating C3 convertase formation or capable of counteracting inhibition of C3 convertase formation.

~~34~~33. An antibody or functional part or nucleic acid molecule or vector or cell for use according to claim ~~323~~ wherein said agent is a CD55 blocking antibody or a CD46 blocking antibody or a CD59 blocking antibody or an antibody against another complement regulatory protein, preferably a CD55 blocking antibody.

~~35~~34. An antibody or functional part or nucleic acid molecule or vector or cell for use according to claim ~~312~~ wherein said agent is a blocking antibody specific for a co-inhibitory T cell molecule, or a small molecule against mutated BRAF, or another chemotherapy agent.

~~36~~35. An antibody or functional part or nucleic acid molecule or vector or cell for use according to claim ~~345~~ 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-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.

~~37~~36. Use of an antibody or functional part according to any one of claims ~~1-910~~ for determining whether a sample comprises CD9-expressing cells.

~~38~~37. Use according to claim ~~36~~7, for determining whether a sample comprises CD9-expressing tumor cells.

~~39~~38. A method for determining whether CD9-expressing cells are present in a sample comprising:

- contacting said sample with an antibody or functional part according to any one of claims 1-~~9~~10, and
- allowing said antibody or functional part to bind CD9-expressing cells, if present, and
- determining whether or not CD9-expressing cells are bound to said antibody or functional part, thereby determining whether or not CD9-expressing cells are present in said sample.

~~40~~39. A method according to claim ~~38~~9, wherein said CD9 expressing cells are CD9 positive tumor cells.

~~41~~40. A method for producing an antibody or functional part according to any one of claims 1-7, the method comprising providing a cell with a nucleic acid molecule or a vector according to claim ~~14~~5 or ~~17~~8, and allowing said cell to translate said nucleic acid molecule or vector, thereby producing said antibody or functional part according to any one of claims 1-7, the method preferably further comprising harvesting, purifying and/or isolating said antibody or functional part according to any one of claims 1-7.

~~42~~41. Use of a therapeutically effective amount of an antibody or functional part according to any one of claims 1-~~9~~10, or a nucleic acid molecule according to claim ~~14~~5, or a vector according to claim ~~17~~8, or a cell according to claim ~~18~~9, or a composition according to claim ~~19~~20 or ~~20~~1 in the manufacture of a medicament for at least in part treating a CD9 positive cancer, osteoporosis, arthritis, lung inflammation, COPD, colitis, a virus infection, a bacterial infection, CMV retinitis, oral candidiasis, Glanzmann thrombasthenia, or diphtheria.

~~43~~42. A use according to claim ~~41~~2, wherein said CD9 positive cancer is selected from the 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 lung carcinoma, bladder cancer and small cell lung cancer.

~~44-43.~~ A use according to claim ~~412~~ or ~~423~~, whereby said antibody or functional part or nucleic acid molecule or vector or cell is to be combined with a therapeutic agent useful in the treatment and/or prevention of a CD9 positive cancer, osteoporosis, arthritis, lung inflammation, COPD, colitis, a virus infection, a bacterial infection, CMV retinitis, oral candidiasis, Glanzmann thrombasthenia, or diphtheria.

~~45-44.~~ A use according to claim ~~434~~ wherein said agent is a complement regulatory protein or an agent capable of stimulating C3 convertase formation or capable of counteracting inhibition of C3 convertase formation.

~~46-45.~~ A use according to claim ~~445~~ wherein said agent is a CD55 blocking antibody or a CD46 blocking antibody or a CD59 blocking antibody or an antibody against another complement regulatory protein, preferably a CD55 blocking antibody.

~~47-46.~~ A use according to claim ~~434~~ wherein said agent is a blocking antibody specific for a co-inhibitory T cell molecule, or a small molecule against mutated BRAF, or another chemotherapy agent.

~~48-47.~~ A use according to claim ~~467~~ 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-SIRP α antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-CD276 antibody, an anti-CD272 antibody, an anti-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.

~~49-48.~~ 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 according to any one of claims 1-~~910~~,
- allowing said antibody or functional part to bind CD9-expressing cells, if present, and
- determining whether or not CD9-expressing cells are bound to said antibody or functional part, thereby determining whether or not said individual is suffering from a CD9-positive cancer.

~~50-49.~~ An antibody according to any one of claims 1-7, or an antibody for use according to any one of claims ~~267-289~~ and ~~301-356~~, or a use according to any one claims ~~367-378~~ and ~~412-478~~, or a method according to any one of claims ~~389-401~~ and ~~489~~, wherein said antibody is an antibody that comprises:

- a heavy chain CDR1 sequence DYAMH; and
- a heavy chain CDR2 sequence GISWNSGSIVYADSVKG; and

- a heavy chain CDR3 sequence AVSGYYPYFDY; and
 - a light chain CDR1 sequence KSSQSVLYSSNNKNYLG; and
 - a light chain CDR2 sequence WASTRES; and
 - a light chain CDR3 sequence QQYYTTP,
- 5 or a functional part ~~thereof~~ said antibody.

501. A synthetic or recombinant antibody, or a functional part thereof, which
comprises a Fab fragment of an antibody according to any one of claims 1-~~940~~ and a
Fab fragment of a CD55 blocking antibody, a CD46 blocking antibody, a CD59
10 blocking antibody, an anti-CTLA4 antibody, an anti-PD-1 antibody, an anti-PD-L1
antibody, an anti-PD-L2 antibody, an anti-SIRP α antibody, an anti-TIM3 antibody,
an anti-LAG3 antibody, an anti-CD276 antibody, an anti-CD272 antibody, an anti-
KIR antibody, an anti-A2AR antibody, an anti-VISTA antibody or an anti-IDO
antibody, preferably an anti-PD-1 antibody or an anti-PD-L1 antibody.