

CLAIMS

1. Use of an activatable antibody (AA) conjugated to an agent in the manufacture of a medicament for treating, alleviating a symptom of, or delaying the progression of cancer, wherein the AA comprises:
 - a. an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 480, and a light chain comprising an amino acid sequence of SEQ ID NO: 240;
 - b. a masking moiety (MM) coupled to the AB, wherein the MM inhibits the binding of the AB to the mammalian CD166 when the AA is in an uncleaved state, wherein the MM comprises the amino acid sequence of SEQ ID NO: 222; and
 - c. a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease, and wherein the CM comprises the amino acid sequence of SEQ ID NO: 76;

wherein the AB is linked to the CM and the MM is linked to the CM such that the AA in an uncleaved state comprises the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; and

wherein the AA conjugated to the agent is at a dose of 0.25 mg/kg to 6 mg/kg, 0.25 mg/kg to 0.5 mg/kg, 0.5 mg/kg to 1 mg/kg, 1 mg/kg to 2 mg/kg, 2 mg/kg to 4 mg/kg, 4 mg/kg to 5 mg/kg, or 5 mg/kg to 6 mg/kg.
2. Use of an activatable antibody (AA) conjugated to an agent in the manufacture of a medicament for inhibiting or reducing the growth, proliferation, or metastasis of cells expressing CD166, wherein the AA comprises:
 - a. an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 480, and a light chain comprising an amino acid sequence of SEQ ID NO: 240;

- b.** a masking moiety (MM) coupled to the AB, wherein the MM inhibits the binding of the AB to the mammalian CD166 when the AA is in an uncleaved state, wherein the MM comprises the amino acid sequence of SEQ ID NO: 222; and
 - c.** a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease, and wherein the CM comprises the amino acid sequence of SEQ ID NO: 76;
 - wherein the AB is linked to the CM and the MM is linked to the CM such that the AA in an uncleaved state comprises the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; and
 - wherein the AA conjugated to the agent is at a dose of 0.25 mg/kg to 6 mg/kg, 0.25 mg/kg to 0.5 mg/kg, 0.5 mg/kg to 1 mg/kg, 1 mg/kg to 2 mg/kg, 2 mg/kg to 4 mg/kg, 4 mg/kg to 5 mg/kg, or 5 mg/kg to 6 mg/kg.
3. The use according to claim 1, wherein the cancer is breast carcinoma, castration-resistant prostate carcinoma, cholangiocarcinoma, endometrial carcinoma, epithelial ovarian carcinoma, head and neck squamous cell carcinoma, or non-small cell lung cancer.
 4. The use according to claim 2, wherein the cells are breast cells, prostate cells, endometrial cells, ovarian cells, head or neck squamous cells, bile duct cells, or lung cells.
 5. The use according to any one of claims 1-4, wherein the agent is a maytansinoid or derivative thereof.
 6. The use according to any one of claims 1-5, wherein the agent is N2'-deacetyl-N2'-(4-mercapto-4-methyl-1-oxopentyl)-maytansine (DM4).
 7. The use according to any one of claims 1-6, wherein the DM4 is conjugated to the AA via a linker, optionally wherein the linker comprises an N-succinimidyl-4-(2-pyridyldithio) butanoate (SPDB) moiety.

8. The use according to any one of claims 1-7, wherein the AA comprises a linking peptide between the MM and the CM and/or wherein the AA comprises a linking peptide between the CM and AB, optionally wherein the MM and/or the CM are linked to the light chain.
9. The use according to claim 8, wherein linking peptide between MM and the CM comprises the amino acid sequence of SEQ ID NO: 479 and/or wherein linking peptide between CM and the AB comprises the amino acid sequence of SEQ ID NO: 15.
10. The use according to any one of claims 1-9, wherein the AA comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the AA in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM.
11. The use according to any one of claims 1-10, wherein the light chain is linked to a spacer at its N-terminus, optionally wherein the spacer comprises the amino acid sequence of SEQ ID NO: 305.
12. The use according to claim 11, wherein the spacer comprises the amino acid sequence of SEQ ID NO: 305, a first linking peptide (LP1) comprises the amino acid sequence of SEQ ID NO: 479, and a second linking peptide (LP2) comprises the amino acid sequence of GGS.
13. The use according to any one of claims 1-12, wherein a light chain of the AA comprises the sequence of SEQ ID NOs: 314 or 246.
14. The use according to any one of claims 1-13, wherein the cancer is in a subject that has one or more characteristics selected from the following criteria: (a) the subject is at least 18 years of age, (b) the subject has an ECOG performance status of 0-1, (c) the subject has a histologically confirmed diagnosis of an active metastatic cancer, (d) the subject has a histologically confirmed diagnosis of a locally advanced unresectable solid tumor, (e) the subject has a life expectancy of at least 3 months at the time of administration, (f) the subject has a breast carcinoma, (g) the breast carcinoma is ER+, (h) the subject has

received prior anti-hormonal therapy and experienced disease progression, (i) the subject has a triple negative breast cancer and has undergone at least two prior lines of therapy, (j) the subject has castration-resistant prostate carcinoma, (k) the subject has received at least one prior therapy, (l) the subject has cholangiocarcinoma, (m) the subject has failed at least one prior line of gemcitabine-containing regimen, (n) the subject has endometrial carcinoma, (o) the subject has received at least one platinum-containing regimen for extra-uterine or advanced disease, (p) the subject has epithelial ovarian carcinoma, (q) the subject has a platinum-resistant carcinoma, (r) the subject has a platinum refractory ovarian carcinoma, (s) the subject has a BRCA mutation and is refractory to or otherwise ineligible for PARP inhibitors, (t) the subject has a non-BRCA mutation, (u) the subject has received at least one platinum-containing regimen, (v) the subject has received at least one PD-1/PD-L1 inhibitor, (w) the subject has non-small cell lung cancer (NSCLC), (x) the subject has received at least one platinum-containing regimen, (y) the subject has received at least one checkpoint inhibitor, and (z) the subject has received at least one PD-1/PD-L1 inhibitor.

15. The use according to any one of claims 1-14, wherein the dose is 0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg, 5 mg/kg, or 6 mg/kg.
16. The use according to any one of claims 1-14, wherein the AA conjugated to the agent is at a fixed dose of 10 mg to 200 mg, 25 mg to 500 mg, 10 mg to 25 mg, 20 mg to 50 mg, 30 mg to 75 mg, 40 mg to 100 mg, 50 mg to 125 mg, 60 mg to 150 mg, 80 mg to 200 mg, 100 mg to 250 mg, 120 mg to 300 mg, 140 mg to 350 mg, 160 mg to 400 mg, 180 mg to 450 mg, or 200 mg to 500 mg.
17. The use according to any one of claims 1-16, wherein the AA conjugated to the agent is to be administered intravenously, optionally wherein the AA conjugated to the agent is to be administered intravenously every 21 days.
18. The use according to any one of claims 1-15 and 17, wherein the AA conjugated to the agent is to be administered at a dosage based on the subject's actual body weight or

wherein the AA conjugated to the agent is to be administered at a dosage based on the subject's adjusted ideal body weight.

- 19.** Use of an activatable antibody (AA) conjugated to an agent in the manufacture of a medicament for treating, alleviating a symptom of, or delaying the progression of cancer, wherein the AA comprises:

a. an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 480, and a light chain comprising an amino acid sequence of SEQ ID NO: 240;

b. a masking moiety (MM) coupled to the AB, wherein the MM inhibits the binding of the AB to the mammalian CD166 when the AA is in an uncleaved state, wherein the MM comprises the amino acid sequence of SEQ ID NO: 222; and

c. a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease, and wherein the CM comprises the amino acid sequence of SEQ ID NO: 76;

wherein the AB is linked to the CM and the MM is linked to the CM such that the AA in an uncleaved state comprises the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM;

wherein the agent is N2'-deacetyl-N2'-(4-mercapto-4-methyl-1-oxopentyl)-maytansine (DM4);

wherein the DM4 is conjugated to the AA via a linker, wherein the linker comprises an N-succinimidyl-4-(2-pyridyldithio) butanoate (SPDB) moiety; and

wherein the AA conjugated to the agent is at a dose of 0.25 mg/kg to 6 mg/kg, 0.25 mg/kg to 0.5 mg/kg, 0.5 mg/kg to 1 mg/kg, 1 mg/kg to 2 mg/kg, 2 mg/kg to 4 mg/kg, 4 mg/kg to 5 mg/kg, or 5 mg/kg to 6 mg/kg.