

CLAIMS

~~It is claimed:~~ Claims

1. An isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

- (1) SEQ ID NOs:177, 46, 47, 178, 112, and 179, respectively;
- (2) SEQ ID NOs:51, 52, 53, 117, 118, and 119, respectively;
- (3) SEQ ID NOs:54, 55, 56, 120, 121, and 122, respectively;
- (4) SEQ ID NOs:57, 58, 59, 123, 124, and 125, respectively;
- (5) SEQ ID NOs:60, 61, 62, 126, 127, and 128, respectively;
- (6) SEQ ID NOs:180, 181, 182, 129, 130, and 131, respectively;
- (7) SEQ ID NOs:72, 73, 74, 138, 139, and 140, respectively;
- (8) SEQ ID NOs:78, 79, 80, 144, 145, and 146, respectively;
- (9) SEQ ID NOs:81, 82, 83, 147, 148, and 149, respectively;
- (10) SEQ ID NOs:84, 85, 86, 150, 151, and 152, respectively;
- (11) SEQ ID NOs:87, 88, 89, 153, 154, and 155, respectively;
- (12) SEQ ID NOs:90, 91, 92, 156, 157, and 158, respectively;
- (13) SEQ ID NOs:93, 94, 95, 159, 160, and 161, respectively;
- (14) SEQ ID NOs:96, 97, 98, 162, 163, and 164, respectively;
- (15) SEQ ID NOs:99, 100, 101, 165, 166, and 167, respectively;
- (16) SEQ ID NOs:102, 103, 104, 168, 169, and 170, respectively;
- (17) SEQ ID NOs:105, 106, 107, 171, 172, and 173, respectively;
- (18) SEQ ID NOs:108, 109, 110, 174, 175, and 176, respectively; or
- (19) SEQ ID NOs:201, 202, 203, 204, 205, and 206, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds CD47, ~~preferably human CD47.~~

2. The isolated monoclonal antibody or antigen-binding fragment of claim 1, comprising:
 - (a) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, or

43, ~~or~~ and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:2₁;

(b) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:3, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:4₁;

(c) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:5, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:6₁;

(d) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:7, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:8₁;

(e) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:9, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:10₁;

(f) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:11, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:12₁;

(g) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:13, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:14₁;

(h) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:15, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:16₁;

(i) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:17, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:18₁;

(j) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:19, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:20₁;

- (k) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:21, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:22;
- (l) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:23, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:24;
- (m) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:25, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:26;
- (n) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:27, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:28;
- (o) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:29, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:30;
- (p) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:31, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:32;
- (q) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:33, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:34;
- (r) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:35, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:36;
- (s) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:37, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:38;
- (t) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:39, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:40;

(u) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:41, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:42; or

(v) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:43, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:44;

(w) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:183, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:191;

(x) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:183, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:192;

(y) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:183, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:193;

(z) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:184, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:190;

(aa) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:184, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:192;

(bb) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:184, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:193;

(cc) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:185, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:190;

(dd) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:185, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:191;

(ee) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:185, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:193;

(ff) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:185, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:198;

(gg) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:187, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:194;

(hh) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:188, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:194;

(ii) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:188, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:196;

(jj) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:188, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:197; or

(kk) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:199, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:200.

3. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 1 or 2, comprising:

- a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;
- b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;
- c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;
- d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

- e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;
- f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;
- g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;
- h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;
- i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;
- j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;
- k. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22;
- l. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24;
- m. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25, and a light chain variable region having the polypeptide sequence of SEQ ID NO:26;
- n. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:27, and a light chain variable region having the polypeptide sequence of SEQ ID NO:28;
- o. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29, and a light chain variable region having the polypeptide sequence of SEQ ID NO:30;
- p. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31, and a light chain variable region having the polypeptide sequence of SEQ ID NO:32;
- q. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:33, and a light chain variable region having the polypeptide sequence of SEQ ID NO:34;
- r. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35, and a light chain variable region having the polypeptide sequence of SEQ ID NO:36;
- s. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37, and a light chain variable region having the polypeptide sequence of SEQ ID NO:38;

- t. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:39,
and a light chain variable region having the polypeptide sequence of SEQ ID NO:40;
- u. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:41,
and a light chain variable region having the polypeptide sequence of SEQ ID NO:42;
- ~~or~~
- v. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:43,
and a light chain variable region having the polypeptide sequence of SEQ ID NO:44;
- w. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:191;
- x. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:192;
- y. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:193;
- z. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:190;
- aa. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:192;
- bb. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:193;
- cc. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:190;
- dd. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:191;

- ee. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:193;
 - ff. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:198;
 - gg. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:187,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:194;
 - hh. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:194;
 - ii. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:196;
 - jj. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:197; or
 - kk. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:199,
and a light chain variable region having the polypeptide sequence of SEQ ID NO:200.
4. The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1 to 3, wherein the antibody or antigen-binding fragment thereof ~~inhibits the interaction of CD47 and thrombospondin 1 (TSP-1) and/or that of CD47 and SIRP α .~~ is chimeric and/or human or humanized.
5. ~~The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1 to 4, wherein the antibody or antigen-binding fragment thereof is chimeric.~~
6. ~~The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1 to 5, wherein the antibody or antigen-binding fragment thereof is human or humanized.~~
7. ~~The isolated monoclonal antibody or antigen-binding fragment thereof of claim 6, wherein the antibody or antigen-binding fragment thereof comprises:~~

- a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:191;
- b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:192;
- c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:193;
- d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:190;
- e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:192;
- f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:193;
- g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:190;
- h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:191;
- i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:193;
- j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185,
and a light chain variable region having the polypeptide sequence of SEQ ID
NO:198;

- ~~k. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:187, and a light chain variable region having the polypeptide sequence of SEQ ID NO:194;~~
 - ~~l. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:194;~~
 - ~~m. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:196; or~~
 - ~~n. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:197~~
 - ~~o. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:199, and a light chain variable region having the polypeptide sequence of SEQ ID NO:200.~~
- ~~8.5. The isolated monoclonal antibody or antigen-binding fragment of any one of claims 1 to 7, wherein the antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to thrombospondin 1 (TSP1) and/or to signal regulatory protein alpha (SIRP α)- α , inducing macrophage-mediated phagocytosis of cancer cells, and/or binding cancer cells with minimal to undetectable binding to red blood cells.~~
- ~~9. The isolated A bispecific antibody comprising the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1 to 7, wherein the antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells.~~
- ~~10.6. The isolated monoclonal antibody or antigen-binding fragment of any one of claims 1 to 7, wherein the antibody or antigen-binding fragment thereof is capable of binding cancer cells with minimal to undetectable binding to red blood cells~~5.
- ~~11.7. An isolated nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof of any of claims 1 to 10~~5.
- ~~12.8. A vector comprising the isolated nucleic acid of claim 11~~7.
- ~~13.9. A host cell comprising the vector of claim 12~~8.
- ~~14.10. A pharmaceutical composition, comprising the isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1 to 10~~5 and a pharmaceutically acceptable carrier.

15. — A method of blocking binding of CD47 to thrombospondin-1 (TSP1) in a subject in need thereof, comprising administering to the subject the A pharmaceutical composition of claim 14.

16. — A method of blocking binding of CD47 to signal regulatory protein α (SIRP α) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 14.

17. — A method of treating 10 for use in the treatment of cancer in a subject in need thereof, comprising administering to the subject wherein the pharmaceutical composition of claim 14.

18. — A method of treating an inflammatory disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 14.

19. — A method of treating an infectious disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 14.

20. — A method of treating atherosclerosis in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 14.

21. — A method of treating a cardiovascular disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 14.

22. — A method of treating a metabolic disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 14.

23. — A method of treating a radiation-induced injury in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 14.

24. 11. A method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 14 is to be administered to the subject.

25. 12. A method of determining a level of CD47 in a subject, the method comprising:

- a. obtaining a sample from the subject;
- b. contacting the sample with an antibody or antigen-binding fragment thereof of any one of claims 1 to 10-5;
- c. determining a level of CD47 in the subject.

26. — The method of claim 25 12, wherein the sample is a tissue sample

27. — The method of claim 26, wherein the tissue sample is a cancer tissue sample.

28. 13. The method of claim 25, wherein the sample is or a blood sample.

~~29.14.~~ 14. A method of producing the monoclonal antibody or antigen-binding fragment thereof of any one of claims ~~1 to 10~~ 1-5, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof under conditions to produce the monoclonal antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof from the cell or culture.

~~30.15.~~ 15. A method of producing a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment thereof of any one of claims ~~1 to 10~~ 1-5, comprising combining the monoclonal antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

16. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-5, wherein the monoclonal antibody or antigen-binding fragment thereof specifically binds human CD47.

17. The method of claim 13, wherein the tissue sample is a cancer tissue sample.

18. Use of a pharmaceutical composition of claim 10 in the manufacture of a medicament for the treatment of cancer in a subject in need thereof, wherein the pharmaceutical composition is to be administered to the subject.