

# **MULTIFUNCTIONAL MOLECULES THAT BIND TO CALRETICULIN AND USES THEREOF**

## **RELATED APPLICATIONS**

5           This application claims priority to U.S. Provisional Application 62/808,779 filed on February 21, 2019, U.S. Provisional Application 62/818,427 filed on March 14, 2019, and U.S. Provisional Application 62/956,866 filed on January 3, 2020, the entire contents of each of which are hereby incorporated by reference.

## **SEQUENCE LISTING**

10           The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 19, 2020, is named E2070-7021WO\_SL.txt and is 1,760,247 bytes in size.

## **BACKGROUND**

15           Myeloproliferative neoplasms (MPNs) are a group of conditions that cause blood cells to grow abnormally in the bone marrow. Common myeloproliferative neoplasms include primary or idiopathic myelofibrosis (MF), essential thrombocytosis (ET), polycythemia vera (PV), and  
20           chronic myelogenous leukemia (CML). Primary myelofibrosis is a chronic blood cancer in which excessive scar tissue forms in the bone marrow and impairs its ability to produce normal blood cells. Given the ongoing need for improved treatment of myeloproliferative neoplasms such as myelofibrosis, new compositions and treatments targeting myeloproliferative neoplasms are highly desirable.

## **SUMMARY**

25           In one aspect, the present disclosure refers to a multifunctional molecule comprising:

          (i) a first antigen binding domain that binds to a calreticulin protein,  
          and

30           (ii) a second antigen binding domain that binds to a T cell receptor beta variable chain (TCR $\beta$ V) and activates a T cell.

We claim:

1. A multifunctional molecule comprising:
  - (i) a first antigen binding domain that binds to a calreticulin protein, and
  - (ii) a second antigen binding domain that binds to a T cell receptor beta variable chain (TCR $\beta$ V) and activates a T cell.
2. The multifunctional molecule of claim 1, further comprising a cytokine molecule.
3. The multifunctional molecule of claim 1 or 2, further comprising a stromal modifying moiety selected from a hyaluronidase molecule, a collagenase molecule, a chondroitinase molecule, a matrix metalloproteinase molecule, a hyaluronan degrading enzyme, an agent that inhibits hyaluronan synthesis, and an antibody molecule against hyaluronic acid.
4. The multifunctional molecule of any one of claims 1-3, further comprising a TGF- $\beta$  inhibitor.
5. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6358, a VHCDR2 amino acid sequence of SEQ ID NO: 6360, and a VHCDR3 amino acid sequence of SEQ ID NO: 227; and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 251, a VHCDR2 amino acid sequence of SEQ ID NO: 246, and a VLCDR3 amino acid sequence of SEQ ID NO: 248.
6. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 6253, a VHCDR2 amino acid sequence of SEQ ID NO: 6254, and a VHCDR3 amino acid sequence of SEQ ID NO: 6255, and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6259, a VLCDR2 amino acid sequence of SEQ ID NO: 6260, and a VLCDR3 amino acid sequence of SEQ ID NO: 6261.
7. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO:

6253, a VHCDR2 amino acid sequence of SEQ ID NO: 243, and a VHCDR3 amino acid sequence of SEQ ID NO: 6255, and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6259, a VLCDR2 amino acid sequence of SEQ ID NO: 6260, and a VLCDR3 amino acid sequence of SEQ ID NO: 6261.

8. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 6256, a VHCDR2 amino acid sequence of SEQ ID NO: 6257, and a VHCDR3 amino acid sequence of SEQ ID NO: 6258, and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6259, a VLCDR2 amino acid sequence of SEQ ID NO: 6260, and a VLCDR3 amino acid sequence of SEQ ID NO: 6261.

9. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 6256, a VHCDR2 amino acid sequence of SEQ ID NO: 6257, and a VHCDR3 amino acid sequence of SEQ ID NO: 6262, and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6259, a VLCDR2 amino acid sequence of SEQ ID NO: 6260, and a VLCDR3 amino acid sequence of SEQ ID NO: 6261.10. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL) comprising sequences with at least 75% sequence identity to sequences of SEQ ID NOs: 6247 and 6249, respectively.

11. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a VH and a VL comprising sequences with at least 75% sequence identity to sequences of SEQ ID NOs: 6248 and 6249, respectively.

12. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a VH comprising a sequence with at least 75% sequence identity to a sequence selected from the group consisting of 6250, 6372, 234-237, and 244, and a VL comprising a sequence with at least 75% sequence identity to a sequence selected from the group consisting of 6252, 238-242, and 245.

13. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a VH and a VL comprising sequences with at least 75% sequence identity to sequences of SEQ ID NOs: 6251 and 6252, respectively.

14. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a VH comprising a sequence with at least 75% sequence identity to a sequence selected from the group consisting of 6347 and 6349-6351, and a VL comprising a sequence with at least 75% sequence identity to a sequence selected from the group consisting of 6348 and 6352-6356.

15. The multifunctional molecule of any one of claims 1-9, wherein the first antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising

- (a) a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6232, a VHFWR2 amino acid sequence of SEQ ID NO: 6234, a VHFWR3 amino acid sequence of SEQ ID NO: 6236, and a VHFWR4 amino acid sequence of SEQ ID NO: 6230;
- (b) a VHFWR1 amino acid sequence of SEQ ID NO: 6362, a VHFWR2 amino acid sequence of SEQ ID NO: 6363, a VHFWR3 amino acid sequence of SEQ ID NO: 226, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;
- (c) a VHFWR1 amino acid sequence of SEQ ID NO: 229, a VHFWR2 amino acid sequence of SEQ ID NO: 6369, a VHFWR3 amino acid sequence of SEQ ID NO: 6371, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;
- (d) a VHFWR1 amino acid sequence of SEQ ID NO: 6373, a VHFWR2 amino acid sequence of SEQ ID NO: 6369, a VHFWR3 amino acid sequence of SEQ ID NO: 6371, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;
- (e) a VHFWR1 amino acid sequence of SEQ ID NO: 6263, a VHFWR2 amino acid sequence of SEQ ID NO: 6264, a VHFWR3 amino acid sequence of SEQ ID NO: 6265, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;
- (f) a VHFWR1 amino acid sequence of SEQ ID NO: 6357, a VHFWR2 amino acid sequence of SEQ ID NO: 6359, a VHFWR3 amino acid sequence of SEQ ID NO: 6361, and a VHFWR4 amino acid sequence of SEQ ID NO: 6273;
- (g) a VHFWR1 amino acid sequence of SEQ ID NO: 6266, a VHFWR2 amino acid sequence of SEQ ID NO: 6267, a VHFWR3 amino acid sequence of SEQ ID NO: 6268, and a VHFWR4 amino acid sequence of SEQ ID NO: 6269;

- (h) a VHFWR1 amino acid sequence of SEQ ID NO: 6270, a VHFWR2 amino acid sequence of SEQ ID NO: 6271, a VHFWR3 amino acid sequence of SEQ ID NO: 6272, and a VHFWR4 amino acid sequence of SEQ ID NO: 6273;
  - (i) a VHFWR1 amino acid sequence of SEQ ID NO: 6274, a VHFWR2 amino acid sequence of SEQ ID NO: 6275, a VHFWR3 amino acid sequence of SEQ ID NO: 6276, and a VHFWR4 amino acid sequence of SEQ ID NO: 6273; or
  - (j) a VHFWR1 amino acid sequence of SEQ ID NO: 6362, a VHFWR2 amino acid sequence of SEQ ID NO: 6363, a VHFWR3 amino acid sequence of SEQ ID NO: 226, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;
- and/or
- (ii) a light chain variable region (VL) comprising
    - (a) a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6238, a VLFWR2 amino acid sequence of SEQ ID NO: 6240, a VLFWR3 amino acid sequence of SEQ ID NO: 6242, and a VLFWR4 amino acid sequence of SEQ ID NO: 6244;
    - (b) a VLFWR1 amino acid sequence of SEQ ID NO: 250, a VLFWR2 amino acid sequence of SEQ ID NO: 252, a VLFWR3 amino acid sequence of SEQ ID NO: 254, and a VLFWR4 amino acid sequence of SEQ ID NO: 256;
    - (c) a VLFWR1 amino acid sequence of SEQ ID NO: 257, a VLFWR2 amino acid sequence of SEQ ID NO: 259, a VLFWR3 amino acid sequence of SEQ ID NO: 261, and a VLFWR4 amino acid sequence of SEQ ID NO: 263;
    - (d) a VLFWR1 amino acid sequence of SEQ ID NO: 264, a VLFWR2 amino acid sequence of SEQ ID NO: 266, a VLFWR3 amino acid sequence of SEQ ID NO: 268, and a VLFWR4 amino acid sequence of SEQ ID NO: 270;
    - (e) a VLFWR1 amino acid sequence of SEQ ID NO: 271, a VLFWR2 amino acid sequence of SEQ ID NO: 273, a VLFWR3 amino acid sequence of SEQ ID NO: 275, and a VLFWR4 amino acid sequence of SEQ ID NO: 277;
    - (f) a VLFWR1 amino acid sequence of SEQ ID NO: 278, a VLFWR2 amino acid sequence of SEQ ID NO: 280, a VLFWR3 amino acid sequence of SEQ ID NO: 282, and a VLFWR4 amino acid sequence of SEQ ID NO: 284;

- (g) a VLFWR1 amino acid sequence of SEQ ID NO: 6277, a VLFWR2 amino acid sequence of SEQ ID NO: 6278, a VLFWR3 amino acid sequence of SEQ ID NO: 6279, and a VLFWR4 amino acid sequence of SEQ ID NO: 6280;
  - (h) a VLFWR1 amino acid sequence of SEQ ID NO: 6374, a VLFWR2 amino acid sequence of SEQ ID NO: 6375, a VLFWR3 amino acid sequence of SEQ ID NO: 247, and a VLFWR4 amino acid sequence of SEQ ID NO: 249; or
  - (i) a VLFWR1 amino acid sequence of SEQ ID NO: 6281, a VLFWR2 amino acid sequence of SEQ ID NO: 6282, a VLFWR3 amino acid sequence of SEQ ID NO: 6283, and a VLFWR4 amino acid sequence of SEQ ID NO: 6284.
16. The multifunctional molecule of any one of claims 1-15, wherein the second antigen binding domain binds to TCR $\beta$ V12, TCR $\beta$ V6, or TCR $\beta$ V5.
17. The multifunctional molecule of any one of claims 1-16, wherein the second antigen binding domain comprises:
- (i) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 3, a VHCDR2 amino acid sequence of SEQ ID NO: 4, and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and
  - (ii) a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6, a VLCDR2 amino acid sequence of SEQ ID NO: 7, and a VLCDR3 amino acid sequence of SEQ ID NO: 8.
18. The multifunctional molecule of any one of claim 1-16, wherein the second antigen binding domain comprises:
- (i) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 17, a VHCDR2 amino acid sequence of SEQ ID NO: 18, and a VHCDR3 amino acid sequence of SEQ ID NO: 19; and
  - (ii) a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 20, a VLCDR2 amino acid sequence of SEQ ID NO: 21, and a VLCDR3 amino acid sequence of SEQ ID NO: 22.
19. The multifunctional molecule of any one of claims 1-16, wherein the second antigen binding domain comprises:

(i) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 1315, a VHCDR2 amino acid sequence of SEQ ID NO: 1316, and a VHCDR3 amino acid sequence of SEQ ID NO: 1317; and

a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 1321, a VLCDR2 amino acid sequence of SEQ ID NO: 1322, and a VLCDR3 amino acid sequence of SEQ ID NO: 1323.

20. The multifunctional molecule of any one of claims 1-16, wherein the second antigen binding domain comprises:

(i) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 1298, a VHCDR2 amino acid sequence of SEQ ID NO: 1299, and a VHCDR3 amino acid sequence of SEQ ID NO: 1300; and

(ii) a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 1305, a VLCDR2 amino acid sequence of SEQ ID NO: 1306, and a VLCDR3 amino acid sequence of SEQ ID NO: 1307.

21. The multifunctional molecule of any one of claims 1-20, wherein the first antigen binding domain binds to a mutant calreticulin protein.

22. The multifunctional molecule of any one of claims 1-21, wherein the first antigen binding domain binds to an epitope located within the C-terminus of the calreticulin protein, optionally, wherein the first antigen binding domain binds to an epitope located within the amino acid sequence of SEQ ID NO: 6285 or 6286.

23. A polynucleotide comprising a sequence encoding the multifunctional molecule of any one of claims 1-22.

24. A medicament comprising the multifunctional molecule of any one of claims 1-22, and a pharmaceutically acceptable carrier, excipient, or stabilizer.

25. Use of a pharmaceutical composition comprising the multifunctional molecule of any one of claims 1-22 in the manufacture of a medicament for treating cancer in a subject in need thereof,

optionally, wherein the cancer is a hematological cancer or a solid tumor cancer.

26. The use of claim 25, wherein the cancer is a myeloproliferative neoplasm,

optionally, wherein the myeloproliferative neoplasm is primary or idiopathic myelofibrosis (MF), essential thrombocytosis (ET), polycythemia vera (PV), or chronic myelogenous leukemia (CML).

27. A pharmaceutical composition comprising the multifunctional molecule of any one of claims 1-22 for use in therapy.