

higher than those of classical F(ab) or Fv fragments (Ghahroudi, M. A. et al. *FEBS Lett.* 414, 521-526 (1997)).

**[0008]** Mice in which the  $\lambda$  (lambda) light (L) chain locus and/or the  $\lambda$  and  $\kappa$  (kappa) L chain loci have been functionally silenced and antibodies produced by such mice are described in U.S. Patent Nos. 7,541,513 and 8,367,888. Recombinant production of heavy chain-only antibodies in mice and rats has been reported, for example, in WO2006008548; U.S. Application Publication No. 20100122358; Nguyen et al., 2003, *Immunology*; 109(1), 93-101; Brüggemann et al., *Crit. Rev. Immunol.*; 2006, 26(5):377-90; and Zou et al., 2007, *J Exp Med*; 204(13): 3271–3283. The production of knockout rats via embryo microinjections of zinc-finger nucleases is described in Geurts et al., 2009, *Science*, 325(5939):433. Soluble heavy chain-only antibodies and transgenic rodents comprising a heterologous heavy chain locus producing such antibodies are described in U. S. Patent Nos. 8,883,150 and 9,365,655. CAR-T structures comprising single-domain antibodies as binding (targeting) domain are described, for example, in Iri-Sofla et al., 2011, *Experimental Cell Research* 317:2630-2641 and Jamnani et al., 2014, *Biochim Biophys Acta*, 1840:378-386.

### SUMMARY

**[0009]** Examples of the disclosure relate to heavy chain antibodies, including but not limited to UniAbs™, with binding affinity to PSMA. Further examples of the disclosure relate to methods of making such antibodies, compositions comprising such antibodies, and their use in the treatment of disorders that are characterized by the expression of PSMA.

**[0009a]** In one aspect, the present disclosure refers to a multi-specific antibody that binds to to a CD3 protein and a PSMA protein, comprising:

- (a) a heavy chain variable region of an anti-PSMA heavy chain antibody comprising:
  - (i) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; or
  - (ii) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 18; or
  - (iii) a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; and
- (b) a CD3-binding VH domain that is paired with a light chain variable domain, wherein the CD3-binding VH domain comprises a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and a CDR3 sequence of SEQ ID NO: 61 and the light chain variable domain comprises a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and a CDR3 sequence of SEQ ID NO: 64.

## CLAIMS:

1. A multi-specific antibody that binds to a CD3 protein and a PSMA protein, comprising:
  - (a) a heavy chain variable region of an anti-PSMA heavy chain antibody comprising:
    - (i) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; or
    - (ii) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 12, and a CDR3 sequence of SEQ ID NO: 18; or
    - (iii) a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18; and
  - (b) a CD3-binding VH domain that is paired with a light chain variable domain, wherein the CD3-binding VH domain comprises a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and a CDR3 sequence of SEQ ID NO: 61 and the light chain variable domain comprises a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and a CDR3 sequence of SEQ ID NO: 64.
2. The multi-specific antibody of claim 1, which is bispecific.
3. The multi-specific antibody of claim 1 or claim 2, wherein:
  - the CD3-binding VH domain comprises a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and a CDR3 sequence of SEQ ID NO: 61, in a human VH framework; and
  - the light chain variable domain comprises a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and a CDR3 sequence of SEQ ID NO: 64, in a human VL framework.
4. The multi-specific antibody of any one of claims 1 to 3, wherein:
  - the CD3-binding VH domain comprises a sequence having at least 95% sequence identity to the heavy chain variable region sequence of SEQ ID NO: 65; and/or
  - the light chain variable domain comprises a sequence having at least 95% sequence identity to the light chain variable region of SEQ ID NO: 66.
5. The multi-specific antibody of any one of claims 1 to 4, wherein:
  - the CD3-binding VH domain comprises the heavy chain variable region sequence of SEQ ID NO: 65, and/or
  - the light chain variable domain comprises the light chain variable region of SEQ ID NO: 66.

6. The multi-specific antibody of any one of claims 1 to 5, wherein the heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18.
7. The multi-specific antibody of any one of claims 1 to 6, wherein the heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18 in a monovalent configuration.
8. The multi-specific antibody of any one of claims 1 to 7, wherein the heavy chain variable region comprises a sequence having at least 95% sequence identity to the heavy chain variable region sequence of SEQ ID NO: 25.
9. The multi-specific antibody of any one of claims 1 to 8, wherein the heavy chain variable region comprises the heavy chain variable region sequence of SEQ ID NO: 25.
10. The multi-specific antibody of any one of claims 1 to 9, further comprising a heavy chain constant region sequence in the absence of a CH1 sequence.
11. The multi-specific antibody of any one of claims 1 to 10, further comprising a silenced Fc region.
12. A multi-specific antibody of claim 2, wherein:
  - (i) the CD3-binding VH domain comprises a CDR1 sequence of SEQ ID NO: 59, a CDR2 sequence of SEQ ID NO: 60, and a CDR3 sequence of SEQ ID NO: 61, in a human VH framework;
  - (ii) the light chain variable domain comprises a CDR1 sequence of SEQ ID NO: 62, a CDR2 sequence of SEQ ID NO: 63, and a CDR3 sequence of SEQ ID NO: 64, in a human VL framework; and
  - (iii) the heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 11, and a CDR3 sequence of SEQ ID NO: 18, in a human VH framework.
13. The multi-specific antibody of claim 12, further comprising a silenced Fc region.
14. The multi-specific antibody of any one of claims 1 to 13, which is a three-chain antibody-like molecule.

15. The multi-specific antibody of claim 14, comprising:  
a first polypeptide comprising SEQ ID NO: 86;  
a second polypeptide comprising SEQ ID NO: 87; and  
a third polypeptide comprising SEQ ID NO: 88 or 89.
16. The multi-specific antibody of claim 15, wherein the third polypeptide comprises SEQ ID NO: 88.
17. The multi-specific antibody of any one of claims 14 to 16, comprising:  
a first polypeptide consisting of SEQ ID NO: 86;  
a second polypeptide consisting of SEQ ID NO: 87; and  
a third polypeptide consisting of SEQ ID NO: 88.
18. A pharmaceutical composition comprising:  
a multi-specific antibody of any one of claims 1 to 17; and  
a pharmaceutically acceptable carrier.
19. Use of a multi-specific antibody of any one of claims 1 to 17, in the preparation of a medicament for the treatment of a disorder characterized by expression of PSMA.
20. The use of claim 19, wherein the disorder is prostate cancer.
21. The use of claim 19, wherein the disorder is a solid tumor.
22. A polynucleotide encoding a multi-specific antibody of any one of claims 1 to 17.
23. A vector comprising a polynucleotide of claim 22.
24. A cell comprising a vector of claim 23.
25. A method of producing a multi-specific antibody of any one of claims 1 to 17, comprising growing a cell according to claim 24 under conditions permissive for expression of the multi-specific antibody, and isolating the multi-specific antibody from the cell.

26. The multi-specific antibody of any one of claims 1 to 17 for use in therapy.