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(54) Title: MULTISPECIFIC ANTIGEN-BINDING MOLECULES AND USES THEREOF

(57) **Abstract:** The present disclosure provides multispecific antigen-binding molecules and uses thereof. The multispecific antigen-binding molecules comprise a first antigen-binding domain that specifically binds a target molecule, and a second antigen-binding domain that specifically binds an internalizing effector protein. The multispecific antigen-binding molecules of the present disclosure can, in some embodiments, be bispecific antibodies that are capable of binding both a target molecule and an internalizing effector protein. In certain embodiments of the disclosure, the simultaneous binding of the target molecule and the internalizing effector protein by the multispecific antigen-binding molecule of the present disclosure results in the attenuation of the activity of the target molecule to a greater extent than the binding of the target molecule alone. In other embodiments of the disclosure, the target molecule is a tumor associated antigen, and the simultaneous binding of the tumor associated antigen and the internalizing effector protein by the multispecific antigen-binding molecule of the present disclosure causes or facilitates the targeted killing of tumor cells.



AMENDED CLAIMS

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1. A method of inhibiting the growth of a tumor cell or promoting the killing of a tumor cell, the method comprising contacting a tumor cell with a multispecific antigen-binding protein, wherein:

(a) the multispecific antigen-binding protein comprises a first antigen-binding domain that specifically binds a tumor target (T), and a second antigen-binding domain that specifically binds an internalizing effector protein (E);

(b) said tumor cell expresses both T and E;

(c) said T and said E each are expressed at a low level on said cell;

(d) said T in the absence of said multispecific antigen-binding protein does not undergo rapid lysosomal trafficking;

(e) said E in the absence of said multispecific antigen-binding protein undergoes rapid lysosomal trafficking; and

(f) said T undergoes rapid lysosomal targeting and degradation in the presence of said multispecific antigen-binding protein.

2. The method of claim 1 further comprising contacting said tumor cell with a cytotoxic agent.

3. The method of claim 2 wherein the cytotoxic agent is an antibody drug conjugate (ADC) comprising a drug, toxin or radioisotope conjugated to an antigen-binding protein that specifically binds said T.

4. The method of claim 3 wherein the antigen-binding portion of the ADC binds an epitope on T that does not overlap with the epitope on T recognized by the first antigen-binding domain of the multispecific antigen-binding domain.

5. The method of claim 3 or 4, wherein said antigen-binding protein of said ADC is an antibody.

6. The method of claim 2, wherein the cytotoxic agent is a radioisotope, a toxin, or a drug.

7. The method of any one of claims 3-6, wherein said drug is selected from the group consisting of a calicheamicin, auristatin or a maytansine-based drug.

8. The method of any one of claims 1-7, wherein the binding affinity of the multispecific antigen-binding protein for E is less than the binding affinity of the multispecific antigen-binding protein for T.

9. The method of any one of claims 1-8, wherein said target molecule is a membrane protein or a soluble protein.

10. The method of any one of claims 1-9, wherein the multispecific antigen-binding protein is a bispecific antibody.

11. The method of any one of claims 1-10, wherein the target molecule is selected from the group consisting of an allergen, an allergen-binding protein, a cytokine, a cytokine-binding protein, a cytokine receptor, a wnt protein, a wnt-binding protein, a frizzled protein, a growth factor, a growth factor-binding protein, a growth factor receptor, a G protein-coupled receptor, a lipoprotein, a lipoprotein-binding protein, a lipoprotein receptor, a hormone, a hormone-binding protein, a hormone receptor, a membrane channel, a ligand-gated ion channel, a voltage-gated ion channel, a pathogen or fragment thereof, and a GPI-linked protein.

12. The method of any one of claims 1-11, wherein the target molecule is a tumor-associated target selected from the group consisting of AFP, ALK, BAGE proteins, β -catenin, bcr-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CD40, CDK4, CEA, CTLA4, cyclin-B1, CYP1 B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EphA2, Fra-1, FOLR1, GAGE proteins (e.g., GAGE-1, -2), GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE A-3, hTERT, LMP2, MAGE proteins (e.g., MAGE-1, -2, -3, -4, -6, and -12), MART-1, mesothelin, ML-IAP, Mud, Muc16 (CA-125), MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ES01, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PLAC1, PRLR, PRAME, PSMA (FOLH1), RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, survivin, TAG-72, TGF- β , TMPRSS2, Tn, TRP-1, TRP-2, tyrosinase, and uroplakin-3.

13. The method of any one of claims 1-12, wherein the internalization effector comprises a cytoplasmic domain comprising one or more di-leucine motifs.

14. The method of claim 13, wherein the internalization effector comprises a cytoplasmic domain comprising an amino acid sequence of SEQ ID NO: 12.

15. The method of claim 13 or 14, wherein the internalization effector is prolactin receptor (PRLR).

16. The method of claim 15, wherein the target molecule is a receptor tyrosine-protein kinase.

17. The method of claim 16, wherein the target molecule is HER2.

18. The method of claim 16, wherein the multispecific antigen-binding protein is an anti-HER2 x PRLR bispecific antibody.

19. The method of any one of claims 1-18, wherein said ADC comprises a maytansine-based drug conjugated to an anti-HER2 bivalent monospecific antibody or to an anti-PRLR bivalent monospecific antibody.

20. The method of any one of claims 1-19, wherein said tumor cell is a breast cancer cell.

21. The method of claim 20, wherein said breast cancer cell is a T47D cell.

22. A pharmaceutical composition comprising:

(a) a multispecific antigen-binding protein that binds to (i) a rapidly internalized cell surface receptor and (ii) a slowly internalized cell surface receptor, wherein said rapidly internalized cell surface receptor and said slowly internalized cell surface receptor are expressed on the same cell; and

(b) a cytotoxic agent.

23. The pharmaceutical composition of claim 22, wherein the multispecific antigen-binding protein is a bispecific antibody.

24. The pharmaceutical composition of claim 22 or 23, wherein said rapidly internalized cell surface receptor comprises a cytoplasmic domain comprising one or more dileucine motifs.

25. The pharmaceutical composition of claim 24, wherein said rapidly internalized cell surface receptor comprises a cytoplasmic domain comprising an amino acid sequence of SEQ ID NO:12.

26. The pharmaceutical composition of claim 25, wherein said rapidly internalized cell surface receptor is prolactin receptor (PRLR).

27. The pharmaceutical composition of any one of claims 22-26, wherein said slowly internalized cell surface receptor is a receptor tyrosine-protein kinase.

28. The pharmaceutical composition of claim 27, wherein said receptor tyrosine-protein kinase is HER2.

29. The pharmaceutical composition of claim 28, wherein said multispecific antigen-binding protein is an anti-HER2 x PRLR bispecific antibody.

30. The pharmaceutical composition of any one of claims 22-29, wherein said cytotoxic agent comprises a maytansinoid-based drug conjugated to an anti-HER2 bivalent monospecific antibody or to an anti-PRLR bivalent monospecific antibody.