

BINDING MOIETY FOR CONDITIONAL ACTIVATION OF IMMUNOGLOBULIN MOLECULES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application Nos. 62/671,344 filed May 14, 2018; 62/671,349 filed May 14, 2018; 62/756,429 filed Nov 6, 2018; and 62/756,453 filed Nov 6, 2018, each of which is incorporated by reference herein in its entirety.

INCORPORATION BY REFERENCE

[0002] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference, and as if set forth in their entireties.

BACKGROUND OF THE INVENTION

[0003] T cell engagers transiently tether T cells to tumor cells and mediate T cell-directed tumor killing. T cell engagers, such as blinatumomab (BLINCYTO®), have demonstrated clinical activity in several hematological malignancies. Adoption of T cell engagers in solid tumors is limited by the scarcity of tumor antigens with sufficient differential expression between tumor and normal tissue. T cell engagers that are preferentially active in the tumor microenvironment may enable the safe targeting of more solid tumor antigens.

[0004] There is a need to extend the half-life of a therapeutic, diagnostic, or imaging molecule in circulation and also improve its ability to reach its target within an intended location (*e.g.*, a tumor cell) without non-specific binding.

SUMMARY OF THE INVENTION

[0004a] In one aspect, One embodiment provides herein is a binding moiety comprising a non-CDR loop and a cleavable linker, wherein the non-CDR loop provides a binding site for binding to an immunoglobulin molecule and binding moiety is capable of masking the binding of the immunoglobulin molecule to its target and wherein the non-CDR loop is not located in a DE loop of the binding moiety.

[0004b] In another aspect, provided herein is a conditionally active binding protein comprising a binding moiety (M) which comprises a non-CDR loop, a cleavable linker (L), a first target antigen binding domain (T1), and a second target antigen binding domain (T2), wherein the first target antigen binding domain (T1) comprises an immunoglobulin molecule, wherein the non-CDR loop comprises a binding site specific for the first target antigen

binding domain (T1), wherein the binding moiety (M) is capable of masking the binding of the first target antigen binding domain (T1) to the target antigen, wherein the non-CDR loop is not located in a DE loop of the binding moiety and wherein the domains are organized in any one of the following orders: M:L:T1:T2; M:L:T2:T1; T2:T1:L:M; T1:T2:L:M.

[0005] One embodiment provides a binding moiety comprising a non-CDR loop and a cleavable linker, wherein the moiety is capable of masking the binding of binding molecule to its target, wherein the binding molecule comprises an immunoglobulin molecule or a non-immunoglobulin molecule. In some embodiments, the moiety is a natural peptide, a synthetic peptide, an engineered scaffold, or an engineered bulk serum protein. In some embodiments, the engineered scaffold comprises a sdAb, a scFv, a Fab, a VHH, a fibronectin type III domain, immunoglobulin-like scaffold, DARPin, cystine knot peptide, lipocalin, three-helix bundle scaffold, protein G-related albumin-binding module, or a DNA or RNA aptamer scaffold. In some embodiments, the moiety is capable of binding to a bulk serum protein. In some embodiments, the non-CDR loop is from a variable domain, a constant domain, a C1-set domain, a C2-set domain, an I-domain, or any combinations thereof. In some embodiments, the moiety further comprises complementarity determining regions (CDRs). In some embodiments,

CLAIMS

WHAT IS CLAIMED IS:

1. A binding moiety comprising a non-CDR loop and a cleavable linker, wherein the non-CDR loop provides a binding site for binding to an immunoglobulin molecule and binding moiety is capable of masking the binding of ~~an~~the immunoglobulin molecule to its target and wherein the non-CDR loop is not located in a DE loop of the binding moiety.
2. The binding moiety of claim 1, wherein the binding moiety further comprises complementarity determining regions (CDRs) and wherein the CDRs are is capable of binding to a bulk serum protein, wherein the bulk serum protein is albumin, transferrin, IgG1, IgG2, IgG4, IgG3, IgA monomer, Factor XIII, Fibrinogen, IgE, or pentameric IgM.
- ~~3.~~ The binding moiety of claim 2, wherein the bulk serum protein is albumin, transferrin, IgG1, IgG2, IgG4, IgG3, IgA monomer, Factor XIII, Fibrinogen, IgE, or pentameric IgM.
- ~~4.~~3. The binding moiety of claim 1, wherein the immunoglobulin molecule comprises a target antigen binding domain.
- ~~5.~~4. The binding moiety of claim ~~3~~4, wherein the binding moiety is bound to the target antigen binding domain.
- ~~6.~~5. The binding moiety of claim ~~4~~5, wherein the binding moiety is covalently linked to the target antigen binding domain.
- ~~7.~~6. The binding moiety of claim ~~3~~4, wherein the binding moiety is capable of masking the binding of the target antigen binding domain to ~~its~~the target antigen via specific intermolecular interactions between the binding moiety and the target antigen binding domain.
- ~~8.~~ The binding moiety of claim 4, wherein the non-CDR loop provides a binding site specific for the binding of the binding moiety to the target antigen binding domain.
- ~~9.~~7. The binding moiety of claim ~~3~~4, wherein upon cleavage of the cleavable linker, the binding moiety is separated from the target antigen binding domain and the target antigen binding domain binds to ~~its~~the target antigen.
- ~~10.~~8. The binding moiety of claim ~~3~~4, wherein the target antigen binding domain binds to a tumor antigen.
- ~~11.~~9. The binding moiety of claim ~~8~~10, wherein the tumor antigen is selected from the group consisting of: EpCAM, EGFR, HER-2, HER-3, c-Met, FoIR, PSMA, CD38, BCMA, CEA, 5T4, AFP, B7-H3, CDH-6, CAIX, CD117, CD123, CD138, CD166, CD19, CD20, CD205, CD22, CD30, CD33, CD352, CD37, CD44, CD52, CD56, CD70, CD71, CD74, CD79b, DLL3, EphA2, FAP, FGFR2, FGFR3, GPC3, gpA33, FLT-3, gpNMB, HPV-16 E6, HPV-16 E7,

ITGA2, ITGA3, SLC39A6, MAGE, mesothelin, Muc1, Muc16, NaPi2b, Nectin-4, CDH-3, CDH-17, EPHB2, ITGAV, ITGB6, NY-ESO-1, PRLR, PSCA, PTK7, ROR1, SLC44A4, SLITRK5, SLITRK6, STEAP1, TIM1, Trop2, and WT1.

12.10. The binding moiety of claim 34, wherein the target antigen binding domain binds to CD3.

13.11. A conditionally active binding protein comprising a binding moiety (M) which comprises a non-CDR loop, a cleavable linker (L), a first target antigen binding domain (T1), and a second target antigen binding domain (T2), wherein the first target antigen binding domain (T1) comprises an immunoglobulin molecule, wherein the non-CDR loop comprises a binding site specific for the first target antigen binding domain (T1), ~~and~~ wherein the binding moiety (M) is capable of masking the binding of the first target antigen binding domain (T1) to ~~its~~ the target antigen, wherein the non-CDR loop is not located in a DE loop of the binding moiety and wherein the domains are organized in any one of the following orders: M:L:T1:T2; M:L:T2:T1; T2:T1:L:M; T1:T2:L:M.

14.12. The conditionally active binding protein of claim 113, wherein the binding moiety further comprises complementarity determining regions (CDRs) and wherein the CDRs are is capable of binding to a bulk serum protein, wherein the bulk serum protein is albumin, transferrin, IgG1, IgG2, IgG4, IgG3, IgA monomer, Factor XIII, Fibrinogen, IgE, or pentameric IgM.

15.13. The conditionally active binding protein of claim 113, wherein the first target antigen binding domain (T1) binds to a human CD3.

16.14. The conditionally active binding protein of claim 113, wherein the binding moiety (M), the cleavable linker (L), the first target antigen binding domain (T1), and the second target antigen binding domain (T2) are in one of the following configurations from N-terminal to C-terminal: M: L:T1:T2, and T2:T1:L:M.

17.15. The conditionally active binding protein of claim 113, wherein the cleavable linker (L) comprises a cleavage site.

18.16. The conditionally active binding protein of claim 157, wherein upon cleavage of the cleavage site in a tumor microenvironment, the conditionally active binding protein is activated by separation of the binding moiety.

19.17. The conditionally active binding protein of claim 135, wherein the binding site comprises at least one of the following motifs: LDGNEE, QDGNE, QDGNEE, DGNE, and DGNEE.

20.18. The binding moiety of claim 102, wherein the binding moiety comprises a binding site specific for the target antigen binding domain that binds to a human CD3, and

wherein the binding site comprises at least one of the following motifs: LDGNEE, QDGNE, QDGNEE, DGNE, and DGNEE.

~~21.19.~~ The conditionally active binding protein of claim [113](#), wherein the second target antigen binding domain (T2) binds to a tumor antigen.

~~22.20.~~ The conditionally active binding protein of claim [1921](#), wherein the tumor antigen is selected from the group consisting of: EpCAM, EGFR, HER-2, HER-3, c-Met, FoIR, PSMA, CD38, BCMA, CEA, 5T4, AFP, B7-H3, CDH-6, CAIX, CD117, CD123, CD138, CD166, CD19, CD20, CD205, CD22, CD30, CD33, CD352, CD37, CD44, CD52, CD56, CD70, CD71, CD74, CD79b, DLL3, EphA2, FAP, FGFR2, FGFR3, GPC3, gpA33, FLT-3, gpNMB, HPV-16 E6, HPV-16 E7, ITGA2, ITGA3, SLC39A6, MAGE, mesothelin, Muc1, Muc16, NaPi2b, Nectin-4, CDH-3, CDH-17, EPHB2, ITGAV, ITGB6, NY-ESO-1, PRLR, PSCA, PTK7, ROR1, SLC44A4, SLITRK5, SLITRK6, STEAP1, TIM1, Trop2, and WT1.

~~23.21.~~ The conditionally active binding protein of claim [113](#), wherein the binding moiety comprises a sequence selected from the group consisting of SEQ ID Nos. 50 and 259-301.

~~24.22.~~ The binding moiety of claim [38](#), wherein the binding site comprises a sequence selected from the group consisting of SEQ ID Nos. 50 and 259-301.