Selby et al. Cancer Immunol Res., 1(1);32-42, 2013.] Unfortunately, ipilimumab causes generalized (not tumor-specific) activation of T-cell dependent immune responses that leads to immune-related adverse effects which can be life-threatening and are often dose and treatment duration-limiting (Weber, J.S., et al., 2008. Phase I/II study of ipilimumab for patients with metastatic melanoma. Journal of Clinical Oncology 26:5950-5956). These include enterocolitis, dermatitis, hypophysitis, uveitis, hepatitis, nephritis and death. Enterocolitis is the most common major toxicity (affecting approximately 20% of patients). The severe safety risks related to immune-mediated adverse reactions prompted the FDA to approve ipilimumab with a Risk Evaluation and Mitigation Strategy (REMS). Recently, coadministration of ipilimumab and a second immune checkpoint modulator targeting PD1 (e.g., nivolumab) has been shown to significantly increase efficacy of immunotherapy of melanoma when compared to ipilimumab alone. This gain, however, was associated with increased frequencies of grade 3/4 adverse effects, which affected more than 50% of patients receiving combination treatment (Wolchok, J.D., et al. 2013. Nivolumab plus Ipilimumab in Advanced Melanoma. N Engl J Med).

[0005] These findings illustrate the need for developing anti-CTLA4 protein therapeutics that effectively target tumors without the side effects associated with systemic immune activation. Provided herein are anti-CTLA binding proteins, compositions thereof and methods of use thereof for addressing this need.

[0006] All references cited herein, including patent applications, patent publications, and scientific literature, are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0006a] In one aspect, the present disclosure refers to a masked antibody, comprising: a) an anti-CTLA4 antibody or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain; and b) a masking peptide comprising the amino acid sequence of SEQ ID NO: 5; wherein the masking peptide is linked via a linker comprising a cleavable peptide to an amino-terminus of the VL domain; wherein the linker

comprising a cleavable peptide comprises a cleavable peptide comprising the amino acid sequence of SEQ ID NO: 86; wherein (a) the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 408, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 409, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 410; and the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 411, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 412, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 413; or (b) the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 438, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 439, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 440; and the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 441, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 442, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 443.

In another aspect, the present disclosure refers to a masked antibody, comprising: a) an anti-CTLA4 antibody or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain; and b) a masking peptide comprising the amino acid sequence of SEQ ID NO: 19; wherein the masking peptide is linked via a linker comprising a cleavable peptide to an amino-terminus of the VL domain; wherein the linker comprising a cleavable peptide comprises a cleavable peptide comprising the amino acid sequence of SEQ ID NO: 50; wherein (a) the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 408, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 409, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 410; and the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 411, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 412, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 413; or (b) the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 438, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 439, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 440; and the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 441, a CDR-H2 comprising the amino

acid sequence of SEQ ID NO: 442, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 443.

In another aspect, the present disclosure refers to a masked anti-CTLA4 antibody, comprising: (a) an anti-CTLA4 antibody comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein the VL domain comprises (i) a light chain complementarity determining region 1 (CDR-L1) defined by the amino acid sequence of SEQ ID NO: 438, (ii) a CDR-L2 defined by the amino acid sequence of SEQ ID NO: 439, and (iii) a CDR-L3 defined by the amino acid sequence of SEQ ID NO: 440; and the VH domain comprises (i) a heavy chain complementarity determining region 1 (CDR-H1) defined by the sequence of SEQ ID NO: 441, (ii) a CDR-H2 defined by the amino acid sequence of SEQ ID NO: 442, and (iii) a CDR-H3 defined by the amino acid sequence of SEQ ID NO: 443, and a heavy chain constant (CH) domain comprising amino acid substitutions of S239D and I332E, wherein the amino acids are numbered according to EU index as in Kabat; and (b) a masking peptide that binds to the antibody.

In another aspect, the present disclosure refers to a masked antibody, comprising: a) an anti-CTLA4 antibody or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain; and b) a masking peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-46; wherein the masking peptide is linked via a linker comprising a cleavable peptide to an amino-terminus or carboxy-terminus of the VL domain or the VH domain.In another aspect, the present disclosure refers to a masked bispecific antibody comprising the masked antibody as described herein.

In another aspect, the present disclosure refers to a nucleic acid encoding the masked antibody as described herein or the masked bispecific antibody as described herein.

In another aspect, the present disclosure refers to a vector comprising the nucleic acid as described herein.

In another aspect, the present disclosure refers to a host cell comprising the nucleic acid as described herein.

In another aspect, the present disclosure refers to a method of producing a masked antibody, comprising culturing the host cell as described herein under conditions that produce the masked antibody or the masked bispecific antibody.

In another aspect, the present disclosure refers to a masked antibody, or a masked bispecific antibody produced by the method as described herein.

In another aspect, the present disclosure refers to a composition comprising the masked antibody as described herein, or the masked bispecific antibody as described herein.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the masked antibody as described herein, or the masked bispecific antibody as described herein, and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure refers to a kit comprising the masked antibody as described herein, or the masked bispecific antibody as described herein.

In another aspect, the present disclosure refers to the use of the masked antibody as described herein, or the masked bispecific antibody as described herein in the manufacture of a medicament for treating or preventing a neoplastic disease in a subject.

In another aspect, the present disclosure refers to the use of the masked antibody as described herein, or the masked bispecific antibody as described herein, in the manufacture of a medicament for treating a neoplastic disease.

[0007] Provided herein are activatable masked anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) binding proteins, compositions comprising thereof, and methods of using the same.

[0008] Provided herein is a masked antibody containing an antibody or antigen-binding fragment thereof that binds to CTLA4, wherein the antibody or antigen-binding fragment

CLAIMS

1. A masked antibody, comprising:

a) an anti-CTLA4 antibody or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain; and

b) a masking peptide comprising the amino acid sequence of SEQ ID NO: 5;

wherein the masking peptide is linked via a linker comprising a cleavable peptide to an amino-terminus of the VL domain;

wherein the linker comprising a cleavable peptide comprises a cleavable peptide comprising the amino acid sequence of SEQ ID NO: 86;

wherein (a) the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 408, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 409, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 410; and the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 411, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 412, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 413; or (b) the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 438, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 439, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 440; and the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 441, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 442, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 443.

2. A masked antibody, comprising:

a) an anti-CTLA4 antibody or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain; and

b) a masking peptide comprising the amino acid sequence of SEQ ID NO: 19;

wherein the masking peptide is linked via a linker comprising a cleavable peptide to an amino-terminus of the VL domain;

wherein the linker comprising a cleavable peptide comprises a cleavable peptide comprising the amino acid sequence of SEQ ID NO: 50;

wherein (a) the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 408, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 409, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 410; and the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 411, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 412, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 413; or (b) the VL

domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 438, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 439, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 440; and the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 441, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 442, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 443.

3. A masked anti-CTLA4 antibody, comprising:

(a) an anti-CTLA4 antibody comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein the VL domain comprises (i) a light chain complementarity determining region 1 (CDR-L1) defined by the amino acid sequence of SEQ ID NO: 438, (ii) a CDR-L2 defined by the amino acid sequence of SEQ ID NO: 439, and (iii) a CDR-L3 defined by the amino acid sequence of SEQ ID NO: 440; and the VH domain comprises (i) a heavy chain complementarity determining region 1 (CDR-H1) defined by the sequence of SEQ ID NO: 441, (ii) a CDR-H2 defined by the amino acid sequence of SEQ ID NO: 442, and (iii) a CDR-H3 defined by the amino acid sequence of SEQ ID NO: 443, and a heavy chain constant (CH) domain comprising amino acid substitutions of S239D and I332E, wherein the amino acids are numbered according to EU index as in Kabat; and

(b) a masking peptide that binds to the antibody.

4. The masked anti-CTLA4 antibody of claim 3, wherein the masking peptide is defined by the amino acid sequence of any one of SEQ ID NOs: 1-46.

5. The masked anti-CTLA4 antibody of claim 4, wherein the masking peptide is defined by the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 19.

6. A masked antibody, comprising:

a) an anti-CTLA4 antibody or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain; and

b) a masking peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-46;

wherein the masking peptide is linked via a linker comprising a cleavable peptide to an amino-terminus or carboxy-terminus of the VL domain or the VH domain.

7. The masked antibody of any one of claims 3-6, wherein the linker comprising a cleavable peptide comprises a cleavable peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 47-88, 464-469, and 479-508, optionally

wherein the cleavable peptide comprises an amino-terminus and a carboxy-terminus, and the linker comprising a cleavable peptide comprises a first spacer linker

and a second spacer linker, wherein the first spacer linker is linked to the amino-terminus of the cleavable peptide and comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-112 and 415-420, and the second spacer linker is linked to the carboxy-terminus of the cleavable peptide and comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-112 and 415- 420.

8. The masked antibody of any one of claims 3-7, wherein the linker comprising a cleavable peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 454-462.

9. The masked antibody of any one of claims 3 to 8, wherein the masked antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 113-231 and 444-453.

10. The masked antibody of any one of claims 1 to 9, wherein the anti-CTLA4 antibody or antigen-binding fragment thereof is a humanized antibody, a chimeric antibody, or a human antibody.

11. The masked antibody of any one of claims 6 to 10, wherein:

a) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 402, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 403, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 404; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 405, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 406, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 407; or

b) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 408, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 409, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 410; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 411, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 412, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 413; or

c) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 432, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 433, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 434; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 435, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 436, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 437; or

d) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 438, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 439, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 440; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 441, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 442, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 443.

12. The masked antibody of any one of claims 6 to 11, wherein:

a) the VL domain comprises the amino acid sequence of SEQ ID NO: 321, and the VH domain comprises the amino acid sequence of SEQ ID NO: 323; or

b) the VL domain comprises the amino acid sequence of SEQ ID NO: 322, and the VH domain comprises the amino acid sequence of SEQ ID NO: 324.

13. The masked antibody of any one of claims 6 to 12, wherein the VL domain is contained within a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 327-341, and the VH domain is contained within a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 366-380 and 421.

14. The masked antibody of any one of claims 6 to 13, wherein the masked antibody comprises the amino acid sequence of SEQ ID NO: 421, and comprises an amino acid sequence that is selected from the group consisting of SEQ ID NOs: 358 and 422-431.

15. The masked antibody of any one of claims 6 to 14, wherein the masking peptide comprises the amino acid sequence of SEQ ID NO: 5; and wherein the masking peptide is linked via the linker to the amino-terminus of the VL domain; and wherein the cleavable peptide comprises the amino acid sequence of SEQ ID NO: 86.

16. The masked antibody of any one of claims 6 to 14, wherein the masking peptide comprises the amino acid sequence of SEQ ID NO: 19; and wherein the masking peptide is linked via the linker to the amino-terminus of the VL domain; and wherein the cleavable peptide comprises the amino acid sequence of SEQ ID NO: 50.

17. The masked antibody of any one of claims 1 to 16, wherein the anti-CTLA4 antibody or antigen-binding fragment thereof is conjugated to an agent, optionally wherein the agent is an inhibitor of tubulin polymerization, a DNA damaging agent, or a DNA synthesis inhibitor, or wherein the agent is a maytansinoid, an auristatin, a pyrrolobenzodiazepine (PBD) dimer, a calicheamicin, a duocarmycin, a indo-linobenzodiazepine dimer, or exatecan derivative Dxd.

18. A masked bispecific antibody comprising the masked antibody of any one of claims 1-17.

19. A nucleic acid encoding the masked antibody of any one of claims 1 to 17 or the masked bispecific antibody of claim 18.

20. A vector comprising the nucleic acid of claim 19.

21. A host cell comprising the nucleic acid of claim 19.

22. A method of producing a masked antibody, comprising culturing the host cell of claim 21 under conditions that produce the masked antibody or the masked bispecific antibody.

23. A masked antibody, or a masked bispecific antibody produced by the method of claim 22.

24. A composition comprising the masked antibody of any one of claims 1 to 17, or the masked bispecific antibody of claim 18.

25. A pharmaceutical composition comprising the masked antibody of any one of claims 1 to 17, or the masked bispecific antibody of claim 18, and a pharmaceutically acceptable carrier.

26. A kit comprising the masked antibody of any one of claims 1 to 17, or the masked bispecific antibody of claim 18.

27. Use of the masked antibody of any one of claims 1 to 17, or the masked bispecific antibody of claim 18 in the manufacture of a medicament for treating or preventing a neoplastic disease in a subject.

28. The masked antibody of any one of claims 1 to 17, or the masked bispecific antibody of claim 18, for use in therapy.

29. Use of the masked antibody of any one of claims 1 to 17, or the masked bispecific antibody of claim 18, in the manufacture of a medicament for treating a neoplastic disease.