proteins for treating cancer that have adequate therapeutic efficacy, a format straightforward to manufacture, and favorable pharmacokinetic properties such as a longer half-life.

SUMMARY

[0009] Described herein, in certain examples, are methods of treating an individual in need thereof having a CD19 low expressing cancer, comprising administering to the individual a multi-specific binding protein comprising: a) a first antigen-binding site that binds human CD19 and comprising a heavy chain variable domain (VH) comprising complementarity determining regions HCDR1, HCDR2, and HCDR3 and a light chain variable domain (VL) comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 4, 5, 7, 8, 9, and 10, respectively; and b) a second antigen-binding domain that binds CD3 and comprising a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3, and a VL comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 15, 16, 18, 19, 20, and 21, respectively.

[0009a] In one aspect, the disclosure refers to use of a multi-specific binding protein in the manufacture of a medicament for treatment of relapsed and/or refractory Non-Hodgkin Lymphoma (NHL), the multi-specific binding protein comprising: a) a first antigen-binding site that binds human CD19 and comprising a heavy chain variable domain (VH) comprising complementarity determining regions HCDR1, HCDR2, and HCDR3 and a light chain variable domain (VL) comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 4, 5, 7, 8, 9, and 10, respectively; and b) a second antigen-binding domain that binds CD3 and comprising a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3, and a VL comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 15, 16, 18, 19, 20, and 21, respectively.

[0009b] In another aspect, the disclosure refers to use of a multi-specific binding protein in the manufacture of a medicament for the treatment of CD19 low expressing cancer, the multi-specific binding protein comprising: a) a first antigen-binding site that binds human CD19 and

comprising a heavy chain variable domain (VH) comprising complementarity determining regions HCDR1, HCDR2, and HCDR3 and a light chain variable domain (VL) comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 4, 5, 7, 8, 9, and 10, respectively; and b) a second antigen-binding domain that binds CD3 and comprising a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3, and a VL comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 15, 16, 18, 19, 20, and 21, respectively.

[0010] In some examples, the CD19 low expressing cancer is classified as low expressing by flow cytometry. In some examples, the CD19 low expressing cancer is classified by having about 325 to about 17,000 CD19 molecules per cell. In some examples, the CD19 low expressing cancer is classified by having less than about 3000 CD19 molecules per cell.

[0011] In some examples, the VH of the first antigen-binding domain comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 1, and the VL of the first antigen-binding domain comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 2. In some examples, the VH of the first antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 1, and the VL of the first antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 2. In some examples, the first antigen-binding site comprises an amino acid sequence at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 11. In some examples, the first antigen-binding site comprises the amino acid sequence of SEQ ID NO: 11.

[0012] In some examples, the VH of the second antigen-binding site comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 12, and the VL of the second antigen-binding site comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 13. In some examples, the VH of the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 12, and the VL of the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 13. In some examples, the second antigen-binding site comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 22. In some examples, the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 22.

[0013] In some examples, the multi-specific binding protein further comprises a half-life extension domain. In some examples, the half-life extension domain comprises a third antigen-binding site that binds human serum albumin. In some examples, the half-life extension domain is not disposed between the first antigen-binding site and the second antigen-binding site in a polypeptide chain. In some examples, the third antigen-binding site comprises a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3, wherein the HCDR1, HCDR2, and HCDR3 comprise the amino acid sequences of SEQ ID NOs: 26, 27, and 29, respectively.

[0014] In some examples, the VH of the third antigen-binding site comprises an amino acid sequence at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO: 24. In some examples, the VH of the third antigen-binding site comprises the amino acid sequence of SEQ ID NO: 24. In some examples, the multi-specific binding protein comprises from N-terminus to C-terminus: a) a third antigen-binding site that binds human serum albumin and comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 24; b) a first antigen-binding site that binds CD19 and comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 11; and c) a second antigen-binding site that binds human CD3 and comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 22. In some examples, the multi-specific binding protein comprises from N-terminus to C-terminus: a) a third antigen-binding site that binds human serum albumin and comprises the amino acid sequence of SEQ ID NO: 24; b) a first antigen-binding site that binds CD19 and comprises the amino acid sequence of SEQ ID NO: 11; and c) a second antigen-binding site that binds human CD3 and comprises the amino

acid sequence of SEQ ID NO: 22. In some examples, the multi-specific binding protein comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 41. In some examples, the multi-specific binding protein comprises the amino acid sequence of SEQ ID NO: 41.

[0015] Also described herein, in certain examples, are methods of treating relapsed and/or refractory Non-Hodgkin lymphoma (NHL) in an individual in need thereof, the method comprising administering to the individual a multi-specific binding protein comprising: a) a first antigen-binding site that binds human CD19 and comprising a heavy chain variable domain (VH) comprising complementarity determining regions HCDR1, HCDR2, and HCDR3 and a light chain variable domain (VL) comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 4, 5, 7, 8, 9, and 10, respectively; and b) a second antigen-binding domain that binds CD3 and comprising a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3, and a VL comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 15, 16, 18, 19, 20, and 21, respectively. In some examples, the individual has relapsed following treatment with a CD19-targeting therapy, or is refractory to the CD19-targeting therapy. In some examples, the CD19-targeting therapy comprises blinatumomab. In some examples, the CD19-targeting therapy comprises a CD19 CAR-T cell therapy. In some examples, the VH of the first antigen-binding domain comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 1, and the VL of the first antigen-binding domain comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 2. In some examples, the VH of the first antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 1, and the VL of the first antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 2. In some examples, the first antigen-binding site comprises an amino acid sequence at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 11. In some examples, the first antigen-binding site comprises the amino acid sequence of SEQ ID NO: 11. In some examples, the VH of the second antigen-binding site comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 12, and the VL of the second antigen-binding site comprises an amino acid sequence

at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 13. In some examples, the VH of the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 12, and the VL of the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 13. In some examples, the second antigen-binding site comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 22. In some examples, the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 22. In some examples, the multi-specific binding protein further comprises a half-life extension domain. In some examples, the half-life extension domain comprises a third antigen-binding site that binds human serum albumin. In some examples, the half-life extension domain is not disposed between the first antigen-binding site and the second antigen-binding site in a polypeptide chain. In some examples, the third antigen-binding site comprises a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3, wherein the HCDR1, HCDR2, and HCDR3 comprise the amino acid sequences of SEQ ID NOs: 26, 27, and 29, respectively. In some examples, the VH of the third antigen-binding site comprises an amino acid sequence at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO: 24. In some examples, the VH of the third antigen-binding site comprises the amino acid sequence of SEQ ID NO: 24. In some examples, the multi-specific binding protein comprises from N-terminus to C-terminus: a) a third antigen-binding site that binds human serum albumin and comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 24; b) a first antigen-binding site that binds CD19 and comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 11; and c) a second antigen-binding site that binds human CD3 and comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 22. In some examples, the multi-specific binding protein comprises from N-terminus to C-terminus: a) a third antigen-binding site that binds human serum albumin and comprises the amino acid sequence of SEQ ID NO: 24; b) a first antigen-binding site that binds CD19 and comprises the amino acid sequence of SEQ ID NO: 11; and c) a second antigen-binding site that binds human CD3 and comprises the amino acid sequence of SEQ ID NO: 22. In some examples, the multi-specific binding protein comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 41. In some examples, the multi-specific binding protein comprises the amino acid sequence of SEQ ID NO: 41.

[0016] In some examples, the administration produces B cell depletion in said subject within 96 hours of administration of said multi-specific binding protein. In some examples, the administration produces a persistent B-cell depletion that is sustained to at least 90 days after administration of said multi-specific binding protein. In some examples, the administration produces a persistent B-cell depletion that is sustained to at least 90 days after a last administration of said multi-specific binding protein.

[0017] In some examples, the subject is administered a dose of at least about 30μg said multi-specific binding protein. In some examples, the subject is administered a dose of at least about 40μg said multi-specific binding protein. In some examples, the subject is administered a dose of at least about 50μg said multi-specific binding protein. In some examples, the subject is administered a dose of at least 60μg said multi-specific binding protein.

[0018] In some examples, the multi-specific binding protein is administered once per week. In some examples, the multi-specific binding protein is administered once every two weeks.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1A** is a schematic representation of six domain arrangements of single-chain multi-specific binding proteins. The CD19 binding domain in the form of a scFv, the CD3 binding domain in the form of a scFv, and the HSA binding domain in the form of an sdAb are linked in different orientations. The top of each construct represents the N-terminus and the bottom of each construct represents the C-terminus of a given polypeptide chain. **FIG. 1B** depicts a multi-specific binding protein design according to an aspect of this disclosure.

[0020] **FIGs. 2A-2D** depict *in vitro* data comparing tAB0050 and blinatumomab in ability to redirect lysis. **FIG. 2A** depicts CD19 expression in EMT6 cell lines. The parental line, a CD19 low line (clone 6), and a CD19 high line (clone 5) were characterized by flow cytometry. **FIGs. 2B-2D** demonstrate cytotoxicity data in the parental line (**FIG. 2B**), the low CD19 cell line (**FIG. 2C**), and the high CD19 cell line (**FIG. 2D**).

[0021] **FIGs. 3A-3D** depict activity of tAB0050 in co-cultures of human T cells with low CD19 expression. **FIG. 3A** depicts *in vitro* data of tAB0050 in redirecting lysis of low CD19-expressing cell lines. **FIG. 3B** depicts CD69 and CD25 expression. **FIGs. 3C and 3D** depict TNFα (**FIG. 3C**) and IFNγ (**FIG. 3D**) expression.

WHAT IS CLAIMED IS:

1. Use of a multi-specific binding protein in the manufacture of a medicament for treatment of relapsed and/or refractory Non-Hodgkin Lymphoma (NHL), the multi-specific binding protein comprising:

a) a first antigen-binding site that binds human CD19 and comprising a heavy chain variable domain (VH) comprising complementarity determining regions HCDR1, HCDR2, and HCDR3 and a light chain variable domain (VL) comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 4, 5, 7, 8, 9, and 10, respectively; and

b) a second antigen-binding domain that binds CD3 and comprising a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3, and a VL comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 15, 16, 18, 19, 20, and 21, respectively.

2. Use of a multi-specific binding protein in the manufacture of a medicament for the treatment of CD19 low expressing cancer, the multi-specific binding protein comprising:

a) a first antigen-binding site that binds human CD19 and comprising a heavy chain variable domain (VH) comprising complementarity determining regions HCDR1, HCDR2, and HCDR3 and a light chain variable domain (VL) comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 4, 5, 7, 8, 9, and 10, respectively; and

b) a second antigen-binding domain that binds CD3 and comprising a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3, and a VL comprising complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 15, 16, 18, 19, 20, and 21, respectively.

3. The use of claim 1 or 2, wherein the VH of the first antigen-binding domain comprises an amino acid sequence at least 85%identical to SEQ ID NO: 1, and the VL of the first antigen-binding domain comprises an amino acid sequence at least 85%identical to SEQ ID NO: 2.

4. The use of any one of claims 1 to 3, wherein the first antigen-binding site comprises an amino acid sequence at least 85%identical to the amino acid sequence of SEQ ID NO: 11.

5. The use of any one of claims 1 to 4, wherein the VH of the second antigen-binding site comprises an amino acid sequence at least 85%identical to SEQ ID NO: 12, and the VL of the second antigen-binding site comprises an amino acid sequence at least 85%identical to SEQ ID NO: 13.

6. The use of any one of claims 1 to 5, wherein the second antigen-binding site comprises an amino acid sequence at least 85% identical to SEQ ID NO: 22.

7. The use of any one of claims 1 to 6, wherein the multi-specific binding protein further comprises a half-life extension domain, optionally wherein the half-life extension domain comprises a third antigen-binding site that binds human serum albumin.

8. The use of claim 7, wherein the half-life extension domain is not disposed between the first antigen-binding site and the second antigen-binding site in a polypeptide chain.

9. The use of claim 7 or 8, wherein the third antigen-binding site comprises a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3, wherein the HCDR1, HCDR2, and HCDR3 comprise the amino acid sequences of SEQ ID NOs: 26, 27, and 29, respectively.

10. The use of any one of claims 7 to 9, wherein the VH of the third antigen-binding site comprises an amino acid sequence at least 85% identical to SEQ ID NO: 24.

11. The use of any one of claims 7 to 10, wherein the multi-specific binding protein comprises from N-terminus to C-terminus:

a) a third antigen-binding site that binds human serum albumin and comprises an amino acid sequence at least 85% identical to SEQ ID NO: 24;

b) a first antigen-binding site that binds CD19 and comprises an amino acid sequence at least 85% identical to SEQ ID NO: 11; and

c) a second antigen-binding site that binds human CD3 and comprises an amino acid sequence at least 85% identical to SEQ ID NO: 22.

12. The use of any one of claims 1 to 11, wherein the multi-specific binding protein comprises an amino acid sequence at least 85% identical to SEQ ID NO: 41.

13. The use of any one of claims 1 to 12, wherein the medicament is to be administered at a dose of at least 20µg at least once per week.

14. The use of any one of claims 1 to 12, wherein the medicament is to be administered at a dose of least 20µg at least once every two weeks.