

## METHODS OF USE OF MULTI-SPECIFIC BINDING PROTEINS

### RELATED APPLICATIONS

[0001] The present patent application claims the priority benefit of U.S. Provisional Patent Application Ser. No. 63/494970, filed April 7, 2023; U.S. Provisional Patent Application Ser. No. 63/494973, filed April 7, 2023; U.S. Provisional Patent Application Ser. No. 63/464258, filed May 5, 2023; U.S. Provisional Patent Application Ser. No. 63/464259, filed May 5, 2023; U.S. Provisional Patent Application Ser. No. 63/511125, filed June 29, 2023; U.S. Provisional Patent Application Ser. No. 63/581767, filed September 11, 2023; U.S. Provisional Patent Application Ser. No. 63/591594, filed October 19, 2023; and U.S. Provisional Patent Application Ser. No. 63/605804, filed December 4, 2023 the content of each is hereby incorporated by reference in its entirety into this disclosure.

### INCORPORATION BY REFERENCE OF AN ELECTRONIC SEQUENCE LISTING

[0002] This application contains a Sequence listing that has been electronically submitted in a computer readable format and is hereby incorporated by reference in its entirety. The computer readable file, created on March 16, 2024, is named 67300WO01\_SequenceListing.xml and is 45,643 bytes in size.

### FIELD OF THE DISCLOSURE

[0003] The disclosure relates to anti-CD19 antibodies and multi-specific binding proteins that bind CD19, CD3, and, optionally, serum albumin for treating cancers.

### BACKGROUND

[0004] Bispecific molecules such as BiTE<sup>®</sup> (bispecific T-cell engager) constructs are recombinant protein constructs made from two flexibly linked antibody-derived binding domains. One binding domain of BiTE<sup>®</sup> constructs is specific for a selected tumor-associated surface antigen on target cells, and the second binding domain is specific for CD3, a subunit of the T cell receptor complex on T cells. By this design, BiTE<sup>®</sup> constructs can transiently connect T cells with target cells and, at the same time, potentially activate the inherent cytolytic potential of T cells against target cells.

[0005] The CD3 receptor complex is a protein complex composed of four polypeptide chains. In mammals, the complex contains a CD3 $\gamma$  (gamma) chain, a CD3 $\delta$  (delta) chain, and

two CD3 $\epsilon$  (epsilon) chains. The CD3 $\gamma$  (gamma), CD3 $\delta$  (delta), and CD3 $\epsilon$  (epsilon) chains are highly related cell-surface proteins of the immunoglobulin superfamily containing a single extracellular immunoglobulin domain. These chains associate with the T cell receptor (TCR) to form a TCR-CD3 complex and to generate an activation signal in T lymphocytes upon antigen engagement. About 95% of T cells express  $\alpha\beta$  TCR, which contains an  $\alpha$  (alpha) chain and a  $\beta$  (beta) chain. Two TCR $\zeta$  (zeta) chains are also present in the TCR-CD3 complex. The  $\alpha\beta$  TCR is responsible for recognizing antigens presented by a major histocompatibility complex (MHC). When the TCR engages with antigenic peptide and MHC complex, the T lymphocyte is activated through a series of biochemical events mediated by associated enzymes, co-receptors, specialized adaptor molecules, and activated or released transcription factors.

**[0006]** CD19, also known as B-cell surface antigen B4 or Leu-12, is a transmembrane protein expressed on B lymphocytes and follicular dendritic cells. CD19 is a co-receptor for the B-cell antigen receptor complex on B lymphocytes (see, Carter *et al.* (2002) *Science*, 256: 105-7; van Zelm *et al.* (2006) *N. Eng. J. Med.*, 354: 1901-12). Together with the B cell receptor (BCR), CD19 modulates intrinsic and antigen receptor-induced signaling thresholds critical for clonal expansion of B cells and humoral immunity. CD19 is a human B-cell surface marker that is expressed from early stages of pre-B cell development through terminal differentiation into plasma cells. It is also expressed on many non-Hodgkin lymphoma (NHL) cells and certain leukemias. Antibodies that bind CD19 have been developed and tested in clinical studies against cancers of lymphoid origin such as B-cell malignancies (see, *e.g.*, Hekman *et al.* (1991) *Cancer Immunol. Immunother.*, 32: 364-72; Vlasfeld *et al.* (1995) *Cancer Immunol. Immunother.*, 40: 37-47; Corny *et al.* (1995) *J. Immunother. Emphasis Tumor Immunol.*, 18: 231-41; and Manzke *et al.* (2001) *Int. J. Cancer*, 91: 516-22). Furthermore, a BiTE<sup>®</sup> construct called blinatumomab has been developed for clinical use.

**[0007]** BiTE<sup>®</sup> constructs are believed to suffer from rapid clearance from the body. Therefore, whilst they are able to rapidly penetrate many areas of the body, are quick to produce, and are easier to handle, there *in vivo* applications may be limited by their brief persistence *in vivo*. Prolonged administration by continuous intravenous infusions may be required to achieve therapeutic effects of blinatumomab and solitomab because of their short *in vivo* half-life. However, such continuous intravenous infusions are inconvenient for patients and may increase the costs of treatment.

**[0008]** Although significant developments have been made in constructing anti-CD19 antibodies and multi-specific binding proteins, there remains a need for new and useful

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 OF THE DISCLOSURE**

**[0009]** Described herein, in certain embodiments, 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.

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

**[0011]** In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the first antigen-binding site comprises the amino acid sequence of SEQ ID NO: 11.

**[0012]** In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 22.

**[0013]** In some embodiments, the multi-specific binding protein further comprises a half-life extension domain. In some embodiments, the half-life extension domain comprises a third antigen-binding site that binds human serum albumin. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the VH of the third antigen-binding site comprises the amino acid sequence of SEQ ID NO: 24. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the multi-specific binding protein comprises the amino acid sequence of SEQ ID NO: 41.

**[0015]** Also described herein, in certain embodiments, 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 embodiments, the individual has relapsed following treatment with a CD19-targeting therapy, or is refractory to the CD19-targeting therapy. In some embodiments, the CD19-targeting therapy comprises blinatumomab. In some embodiments, the CD19-targeting therapy comprises a CD19 CAR-T cell therapy. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the first antigen-binding site comprises the amino acid sequence of SEQ ID NO: 11. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 22. In some embodiments, the multi-specific binding protein further comprises a half-life extension domain. In some embodiments, the half-life extension domain comprises a third antigen-binding site that binds human serum albumin. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the VH of the third antigen-binding site comprises the amino acid sequence of SEQ ID NO: 24. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the multi-specific binding protein comprises the amino acid sequence of SEQ ID NO: 41.

[0016] In some aspects, the administration produces B cell depletion in said subject within 96 hours of administration of said multi-specific binding protein. In some aspects, 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 aspects, 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 aspects, the subject is administered a dose of at least about 30µg said multi-specific binding protein. In some aspects, the subject is administered a dose of at least about 40µg said multi-specific binding protein. In some aspects, the subject is administered a dose of at least about 50µg said multi-specific binding protein. In some aspects, the subject is administered a dose of at least 60µg said multi-specific binding protein.

[0018] In some aspects, the multi-specific binding protein is administered once per week. In some aspects, 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.

[0022] **FIGs. 4A-4F** depict tAB0050 T cell induction and T cell-dependent cytotoxicity (TDCC). Chemically-induced CD19-expressing CHO cells co-cultured with isolated T cells at an E:T ratio of 10:1, in the presence or absence of the indicated concentrations of tAB0050 for 68 hr. (n=4). **FIG. 4A** depicts lysis curves, as flow cytometrically assessed by 7-AAD uptake in CD19-expressing CHO cells. **FIG. 4B** depicts CD25 and **FIG. 4C** depicts Ki67 expression profiles as marker for activation of CD8+ T cells. **FIG. 4D** depicts supernatants from co-cultures of CD19-expressing CHO cells and PBMC analyzed for INF $\gamma$ . **FIG. 4E** depicts cytotoxicity or INF $\gamma$  production at 225 pM concentration of tAB0050 or blinatumomab at 72 hr. (n=10). **FIG. 4F** depicts Wilcoxon signed-rank test.

[0023] **FIGs. 5A** and **5B** depict the design and characterization of tAB0050 according to an aspect of the disclosure. **FIG. 5A** is a summary of Biacore binding data of tAB0050 against targeted proteins. **FIG. 5B** is a binding of tAB0050 to human, cynomolgus monkey and mouse peripheral blood mononuclear cells (n=3 donors).

[0024] **FIG. 6** depicts the binding of tAB0050 in the presence or absence of serum albumin.

[0025] **FIGs. 7A-7E** depict the potential inducement of T cell activation and TDCC following administration of tAB0050. **FIGs. 7A** and **7B** show RAMOS cells co-cultured with PBMC from six healthy donors at an E:T ratio of 10:1, in the absence of albumin, at the indicated concentrations of tAB0050 for 48h. Shown are **FIG. 7A** lysis curves, as flow cytometrically assessed by 7-AAD uptake in RAMOS cells and **FIG. 7B** CD25 and CD69 expression on CD4+ and CD8+ T cells. **FIG. 7C** shows RAMOS cells co-cultured with PBMC at an E:T ratio of 10:1 and the indicated concentrations of tAB0050, in the presence or absence of human serum albumin, for 48h (n=10). **FIGs. 7D** and **7E** show supernatants from co-cultures of RAMOS cells and PBMC as in 2C in the presence of albumin were analyzed for the indicated cytokines by Luminex.

[0026] **FIG. 8A** shows Raji cells co-cultured with PBMC at an E:T ratio of 10:1, in the presence or absence of the indicated concentrations of tAB0050 and human albumin for 48h (n=8). Shown are lysis curves, as flow cytometrically assessed by 7-AAD uptake in RAMOS cells and CD69 expression profiles as marker for activation of CD4+ and CD8+ T cells. **FIG. 8B** shows tAB0050 redirected lysis of endogenous B cells in the presence or absence of human albumin. **FIG. 8C** shows T cell activation by tAB0050 in the presence of lymphoma target cell lines Ramos and Raji.

[0027] **FIGs. 9A-9E** depicts *in vivo* efficacy of tAB0050. **FIG. 9A** shows hCD3ε-expressing BALB/c mice were inoculated with A20 cells expressing hCD19. Mice were treated once IV when tumor volume reached ~100mm<sup>3</sup>. **FIG. 9B** shows immunodeficient NCG mice were IV engrafted with 2x10<sup>5</sup> Raji cells, then implanted IP with 2x10<sup>7</sup> PBMCs the following day. Mice were treated IV weekly starting on day 1. Statistics were calculated versus vehicle or PBS using ANOVA with multiple comparisons test on d10 in A and d15 in **FIG. 9B**. **FIG. 9C** shows immunodeficient NCG mice were IV engrafted with 2x10<sup>5</sup> Raji cells. On the following day (Day 1), 2x10<sup>7</sup> PBMCs from a healthy donor were implanted IP. tAB0050 was dosed either IV or SC weekly starting on Day 1. All treatment conditions with tAB0050 imparted statistically significant responses relative to controls by ANOVA at d14 (p < 0.0001). **FIG. 9D** depicts representative luminescent images of **FIG. 9C**. **FIG. 9E** depicts graphs showing hCD3ε-expressing BALB/c mice were inoculated with A20 cells expressing varying levels of hCD19. Mice were treated once weekly IV with 0.1 mg/kg tAB0050 or control when tumor volume reached ~100mm<sup>3</sup>.

[0028] **FIGs. 10A-10C** depict flow cytometry quantification. **FIG. 10A** shows a reduction in normal B cells and tumor cells in peripheral blood after treatment with tAB0050 in the huPBMC Raji B.luc mouse model. **FIG. 10B** shows the number of total T cells and frequency of T cell activation in peripheral blood after treatment with tAB0050 in the huPBMC Raji B.luc mouse model. **FIG. 10C** shows pharmacodynamic changes in the bone marrow after a single dose of tAB0050 in the huPBMC Raji B.luc mouse model.

[0029] **FIGs. 11A and 11B** show cytokine induction in peripheral blood after treatment with tAB0050 in the huPBMC Raji B.luc mouse model.

[0030] **FIG. 12A** is a PK profile of tAB0050 after a single dose in the huPBMC Raji B.luc mouse model. **FIG. 12B** is a table showing PK parameters of tAB0050 after a single treatment of tAB0050 in the huPBMC Raji B.luc mouse model.

[0031] **FIG. 13** depicts relative bioactivity of tAB0050 following incubation in human serum as measured by a T cell activation reporter assay in the presence of Raji target cells.

[0032] **FIGs. 14A-14E** depicts study design, pharmacokinetics, B cell depletion, T cell redistribution and cytokine release in cynomolgus monkeys in response to a single IV- or SC-administered tAB0050 dose. Female monkeys (n=2) were dosed at 0.1 or 1 mg/kg either IV (blue bars) or SC (red bars). **FIG. 14A** depicts the study design. Blood samples were collected at predetermined timepoints. After a single IV or SC administration (**FIG. 14B**), absolute B

cells (**FIG. 14C**), absolute T cells (**FIG. 14D**), and cytokines (**FIG. 14E**) as measured by Luminex.

**[0033]** **FIG. 15A** depicts CLN-978 (tAB0050) serum concentration-time profiles in female cynomolgus monkeys by IV route (0.1 mg/kg). **FIG. 15B** depicts CLN-978 (tAB0050) serum concentration-time profiles in female cynomolgus monkeys by IV route (1 mg/kg). **FIG. 15C** depicts CLN-978 (tAB0050) serum concentration-time profiles in female cynomolgus monkeys by SC route (0.1 mg/kg). **FIG. 15D** depicts CLN-978 (tAB0050) serum concentration-time profiles in female cynomolgus monkeys by SC route (1 mg/kg).

**[0034]** **FIG. 16A** is a table showing toxicokinetic parameters for CLN-978 (tAB0050) in serum of female cynomolgus monkeys administrated by IV route; primary parameters. **FIG. 16B** is a table showing toxicokinetic parameters for CLN-978 (tAB0050) in serum of female cynomolgus monkeys administrated by SC route; primary parameters.

**[0035]** **FIG. 17** is an illustration of a human model diagram. The T-cell engager (TCE) is administered SC and absorbed into the central compartment, where it can distribute to the peripheral or tumor compartments, be eliminated, or bind to and crosslink to CD3 or CD19 on T cells and normal or malignant B cells, respectively. CD3 and CD19 are synthesized and internalized in each compartment.

**[0036]** **FIGS. 18A and 18B** depict dose ranging simulations to illustrate starting and efficacious dose predictions. **FIG. 18A** depicts Avg TpT in tumor projections. Black dashed lines are 60 TpT (bottom line, starting dose criterium) and 472 TpT (top line, efficacious dose criterium). Note that 240 µg (blue) crosses the bottom black line at 28 days and 1850 µg (cyan) crosses the top black line at day 28, achieving the criteria for starting and efficacious dose, respectively. **FIG. 18B** depicts projected PK profiles.

**[0037]** **FIG. 19** is an illustration of a phase 1, open-label, multi-center, first-in-human, dose escalation, and dose expansion study of tAB0050 in patients with relapsed or refractory (R/R) B cell Non-Hodgkin Lymphoma (B-NHL).

**[0038]** **FIG. 20** is a graph showing preliminary clinical pharmacokinetics (PK) for CLN-978 overlaid with a simulated PK from a preclinical PK model.

**[0039]** **FIGS. 21A – 21F** are graphs showing B-cell depletion, T-cell activation and cytokine analysis in a subject treated with CLN-978.

[0040] FIGs. 22A – 22C are graphs showing deep B cell depletion in bone marrow (FIG. 22A), spleen (FIG. 22B), and lymphoid tissues (FIG. 22C) following SC administration of CLN-97 in cynomolgus monkeys.

[0041] FIG. 23 is a table summarizing the clinical observations from three patients treated 30 µg of CLN-978 administered subcutaneously weekly.

[0042] FIG. 24 are images from a patient treated 30 µg of CLN-978 administered subcutaneously weekly showing response the CLN-978 treatment.

#### DETAILED DESCRIPTION

[0043] Provided herein are multi-specific binding proteins comprising a first domain that binds CD19 (e.g., human CD19), a second domain that binds CD3 (e.g., human CD3), and optionally a half-life extension domain, which can be a third domain that binds serum albumin (e.g., human serum albumin) for treating diseases and disorders associated with aberrant cells expressing CD19, such as certain B-cell hematologic malignancies. FIG. 1A is a schematic representation of six domain arrangements of single-chain multi-specific binding proteins. FIG. 1B depicts a multi-specific binding protein design according to an aspect of this disclosure. In an embodiment, the multi-specific binding protein is tAB0050.

[0044] To facilitate an understanding of the present disclosure, a number of terms and phrases are defined below.

[0045] The term “multi-specific binding protein” refers to a protein or protein conjugate capable of binding two or more different targets (e.g., two or more different antigens or two or more different epitopes of the same antigen). For example, the multi-specific binding protein can bind two or more different targets through two or more different binding domains. The structure and/or function of the multi-specific binding protein can be based on the structure and/or function of an antibody, e.g., a full-length or whole immunoglobulin molecule, an antibody heavy chain variable domain (VH) and/or light chain variable domain (VL), and/or a single chain antibody. In one example, each one of the binding domains of a multi-specific binding protein according to the disclosure comprises the minimum structural requirements of an antibody which allow for the target binding. This minimum requirement may be, e.g., defined by the presence of at least the three heavy chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VH domain) and/or the three light chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VL domain). An alternative approach to defining the minimal structural requirements of an antibody is defining the epitope of a specific target to which the antibody binds, or by referring to a known antibody with which the antibody competes to bind to the same epitope

that the known antibody binds. The antibodies on which the constructs according to the disclosure are based include for example monoclonal, recombinant, chimeric, deimmunized, humanized and human antibodies.

**[0046]** Any one of the binding domains of a multi-specific binding protein according to the disclosure may comprise the above referred groups of CDRs. Those CDRs may be comprised in the framework of a VH and/or VL. Fd fragments, for example, have two VH domains and often retain some antigen-binding function of the intact antigen-binding domain. Additional examples for formats of antibody fragments, antibody variants or binding domains include: (1) a Fab fragment, a monovalent fragment having the VL, VH, CL and CH1 domains; (2) a F(ab')<sub>2</sub> fragment, a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; (3) an Fd fragment having the two VH and CH1 domains; (4) an Fv fragment having the VL and VH domains of a single arm of an antibody; (5) a dAb fragment (Ward *et al.*, (1989) Nature 341:544-546), which has a VH domain; (6) an isolated complementarity determining region (CDR); and (7) a single chain Fv (scFv), which may be derived, for example, from an scFv-library.

**[0047]** Multi-specific binding proteins according to the disclosure may also comprise modified fragments of antibodies, also called antibody variants, such as di-scFv or bi(s)-scFv, scFv-Fc, scFv-zipper, scFab, Fab<sub>2</sub>, Fab<sub>3</sub>, diabodies, single chain diabodies, tandem diabodies (Tandab's), tandem di-scFv, tandem tri-scFv, "multibodies" such as triabodies or tetrabodies, or single-domain antibodies such as nanobodies or single variable domain antibodies comprising a single variable domain, which might be VH (also called VHH in the context of an sdAb) or VL, that specifically bind an antigen or epitope independently of other V regions or domains.

**[0048]** As used herein, the terms "single-chain Fv," "single-chain antibody," and "scFv" refer to a single-polypeptide-chain antibody fragment that comprise the variable regions from both the heavy and light chains, but lack the constant regions. Generally, a single-chain antibody further comprises a peptide linker connecting the VH and VL domains which enables it to form the desired structure to bind to antigen. In specific embodiments, single-chain antibodies can also be bispecific, multispecific, human, humanized and/or synthetic.

**[0049]** Furthermore, the "multi-specific binding protein" described herein can be a monovalent, bivalent or polyvalent/multivalent construct. Moreover, the "multi-specific binding protein" described herein can include a molecule consisting of only one polypeptide chain, or a molecules consisting of more than one polypeptide chain, wherein the chains can

be either identical (homodimers, homotrimers or homo oligomers) or different (heterodimer, heterotrimer or heterooligomer).

**[0050]** The domains of the multi-specific binding protein of the present disclosure may be connected through one or more peptide bonds and/or peptide linkers. The term “peptide linker” comprises in accordance with the present disclosure an amino acid sequence linking two domains. The peptide linkers can also be used to fuse the third domain to the other domains of the multi-specific binding protein of the disclosure. An essential technical feature of such peptide linker is that it does not comprise any polymerization activity.

**[0051]** The term “binding domain” or “domain that binds (an antigen)” characterizes in connection with the present disclosure a domain which (specifically) binds to or interacts with a given target epitope or a given target side on the target molecules (antigens), *e.g.* CD19, serum albumin, and CD3, respectively. The structure and function of the first binding domain, the second binding domain, and/or the third binding domain can be based on the structure and/or function of an antibody, *e.g.* of a full-length or whole immunoglobulin molecule. A binding domain can be drawn from the VH and/or VL or VHH domain of an antibody or fragment thereof. For example, a binding domain can include three light chain CDRs (*i.e.*, CDR1, CDR2 and CDR3 of the VL domain) and/or three heavy chain CDRs (*i.e.*, CDR1, CDR2 and CDR3 of the VH domain). A binding domain can also include VHH CDRs (*i.e.*, CDR1, CDR2 and CDR3 of the VHH region).

**[0052]** The terms “variable domain” and “variable region” are used interchangeably and refer to the portions of the antibody or immunoglobulin domains that exhibit variability in their sequence and that are involved in determining the specificity and binding affinity of a particular antibody. Variability is not evenly distributed throughout the variable domains of antibodies; it is concentrated in sub-domains of each of the heavy and light chain variable regions. These sub-domains are called “hypervariable regions” or “complementarity determining regions” (CDRs). The more conserved (*i.e.*, non-hypervariable) portions of the variable domains are called the “framework” regions (FRM or FR) and provide a scaffold for the six CDRs in three-dimensional space to form an antigen-binding surface.

**[0053]** In the present disclosure, any one of the binding domains of the multi-specific binding protein may comprise a single domain antibody (sdAb). A single domain antibody comprises a single, monomeric antibody variable domain that is able to bind selectively to a specific antigen, independently of other variable regions or domains. The first single domain antibodies were engineered from heavy chain antibodies found in camelids, and these are called VHH fragments. Cartilaginous fishes also have heavy chain antibodies (IgNAR) from

which single domain antibodies called V<sub>NAR</sub> fragments can be obtained. An alternative approach is to split the dimeric variable domains from common immunoglobulins *e.g.*, from humans or rodents into monomers, hence obtaining VH or VL as a single domain antibody. Although most research into single domain antibodies is currently based on heavy chain variable domains, nanobodies derived from light chains have also been shown to bind specifically to target epitopes. Examples of single domain antibodies include nanobodies and single variable domain antibodies.

**[0054]** As used herein, the term “antigen-binding site” refers to the part of an immunoglobulin molecule or a derivative or variant thereof that participates in antigen binding. In human antibodies, the antigen binding site is formed by amino acid residues of the N-terminal variable (“V”) regions of the heavy (“H”) and light (“L”) chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as “hypervariable regions” which are interposed between more conserved flanking stretches known as “framework regions,” or “FR.” Thus the term “FR” refers to amino acid sequences which are naturally found between and adjacent to hypervariable regions in immunoglobulins. In a human antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three-dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as “complementarity-determining regions,” or “CDRs.” In certain animals, such as camels and cartilaginous fish, the antigen-binding site is formed by a single antibody chain providing a “single domain antibody.” Antigen-binding sites can exist in an intact antibody, in an antigen-binding fragment of an antibody that retains the antigen-binding surface, or in a recombinant polypeptide such as an scFv, using a peptide linker to connect the heavy chain variable domain to the light chain variable domain in a single polypeptide.

**[0055]** As used herein, the term “antibody” refers to a protein or a protein conjugate that comprises an antigen-binding site. An antibody can be monospecific or multi-specific (*e.g.*, bispecific).

**[0056]** The terms “a” and “an” as used herein mean “one or more” and include the plural unless the context is inappropriate.

**[0057]** The terms “recipient”, “individual”, “subject”, “host”, and “patient”, are used interchangeably herein and in some embodiments, refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. “Mammal” for purposes of

treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and laboratory, zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, mice, rats, rabbits, guinea pigs, monkeys etc. In some embodiments, the mammal is human. None of these terms require the supervision of medical personnel.

**[0058]** As used herein, the term “CD19 low expressing” refers to low CD19 expression in a cancer. A cancer can be classified as “CD19 low expressing” if a number of CD19 molecules per cell is less than or equal to about 17000, more specifically less than or equal to about 3000. The number of CD19 molecules can be determined by flow cytometry.

**[0059]** As used herein, the term “effective amount” refers to the amount of a compound (*e.g.*, a compound of the present disclosure) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route. As used herein, the term “treating” includes any effect, *e.g.*, lessening, reducing, modulating, ameliorating or eliminating, that results in the improvement of the condition, disease, disorder, and the like, or ameliorating a symptom thereof.

**[0060]** As used herein, the term “pharmaceutical composition” refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

**[0061]** As used herein, the term “pharmaceutically acceptable carrier” refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, *see e.g.*, Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA (1975).

**[0062]** Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present disclosure that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present disclosure that consist essentially of, or consist of, the recited processing steps.

**[0063]** As a general matter, compositions specifying a percentage are by weight unless otherwise specified. Further, if a variable is not accompanied by a definition, then the previous definition of the variable controls.

## **I. MULTI-SPECIFIC BINDING PROTEINS**

[0064] In some embodiments, the present disclosure provides a multi-specific binding protein that comprises a first domain (*e.g.*, a first antigen-binding site) that binds CD19 (*e.g.*, human CD19); a second domain (*e.g.*, a second antigen-binding site) that binds CD3 (*e.g.*, human and/or Macaca CD3), such as CD3 $\epsilon$  (epsilon), CD3 $\delta$  (delta), and/or CD3 $\gamma$  (gamma); and optionally a half-life extension domain. The multi-specific binding protein is configured to bring CD19-expressing cells, such as B cells, into spatial proximity with CD3-expressing cells, such as T cells, to enhance cytotoxicity of the CD3-expressing cells against the CD19-expressing cells. The optional half-life extension domain can be a third domain (*e.g.*, a third antigen-binding site) that binds serum albumin (*e.g.*, HSA).

[0065] Each of the antigen-binding sites of the multi-specific binding protein can take various forms, such as single-chain variable fragment (scFv), Fab fragment, or single domain antibody (sdAb). In some embodiments, the first antigen-binding site comprises an scFv. In some embodiments, the second antigen-binding site comprises an scFv. In some embodiments, the third antigen-binding site comprises an sdAb.

[0066] In some embodiments, the multi-specific binding protein further comprises an antibody Fc region. The presence of an Fc region may increase the serum half-life of the multi-specific binding protein. Depending on the specific Fc subtype and variant used, the Fc region may also alter the activity (*e.g.*, cytotoxic activity) of the multi-specific binding protein.

[0067] In some embodiments, the multi-specific binding protein does not comprise an antibody Fc region. The absence of Fc contributes to a smaller size of the multi-specific binding protein, which can exhibit improved tissue penetration and pharmacokinetic properties. In some embodiments, the multi-specific binding proteins consists of or consists essentially of the first, second, and third antigen-binding sites and the linkers between them. In some embodiments, the multi-specific binding proteins consists essentially of the first, second, and third antigen-binding sites.

[0068] In some embodiments, the multi-specific binding protein binds CD19, CD3, and/or serum albumin monovalently. The exclusion of additional binding domains reduces the risk of non-specific immune cell activation and decreases the size of the multi-specific binding protein.

### **A. Antigen-Binding Site That Binds CD19**

[0069] The present disclosure provides, in some embodiments, an antigen-binding site that binds CD19 (*e.g.*, human CD19). The present disclosure also provides an antibody comprising

the antigen-binding site. The CDR sequences are identified under the Kabat numbering scheme unless indicated by an asterisk (\*).

Table 1. CD19 Antibody Sequences

| Antibody                                                                                                                                                                                                                                                                    | VH and HCDRs                                                                                                                              | VL and LCDRs                                                                                                              |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| CNG-CD19-701                                                                                                                                                                                                                                                                | QVQLQESGPGLVKPSQTLSTLTCTVSGGSISTSTMGVGWIRQHPGKGLEWIGFIWWDDDKRYNPNLKSRVTMSVDTSKNQFSLKLSSVT AADTAVYYCARMELWSYYFDYWGGQGLVTVSS (SEQ ID NO: 1) | EIVLTQSPATLSLSPGERATLSCSASSSVGYMHWYQQKPGQAPRLLIYDTSKLASGIPARFSGSGSGTDFTLTISSELPEDFAVYYCFQGSVYPFTFGQGTKLEIK (SEQ ID NO: 2) |
|                                                                                                                                                                                                                                                                             | HCDR1*: GSISTSTMGVG (SEQ ID NO: 3)                                                                                                        | LCDR1: SASSSVGYMH (SEQ ID NO: 8)                                                                                          |
|                                                                                                                                                                                                                                                                             | HCDR1: TSTMGVG (SEQ ID NO: 4)                                                                                                             | LCDR2: DTSKLAS (SEQ ID NO: 9)                                                                                             |
|                                                                                                                                                                                                                                                                             | HCDR2: FIWWDDDKRYNPNLKS (SEQ ID NO: 5)                                                                                                    | LCDR3: FQGSVYPFT (SEQ ID NO: 10)                                                                                          |
|                                                                                                                                                                                                                                                                             | HCDR3*: ARMELWSYYFDY (SEQ ID NO: 6)                                                                                                       |                                                                                                                           |
|                                                                                                                                                                                                                                                                             | HCDR3: MELWSYYFDY (SEQ ID NO: 7)                                                                                                          |                                                                                                                           |
| scFv:<br>EIVLTQSPATLSLSPGERATLSCSASSSVGYMHWYQQKPGQAPRLLIYDTSKLASGIPARFSGSGSGTDFTLTISSELPEDFAVYYCFQGSVYPFTFGQGTKLEIKGGGGSGGGGSGGGGSQVQLQESGPGLVKPSQTLSTLTCTVSGGSISTSTMGVGWIRQHPGKGLEWIGFIWWDDDKRYNPNLKSRVTMSVDTSKNQFSLKLSSVTAADTAVYYCARMELWSYYFDYWGGQGLVTVSS (SEQ ID NO: 11) |                                                                                                                                           |                                                                                                                           |

[0070] In some embodiments, the antigen-binding site that binds CD19 is in the form of an scFv. In certain embodiments, the VH is positioned C-terminal to the VL. In some embodiments, the VH is positioned N-terminal to the VL. In some embodiments, the VH and the VL are linked by a peptide linker, for example, a linker disclosed in subsection E below titled “Linkers.” To stabilize the scFv, the amino acid residues at position 44 of the VH and at position 100 of the VL (under Kabat numbering) can be substituted by Cys, thereby facilitating the formation of a disulfide bond between the VH and the VL. Accordingly, In some embodiments, the VH and VL comprise Cys at positions 100 and 44, respectively.

[0071] In some embodiments, the antigen-binding site that binds CD19 of the present disclosure comprises a VH that comprises an amino acid sequence at least 60% (e.g., at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the VH of an antibody disclosed in Table 1, and a VL that comprises an amino acid sequence at least 60% (e.g., at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the VL of the same antibody disclosed in Table 1. In some

embodiments, the antigen-binding site comprises the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, determined under Kabat (see Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No. 91-3242, Bethesda), Chothia (see, *e.g.*, Chothia C & Lesk A M, (1987), J Mol Biol 196: 901-917), MacCallum (see MacCallum R M et al., (1996) J Mol Biol 262: 732-745), IMGT (see Lefranc, (1999) The Immunologist, 7, 132-136), or any other CDR determination method known in the art, of the VH and VL sequences of an antibody disclosed in Table 1.

**[0072]** In some embodiments, the antigen-binding site that binds CD19 is derived from CNG-CD19-701. In some embodiments, the antigen-binding site comprises a VH comprising HCDR1, HCDR2, and HCDR3 sequences set forth in SEQ ID NOs: 3, 5, and 6, respectively, and a VL comprising LCDR1, LCDR2, and LCDR3 sequences set forth in SEQ ID NOs: 8, 9, and 10, respectively. In some embodiments, the antigen-binding site comprises a VH comprising HCDR1, HCDR2, and HCDR3 sequences set forth in SEQ ID NOs: 4, 5, and 7, respectively, and a VL comprising LCDR1, LCDR2, and LCDR3 sequences set forth in SEQ ID NOs: 8, 9, and 10, respectively. In some embodiments, the antigen-binding site comprises a VH comprising an amino acid sequence at least 60% (*e.g.*, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 1, and a VL that comprising an amino acid sequence at least 60% (*e.g.*, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 2. In some embodiments, the VH and the VL comprise the amino acid sequences of SEQ ID NOs: 1 and 2, respectively.

**[0073]** In some embodiments, the antigen-binding site binds human CD19 or an extracellular fragment thereof with a  $K_D$  lower than or equal to 2 nM, 1 nM, or 0.5 nM, as measured by surface plasmon resonance (SPR) when the antigen-binding site is present as a monomer. In some embodiments, the antigen-binding site binds human CD19 or an extracellular fragment thereof with a  $K_D$  in the range of 0.5-2 nM, in the range of 0.5-1 nM, or in the range of 0.5-0.5 nM, as measured by SPR when the antigen-binding site is present as a monomer.

**[0074]** In some embodiments, the antigen-binding site derived from CNG-CD19-701 binds human CD19 or an extracellular fragment thereof with a  $K_D$  lower than or equal to 2 nM, 1 nM, or 0.5 nM, as measured by surface plasmon resonance (SPR) when the antigen-binding site is present as a monomer. In some embodiments, the antigen-binding site derived from

CNG-CD19-701 binds human CD19 or an extracellular fragment thereof with a  $K_D$  in the range of 0.5-2 nM, in the range of 0.5-1 nM, or in the range of 0.5-0.5 nM, as measured by SPR when the antigen-binding site is present as a monomer.

[0075] In some embodiments, the antigen-binding site derived from CNG-CD19-701 binds human CD19 or an extracellular fragment thereof with a  $K_D$  lower than or equal to 0.4 nM, 0.3 nM, 0.2 nM, or 0.1 nM, as measured by SPR when the antigen-binding site is present as a monomer. In some embodiments, the antigen-binding site derived from CNG-CD19-701 binds human CD19 or an extracellular fragment thereof with a  $K_D$  in the range of 0.5-0.4 nM, 0.5-0.3 nM, 0.5-0.2 nM, or 0.5-0.1 nM, as measured by SPR when the antigen-binding site is present as a monomer.

[0076] In some embodiments, the antigen-binding site derived from CNG-CD19-701 binds cynomolgus CD19 with a  $K_D$  lower than or equal to 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, or 3 nM, as measured by SPR when the antigen-binding site is present as a monomer. In some embodiments, the antigen-binding site derived from CNG-CD19-701 binds cynomolgus CD19 with a  $K_D$  in the range of 1-8 nM, 1-7 nM, 1-6 nM, 1-5 nM, 1-4 nM, or 1-3 nM, as measured by SPR when the antigen-binding site is present as a monomer.

[0077] The present disclosure also provides an antigen-binding site that competes for binding CD19 (*e.g.*, human CD19) with an antibody or antigen-binding site comprising the VH, VL and/or scFv sequences provided in Table 1.

#### **B. Antigen-Binding Site That Binds CD3**

[0078] The second antigen-binding site of the multi-specific binding protein binds CD3 (*e.g.*, human CD3 and/or Macaca CD3). In some embodiments, the second antigen-binding site binds CD3 $\epsilon$  (epsilon). In some embodiments, the second antigen-binding site binds CD3 $\delta$  (delta). In some embodiments, the second antigen-binding site binds CD3 $\gamma$  (gamma).

[0079] In some embodiments, the second antigen-binding site of the multi-specific binding protein binds an epitope at the N-terminus of CD3 $\epsilon$  chain. In some embodiments, the second antigen-binding site binds an epitope localized in amino acid residues 1-27 of human CD3 $\epsilon$  extracellular domain. This epitope or a homologous variant thereof is also present in certain non-human primates. Accordingly, in certain embodiments, the second antigen-binding site binds CD3 in different primates, for example, human, new world primates (such as *Callithrix jacchus*, *Saguinus Oedipus*, or *Saimiri sciureus*), old world primates (such as baboons and macaques), gibbons, and non-human homininae. *Callithrix jacchus* and *Saguinus oedipus* are new world primates belonging to the family of Callitrichidae, while *Saimiri sciureus* is a new world primate belonging to the family of Cebidae. In some embodiments, the second antigen-

binding site binds human CD3ε and/or *Macaca* CD3ε. In some embodiments, the second antigen-binding site further binds *Callithrix jacchus*, *Saguinus Oedipus*, and/or *Saimiri sciureus* CD3ε.

Table 2. CD3 Antibody Sequences

| Antibody                                                                                                                                                                                                                                                                                                               | VH and HCDRs                                                                                                                                         | VL and LCDRs                                                                                                                                    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| CNG-CD3-1                                                                                                                                                                                                                                                                                                              | QVQLVQSGAEVKKPGASVKVSCASGFNIKDY<br>YMHWVRQAPGQRLEWMGWIDLENANTIYD<br>AKFQGRVTITRDTASTAYMELSSLRSEDTAVY<br>YCARDAYGRYFYDVWGQGLTVTVSS (SEQ ID<br>NO: 12) | DIVMTQSPDSLAVSLGERATINCKSSQSLL<br>NARTGKNYLAWYQQKPGQPPKLLIYWAS<br>TRESGVPDRFSGSGSGTDFTLTISSLQAED<br>VAVYYCKQSYSRRTFGGGTKVEIK (SEQ ID<br>NO: 13) |
|                                                                                                                                                                                                                                                                                                                        | HCDR1*: FNIKDYMH (SEQ ID NO: 14)                                                                                                                     | LCDR1: KSSQSLLNARTGKNYLA (SEQ ID<br>NO: 19)                                                                                                     |
|                                                                                                                                                                                                                                                                                                                        | HCDR1: DYMH (SEQ ID NO: 15)                                                                                                                          | LCDR2: WASTRES (SEQ ID NO: 20)                                                                                                                  |
|                                                                                                                                                                                                                                                                                                                        | HCDR2: WIDLENANTIYDAKFQG (SEQ ID<br>NO: 16)                                                                                                          | LCDR3: KQSYSRRT (SEQ ID NO: 21)                                                                                                                 |
|                                                                                                                                                                                                                                                                                                                        | HCDR3*: ARDAYGRYFYDV (SEQ ID NO: 17)                                                                                                                 |                                                                                                                                                 |
|                                                                                                                                                                                                                                                                                                                        | HCDR3: DAYGRYFYDV (SEQ ID NO: 18)                                                                                                                    |                                                                                                                                                 |
| scFv:<br>DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKNYLAWYQQKPGQPPKLLIYWASTRESGV<br>PDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSYSRRTFGGGTKVEIKGGGGSGGGSGGGSGG<br>GGGSQVQLVQSGAEVKKPGASVKVSCASGFNIKDYMHWVRQAPGQRLEWMGWIDLENA<br>NTIYDAKFQGRVTITRDTASTAYMELSSLRSEDTAVYYCARDAYGRYFYDVWGQGLTVTVSS<br>(SEQ ID NO: 22)                        |                                                                                                                                                      |                                                                                                                                                 |
| scFv with Cys substitutions:<br>DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKNYLAWYQQKPGQPPKLLIYWASTRESGV<br>PDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSYSRRTFGCGTKVEIKGGGGSGGGSGGGSGG<br>GGGSQVQLVQSGAEVKKPGASVKVSCASGFNIKDYMHWVRQAPGQCLEWMGWIDLENA<br>NTIYDAKFQGRVTITRDTASTAYMELSSLRSEDTAVYYCARDAYGRYFYDVWGQGLTVTVSS<br>(SEQ ID NO: 23) |                                                                                                                                                      |                                                                                                                                                 |

Where the VL and LCDR sequences are noted as “N/A,” the antigen-binding site is an sdAb having a VH (e.g., VHH) only.

[0080] In some embodiments, the second antigen-binding site comprises a VH that comprises an amino acid sequence at least 60% (e.g., at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the VH of an antibody disclosed in Table 2, and a VL that comprises an amino acid sequence at least 60% (e.g., at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the VL of the same antibody disclosed in Table 2. In some embodiments, the antigen-binding site

comprises the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, determined under Kabat (see Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No. 91-3242, Bethesda), Chothia (see, *e.g.*, Chothia C & Lesk A M, (1987), J Mol Biol 196: 901-917), MacCallum (see MacCallum R M et al., (1996) J Mol Biol 262: 732-745), IMGT (see Lefranc, (1999) The Immunologist, 7, 132-136), or any other CDR determination method known in the art, of the VH and/or VL sequences of an antibody disclosed in Table 2. In some embodiments, the antigen-binding site comprises the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 sequences of an antibody disclosed in Table 2. In some embodiments, the antigen-binding site comprises the VH and VL sequences of an antibody disclosed in Table 2.

**[0081]** In some embodiments, the second antigen-binding site that binds CD3 is derived from CNG-CD3-1. In some embodiments, the second antigen-binding site comprises a VH comprising HCDR1, HCDR2, and HCDR3 sequences set forth in SEQ ID NOs: 14, 16, and 17, respectively, and a VL comprising LCDR1, LCDR2, and LCDR3 sequences set forth in SEQ ID NOs: 19, 20, and 21, respectively. In some embodiments, the second antigen-binding site comprises a VH comprising HCDR1, HCDR2, and HCDR3 sequences set forth in SEQ ID NOs: 15, 16, and 18, respectively, and a VL comprising LCDR1, LCDR2, and LCDR3 sequences set forth in SEQ ID NOs: 19, 20, and 21, respectively. In some embodiments, the second antigen-binding site comprises a VH comprising an amino acid sequence at least 60% (*e.g.*, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 12, and a VL that comprising an amino acid sequence at least 60% (*e.g.*, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 13. In some embodiments, the VH and the VL of the second antigen-binding site comprise the amino acid sequences of SEQ ID NOs: 12 and 13, respectively.

**[0082]** Such antigen-binding site may take the form of scFv. In certain embodiments, the VH is positioned C-terminal to the VL. In some embodiments, the VH is positioned N-terminal to the VL. In some embodiments, the VH and the VL are linked by a peptide linker, for example, a linker disclosed in subsection E below titled "Linkers." In some embodiments, the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 22. To stabilize the scFv, the amino acid residues at position 44 of the VH and at position 100 of the VL (under Kabat numbering) can be substituted by Cys, thereby facilitating the formation of

a disulfide bond between the VH and the VL. Accordingly, in some embodiments, the VH and VL comprise Cys at positions 100 and 44, respectively.

**[0083]** In some embodiments, the second antigen-binding site comprises an sdAb comprising a VH comprising complementarity determining regions HCDR1, HCDR2, and HCDR3. In some embodiments, the VH comprises an amino acid sequence at least 60% (*e.g.*, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the VH of an sdAb antibody provided in Table 2. In some embodiments, the VH comprises the HCDR1, HCDR2, and HCDR3 sequences of the antibody provided in Table 2. In some embodiments, the VH comprises the amino acid sequence of the VH of an sdAb provided in Table 2.

**[0084]** In some embodiments, the second antigen-binding site competes for binding CD3 (*e.g.*, human CD3 and/or Macaca CD3) with an antibody or antigen-binding fragment thereof comprising the VH, VL and/or scFv sequences provided in Table 2.

**[0085]** In some embodiments, the second antigen-binding site of the multi-specific binding protein binds CD3 (*e.g.*, human CD3 and/or Macaca CD3) with a dissociation constant ( $K_D$ ) of about 0.1 nM – about 1  $\mu$ M. The  $K_D$  can be measured by a method known in the art. In some embodiments, the  $K_D$  is measured by SPR to CD3 or an extracellular fragment thereof immobilized on a chip. In some embodiments, the  $K_D$  is measured by flow cytometry to CD3 expressed on the surface of cells.

**[0086]** In some embodiments, the second antigen-binding site binds CD3 with a  $K_D$ , as measured by SPR, lower than or equal to 20 nM, 15 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 90 pM, 80 pM, 70 pM, 60 pM, 50 pM, 40 pM, 30 pM, 20 pM, or 10 pM. For example, in certain embodiments, the first antigen-binding site binds CD3 with a  $K_D$ , as measured by SPR, within the range of about 10 pM – about 1 nM, about 10 pM – about 0.9 nM, about 10 pM – about 0.8 nM, about 10 pM – about 0.7 nM, about 10 pM – about 0.6 nM, about 10 pM – about 0.5 nM, about 10 pM – about 0.4 nM, about 10 pM – about 0.3 nM, about 10 pM – about 0.2 nM, about 10 pM – about 0.1 nM, about 10 pM – about 50 pM, 0.1 nM – about 10 nM, about 0.1 nM – about 9 nM, about 0.1 nM – about 8 nM, about 0.1 nM – about 7 nM, about 0.1 nM – about 6 nM, about 0.1 nM – about 5 nM, about 0.1 nM – about 4 nM, about 0.1 nM – about 3 nM, about 0.1 nM – about 2 nM, about 0.1 nM – about 1 nM, about 0.1 nM – about 0.5 nM, about 0.5 nM – about 10 nM, about 0.5 nM – about 9 nM, about 0.5 nM – about 8 nM, about 0.5 nM – about 7 nM, about 0.5 nM – about 6 nM, about 0.5 nM

– about 5 nM, about 0.5 nM – about 4 nM, about 0.5 nM – about 3 nM, about 0.5 nM – about 2 nM, about 0.5 nM – about 1 nM, about 1 nM – about 10 nM, about 1 nM – about 9 nM, about 1 nM – about 8 nM, about 1 nM – about 7 nM, about 1 nM – about 6 nM, about 1 nM – about 5 nM, about 1 nM – about 4 nM, about 1 nM – about 3 nM, about 1 nM – about 2 nM, about 2 nM – about 10 nM, about 3 nM – about 10 nM, about 4 nM – about 10 nM, about 5 nM – about 10 nM, about 6 nM – about 10 nM, about 7 nM – about 10 nM, about 8 nM – about 10 nM, or about 9 nM – about 10 nM.

**[0087]** In some embodiments, the second antigen-binding site binds CD3 with a  $K_D$ , as measured by BLI, lower than or equal to 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, or 0.1 nM. For example, in certain embodiments, the first antigen-binding site binds CD3 with a  $K_D$ , as measured by BLI, within the range of about 0.1 nM – about 20 nM, about 0.1 nM – about 19 nM, about 0.1 nM – about 18 nM, about 0.1 nM – about 17 nM, about 0.1 nM – about 16 nM, about 0.1 nM – about 15 nM, about 0.1 nM – about 14 nM, about 0.1 nM – about 13 nM, about 0.1 nM – about 12 nM, about 0.1 nM – about 11 nM, about 0.1 nM – about 10 nM, about 0.1 nM – about 9 nM, about 0.1 nM – about 8 nM, about 0.1 nM – about 7 nM, about 0.1 nM – about 6 nM, about 0.1 nM – about 5 nM, about 0.1 nM – about 4 nM, about 0.1 nM – about 3 nM, about 0.1 nM – about 2 nM, about 0.1 nM – about 1 nM, about 0.1 nM – about 0.5 nM, about 1 nM – about 50 nM, about 1 nM – about 40 nM, about 1 nM – about 30 nM, about 1 nM – about 20 nM, about 1 nM – about 19 nM, about 1 nM – about 18 nM, about 1 nM – about 17 nM, about 1 nM – about 16 nM, about 1 nM – about 15 nM, about 1 nM – about 14 nM, about 1 nM – about 13 nM, about 1 nM – about 12 nM, about 1 nM – about 11 nM, about 1 nM – about 10 nM, or about 1 nM – about 5 nM.

**[0088]** In some embodiments, the second antigen-binding site binds CD3 (*e.g.*, human CD3, *e.g.*, human CD3ε) with a  $K_D$ , as measured by SPR, greater than or equal to 1 nM, 2 nM, 3 nM, 4 nM, 5 nM, 6 nM, 7 nM, 8 nM, 9 nM, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, or 100 nM. In some embodiments, the second antigen-binding site binds CD3 with a  $K_D$ , as measured by SPR, within the range of about 1 nM – about 100 nM, about 1 nM – about 90 nM, about 1 nM – about 80 nM, about 1 nM – about 70 nM, about 1 nM – about 60 nM, about 1 nM – about 50 nM, about 1 nM – about 40 nM, about 1 nM – about 30 nM, about 1 nM – about 20 nM, about 1 nM – about 10 nM, about 10 nM – about 100 nM, about 10 nM – about 90 nM, about 10 nM – about 80 nM, about 10 nM – about 70 nM, about 10 nM – about 60 nM, about 10 nM – about 50 nM, about 10 nM – about 40 nM,

about 10 nM – about 30 nM, or about 10 nM – about 20 nM. In some embodiments, the second antigen-binding site binds CD3 (*e.g.*, human CD3, *e.g.*, human CD3ε) with a  $K_D$ , as measured by BLI, greater than or equal to 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, 100 nM, 200 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, or 1 μM. In some embodiments, the second antigen-binding site binds CD3 with a  $K_D$ , as measured by BLI, within the range of about 10 nM – about 1 μM, about 10 nM – about 900 nM, about 10 nM – about 800 nM, about 10 nM – about 700 nM, about 10 nM – about 600 nM, about 10 nM – about 500 nM, about 10 nM – about 400 nM, about 10 nM – about 300 nM, about 10 nM – about 200 nM, about 10 nM – about 100 nM, about 100 nM – about 1 μM, about 100 nM – about 900 nM, about 100 nM – about 800 nM, about 100 nM – about 700 nM, about 100 nM – about 600 nM, about 100 nM – about 500 nM, about 100 nM – about 400 nM, about 100 nM – about 300 nM, or about 100 nM – about 200 nM.

**[0089]** In some embodiments, the second antigen-binding site, when present in the form of a Fab, has a melting temperature of at least 60 °C, at least 65 °C, at least 70 °C, at least 75 °C, or at least 80 °C. In some embodiments, the second antigen-binding site, when present in the form of an Fab, has a melting temperature in the range of 60-85 °C, 60-80 °C, 60-75 °C, 60-70 °C, 60-65 °C, 65-85 °C, 65-80 °C, 65-75 °C, 65-70 °C, 70-85 °C, 70-80 °C, 70-75 °C, 75-85 °C, 75-80 °C, or 80-85 °C.

### **C. Half-life Extension Domain**

**[0090]** In some embodiments, the multi-specific binding protein comprises a half-life extension domain. As used herein, the term “half-life extension domain” refers to a protein domain that prolongs the half-life of a protein to which it is fused, within a subject (*e.g.*, the blood of the subject). Exemplary half-life extension domains include Fc domains, serum albumin domains, and protein domains that bind serum albumin. In some embodiments, the half-life extension domain in the multi-specific binding protein comprises a third antigen-binding site that binds serum albumin (*e.g.*, HSA). It is contemplated that a serum albumin binding domain may facilitate recycling of the multi-specific binding protein through binding to neonatal Fc receptor (FcRn), thereby extending the serum half-life of the multi-specific binding protein. Accordingly, in certain embodiments, the third antigen-binding site does not bind the D-III domain of HSA (the domain that mediates the interaction between HSA and FcRn). In some embodiments, the third antigen-binding site extends the serum half-life of the multi-specific binding protein.

**[0091]** In some embodiments, the third antigen-binding site is an antigen-binding site that binds serum albumin (*e.g.*, human serum albumin (HSA)) derived from the single domain

antibodies listed in Table 3. The CDR sequences are identified under the Kabat numbering scheme unless indicated by an asterisk (\*).

**Table 3. Serum Albumin Antibody Sequences**

| Antibody    | VH and HCDRs                                                                                                                                                                                                                                                                                                                                    |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CNG-HSA-101 | KVQLVESGGGLVQPGGSLRLSCAASGFTFSSFGMTWVRQAPGKGLEWVSSISGSGSDTLYADSVRGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTIGGSLSPSSQGT LVTVSS (SEQ ID NO: 24)<br><br>HCDR1*: FTFSSFGMT (SEQ ID NO: 25)<br><br>HCDR1: SFGMT (SEQ ID NO: 26)<br><br>HCDR2: SISGSGSDTLYADSVRG (SEQ ID NO: 27)<br><br>HCDR3*: TIGGSLSP (SEQ ID NO: 28)<br><br>HCDR3: GGSLSP (SEQ ID NO: 29) |

**[0092]** In some embodiments, the antigen-binding site that binds serum albumin comprises a VH that comprises an amino acid sequence at least 60% (*e.g.*, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to the VH of an antibody disclosed in Table 3. In some embodiments, the antigen-binding site comprises the HCDR1, HCDR2, and HCDR3, determined under Kabat (see Kabat et al., (1991) Sequences of Proteins of Immunological Interest, NIH Publication No. 91-3242, Bethesda), Chothia (see, *e.g.*, Chothia C & Lesk A M, (1987), J Mol Biol 196: 901-917), MacCallum (see MacCallum R M et al., (1996) J Mol Biol 262: 732-745), IMGT (see Lefranc, (1999) The Immunologist, 7, 132-136), or any other CDR determination method known in the art, of the VH sequence of an antibody disclosed in Table 3. In some embodiments, the antigen-binding site comprises the HCDR1, HCDR2, and HCDR3 sequences of an antibody disclosed in Table 3. In some embodiments, the antigen-binding site comprises the VH sequence of an antibody disclosed in Table 3.

**[0093]** In some embodiments, the antigen-binding site that binds serum albumin is derived from CNG-HSA-101. In some embodiments, the antigen-binding site comprises a VH comprising HCDR1, HCDR2, and HCDR3 sequences set forth in SEQ ID NOs: 25, 27, and 28, respectively. In some embodiments, the antigen-binding site comprises a VH comprising HCDR1, HCDR2, and HCDR3 sequences set forth in SEQ ID NOs: 26, 27, and 29, respectively. In some embodiments, the antigen-binding site comprises a VH comprising an

amino acid sequence at least 60% (*e.g.*, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) identical to SEQ ID NO: 24. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO: 24.

**[0094]** In some embodiments, the antigen-binding site binds human serum albumin with a  $K_D$  lower than or equal to 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, or 3 nM, as measured by SPR when the antigen-binding site is present as a monomer. In some embodiments, the antigen-binding site binds human serum albumin with a  $K_D$  in the range of 1-10 nM, 1-9 nM, 1-8 nM, 1-7 nM, 1-6 nM, 1-5 nM, 1-4 nM, or 1-3 nM, as measured by SPR when the antigen-binding site is present as a monomer.

**[0095]** In some embodiments, the antigen-binding site derived from CNG-HSA-101 binds cynomolgus serum albumin with a  $K_D$  lower than or equal to 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, or 3 nM, as measured by SPR when the antigen-binding site is present as a monomer. In some embodiments, the antigen-binding site derived from CNG-HSA-101 binds cynomolgus serum albumin with a  $K_D$  in the range of 1-9 nM, 1-8 nM, 1-7 nM, 1-6 nM, 1-5 nM, 1-4 nM, or 1-3 nM, as measured by SPR when the antigen-binding site is present as a monomer.

**[0096]** In some embodiments, the antigen-binding site derived from CNG-HSA-101 binds mouse serum albumin with a  $K_D$  lower than or equal to 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, or 10 nM, as measured by SPR when the antigen-binding site is present as a monomer. In some embodiments, the antigen-binding site derived from CNG-HSA-101 binds mouse serum albumin with a  $K_D$  in the range of 1-100 nM, 1-90 nM, 1-80 nM, 1-70 nM, 1-60 nM, 1-50 nM, 1-40 nM, 1-30 nM, 1-20 nM, or 1-10 nM, as measured by SPR when the antigen-binding site is present as a monomer.

**[0097]** In some embodiments, the antigen-binding site derived from CNG-HSA-101 binds human serum albumin with a first  $K_D$  and binds mouse serum albumin with a second  $K_D$ , wherein the ratio of the second  $K_D$  to the first  $K_D$  is in the range of 0.5-10, 0.5-9, 0.5-8, 0.5-7, 0.5-6, 0.5-5, 0.5-4, 0.5-3, 0.5-2, 0.9-10, 0.9-9, 0.9-8, 0.9-7, 0.9-6, 0.9-5, 0.9-4, 0.9-3, 0.9-2, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2. It is understood that an antigen-binding site having a ratio closer to 1 has more similar affinity to mouse serum albumin relative to affinity to human serum albumin, which allows assessment of the pharmacokinetics of the antigen-binding site or a protein comprising the same using a mouse model at higher accuracy.

**[0098]** Melting temperature represents the thermostability of the antigen-binding site and can be measured by differential scanning fluorimetry, for example, as described in Durowoju

*et al.* (2017) *J. Vis. Exp.* (121): 55262. The thermostability of an antibody or fragment thereof may be enhanced by grafting CDRs onto stable frameworks, introducing non-canonical disulfide bonds, and other mutagenesis, as described in McConnell *et al.* (2014) *MAbs*, 6(5): 1274-82; and Goldman *et al.* (2017) *Front. Immunol.*, 8: 865. In some embodiments, the antigen-binding site derived from CNG-HSA-101 has a melting temperature greater than or equal to 60 °C, as measured by differential scanning fluorimetry. In some embodiments, the antigen-binding site derived from CNG-HSA-101 has a melting temperature greater than or equal to 65 °C, as measured by differential scanning fluorimetry.

**[0099]** In some embodiments, the third antigen-binding site competes for binding serum (*e.g.*, human serum albumin) and/or competes for binding protein A with an antibody or antigen-binding site comprising the VH sequence provided in Table 3.

**[0100]** In some embodiments, the third antigen-binding site has a melting temperature of at least 50 °C, at least 55 °C, at least 56 °C, at least 57 °C, at least 58 °C, at least 59 °C, at least 60 °C, at least 61 °C, at least 62 °C, at least 63 °C, at least 64 °C, at least 65 °C, at least 70 °C, at least 75 °C, or at least 80 °C. In some embodiments, the third antigen-binding site has a melting temperature in the range of 50-80 °C, 50-70 °C, 50-65 °C, 50-60 °C, 50-55 °C, 55-70 °C, 55-65 °C, 55-60 °C, 56-65 °C, 56-60 °C, 57-65 °C, 57-60 °C, 58-65 °C, 58-60 °C, 59-65 °C, 59-60 °C, 60-80 °C, 60-75 °C, 60-70 °C, 60-65 °C, 65-80 °C, 65-75 °C, 65-70 °C, 70-80 °C, or 70-75 °C.

#### **D. Construct Formats**

**[0101]** The first, second, and third antigen-binding sites may take various forms. In some embodiments, the first, second, and/or third antigen-binding sites comprises two antibody variable domains (*e.g.*, a VH and a VL). The VH and the VL can be mutated to introduce a disulfide bond (*e.g.*, between H44 and L100) that stabilizes the antigen-binding site (see, Zhao *et al.* (2010) *Int. J. Mol. Sci.*, 12(1):1-11). In some embodiments, the first, second, and/or third antigen-binding sites comprises a single antibody variable domain (*e.g.*, an sdAb).

**[0102]** In an antigen-binding site that contains a VH and a VL, the VH and the VL can be linked to form an scFv. The VH can be positioned N-terminal or C-terminal to the VL. The VH and the VL are typically linked through a linker, such as a peptide linker. Exemplary sequences of peptide linkers are provided in subsection E below titled "Linkers." In some embodiments, the VH of an antigen-binding domain is connected to the VL of the antigen-binding domain through a peptide linker having an amino acid sequence listed in Table 4. In particular embodiments, the VH of an antigen-binding domain is connected to the VL of the antigen-binding domain through a peptide linker having the amino acid sequence of SEQ ID

NO: 36, 37, or 40, wherein the VH is positioned N-terminal to the VL. In other particular embodiments, the VH of an antigen-binding domain is connected to the VL of the antigen-binding domain through a peptide linker having the amino acid sequence of SEQ ID NO: 36, 37, or 40, wherein the VH is positioned C-terminal to the VL.

**[0103]** In some embodiments, the VH and the VL is present on separate polypeptide chains, and the formation of a VH-VL complex is facilitated by additional domains, such as antibody constant regions CH1 and CL. Accordingly, in certain embodiments, the multi-specific binding protein comprises a Fab comprising a VH and a VL disclosed herein.

**[0104]** In some embodiments, a multi-specific binding protein of the present disclosure comprises a first antigen-binding site comprising a single antibody variable domain, a second antigen-binding site comprising a single antibody variable domain, and a third antigen-binding site comprising a single antibody variable domain. In some embodiments, the multi-specific binding protein comprises a first antigen-binding site in an sdAb format, a second antigen-binding site in an sdAb format, and a third antigen-binding site in an sdAb format.

**[0105]** In some embodiments, a multi-specific binding protein of the present disclosure comprises a first antigen-binding site comprising a single antibody variable domain, a second antigen-binding site comprising a single antibody variable domain, and a third antigen-binding site comprising two antibody variable domains. In some embodiments, the multi-specific binding protein comprises a first antigen-binding site in an sdAb format, a second antigen-binding site in an sdAb format, and a third antigen-binding site in an scFv format.

**[0106]** In some embodiments, a multi-specific binding protein of the present disclosure comprises a first antigen-binding site comprising a single antibody variable domain, a second antigen-binding site comprising two antibody variable domains, and a third antigen-binding site comprising a single antibody variable domain. In some embodiments, the multi-specific binding protein comprises a first antigen-binding site in an sdAb format, a second antigen-binding site in an scFv format, and a third antigen-binding site in an sdAb format.

**[0107]** In some embodiments, a multi-specific binding protein of the present disclosure comprises a first antigen-binding site comprising a single antibody variable domain, a second antigen-binding site comprising two antibody variable domains, and a third antigen-binding site comprising two antibody variable domains. In some embodiments, the multi-specific binding protein comprises a first antigen-binding site in an sdAb format, a second antigen-binding site in an scFv format, and a third antigen-binding site in an scFv format.

**[0108]** In some embodiments, a multi-specific binding protein of the present disclosure comprises a first antigen-binding site comprising two antibody variable domains, a second

antigen-binding site comprising a single antibody variable domain, and a third antigen-binding site comprising a single antibody variable domain. In some embodiments, the multi-specific binding protein comprises a first antigen-binding site in an scFv format, a second antigen-binding site in an sdAb format, and a third antigen-binding site in an sdAb format.

**[0109]** In some embodiments, a multi-specific binding protein of the present disclosure comprises a first antigen-binding site comprising two antibody variable domains, a second antigen-binding site comprising a single antibody variable domain, and a third antigen-binding site comprising two antibody variable domains. In some embodiments, the multi-specific binding protein comprises a first antigen-binding site in an scFv format, a second antigen-binding site in an sdAb format, and a third antigen-binding site in an scFv format.

**[0110]** In some embodiments, a multi-specific binding protein of the present disclosure comprises a first antigen-binding site comprising two antibody variable domains, a second antigen-binding site comprising two antibody variable domains, and a third antigen-binding site comprising a single antibody variable domain. In some embodiments, the multi-specific binding protein comprises a first antigen-binding site in an scFv format, a second antigen-binding site in an scFv format, and a third antigen-binding site in an sdAb format.

**[0111]** In some embodiments, a multi-specific binding protein of the present disclosure comprises a first antigen-binding site comprising two antibody variable domains, a second antigen-binding site comprising two antibody variable domains, and a third antigen-binding site comprising two antibody variable domains. In some embodiments, the multi-specific binding protein comprises a first antigen-binding site in an scFv format, a second antigen-binding site in an scFv format, and a third antigen-binding site in an scFv format.

**[0112]** The three antigen-binding sites of the multi-specific binding protein can be linked in any one of the following orientations in an amino-to-carboxyl direction:

- (i) the first antigen-binding site (CD19 binding domain) – the second antigen-binding site (CD3 binding domain) – the third antigen-binding site (serum albumin binding domain);
- (ii) the first antigen-binding site (CD19 binding domain) – the third antigen-binding site (serum albumin binding domain) – the second antigen-binding site (CD3 binding domain);
- (iii) the second antigen-binding site (CD3 binding domain) – the first antigen-binding site (CD19 binding domain) – the third antigen-binding site (serum albumin binding domain);
- (iv) the second antigen-binding site (CD3 binding domain) – the third antigen-binding site (serum albumin binding domain) – the first antigen-binding site (CD19 binding domain);
- (v) the third antigen-binding site (serum albumin binding domain) – the first antigen-binding site (CD19 binding domain) – the second antigen-binding site (CD3 binding domain); and

(vi) the third antigen-binding site (serum albumin binding domain) – the second antigen-binding site (CD3 binding domain) – the first antigen-binding site (CD19 binding domain), wherein the dashes above represent a peptide bond and/or a linker (*e.g.*, peptide linker).

**[0113]** In some embodiments, the third antigen-binding site is not positioned between the first antigen-binding site and the second antigen-binding site. It is contemplated that constructs having such formats have favorable therapeutic efficacy and *in vivo* half-life. In some embodiments, the third antigen-binding site is positioned N-terminal to both the first antigen-binding site and the second antigen-binding site or C-terminal to both the first antigen-binding site and the second antigen-binding site. In some embodiments, the third antigen-binding site is positioned N-terminal to both the first antigen-binding site and the second antigen-binding site. In some embodiments, the third antigen-binding site is positioned C-terminal to both the first antigen-binding site and the second antigen-binding site.

**[0114]** The position (N-terminal or C-terminal) of one antigen-binding site relative to another is determined under the definitions of “N-terminal” and “C-terminal” as known in the art if a single polypeptide chain comprises both antigen-binding sites. It is understood that if an antigen-binding site comprises two separate polypeptide chains, its position (N-terminal or C-terminal) relative to another antigen-binding site (either having a single polypeptide chain or two polypeptide chains) can be similarly determined if a single polypeptide chain comprises at least one polypeptide chain of the former and at least one polypeptide chain of the latter. It is further understood that if antigen-binding site A is N-terminal to antigen-binding site B and antigen-binding site B is N-terminal to antigen-binding site C, it is deemed that antigen-binding site A is positioned N-terminal to antigen-binding site C even if antigen-binding sites A and C are not present in any single, common polypeptide chain. More complex structures of multi-specific binding proteins are also contemplated, some of which may have orientations difficult to characterize using the terms of “N-terminal” and “C-terminal” as described above due to, for example, different relative positions of two antigen-binding sites on one polypeptide chain versus another polypeptide chain, or the presence of a loop structure.

**[0115]** According to the present disclosure, the multi-specific binding proteins and its constituent binding domains are in the form of one or more polypeptides. Such polypeptides may include proteinaceous parts and non-proteinaceous parts (*e.g.*, chemical linkers or chemical cross-linking agents such as glutaraldehyde). In some embodiments, a multi-specific binding protein of the present disclosure includes a first antigen-binding site, a second antigen-binding site, and a third antigen-binding site, all of which are linked together to form a single polypeptide chain. In some embodiments, the first, second, and third antigen-binding sites

take the forms of scFv and/or sdAb, for example, in a combination as described above, to form a single polypeptide chain.

#### **E. Linkers**

**[0116]** As noted above, the antigen-binding sites of the multi-specific binding proteins of the present disclosure can be linked through a peptide bond or a linker (*e.g.*, peptide linker). In some embodiments, at least two adjacent antigen-binding sites are connected by a linker (*e.g.*, peptide linker). In some embodiments, each two adjacent antigen-binding sites are connected by a linker (*e.g.*, peptide linker).

**[0117]** In some embodiments, the three antigen-binding sites of the multi-specific binding protein can be linked by linkers (*e.g.*, peptide linkers) denoted as  $L_1$  and  $L_2$  in any one of the following orientations in an amino-to-carboxyl direction:

- (i) the first antigen-binding site (CD19 binding domain) –  $L_1$  – the second antigen-binding site (CD3 binding domain) –  $L_2$  – the third antigen-binding site (serum albumin binding domain);
- (ii) the first antigen-binding site (CD19 binding domain) –  $L_1$  – the third antigen-binding site (serum albumin binding domain) –  $L_2$  – the second antigen-binding site (CD3 binding domain);
- (iii) the second antigen-binding site (CD3 binding domain) –  $L_1$  – the first antigen-binding site (CD19 binding domain) –  $L_2$  – the third antigen-binding site (serum albumin binding domain);
- (iv) the second antigen-binding site (CD3 binding domain) –  $L_1$  – the third antigen-binding site (serum albumin binding domain) –  $L_2$  – the first antigen-binding site (CD19 binding domain);
- (v) the third antigen-binding site (serum albumin binding domain) –  $L_1$  – the first antigen-binding site (CD19 binding domain) –  $L_2$  – the second antigen-binding site (CD3 binding domain); and (vi) the third antigen-binding site (serum albumin binding domain) –  $L_1$  – the second antigen-binding site (CD3 binding domain) –  $L_2$  – the first antigen-binding site (CD19 binding domain). It is appreciated that in a given construct,  $L_1$ ,  $L_2$ , or both  $L_1$  and  $L_2$  may be replaced with a peptide bond.

**[0118]** It is understood that if a single polypeptide chain comprises two adjacent antigen-binding sites, the peptide linker connecting the two antigen-binding sites represents the amino acid sequence between them. If an antigen-binding site comprises two separate polypeptide chains, one of which is present in a single, common polypeptide as an adjacent antigen-binding site or a polypeptide chain thereof, the peptide linker connecting the two antigen-binding sites represents the amino acid sequence between them in the common, single polypeptide.

**[0119]** In some embodiments, the linkers  $L_1$  and  $L_2$  are peptide linkers. Suitable lengths of  $L_1$  and  $L_2$  can be independently selected. For example, in certain embodiments,  $L_1$  and/or  $L_2$  are about 50 or less amino acid residues in length. In some embodiments,  $L_1$  consists of about 50 or less amino acid residues. In some embodiments,  $L_1$  consists of about 20 or less amino acid residues. In some embodiments,  $L_2$  consists of about 50 or less amino acid residues. In some embodiments,  $L_2$  consists of about 20 or less amino acid residues. In some embodiments,  $L_1$  and  $L_2$  independently consist of about 50 or less amino acid residues. In some embodiments,  $L_1$  and  $L_2$  independently consist of about 20 or less amino acid residues.

**[0120]** In some embodiments, peptide linkers  $L_1$  and  $L_2$  have an optimized length and/or amino acid composition. In some embodiments,  $L_1$  and  $L_2$  are of the same length and have the same amino acid composition. In some embodiments,  $L_1$  and  $L_2$  are different. In some embodiments,  $L_1$  and/or  $L_2$  are “short,” *i.e.*, consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 amino acid residues. Thus, in certain instances, the linkers consist of about 12 or less amino acid residues. In some embodiments,  $L_1$  and/or  $L_2$  are “long,” *e.g.*, consist of 15, 20 or 25 amino acid residues. In some embodiments,  $L_1$  and/or  $L_2$  consist of about 3 to about 15, for example 8, 9 or 10 contiguous amino acid residues.

**[0121]** Regarding the amino acid composition of  $L_1$  and  $L_2$ , peptides are selected with properties that confer flexibility to multi-specific binding protein of the present disclosure, do not interfere with the binding domains as well as resist cleavage from proteases. For example, glycine and serine residues generally provide protease resistance. Examples of the linkers suitable for linking the domains in the multi-specific binding protein include but are not limited to  $(GS)_n$ ,  $(GGS)_n$ ,  $(GGGS)_n$ ,  $(GGSG)_n$ ,  $(GGS GG)_n$ , and  $(GGGGS)_n$ , wherein  $n$  is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments,  $L_1$  and/or  $L_2$  are independently selected from the peptide sequences listed in Table 4. In some embodiments,  $L_1$  and/or  $L_2$  are independently selected from SEQ ID NOs: 30-40. In some embodiments,  $L_1$  and/or  $L_2$  are independently selected from SEQ ID NOs: 36, 37, or 40. In some embodiments,  $L_1$  and/or  $L_2$  comprise the amino acid sequence of SEQ ID NO: 36, 37, or 40. In some embodiments,  $L_1$  and/or  $L_2$  consist of the amino acid sequence of 36, 37, or 40. In some embodiments,  $L_1$  and  $L_2$  each comprise the amino acid sequence of SEQ ID NO: 36, 37, or 40. In some embodiments,  $L_1$  and  $L_2$  each consist of the amino acid sequence of SEQ ID NO: 36, 37, or 40.

**Table 4. Sequences of Exemplary Peptide Linkers**

[illegible]

**[0122]** A linker, such as a peptide linker disclosed herein, can also be used to connect the VH and VL of a scFv, as mentioned in subsection D above titled “Construct Formats.”

### F. Multi-Specific Binding Proteins

**[0123]** Listed below in Table 5 is multi-specific binding proteins comprising a scFv that binds CD19, an scFv that binds CD3, and an sdAb that binds serum albumin. In some aspects, tAB0050 may also be referred to as CLN-978.

### Table 5. Multi-Specific Binding Proteins

| Construct               | Format <sup>1</sup> | CD19 Binder                  | CD3 Binder <sup>2</sup>   | HSA Binder                  |
|-------------------------|---------------------|------------------------------|---------------------------|-----------------------------|
| tAB0050 (SEQ ID NO: 41) | HSA:CD19:CD3        | CNG-CD19-701 (SEQ ID NO: 11) | CNG-CD3-1 (SEQ ID NO: 22) | CNG-HSA-101 (SEQ ID NO: 24) |

<sup>1</sup> The multi-specific binding proteins in this table are present as a single polypeptide. The format shows the order of CD19-binding scFv, the CD3-binding scFv, and the HSA-binding sdAb, from the N-terminus to the C-terminus.

<sup>2</sup> The CD3-binding scFv sequences can contain or lack Cys substitutions at position 44 of VH and position 100 of VL. As a result, two sequences are provided for scFv derived from each of the antibodies CNG-CD3-1 (see also Table 2).

tAB0050 (SEQ ID NO: 41)

KVQLVESGGGLVQPGGSLRLSCAASGFTFSSFGMTWVRQAPGKGLEWVSSISGSGSDTLYA  
DSVRGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTIGGSLSPSSQGTLLTVSSGGGGSGGG  
SEIVLTQSPATLSLSPGERATLSCSASSSVGYMHYQQKPGQAPRLLIYDTSKLASGIPARFS  
GSGSGTDFTLTISSLEPEDFAVYYCFQGSVYPFTFGQGTKLEIKGGGSGGGSGGGSGGVQ  
LQESGPGLVKPSQTLSTCTVSGGSISTSTMGVGWIRQHPGKGLEWIGFIWDDDKRYNP  
LKSRVTMSVDTSKNQFSLKLSVTAADTAVYYCARMELWSYFYDYWGQGTLLTVSSGGG  
GSGGSDIVMTQSPDSLAVSLGERATINCKSSQSLNARTGKNYLAWYQQKPGQPPKLLIY  
WASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSYSRRTFGGGTKVEIKGGGGS  
GGGSGGGSGGGSGGVQQLVQSGAEVKKPGASVKVSCKASGFNIKDYMHVWRQAPGQR  
LEWMGWIDLENANTIYDAKFQGRVTITRDTASTAYMELSSLRSEDVAVYYCARDAYGRYF  
YDVWGQGTLLTVSS

**[0124]** The antigen-binding sites listed in a given row of Table 5 can be linked, in the orientation specified in the row, through peptide linkers described in subsection E above. In some embodiments, at least two adjacent antigen-binding sites are linked by a peptide linker having the amino acid sequence of SEQ ID NO: 40. In some embodiments, each two adjacent antigen-binding sites are linked by a peptide linker having the amino acid sequence of SEQ ID NO: 40, thereby forming a multi-specific binding protein present in a single polypeptide.

**[0125]** In some embodiments, the multi-specific binding protein comprises, from N-terminus to C-terminus, SEQ ID NOs: 24, 11, and 22. In some embodiments, the multi-specific binding protein comprises the amino acid sequence of SEQ ID NO: 41.

**[0126]** In some embodiments, the multi-specific binding protein comprises an antigen-binding site that binds CD19 as disclosed herein, an antigen-binding site that binds CD3, and a half-life extension domain comprising an antibody Fc region. The multi-specific binding protein can take various formats to combine the antigen-binding sites and the Fc region.

**[0127]** In some embodiments, the multi-specific binding protein comprises an anti-CD19 antibody in an IgG antibody format fused with a CD3-binding scFv at the C-terminus of the IgG Fc region. In some embodiments, the multi-specific binding protein comprises a first polypeptide chain comprising, from the N-terminus to the C-terminus, the VH of an antigen-binding site that binds CD19, CH1 domain, hinge, CH2 domain, and CH3 domain of an IgG antibody (*e.g.*, human IgG1, IgG2, IgG3, or IgG4), and an scFv that binds CD3; and a second polypeptide chain comprising, from the N-terminus to the C-terminus, the VL of the antigen-binding site that binds CD19 and light chain constant (CL) domain of the IgG antibody. In some embodiments, the scFv that binds CD3 comprises a VL domain positioned N-terminal to a VH domain. In some embodiments, the IgG antibody is a human IgG1 antibody. In some embodiments, the multi-specific binding protein comprises two of the first polypeptide chain and two of the second polypeptide chain, thereby forming a dimeric antibody Fc region.

### G. Therapeutic Activities

[0128] The multi-specific binding protein disclosed herein is designed to simultaneously bind B cells and T cells. Recruitment of T cells facilitates lysis of the B cells involving cytolytic synapse formation and delivery of perforin and granzymes. The engaged T cells are capable of serial target cell lysis, and are not affected by immune escape mechanisms interfering with peptide antigen processing and presentation, or clonal T cell differentiation; see, for example, WO2007042261A2. Accordingly, binding of the multi-specific binding proteins to the target B cells destroys the target cells and/or impairs the progression of B cell related diseases. In specific embodiments of the present invention, the B cell related disease is relapsed and/or refractory Non-Hodgkin lymphoma (NHL).

[0129] Cytotoxicity mediated by multi-specific binding proteins of the disclosure can be measured in various ways *in vitro*. Effector cells can be *e.g.*, stimulated enriched (human) CD8 positive T cells or unstimulated (human) peripheral blood mononuclear cells (PBMC). If the target cells are of macaque origin or express or are transfected with macaque target cell surface antigen which is bound by the first domain, the effector cells should also be of macaque origin such as a macaque T cell line, *e.g.*, 4119LnPx. The target cells should express CD19, *e.g.*, human or macaque CD19. The target cells can be a cell line (such as CHO) which is stably or transiently transfected with CD19. Alternatively, the target cells can be a cell line naturally expressing CD19, such as B lymphocytes. The effector to target cell (E:T) ratio is usually about 10:1, but can also vary. Killing of the target cells can be measured in a  $^{51}\text{Cr}$ -release assay (incubation time of about 18 hours) or in a FACS-based cytotoxicity assay (incubation time of about 48 hours). Other methods of measuring cell death are well-known to the skilled person, such as MTT or MTS assays, ATP-based assays including bioluminescent assays, the sulforhodamine B (SRB) assay, WST assay, clonogenic assay and the ECIS technology.

[0130] In some embodiments, the cytotoxic activity mediated by the multi-specific binding protein disclosed herein is measured in a cell-based cytotoxicity assay described above. It is represented by the  $\text{EC}_{50}$  value, which corresponds to the half maximal effective concentration (concentration of the multi-specific binding protein which induces a cytotoxic response halfway between the baseline and maximum). In some embodiments, the  $\text{EC}_{50}$  value of the multi-specific binding proteins is  $\leq 5000$  pM, for example,  $\leq 4000$  pM,  $\leq 3000$  pM,  $\leq 2000$  pM,  $\leq 1000$  pM,  $\leq 500$  pM,  $\leq 400$  pM,  $\leq 300$  pM,  $\leq 200$  pM,  $\leq 100$  pM,  $\leq 50$  pM,  $\leq 20$  pM,  $\leq 10$  pM,  $\leq 5$  pM,  $\leq 4$  pM,  $\leq 3$  pM,  $\leq 2$  pM, or  $\leq 1$  pM.

**[0131]** It is understood that an  $EC_{50}$  value is generally lower when stimulated/enriched  $CD8^+$  T cells are used as effector cells, compared with unstimulated PBMC. It is further understood that the  $EC_{50}$  value is generally lower when the target cells express a high level of the target cell surface antigen compared with a low level of the target antigen. For example, when stimulated/enriched human  $CD8^+$  T cells are used as effector cells (and either target cell surface antigen transfected cells such as CHO cells or target cell surface antigen positive human cell lines are used as target cells), the  $EC_{50}$  value of multi-specific binding protein is  $\leq 1000$  pM, for example,  $\leq 500$  pM,  $\leq 250$  pM,  $\leq 100$  pM, 50 pM,  $\leq 10$  pM, or  $\leq 5$  pM. When human PBMCs are used as effector cells, the  $EC_{50}$  value of the multi-specific binding protein is  $\leq 5000$  pM, for example,  $\leq 4000$  pM,  $\leq 2000$  pM,  $\leq 1000$  pM,  $\leq 500$  pM,  $\leq 200$  pM,  $\leq 150$  pM,  $\leq 100$  pM,  $\leq 50$  pM,  $\leq 10$  pM, or  $\leq 5$  pM. When a macaque T cell line such as LnPx4119 is used as effector cells, and a macaque target cell surface antigen transfected cell line such as CHO cells is used as target cell line, the  $EC_{50}$  value of the multi-specific binding protein is  $\leq 2000$  pM, for example,  $\leq 1500$  pM,  $\leq 1000$  pM,  $\leq 500$  pM,  $\leq 300$  pM,  $\leq 250$  pM,  $\leq 100$  pM,  $\leq 50$  pM,  $\leq 10$  pM, or  $\leq 5$  pM.

**[0132]** Accordingly, in certain embodiments, the  $EC_{50}$  value is measured using stimulated/enriched human  $CD8^+$  T cells as effector cells. In some embodiments, the  $EC_{50}$  value is measured using human PBMCs as effector cells. In some embodiments, the  $EC_{50}$  value is measured using a macaque T cell line such as LnPx4119 as effector cells and cells (*e.g.*, CHO cells) engineered to express macaque CD19 as target cells.

**[0133]** In some embodiments, the multi-specific binding protein of the present disclosure does not induce or mediate lysis of cells that do not express CD19. The term “does not induce lysis” or “does not mediate lysis,” or grammatical equivalents thereof, means that the multi-specific binding protein, at a concentration of up to 500 nM, does not induce or mediate lysis of more than 30%, for example, no more than 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5% of cells that do not express CD19, whereby lysis of a cell line that expresses CD19 is set to be 100%.

**[0134]** In some embodiments, a multi-specific binding protein disclosed herein is more effective in killing CD19-expressing cells (*e.g.*, cancer cells) than the corresponding respective anti-CD19 or anti-CD3 monoclonal antibody at the same molar concentration. In some embodiments, the multi-specific binding protein is more effective in killing CD19-expressing cells (*e.g.*, cancer cells) than a combination of the corresponding respective anti-CD19 and anti-CD3 monoclonal antibodies each at the same molar concentration.

**[0135]** The cytotoxic activity of the multi-specific binding protein can be measured in the presence or absence of serum albumin (*e.g.*, HSA). In some embodiments, the cytotoxic activity disclosed above is measured in the absence of serum albumin (*e.g.*, HSA). In some embodiments, the cytotoxic activity disclosed above is measured in substantial absence of serum albumin (*e.g.*, HSA). In some embodiments, the cytotoxic activity disclosed above is measured in the presence of serum albumin (*e.g.*, HSA), for example, in the presence of about 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL, 40 mg/mL, 45 mg/mL, or 50 mg/mL serum albumin (*e.g.*, HSA).

**[0136]** In some embodiments, the multi-specific binding protein of the present disclosure kills CD19-expressing cells with a similar EC<sub>50</sub> value in the presence of serum albumin to that in the absence or substantial absence of serum albumin. In some embodiments, the EC<sub>50</sub> value of the multi-specific binding protein for killing CD19-expressing cells in the presence of serum albumin is increased by no more than 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 25 fold, 30 fold, 35 fold, 40 fold, 45 fold, or 50 fold compared to that in the absence or substantial absence of serum albumin. It is understood that the presence of serum albumin (*e.g.*, about 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL, 40 mg/mL, 45 mg/mL, or 50 mg/mL serum albumin) may also alter the EC<sub>50</sub> value of a multi-specific binding protein nonspecifically. The nonspecific effect can be assessed by comparing the EC<sub>50</sub> values of a control protein, which does not contain a serum albumin binding domain, in the presence and absence of serum albumin. In some embodiments, the fold change is offset by the nonspecific effect of serum albumin on a control protein, such as a bispecific protein that binds CD19 and CD3.

#### **H. Construct Size**

**[0137]** In some embodiments, the molecular weight of the multi-specific binding protein is from about 40 kD to about 100 kD. In some embodiments, the molecular weight of the multi-specific binding protein is at least 60 kD, at least 65 kD, at least 70 kD, at least 75 kD, at least 80 kD, at least 85 kD, at least 90 kD, or at least 95 kD. It is understood that smaller size generally contributes to faster diffusion and tissue penetration, but size reduction may not be as critical for the purpose of treating the indications with substantial presence of target cells (*e.g.*, cancer cells) in the blood.

**[0138]** In some embodiments, the molecular weight of the multi-specific binding protein is from about 40 kD to about 90 kD, from about 40 kD to about 80 kD, from about 40 kD to about 70 kD, from about 40 kD to about 60 kD, from about 40 kD to about 50 kD, from about 50 kD to about 100 kD, from about 50 kD to about 90 kD, from about 50 kD to about 80 kD,

from about 50 kD to about 70 kD, from about 50 kD to about 60 kD, from about 60 kD to about 100 kD, from about 60 kD to about 90 kD, from about 60 kD to about 80 kD, from about 60 kD to about 70 kD, from about 65 kD to about 100 kD, from about 65 kD to about 90 kD, from about 65 kD to about 80 kD, from about 65 kD to about 70 kD, from about 70 kD to about 100 kD, from about 70 kD to about 90 kD, from about 70 kD to about 80 kD, from about 80 kD to about 100 kD, from about 80 kD to about 90 kD, or from about 90 kD to about 100 kD. In some embodiments, the multi-specific binding protein is lower than 40 kD. In some embodiments, the multi-specific binding protein is about 50 kD – about 90 kD, about 50 kD – about 80 kD, about 50 kD – about 70 kD, about 50 kD – about 60 kD, about 60 kD – about 90 kD, about 60 kD – about 80 kD, about 60 kD – about 70 kD, about 65 kD – about 90 kD, about 65 kD – about 80 kD, about 65 kD – about 70 kD, about 70 kD – about 90 kD, or about 70 kD – about 80 kD.

### ***I. Serum Half-life***

**[0139]** Fusion proteins have been developed to increase the *in vivo* half-life of a small protein, particularly an antibody fragment. For example, fusion with a heterodimeric antibody Fc region, such as an Fc with one or more mutations that extend the *in vivo* half-life, is described in U.S. Patent Application Publication Nos. US20140302037A1, US20140308285A1, and PCT Publication Nos. WO2014144722A2, WO2014151910A1 and WO2015048272A1. An alternative strategy is fusion with human serum albumin (HSA) or an HSA-binding peptide (see, *e.g.*, PCT Publication Nos. WO2013128027A1 and WO2014140358A1). The neonatal Fc receptor (FcRn) appears to be involved in prolonging the life-span of albumin in circulation (see, Chaudhury *et al.* (2003) J. Exp. Med., 3: 315-22). Albumin and IgG bind noncooperatively to distinct sites of FcRn and form a tri-molecular (see *id.*). Binding of human FcRn to HSA and to human IgG is pH dependent, stronger at acidic pH and weaker at neutral or physiological pH (see *id.*). This observation suggests that proteins and protein complexes containing albumin, similar to those containing IgG (particularly Fc), are protected from degradation through pH-sensitive interaction with FcRn (see *id.*). Using surface plasmon resonance (SPR) to measure the capacity of individual HSA domains to bind immobilized soluble human FcRn, it has been shown that FcRn and albumin interact via the D-III domain of albumin in a pH-dependent manner, on a site distinct from the IgG binding site (see, Chaudhury *et al.* (2006) Biochemistry 45:4983-90 and PCT Publication No. WO2008068280A1).

**[0140]** The present disclosure provides multi-specific binding proteins with extended half-life. In some embodiments, the multi-specific binding protein has a serum half-life of at least

24, 36, 48, 60, 72, 84, or 96 hours. In some embodiments, the multi-specific binding protein has a serum half-life of at least about 50 hours. In some embodiments, the multi-specific binding protein has a serum half-life of at least about 100 hours. Methods of measuring serum half-life are known in the art. In some embodiments, the serum half-life is measured in a non-human primate. In some embodiments, the serum half-life is measured in human.

**[0141]** In some embodiments, 50 hours after intravenous administration to a subject, the serum concentration of the multi-specific binding protein is at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% of the serum concentration of the multi-specific binding protein 1 hour after the administration in said subject.

**[0142]** In some embodiments, the multi-specific binding protein has a serum half-life that is at least 20% longer than a control multi-specific binding protein, wherein the control multi-specific binding protein includes a first domain identical to the first antigen-binding site of the multi-specific binding protein, a second domain identical to the second antigen-binding site of the multi-specific binding protein, but not a third domain identical or substantially identical to the third antigen-binding site of the multi-specific binding protein. In some embodiments, the control multi-specific binding protein is identical to the multi-specific binding protein but for the absence of the half-life extension domain. In some embodiments, the serum half-life of the multi-specific binding protein is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% longer than the serum half-life of the control multi-specific binding protein. In some embodiments, the serum half-life of the multi-specific binding protein is longer than the serum half-life of the control multi-specific binding protein by at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, or at least 10 fold.

## **II. METHODS OF PREPARATION**

**[0143]** The antibodies and multi-specific binding proteins described above can be made using recombinant DNA technology well known to a skilled person in the art. For example, one or more isolated polynucleotides encoding the antibody or the multi-specific binding protein can be ligated to other appropriate nucleotide sequences, including, for example, constant region coding sequences, and expression control sequences, to produce conventional gene expression constructs (*i.e.*, expression vectors) encoding the desired antibodies or multi-specific binding proteins. Production of defined gene constructs is within routine skill in the art.

[0144] Nucleic acids encoding desired antibodies or multi-specific binding proteins can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and myeloma cells that do not otherwise produce IgG protein. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the antibodies or multi-specific binding proteins.

[0145] Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, *e.g.*, Trp or Tac, and a prokaryotic signal sequence. The expressed protein may be secreted. The expressed protein may accumulate in refractile or inclusion bodies, which can be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the protein may be refolded and/or cleaved by methods known in the art.

[0146] If the engineered gene is to be expressed in eukaryotic host cells, *e.g.*, CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, a poly A sequence, and a stop codon. Optionally, the vector or gene construct may contain enhancers and introns. In embodiments involving fusion proteins comprising an antibody or portion thereof, the expression vector optionally contains sequences encoding all or part of a constant region, enabling an entire, or a part of, a heavy or light chain to be expressed. The gene construct can be introduced into eukaryotic host cells using conventional techniques.

[0147] The antibodies or multi-specific binding protein disclosed herein may comprise a single polypeptide chain. In this instance, a host cell can be transfected with a single vector expressing the polypeptide (*e.g.*, containing an expression control sequence operably linked to a nucleotide sequence encoding the polypeptide). Alternatively, the antibodies or multi-specific binding proteins disclosed herein may comprise two or more polypeptides. In this instance, a host cell can be co-transfected with more than one expression vector, for example, one expression vector expressing each polypeptide. A host cell can also be transfected with a single expression vector that expresses the two or more polypeptides. For example, the coding sequences of the two or more polypeptides can be operably linked to different expression control sequences (*e.g.*, promoter, enhancer, and/or internal ribosome entry site (IRES)). The

coding sequences of the two or more polypeptides can also be separated by a ribosomal skipping sequence or self-cleaving sequence, such as a 2A peptide.

**[0148]** In some embodiments, in order to express an antibody or multi-specific binding protein, an N-terminal signal sequence is included in the protein construct. Exemplary N-terminal signal sequences include signal sequences from interleukin-2, CD-5, IgG kappa light chain, trypsinogen, serum albumin, and prolactin.

**[0149]** After transfection, single clones can be isolated for cell bank generation using methods known in the art, such as limited dilution, ELISA, FACS, microscopy, or Clonepix. Clones can be cultured under conditions suitable for bio-reactor scale-up and maintained expression of the antibodies or multi-specific binding proteins.

**[0150]** The antibodies or multi-specific binding proteins can be isolated and purified using methods known in the art including centrifugation, depth filtration, cell lysis, homogenization, freeze-thawing, affinity purification, gel filtration, ion exchange chromatography, hydrophobic interaction exchange chromatography, and mixed-mode chromatography.

### **III. PHARMACEUTICAL COMPOSITIONS**

**[0151]** The present disclosure also features pharmaceutical compositions that contain a therapeutically effective amount of the antibodies or multi-specific binding proteins described herein. The composition can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the composition for proper formulation. Suitable formulations for use in the present disclosure are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed., 1985. For a brief review of methods for drug delivery, *see, e.g.*, Langer (Science 249:1527-1533, 1990).

**[0152]** In some embodiments, a pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In such embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers;

monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants (see, *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990).

**[0153]** In some embodiments, a pharmaceutical composition may contain nanoparticles, *e.g.*, polymeric nanoparticles, liposomes, or micelles (See Anselmo *et al.* (2016) *BIOENG. TRANSL. MED.* 1: 10-29).

**[0154]** In some embodiments, a pharmaceutical composition may contain a sustained- or controlled-delivery formulation. Techniques for formulating sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. Sustained-release preparations may include, *e.g.*, porous polymeric microparticles or semipermeable polymer matrices in the form of shaped articles, *e.g.*, films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides, copolymers of L-glutamic acid and gamma ethyl-L-glutamate, poly (2-hydroxyethyl-methacrylate), ethylene vinyl acetate, or poly-D(-)-3-hydroxybutyric acid. Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art.

**[0155]** Pharmaceutical compositions containing an antibody or a multi-specific binding protein disclosed herein can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. Examples of routes of administration are intravenous (IV), intradermal, inhalation, transdermal, topical, transmucosal, intrathecal and rectal administration. In some embodiments, an antibody or a multi-specific binding protein disclosed herein is administered by IV infusion. In some embodiments, an antibody or a multi-specific binding protein disclosed herein is administered by intratumoral injection. Useful

formulations can be prepared by methods known in the pharmaceutical art. For example, see *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990). Formulation components suitable for parenteral administration include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

**[0156]** For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof.

**[0157]** An intravenous drug delivery formulation may be contained in a syringe, pen, or bag. In some embodiments, the bag is connected to a channel comprising a tube and/or a needle. In some embodiments, the formulation is a lyophilized formulation or a liquid formulation. In some embodiments, the formulation may freeze-dried (lyophilized) and contained in about 12-60 vials. In some embodiments, the formulation is freeze-dried and 45 mg of the freeze-dried formulation is contained in one vial. In some embodiments, the about 40 mg – about 100 mg of freeze-dried formulation is contained in one vial. In some embodiments, freeze dried formulation from 12, 27, or 45 vials are combined to obtain a therapeutic dose of the protein in the intravenous drug formulation. In some embodiments, the formulation is a liquid formulation and stored as about 250 mg/vial to about 1,000 mg/vial. In some embodiments, the formulation is a liquid formulation and stored as about 600 mg/vial. In some embodiments, the formulation is a liquid formulation and stored as about 250 mg/vial.

**[0158]** These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as-is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents. The composition in solid form can also be packaged in a container for a flexible quantity.

[0159] In some embodiments, the present disclosure provides a formulation with an extended shelf life including the protein of the present disclosure, in combination with mannitol, citric acid monohydrate, sodium citrate, disodium phosphate dihydrate, sodium dihydrogen phosphate dihydrate, sodium chloride, polysorbate 80, water, and sodium hydroxide.

[0160] In some embodiments, an aqueous formulation is prepared including the protein of the present disclosure in a pH-buffered solution. The buffer of this disclosure may have a pH ranging from about 4 to about 8, *e.g.*, from about 4.5 to about 6.0, or from about 4.8 to about 5.5, or may have a pH of about 5.0 to about 5.2. Ranges intermediate to the above recited pH's are also intended to be part of this disclosure. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. Examples of buffers that will control the pH within this range include acetate (*e.g.* sodium acetate), succinate (such as sodium succinate), gluconate, histidine, citrate and other organic acid buffers.

[0161] In some embodiments, the formulation includes a buffer system which contains citrate and phosphate to maintain the pH in a range of about 4 to about 8. In some embodiments the pH range is from about 4.5 to about 6.0, or from about pH 4.8 to about 5.5, or in a pH range of about 5.0 to about 5.2. In some embodiments, the buffer system includes citric acid monohydrate, sodium citrate, disodium phosphate dihydrate, and/or sodium dihydrogen phosphate dihydrate. In some embodiments, the buffer system includes about 1.3 mg/ml of citric acid (*e.g.*, 1.305 mg/ml), about 0.3 mg/ml of sodium citrate (*e.g.*, 0.305 mg/ml), about 1.5 mg/ml of disodium phosphate dihydrate (*e.g.*, 1.53 mg/ml), about 0.9 mg/ml of sodium dihydrogen phosphate dihydrate (*e.g.*, 0.86), and about 6.2 mg/ml of sodium chloride (*e.g.*, 6.165 mg/ml). In some embodiments, the buffer system includes 1-1.5 mg/ml of citric acid, 0.25 to 0.5 mg/ml of sodium citrate, 1.25 to 1.75 mg/ml of disodium phosphate dihydrate, 0.7 to 1.1 mg/ml of sodium dihydrogen phosphate dihydrate, and 6.0 to 6.4 mg/ml of sodium chloride. In some embodiments, the pH of the formulation is adjusted with sodium hydroxide.

[0162] A polyol, which acts as a tonicifier and may stabilize the antibody or multi-specific binding protein, may also be included in the formulation. The polyol is added to the formulation in an amount which may vary with respect to the desired isotonicity of the formulation. In some embodiments, the aqueous formulation is isotonic. The amount of polyol added may also be altered with respect to the molecular weight of the polyol. For example, a lower amount of a monosaccharide (*e.g.*, mannitol) is added, compared to a disaccharide (such as trehalose). In some embodiments, the polyol which is used in the formulation as a tonicity

agent is mannitol. In some embodiments, the mannitol concentration is about 5 to about 20 mg/ml. In some embodiments, the concentration of mannitol is about 7.5 to 15 mg/ml. In some embodiments, the concentration of mannitol is about 10-14 mg/ml. In some embodiments, the concentration of mannitol is about 12 mg/ml. In some embodiments, the polyol sorbitol is included in the formulation.

**[0163]** A detergent or surfactant may also be added to the formulation. Exemplary detergents include nonionic detergents such as polysorbates (*e.g.*, polysorbates 20, 80 etc.) or poloxamers (*e.g.*, poloxamer 188). The amount of detergent added is such that it reduces aggregation of the formulated antibody and/or minimizes the formation of particulates in the formulation and/or reduces adsorption. In some embodiments, the formulation may include a surfactant which is a polysorbate. In some embodiments, the formulation may contain the detergent polysorbate 80 or Tween 80. Tween 80 is a term used to describe polyoxyethylene (20) sorbitanmonooleate (see Fiedler, Lexikon der Hfisstoffe, Editio Cantor Verlag Aulendorf, 4th edi., 1996). In some embodiments, the formulation may contain between about 0.1 mg/mL and about 10 mg/mL of polysorbate 80, or between about 0.5 mg/mL and about 5 mg/mL. In some embodiments, about 0.1% polysorbate 80 is added in the formulation.

**[0164]** In embodiments, the protein product of the present disclosure is formulated as a liquid formulation. The liquid formulation may be presented at a 10 mg/mL concentration in either a USP / Ph Eur type I 50R vial closed with a rubber stopper and sealed with an aluminum crimp seal closure. The stopper may be made of elastomer complying with USP and Ph Eur. In some embodiments, the liquid formulation is diluted with 0.9% saline solution.

**[0165]** In some embodiments, the liquid formulation of the disclosure is prepared as a 10 mg/mL concentration solution in combination with a sugar at stabilizing levels. In some embodiments the liquid formulation is prepared in an aqueous carrier. In some embodiments, a stabilizer is added in an amount no greater than that which may result in a viscosity undesirable or unsuitable for intravenous administration. In some embodiments, the sugar is disaccharides, *e.g.*, sucrose. In some embodiments, the liquid formulation may also include one or more of a buffering agent, a surfactant, and a preservative.

**[0166]** In some embodiments, the pH of the liquid formulation is set by addition of a pharmaceutically acceptable acid and/or base. In some embodiments, the pharmaceutically acceptable acid is hydrochloric acid. In some embodiments, the base is sodium hydroxide.

**[0167]** The aqueous carrier of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation. Illustrative carriers include sterile water for injection (SWFI), bacteriostatic

water for injection (BWFI), a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

**[0168]** A preservative may be optionally added to the formulations herein to reduce bacterial action. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation.

**[0169]** The antibody or multi-specific binding protein may be lyophilized to produce a lyophilized formulation including the proteins and a lyoprotectant. The lyoprotectant may be sugar, *e.g.*, disaccharides. In some embodiments, the lyoprotectant is sucrose or maltose. The lyophilized formulation may also include one or more of a buffering agent, a surfactant, a bulking agent, and/or a preservative.

**[0170]** The amount of sucrose or maltose useful for stabilization of the lyophilized drug product may be in a weight ratio of at least 1:2 protein to sucrose or maltose. In some embodiments, the protein to sucrose or maltose weight ratio is of from 1:2 to 1:5. In some embodiments, the pH of the formulation, prior to lyophilization, is set by addition of a pharmaceutically acceptable acid and/or base. In some embodiments the pharmaceutically acceptable acid is hydrochloric acid. In some embodiments, the pharmaceutically acceptable base is sodium hydroxide. Before lyophilization, the pH of the solution containing the protein of the present disclosure may be adjusted between 6 to 8. In some embodiments, the pH range for the lyophilized drug product is from 7 to 8.

**[0171]** Actual dosage levels of the active ingredients in the pharmaceutical compositions of this disclosure may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

**[0172]** The specific dose can be a uniform dose for each patient, for example, 50-5,000 mg of protein. Alternatively, a patient's dose can be tailored to the approximate body weight or surface area of the patient. Other factors in determining the appropriate dosage can include the disease or condition to be treated or prevented, the severity of the disease, the route of administration, and the age, sex and medical condition of the patient. Those skilled in the art, especially in light of the dosage information and assays disclosed herein, may routinely make further refinement of the calculations necessary to determine the appropriate dosage for treatment. The dosage can also be determined through the use of known assays for determining dosages used in conjunction with appropriate dose-response data. An individual patient's dosage can be adjusted as the progress of the disease is monitored. Blood levels of the targetable construct or complex in a patient can be measured to see if the dosage needs to be

adjusted to reach or maintain an effective concentration. Pharmacogenomics may be used to determine which targetable constructs and/or complexes, and dosages thereof, are most likely to be effective for a given individual (Schmitz *et al.*, *Clinica Chimica Acta* 308: 43-53, 2001; Steimer *et al.*, *Clinica Chimica Acta* 308: 33-41, 2001).

**[0173]** In general, dosages based on body weight are from about 0.1 µg to about 100 mg per kg of body weight, such as about 0.1 µg to about 100 mg/kg of body weight, about 0.1 µg to about 50 mg/kg of body weight, about 0.1 µg to about 10 mg/kg of body weight, about 0.1 µg to about 1 mg/kg of body weight, about 0.1 µg to about 100 µg/kg of body weight, about 0.1 µg to about 50 µg/kg of body weight, about 0.1 µg to about 10 µg/kg of body weight, about 0.1 µg to about 1 µg/kg of body weight, about 0.1 µg to about 0.1 µg/kg of body weight, about 0.1 µg to about 100 mg/kg of body weight, about 0.1 µg to about 50 mg/kg of body weight, about 0.1 µg to about 10 mg/kg of body weight, about 0.1 µg to about 1 mg/kg of body weight, about 0.1 µg to about 100 µg/kg of body weight, about 0.1 µg to about 10 µg/kg of body weight, about 0.1 µg to about 1 µg/kg of body weight, about 1 µg to about 100 mg/kg of body weight, about 1 µg to about 50 mg/kg of body weight, about 1 µg to about 10 mg/kg of body weight, about 1 µg to about 1 mg/kg of body weight, about 1 µg to about 100 µg/kg of body weight, about 1 µg to about 50 µg/kg of body weight, about 1 µg to about 10 µg/kg of body weight, about 10 µg to about 100 mg/kg of body weight, about 10 µg to about 50 mg/kg of body weight, about 10 µg to about 10 mg/kg of body weight, about 10 µg to about 1 mg/kg of body weight, about 10 µg to about 100 µg/kg of body weight, about 10 µg to about 50 µg/kg of body weight, about 50 µg to about 100 mg/kg of body weight, about 50 µg to about 50 mg/kg of body weight, about 50 µg to about 10 mg/kg of body weight, about 50 µg to about 1 mg/kg of body weight, about 50 µg to about 100 µg/kg of body weight, about 100 µg to about 100 mg/kg of body weight, about 100 µg to about 50 mg/kg of body weight, about 100 µg to about 10 mg/kg of body weight, about 100 µg to about 1 mg/kg of body weight, about 1 mg to about 100 mg/kg of body weight, about 1 mg to about 50 mg/kg of body weight, about 1 mg to about 10 mg/kg of body weight, about 10 mg to about 100 mg/kg of body weight, about 10 mg to about 50 mg/kg of body weight, about 50 mg to about 100 mg/kg of body weight.

**[0174]** In some embodiments, a subject may be administered a dose of at least 20µg the multi-specific binding protein. In some embodiments, a subject may be administered a dose of at least 30µg the multi-specific binding protein. In some embodiments, a subject may be administered a dose of at least 40µg the multi-specific binding protein. In some embodiments, a subject may be administered a dose of at least 50µg the multi-specific binding protein.

[0175] Doses may be given once or more times daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the targetable construct or complex in bodily fluids or tissues. Administration of the present disclosure could be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, intracavitary, by perfusion through a catheter or by direct intralesional injection. This may be administered once or more times daily, once or more times weekly, once or more times monthly, and once or more times annually. In some embodiments, administration is intravenous. In some embodiments, administration is subcutaneous.

#### IV. THERAPEUTIC APPLICATIONS

[0176] It is contemplated that the antibodies or multi-specific binding proteins can be used either alone or in combination with other therapeutic agents.

##### A. *Indications*

[0177] The present disclosure provides methods for the treatment or amelioration of a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, a viral disease, an allergic reaction, a parasitic reaction, a graft-versus-host disease or a host-versus-graft disease in a subject in need thereof, the method comprising administration of a multi-specific binding protein or an antibody that binds CD19 disclosed herein.

[0178] In some embodiments, the cancer to be treated is non-Hodgkin's lymphoma, such as a B-cell lymphoma. Curative therapy for many patients with relapsed and/or refractory (R/R) NHL remains elusive and an area with unmet need. While there have been numerous advances in treatments including CAR-T and bispecific T cell engagers (TCEs) with ~80-90% response rate, 45-50% of those will relapse. Decreased target expression contributes to resistance post CAR-T or TCE therapy.

[0179] CD19 is a clinically and commercially validated for the treatment of patients with relapsed/refractory B cell acute lymphoblastic leukemia (ALL) and Non-Hodgkin's lymphoma (NHL). The modalities are also well validated as T cell engagers (TCEs) show clinical activity in many B-cell malignancies; e.g., CD19-targeted Blincyto® (ALL), several CD20-targeted TCEs (NHL) and BCMA-targeted TCEs (MM). Additional rationale for the development of tAB0050 includes activity against malignant cells expressing very low levels of CD19, a mechanism of resistance post CD19 CAR-T, off-the-shelf convenience with potential for CAR-T-like complete response (CR) rates, and competitive vis-à-vis CD19-

targeted ADCs and Fc-enhanced IgG1s, which require higher levels of CD19 target expression.

**[0180]** In some embodiments, the non-Hodgkin's lymphoma is a B-cell lymphoma, such as a diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma, follicular lymphoma (FL), small lymphocytic lymphoma, mantle cell lymphoma, marginal zone B-cell lymphoma (e.g., nodal, extranodal, or mucosa-associated), extranodal marginal zone B-cell lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, chronic lymphocytic leukemia, or primary central nervous system lymphoma. In some embodiments, the cancer to be treated is multiple myeloma. In some embodiments, the cancer to be treated is acute lymphoblastic leukemia (ALL). In some embodiments, the ALL is relapsed/refractory adult and pediatric ALL.

**[0181]** In some embodiments, the cancer to be treated is relapsed and/or refractory Non-Hodgkin lymphoma (NHL). In some embodiments, the relapsed and/or refractory Non-Hodgkin lymphoma (NHL) is relapsed and/or refractory B-cell lymphoma, such as diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma, follicular lymphoma (FL), small lymphocytic lymphoma, mantle cell lymphoma, marginal zone B-cell lymphoma (e.g., (nodal, extranodal, or mucosa-associated), extranodal marginal zone B-cell lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, chronic lymphocytic leukemia, or primary central nervous system lymphoma.

**[0182]** In some embodiments, the individual has relapsed following treatment with a CD19-targeting therapy, or is refractory to the CD19-targeting therapy. In some embodiments, the CD19-targeting therapy comprises blinatumomab, coltuximab ravtansine, MOR208 (XmAb-5574), MEDI-551, denintuzumab mafodotin, DI-B4, taplitumomabpaptox, XmAb 5871, MDX-1342, AFM11, MDX-1342, loncastuximab tesirine, SAR3419, Combotox, DT2219ARL, SGN-CD19A, AFM11, or GBR401. In some embodiments, the CD19-targeting therapy comprises blinatumomab. In some embodiments, the CD19-targeting therapy comprises a CD19 CAR-T cell therapy. In some embodiments, the CD19 CAR-T cell therapy comprises tisagenlecleucel, axicabtagene ciloleucel, or brexucabtagene autoleucel.

**[0183]** In some embodiments, the individual has relapsed following treatment with at least two lines of therapies. In some embodiments, the individual has relapsed following treatment with at least three lines of therapies. In some embodiments, the individual has relapsed

following treatment with at least four lines of therapies. In some embodiments, the individual has relapsed following treatment with at least five lines of therapies. In some embodiments, the therapy is a CD19-targeting therapy. In some embodiments, the therapy is a CD20 monoclonal antibody. In some embodiments, the therapy is anthracycline. In some embodiments, the therapy is an alkylating agent. In some embodiments, the at least two lines of therapy comprise a CD19-targeting therapy, a CD20 monoclonal antibody, anthracycline, an alkylating agent, or combinations thereof. In some embodiments, the at least two lines of therapy comprise a CD20 monoclonal antibody and anthracycline. In some embodiments, the at least two lines of therapy comprise a CD20 monoclonal antibody and an alkylating agent.

**[0184]** In some embodiments, the cancer to be treated is progressive non-Hodgkin's lymphoma. In some embodiments, the progressive non-Hodgkin's lymphoma is a progressive B-cell lymphoma, such as diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma, follicular lymphoma (FL), small lymphocytic lymphoma, mantle cell lymphoma, marginal zone B-cell lymphoma (e.g., (nodal, extranodal, or mucosa-associated), extranodal marginal zone B-cell lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, chronic lymphocytic leukemia, or primary central nervous system lymphoma. In some embodiments, the cancer to be treated is progressive multiple myeloma. In some embodiments, the cancer to be treated is progressive acute lymphoblastic leukemia (ALL).

**[0185]** Described herein, in some embodiments, 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 described herein. In some embodiments, the individual has low levels of CD19 expression. In some embodiments, the individual has low levels of CD19 expression as a result of down-regulation due to a cancer therapy (e.g., a CD19-targeting therapy). In some embodiments, the individual is not responsive to a cancer therapy. In some embodiments, the individual has received a cancer therapy but the cancer therapy does not improve the cancer, prevent cancer regression, control the cancer, or combinations thereof. In some embodiments, the individual has a CD19 low expressing cancer that is advanced, metastatic, unresectable, or combinations thereof. In some embodiments, the individual has a CD19 low expressing cancer that is advanced, metastatic, unresectable, or combinations thereof after at least 1, 2, 3, 4, or more than 4 cancer therapies.

**[0186]** In some embodiments, the individual has a CD19 low expressing cancer and has received at least one cancer therapy (e.g., antibody treatment, chemotherapy, radiation). In some embodiments, the individual has a CD19 low expressing cancer and has received at least

two cancer therapies. Anticancer therapies include, but are not limited to, surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, immunotherapy, cytokine therapy, and combinations thereof. In some embodiments, the cancer therapy comprises a CD19-targeting therapy. In some embodiments, the CD19-targeting therapy comprises blinatumomab, coltuximab ravtansine, MOR208 (XmAb-5574), MEDI-551, denintuzumab mafodotin, DI-B4, taplitumomabpaptox, XmAb 5871, MDX-1342, AFM11, MDX-1342, loncastuximab tesirine, SAR3419, Combotox, DT2219ARL, SGN-CD19A, AFM11, or GBR401. In some embodiments, the CD19-targeting therapy comprises blinatumomab. In some embodiments, the CD19-targeting therapy comprises a CD19 CAR-T cell therapy. In some embodiments, the CD19 CAR-T cell therapy comprises tisagenlecleucel, axicabtagene ciloleucel, or brexucabtagene autoleucel. In some embodiments, the therapy is a CD20 monoclonal antibody. In some embodiments, the therapy is anthracycline. In some embodiments, the therapy is an alkylating agent. In some embodiments, the at least two lines of therapy comprise a CD19-targeting therapy, a CD20 monoclonal antibody, anthracycline, an alkylating agent, or combinations thereof. In some embodiments, the at least two lines of therapy comprise a CD20 monoclonal antibody and anthracycline. In some embodiments, the at least two lines of therapy comprise a CD20 monoclonal antibody and an alkylating agent.

**[0187]** Various CD19 low expressing cancers for treatment are contemplated herein. In some embodiments, the CD19 low expressing cancer is a metastatic cancer. In some embodiments, the CD19 low expressing cancer comprises a solid tumor. In some embodiments, the CD19 low expressing cancer is non-Hodgkin's lymphoma, such as a B-cell lymphoma. In some embodiments, the non-Hodgkin's lymphoma is a B-cell lymphoma, such as a diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, follicular lymphoma, small lymphocytic lymphoma, mantle cell lymphoma, marginal zone B-cell lymphoma, extranodal marginal zone B-cell lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, chronic lymphocytic leukemia, or primary central nervous system lymphoma. In some embodiments, the CD19 low expressing cancer to be treated is multiple myeloma. In some embodiments, the CD19 low expressing cancer is acute lymphoblastic leukemia (ALL). In some embodiments, the ALL is relapsed/refractory adult and pediatric ALL. In some embodiments, the CD19 low expressing cancer to be treated is relapsed and/or refractory Non-Hodgkin lymphoma (NHL). In some embodiments, the CD19 low expressing cancer is B cell lymphoma.

[0188] CD19 low expressing cancers, in some embodiments, are classified as low expressing by various methods. In some embodiments, the method comprises immunohistochemistry, immunocytochemistry (ICC), in situ hybridization (ISH), flow cytometry, enzyme immuno-assays (EIA), enzyme linked immuno-assays (ELISA), blotting methods (e.g. Western, Southern, and Northern), or labeling inside electrophoresis systems or on surfaces or arrays. In some embodiments, the method comprises flow cytometry. In some embodiments, the method comprises immunohistochemistry. In some embodiments, the method comprises immunohistochemistry in situ hybridization including, but not limited to, fluorescence in situ hybridization (FISH). In some embodiments, the method comprises sequencing including, but not limited to, next generation sequence (NGS), single molecule real-time sequencing, Polony sequencing, sequencing by ligation, reversible terminator sequencing, proton detection sequencing, ion semiconductor sequencing, nanopore sequencing, electronic sequencing, pyrosequencing, Maxam-Gilbert sequencing, chain termination (e.g., Sanger) sequencing, +S sequencing, and sequencing by synthesis.

[0189] Some of the features of tAB0050 include very high affinity human anti-CD19 scFv efficiently can also target NHL cells expressing very low levels of CD19, a humanized anti-Human Serum Albumin-binding single-domain antibody (VHH) extends half-life, and human scFv binds the invariant CD3 epsilon chain of the T cell receptor (TCR). Some of the pre-clinical data highlights include effective B cell depletion with single administration and robust tumor killing at low levels of CD19 expression.

### ***B. Combination Therapies***

[0190] The methods and compositions described herein can be used alone or in combination with other therapeutic agents and/or modalities. The term administered “in combination,” as used herein, is understood to mean that two (or more) different treatments are delivered to the subject during the course of the subject’s affliction with the disorder, such that the effects of the treatments on the patient overlap at a point in time. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as “simultaneous” or “concurrent delivery.” In some embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, *e.g.*, an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen

if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

**[0191]** In one aspect, the present disclosure provides a method of treating a subject by the administration of a second therapeutic agent in combination with one or more of the multi-specific binding proteins and/or antibodies that bind CD19 disclosed herein.

**[0192]** Exemplary therapeutic agents that may be used as part of a combination therapy in treating cancer, include, for example, radiation, mitomycin, tretinoin, ribomustine, gemcitabine, vincristine, etoposide, cladribine, mitobronitol, methotrexate, doxorubicin, carboquone, pentostatin, nitracrine, zinostatin, cetorelix, letrozole, raltitrexed, daunorubicin, fadrozole, fotemustine, thymalfasin, sobuzoxane, nedaplatin, cytarabine, bicalutamide, vinorelbine, vesnarinone, aminoglutethimide, amsacrine, proglumide, elliptinium acetate, ketanserin, doxifluridine, etretinate, isotretinoin, streptozocin, nimustine, vindesine, flutamide, drogenil, butocin, carmofur, razoxane, sizofilan, carboplatin, mitolactol, tegafur, ifosfamide, prednimustine, picibanil, levamisole, teniposide, improsulfan, enocitabine, lisuride, oxymetholone, tamoxifen, progesterone, mepitiostane, epitiostanol, formestane, interferon-alpha, interferon-2 alpha, interferon-beta, interferon-gamma, colony stimulating factor-1, colony stimulating factor-2, denileukin diftitox, interleukin-2, luteinizing hormone releasing factor and variations of the aforementioned agents that may exhibit differential binding to its cognate receptor, and increased or decreased serum half-life.

**[0193]** An additional class of agents that may be used as part of a combination therapy in treating cancer is immune checkpoint inhibitors. The checkpoint inhibitor may, for example, be selected from a PD-1 antagonist, PD-L1 antagonist, CTLA-4 antagonist, adenosine A2A receptor antagonist, B7-H3 antagonist, B7-H4 antagonist, BTLA antagonist, KIR antagonist, LAG3 antagonist, TIM-3 antagonist, VISTA antagonist or TIGIT antagonist.

**[0194]** In some embodiments, the checkpoint inhibitor is a PD-1 or PD-L1 inhibitor. PD-1 is a receptor present on the surface of T-cells that serves as an immune system checkpoint that inhibits or otherwise modulates T-cell activity at the appropriate time to prevent an overactive immune response. Cancer cells, however, can take advantage of this checkpoint by expressing ligands, for example, PD-L1, that interact with PD-1 on the surface of T-cells to shut down or

modulate T-cell activity. Exemplary PD-1/PD-L1 based immune checkpoint inhibitors include antibody based therapeutics. Exemplary anti-PD-1 antibodies include, for example, nivolumab (Opdivo®, Bristol-Myers Squibb Co.), pembrolizumab (Keytruda®, Merck Sharp & Dohme Corp.), PDR001 (Novartis Pharmaceuticals), and pidilizumab (CT-11, Cure Tech). Exemplary anti-PD-L1 antibodies include, for example, atezolizumab (Tecentriq®, Genentech), duvalumab (AstraZeneca), MEDI4736, avelumab, and BMS 936559 (Bristol Myers Squibb Co.).

**[0195]** In some embodiments, a method or composition described herein is administered in combination with a CTLA-4 inhibitor. In the CTLA-4 pathway, the interaction of CTLA-4 on a T-cell with its ligands (*e.g.*, CD80, also known as B7-1, and CD86) on the surface of an antigen presenting cells (rather than cancer cells) leads to T-cell inhibition. Exemplary CTLA-4 antibodies include ipilimumab or tremelimumab.

**[0196]** In some embodiments, a method or composition described herein is administered in combination with (i) a PD-1 or PD-L1 inhibitor, *e.g.*, a PD-1 or PD-L1 inhibitor disclosed herein, and (ii) CTLA-4 inhibitor, *e.g.*, a CTLA-4 inhibitor disclosed herein.

**[0197]** In some embodiments, a method or composition described herein is administered in combination with an IDO inhibitor. Exemplary IDO inhibitors include 1-methyl-D-tryptophan (known as indoximod), epacadostat (INCB24360), navoximod (GDC-0919), and BMS-986205.

**[0198]** Yet other agents that may be used as part of a combination therapy in treating cancer are monoclonal antibody agents that target non-checkpoint targets (*e.g.*, herceptin) and non-cytotoxic agents (*e.g.*, tyrosine-kinase inhibitors).

**[0199]** Yet other categories of anti-cancer agents include, for example: (i) an inhibitor selected from an ALK Inhibitor, an ATR Inhibitor, an A2A Antagonist, a Base Excision Repair Inhibitor, a Bcr-Abl Tyrosine Kinase Inhibitor, a Bruton's Tyrosine Kinase Inhibitor, a CDC7 Inhibitor, a CHK1 Inhibitor, a Cyclin-Dependent Kinase Inhibitor, a DNA-PK Inhibitor, an Inhibitor of both DNA-PK and mTOR, a DNMT1 Inhibitor, a DNMT1 Inhibitor plus 2-chloro-deoxyadenosine, an HDAC Inhibitor, a Hedgehog Signaling Pathway Inhibitor, an IDO Inhibitor, a JAK Inhibitor, a mTOR Inhibitor, a MEK Inhibitor, a MELK Inhibitor, a MTH1 Inhibitor, a PARP Inhibitor, a Phosphoinositide 3-Kinase Inhibitor, an Inhibitor of both PARP1 and DHODH, a Proteasome Inhibitor, a Topoisomerase-II Inhibitor, a Tyrosine Kinase Inhibitor, a VEGFR Inhibitor, and a WEE1 Inhibitor; (ii) an agonist of OX40, CD137, CD40, GITR, CD27, HVEM, TNFRSF25, or ICOS; and (iii) a cytokine selected from IL-12, IL-15, GM-CSF, and G-CSF.

**[0200]** It is understood that the antibody or multi-specific binding protein disclosed herein, which is designed to activate T lymphocytes, may cause side effects such as neurotoxicity. Accordingly, in certain embodiments, the second therapeutic agent that can be used in combination with the antibody or multi-specific binding protein comprises an agent that mitigates a side effect of the antibody or multi-specific binding protein, *e.g.*, reduces neurotoxicity. In some embodiments, the second therapeutic agent inhibits T cell trafficking, for example, reduces or inhibits immune cells from crossing the blood-brain barrier. Non-limiting examples of such therapeutic agents include antagonists (*e.g.*, antagonistic antibodies) of adhesion molecules on immune cells (*e.g.*,  $\alpha 4$  integrin), such as natalizumab. In some embodiments, the second therapeutic agent increases the internalization of a sphingosine-1-phosphate (SIP) receptor (*e.g.*, S1PR1 or S1PR5), such as fingolimod or ozanimod. In some embodiments, the second therapeutic agent is a nitric oxide synthase (NOS) inhibitor, such as ronoplerin, cindunistat, A- 84643, ONO-1714, L-NOARG, NCX-456, VAS-2381, GW-273629, NXN-462, CKD-712, KD- 7040, or guanidinoethylidissulfide. In some embodiments, the second therapeutic agent is an antagonist of CSF1 or CSF1R, such as pexidartinib, emactuzumab, cabiralizumab, LY-3022855, JNJ-40346527, or MCS110. Additional non-limiting examples of the second therapeutic agents include pentosan polysulfate, minocycline, anti-ICAM-1 antibodies, anti-P-selectin antibodies, anti-CD11a antibodies, anti-CD162 antibodies, and anti-IL-6R antibodies (*e.g.*, tocilizumab).

**[0201]** The invention also relates to a kit comprising the injection device of the invention and instructions for use. The instructions for use may comprise instructions for subcutaneous administration of the pharmaceutical composition or unit dose to the patient. The instructions for use may specify that the injection device, unit dose and/or pharmaceutical composition are for use in the treatment of NHL. The kit may comprise packaging, wherein the packaging is adapted to hold the injection device and the instructions for use. The instructions for use may be attached to the injection device.

**[0202]** The instructions for use may specify that the administration of the pharmaceutical composition to the patient treats NHL in the patient.

**[0203]** The amount of the antibody or multi-specific binding protein and additional therapeutic agent and the relative timing of administration may be selected in order to achieve a desired combined therapeutic effect. For example, when administering a combination therapy to a patient in need of such administration, the therapeutic agents in the combination, or a pharmaceutical composition or compositions comprising the therapeutic agents, may be

administered in any order such as, for example, sequentially, concurrently, together, simultaneously and the like. Further, for example, an antibody or multi-specific binding protein may be administered during a time when the additional therapeutic agent(s) exerts its prophylactic or therapeutic effect, or *vice versa*.

**[0204]** Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present disclosure that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present disclosure that consist essentially of, or consist of, the recited processing steps.

**[0205]** In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

**[0206]** Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present disclosure, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present disclosure and/or in methods of the present disclosure, unless otherwise understood from the context. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and disclosure(s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the disclosure(s) described and depicted herein.

**[0207]** It should be understood that the expression “at least one of” includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression “and/or” in connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

**[0208]** The use of the term “include,” “includes,” “including,” “have,” “has,” “having,” “contain,” “contains,” or “containing,” including grammatical equivalents thereof, should be

understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

[0209] Where the use of the term “about” is before a quantitative value, the present disclosure also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term “about” refers to a  $\pm 10\%$  variation from the nominal value unless otherwise indicated or inferred.

[0210] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present disclosure remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[0211] The use of any and all examples, or exemplary language herein, for example, “such as” or “including,” is intended merely to illustrate better the present disclosure and does not pose a limitation on the scope of the disclosure unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present disclosure.

[0212] The description above describes multiple aspects and embodiments of the disclosure. The patent application specifically contemplates all combinations and permutations of the aspects and embodiments.

#### EXAMPLES

[0213] The disclosure now being generally described, will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present disclosure, and is not intended to limit the disclosure.

##### Example 1: Design and Production of tAB0050

[0214] tAB0050 is a recombinant fusion protein comprised of a single polypeptide chain containing a humanized anti-serum albumin single-domain antibody (sdAb), a humanized anti-CD19 scFv and a human anti-CD3 epsilon scFv. Each binding domain was optimized for affinity, selectivity, species cross-reactivity, thermal stability, and developability using Adimab's yeast-based antibody engineering platform. tAB0050 had an apparent molecular size of 65 kDa and was produced from a clonal Chinese hamster ovary (CHO) cell line. The expressed protein was affinity-purified via protein A chromatography, followed by additional polishing steps to ensure high product purity. **FIG. 1B** depicts an exemplary tAB0050 design.

[0215]

**Example 2: Binding Characteristics of tAB0050**

[0216] Binding of tAB0050 to human CD19, CD3 and serum albumin was determined by surface plasmon resonance (SPR) (**FIG. 5A**). The equilibrium binding constants ( $K_D$ ) of tAB0050 for CD19, CD3, and albumin were 73.8 pM, 7,997 pM, and 596 pM, respectively. Given the binding affinity for albumin and typical serum concentrations of albumin in the range of 500  $\mu$ M, it is assumed that all tAB0050 is bound by albumin *in vivo*. SPR analyses performed in the presence of albumin resulted in a 2-3-fold decrease in the affinity and kinetics of the interaction between tAB0050 and CD3 $\epsilon$  and CD19, which was primarily driven by a reduction in the on-rates (**FIG. 6**).

[0217] To evaluate target binding specificity, tAB0050 was screened for binding against fixed HEK293 cells expressing 6,19 individual full-length human plasma membrane proteins, secreted and cell surface-tethered human secreted proteins, as well as an additional 397 human heterodimers, followed by a series of confirmatory screens (Retrogenix technology; Report RP1885).

[0218] tAB0050 showed specific interactions with CD19, expressed alone or in a heterodimer with CD81, albumin, and with CD3 $\epsilon$ . No interaction with other cell surface or secreted human proteins was identified for the test protein, indicating high specificity of CLN-978 for its primary targets, CD19, CD3 $\epsilon$  and albumin.

[0219] The species cross-reactivity of tAB0050 was evaluated by cell surface binding to CD20+ B cells and CD4+ and CD8+ T cells in human, non-human primate (NHP) and murine peripheral blood mononuclear cells (PBMC) across multiple donors using flow cytometry. Cell surface binding of tAB0050 to B and T cells correlated with the SPR binding. Very low EC50 values were calculated for human CD20+ B cells, with a narrow range between 0.33-0.42  $\mu$ g/mL. Binding to human T cells was more moderate. EC50 values were 7.93  $\mu$ g/mL (6.90-8.67  $\mu$ g/mL) for CD4+ T cells. Binding to CD8+ T cells was variable, with an EC50 value of 99.5  $\mu$ g/mL for one donor, whereas two donor T cells were in the range at 13.55 and 19.52  $\mu$ g/mL. With PBMC from NHPs, the EC50 value for tAB0050 binding to B cells was 1.72  $\mu$ g/mL (1-2.59  $\mu$ g/mL). In the T cell subpopulations, similar EC50 values were observed for CD4+ (28.86  $\mu$ g/mL) and CD8+ T cells (28.66  $\mu$ g/mL). Minimal binding of tAB0050 was observed to mouse B and T cells with half-maximal effective concentrations (EC50) values in excess of 100  $\mu$ g/mL for B cells and CD4+ and CD8+ T cell populations (**FIG. 5B**), and with no saturation achieved.

[0220] Overall tAB0050 exhibited more potent binding to B cells than to T cells, consistent with a higher affinity for CD19 than for CD3. Cross-reactivity with NHP PBMC was also confirmed, with similar EC50 values for cell binding to CD8+ T cells, although ~5-fold and ~3-fold lower for binding to human B cells and CD4+ T cells, respectively.

[0221] The tAB0050 mechanism of action allows T cells to lyse a broad range of CD19-expressing cells. tAB0050 potently triggers redirected lysis of CD19-expressing target cells in vitro and in vivo. There is a very high affinity binding to CD19 to efficiently target malignant B cells expressing very low CD19 levels and binding to serum albumin for extending serum half-life.

### **Example 3: Activity of tAB0050 in Co-cultures of Human T Cells with CD19-expressing Target Cells**

[0222] We evaluated T cell-dependent cellular cytotoxicity (TDCC), T cell activation, and the release of selected cytokines as pharmacodynamic markers. These studies were performed in co-culture experiments with unstimulated human PBMC as effector cells (E) and the human B lymphoma RAMOS cell line as target cells (T) at an E:T ratio of 10:1. RAMOS cells expressed an average of 13,108 CD19 molecules on the cell surface, which is within the range of normal B cells and many primary lymphoma cells. First, the ability of tAB0050 to re-direct lysis and activate T cells was assessed across multiple donors in the absence of albumin. **FIG. 7A** shows the variation of TDCC among six human PBMC donors. While EC50 values for lysis ranged from 0.26 to 5 pM tAB0050, all donors mediated ~80% lysis or greater at 48 hours at a concentration of 2 nM. The expression of the T cell activation markers CD69 and CD25 on CD4+ and CD8+ T cells were simultaneously evaluated. Consistent with TDCC, EC50 values for T cell activation were found to be in the single-digit pM range or lower (0.2-1 pM and 1.6-4.7 pM for CD69 and CD25, respectively), with the majority of CD8+ and CD4+ T cells (80-100%) expressing CD69 and CD25 at 2 nM tAB0050 (**FIG. 7B**). Only two out of six donor CD8+ T cells showed a <50% induction of CD25, and one donor for CD25 on CD4+ T cells. Collectively, these data demonstrate potent activation of the majority of T cells in the co-culture.

[0223] Next, the effect of albumin on tAB0050 bioactivity was evaluated. **FIG. 7C** shows half-maximal lysis of RAMOS cells at 0.44 pM (+/- 0.7 SEM; n = 10) and 2.68 pM tAB0050 (+/- 0.43 SEM; n = 10) at 48 hours, in the absence and presence of HSA, respectively. As anticipated from the observed shift in binding affinity in the presence of HSA, we observed a

minor shift in half-maximal tAB0050 concentration in the presence of HSA (<10-fold), with the EC<sub>50</sub> remaining in the single digit pM range. Complete lysis of RAMOS cells was achieved both in the absence and presence of HSA at tAB0050 concentrations of 55 pM and higher, suggesting that the presence of HSA is unlikely to impact anti-tumor activity of tAB0050 under physiological conditions. Similar results were observed using the Raji cell line (**FIG. 8A**).

[0224] As a pharmacodynamic measure of activated cytotoxic T cells, we studied the release of TNF $\alpha$  and IFN $\gamma$  (**FIG. 7D**) and IL-6 and IL-1 $\beta$  (**FIG. 7E**) in response to a tAB0050 titration in co-cultures of RAMOS human lymphoma cells with human PBMC in the presence of albumin. A dose-dependent release was observed for TNF $\alpha$  and IFN $\gamma$ , albeit with EC<sub>50</sub> values higher than those observed for TDCC and T cell activation. The EC<sub>50</sub> values were 23.3 pM (+/- 9.74 SEM; n = 2) for TNF $\alpha$  release and 17.6 pM (+/- 2.1 SEM; n = 2) for IFN $\gamma$ . Maximal cytokine release was observed at 333 pM tAB0050 for both cytokines. In conclusion, tAB0050 is a very potent inducer of redirected lysis/TDCC, T cell activation and cytokine release by previously unstimulated T cells.

[0225] The impact of TDCC on the endogenous CD19+ B cell population present in the PBMC fraction was also assessed in this assay. High basal levels of B cell death were observed during the duration of the assay. Dose-dependent tAB0050 -induced TDCC could still be observed above the high basal levels (**FIG. 8B**). EC<sub>50</sub> values were in the same pM range as for the lymphoma target cell lines. As seen for lymphoma target cell lines, albumin addition reduced the TDCC of B cells by ~25-fold.

[0226] T cell activation was monitored for CD4+ and CD8+ T cell populations using CD69, an early T cell activation marker, which is detectable within hours of TCR ligation, and CD25, the high-affinity subunit of the IL-2 receptor, which is more slowly induced after T cell activation than CD69.

[0227] tAB0050 treatment resulted in potent T cell activation as measured by increased cell surface expression of both CD25 and CD69 with EC<sub>50</sub> values in the low pM range for both target cell lines (**FIG. 8C**). In the presence of albumin, both curves shifted to the right, demonstrating reduced T cell activation by tAB0050 in the presence of albumin.

#### **Example 4: In vivo Evaluation of tAB0050 Activity**

[0228] The in vivo activity of tAB0050-HIS was compared head-to-head with blinatumomab in two different mouse models (**FIGs. 9A and 9B**). One was a human CD19-

expressing A20 lymphoma model implanted in the flank of BALB/c mice expressing a human CD3e transgene; the other was a disseminated Raji B.luc luciferase NCG mouse model using implanted human PBMC as effector cells. In both models, tAB0050 outperformed blinatumomab. In therapeutic studies with established A20 flank tumors, a single IV dose of 0.1 mg/kg tAB0050 completely prevented tumor outgrowth, whereby an equivalent dose of blinatumomab was ineffective. In the disseminated Raji B.luc model, weekly IV dosing of 0.1 mg/kg prevented an increase in the luciferase signal, while blinatumomab could not prevent leukemic spread.

[0229] FIG. 9E shows the effect on hCD3e-expressing BALB/c mice were inoculated with A20 cells expressing varying levels of hCD19. Mice were treated once weekly IV with 0.1 mg/kg tAB0050 or control when tumor volume reached ~100mm<sup>3</sup>. These data demonstrate efficacy of tAB0050 in lymphoma models expressing very low levels of CD19.

**Example 5: Potency of tAB0050 in redirected lysis *in vitro* in low CD19 expressing cell lines**

[0230] This example is designed to determine the potency for redirected lysis *in vitro* of a multi-specific binding protein (tAB0050) in low CD19 expressing cell lines.

[0231] Briefly, cytotoxicity assays were performed with three A20 clones that expressed on their cell surface 17,000, 3,700 or 325 copies of human CD19.

[0232] As seen in 3A-3D, tAB0050 targets a range of CD19 expression levels in the engineered A20 cell lines evaluated as measured by both the low pM EC50 values of redirected cell lysis and the maximum percentage of lysis. In co-culture TDCC assays, 100% redirected lysis was observed with all three A20 cell clones in the presence of HSA (FIG. 3A). No lysis of non-transfected A20 cells was observed, in accordance with the absence of cross-reactivity of tAB0050 to mouse CD19. While EC50 values for TDCC were similar for the clones expressing >3,000 copies of CD19 (in the range of 14 to 27 pM), the lowest expressing A20 clone with 325 copies of CD19 required 683 pM for half-maximal lysis. However, no differences in maximum lysis were observed among the cell lines. All three hCD19 expressing A20 clones activated CD8+ and CD4+ T cells, as measured by expression of CD25 and CD69 (FIG. 3B), and induced the release of TNFα (FIG. 3C) and IFNγ (FIG. 3D) in a CD19- and dose-dependent fashion.

[0233] The data demonstrates that tAB0050 can be used to treat patient populations with lower levels of CD19 expression and/or patients in which CD19 expression is downregulated as a resistance mechanism to CD19-targeted therapies. The data also shows that the robust

lysis of target cells was dependent on CD19 expression, as the A20 parental cell line, which lacks CD19 expression, was not susceptible to lysis at any of the tAB0050 concentrations tested.

**Example 6: Comparison of tAB0050 with blinatumomab for their potency of redirected lysis *in vitro***

[0234] This example was designed to determine the potency for redirected lysis *in vitro* of a multi-specific binding protein (tAB0050) compared to blinatumomab.

[0235] Briefly, EMT6 cells were stably transfected to express hCD19 (**FIG. 2A**). The parental line, a CD19 low line (clone 6), and a CD19 high line (clone 5) were characterized by flow cytometry. Unstimulated T cells were co-cultured with the indicated EMT6 cell line at a 3:1 E:T ratio for 48 hours, and cytotoxicity was evaluated by flow cytometry as seen in the parental line (**FIG. 2B**), the low CD19 cell line (**FIG. 2C**), and the high CD19 cell line (**FIG. 2D**).

[0236] Taken together, these data demonstrate improved potency for redirected lysis of tAB0050 *in vitro* relative to blinatumomab.

**Example 7: Activity of tAB0050 in Lymphoma Cell Lines with low CD19 Expression**

[0237] This Example describes the activity of tAB0050 in lymphoma cell lines with low CD19 expression.

[0238] To determine the minimal copy number of CD19 on target cells necessary for redirected lysis by tAB0050, a chemically inducible system to tune the level of expression of a CD19 transgene in CHO cells was used. As an epithelial cell line of hamster origin, CHO cells do not express the B cell lineage-specific human CD19 protein. CHO cells transfected with the inducible transgene were incubated with a titration of the chemical inducer, ABA, ranging from 2.4 nM to 10  $\mu$ M, enabling expression levels of CD19 on CHO cells ranging from 78 to 185,223 copies of CD19 per cell after 24 hrs. At that time point, ABA was washed out of the media, and purified CD8+ T cells from four human donors were added to an E:T ratio of 10:1 along with tAB0050 at either 225 or 1,770 pM for 68 hours. For all four T cell donors, signals for TDCC, T cell activation (CD25 expression), proliferation (Ki67), and release of IFN $\gamma$  were observed above the background of wild-type CHO cells (**FIGs. 4A-4D**, representative donor). Observed cytotoxicity was CD19-density dependent at 225 pM tAB0050, ranging from 38% lysis (78 CD19 copies) to 80% lysis (185,000 CD19 copies). At the higher tAB0050 concentration of 1,770 pM, cytolysis was independent of CD19 levels.

tAB0050 at 1,770 pM mediated near-maximal lysis of induced CHO cells even at a level of 78 CD19 molecules per cell (**FIG. 4A**). CD25 and Ki67 expression and IFN $\gamma$  release increased with higher levels of CD19 expression at both 225 M and 1,770 pM tAB0050 (**FIGs. 4B-4D**). In spite of this exquisite sensitivity to low levels of CD19, tAB0050 was also highly specific for CD19 expression, with no activity was observed in the wild-type control cells lacking CD19 expression.

[0239] Similar assays were performed in this inducible system to compare the minimal CD19 expression level required for tAB0050 or blinatumomab to induce cytotoxicity and T cell activation, as measured by IFN $\gamma$  production. As shown in **FIGs. 4E-4F**, tAB0050 demonstrated robust efficacy at lower CD19 expression levels compared to blinatumomab.

#### **Example 8: Comparison of Anti-tumor Activity of IV or SC Administered tAB0050**

[0240] Several T cell engagers have recently been evaluated for SC administration to minimize systemic toxicity and improve patient convenience. Because the formulation of tAB0050 allows for both IV and SC administration of tAB0050, we investigated anti-tumor efficacy by both routes of administration in immunodeficient NCG mice engrafted with Raji B.luc cells and human PBMC.

#### Materials and Methods

##### [0241] tAB0050 generation

[0242] The individual components of tAB0050 were optimized using Adimab's antibody engineering yeast platform. Full-length tAB0050 was assembled by fusing a humanized anti-serum albumin single-domain antibody (sdAb), a humanized anti-CD19 single-chain variable fragment (scFv) and a humanized anti-CD3 epsilon scFv into a single polypeptide chain using glycine/serine linkers. tAB0050-HIS consists of the same sequence as tAB0050 with a 10xHistidine tag at the C-terminal end and was produced in HEK-293 cells solely for initial characterization studies.

##### [0243] Biacore evaluation of tAB0050 binding activities

[0244] To determine affinity for a CD3 $\epsilon\delta$  dimer and albumin, each protein was immobilized on a CAPture chip sensor surface via biotin capture. A multi-cycle kinetic assessment of tAB0050 interactions was performed by injection of an appropriate concentration series of the test sample. To determine affinity for CD19, a human CD19-Fc fusion protein was immobilized on a Series S CM5 sensor chip via anti-human Fc antibody

immobilized on the surface. A single-cycle kinetic assessment of the protein-sample interaction was performed by injection of an appropriate concentration series of the test sample. Each protein-sample interaction was analyzed in triplicate and a Langmuir 1:1 binding model was fit to the data. To determine the impact of albumin binding on tAB0050 affinity, tAB0050 was first precomplexed with HSA or left unbound, then passed over either chip-immobilized, recombinant CD3 $\epsilon$  $\delta$  and the binding measured throughout. A global fit algorithm (Biacore Insight Evaluation) was used to calculate  $K_D$ ,  $k_a$ , and  $k_d$  values.

**[0245]**    Cell binding of tAB0050

**[0246]**    Cryopreserved PBMC from human, cynomolgus monkey or mouse donors were thawed prior to addition of live/dead viability dye, Fc block, and staining solution. Briefly  $0.3 - 1 \times 10^6$  cells were stained with FVS700 for viability according to manufacturer's recommendations and blocked with mouse or human Fc block antibody. Next, cells were incubated with tAB0050 conjugated to APC for 60 minutes at 37°C in the dark and stained with fluorescence-conjugated antibodies. Mouse and NHP cells were diluted in Brilliant Stain Buffer and FBS Stain Buffer (BD Biosciences), whereas human cells were diluted in Brilliant Stain Buffer and PBS (ThermoFisher). After staining, cells were fixed with Fluorofix Buffer (Biolegend) and stored at 2-8°C in the dark until acquisition by flow cytometry. CD4+, CD8+ T cells and CD20+ B cells were identified and gated.

**[0247]**    T cell-dependent cytotoxicity (TDCC) and T cell activation in vitro

**[0248]**    In brief, PBMCs (stained with eFluor450) and target cells (RAMOS, Raji, and A20-CD19) stained with eFluor670 were assessed at a 10:1 E:T ratio with a target cell seeding density of  $\sim 3 \times 10^5$  cells/well in the presence or absence of 0.15 mg/mL human serum albumin. An 8-point dilution of tAB0050 was performed starting at 2 nM with 6-fold dilution steps. Cytotoxicity was assessed using a fixable viability dye (eFluor780) by flow cytometry. T cell activation was determined by flow cytometry (CD4, CD8, CD25 and CD69). Cytokine production (IFN- $\gamma$  and TNF $\alpha$ ) was quantified using Luminex Multiplex Analysis where appropriate. CD19 expression was quantified using Quantibrite beads (BDBiosciences).

**[0249]**    CHO-CD19 inducible cell line generation and in vitro studies

**[0250]**    CHO-K1 cells were transduced with an induction cassette that expresses a chemical-induced proximity (CiP) activation cassette that allows titratable expression of CD19 upon treatment with of the chemical inducer, ABA, enabling expression levels of CD19 on CHO cells ranging from 78 to 185,223 copies of CD19 per cell after 24, 48 or 96 hours.

CD19 expression was quantified by staining with PE anti-CD19 and plotted on a curve generated using Quanti-Brite PE receptor quantification kit.

**[0251]** For TDCC studies with inducible CHO CD19 cells, CHO-K1 ABA CD19 cells were seeded in xCELLigence RTCA plates and treated with a 7-point concentration range (2.4-10  $\mu$ M) of ABA for 24 hours to induce CD19 expression. Isolated T cells and target cells were assessed at a 10:1 E:T ratio with two fixed tAB0050 concentrations (225 pM and 1,770 pM), and 4 individual T cell donors. The comparison to blinatumomab utilized isolated T cells from 10 individual donors at an E:T ratio of 10:1, with 225 pM of either tAB0050 or blinatumomab. CD8+ T cell proliferation was measured via Ki-67 staining and flow cytometry analysis. IFN- $\gamma$  production by the effector cells was measured via ELISA from supernatant.

**[0252]** In vivo efficacy studies

**[0253]** For the A20-CD19 study, hCD3 $\epsilon$ -expressing BALB/c mice were inoculated with A20 cells expressing hCD19 which were produced by stable transfection, and individual clones selected by limiting dilution. Mice were treated once IV with tAB0050-HIS or blinatumomab when tumor volume reached  $\sim$ 100mm<sup>3</sup>. For Raji B.luc studies, immunodeficient NCG mice were IV engrafted with  $1 \times 10^5$  Raji B.luc cells. On the following day (Day 1),  $2 \times 10^7$  PBMC from a normal human donor were implanted intraperitoneally (IP). Starting on Day 1, mice were treated weekly IV with tAB0050-HIS or blinatumomab.

**[0254]** For the comparison of IV and SC administration, mice that received PBMC were then placed into 7 groups: PBS vehicle control (n=9) or PBMC + tAB0050 dosed at 0.3, 3 or 30  $\mu$ g/kg (n=9) administered either IV or SC. tAB0050 was administered once weekly to end of study in a dosing volume of 10 mL/kg, adjusted for body weight. At day 14, the number of Raji cells and B cells in peripheral blood was determined by flow cytometry.

**[0255]** Once weekly IV or SC dosing of tAB0050 at 3 and 30 mg/kg resulted in 10/10 complete responses after 28 days, as was evident from luciferase fluorescence signals equivalent to background (**FIG. 9C, FIG. 9D**). Treatment with 0.3 mg/kg had a minimal effect with SC administration and a modest effect with IV administration. The percentage of human B cells and Raji B.luc cells in the peripheral blood of mice on day 14 was determined by FACS analysis. A highly significant and complete elimination of human B cells and lymphoma cells was observed in peripheral blood samples from mice in all tAB0050 treatment groups regardless of dose level or route of administration (**FIG. 10A**). A trend towards dose-dependent

reduction in T cell count was also observed on Day 14 in the periphery, consistent with T cell evacuation from peripheral circulation upon activation. (**FIG. 10B**).

[0256] A similar dose-dependent PK/PD study was performed in the huPBMC Raji B.luc model after a single dose of tAB0050 administered either IV or SC. Consistent with the multi-dose efficacy study, analysis of Raji cells, normal B cells and T cells in the bone marrow as measured by FACS on Day 8 of the study demonstrated statistically significant decreases in the number of Raji B.luc cells across all dose levels and routes of administration (**FIG. 10C**), and a trend in the decrease in number of normal B cells with both routes of administration. Additionally, dose dependent increases in both CD8+ and CD4+ T cell activation, as measured by CD69, were observed with both routes of administration.

[0257] Cytokine levels were measured at 2, 6, and 24 hours after the first dose of tAB0050. While robust human cytokine responses can be difficult to observe in huPBMC efficacy models, a trend of dose-dependent increases in IL-6, IL-8, and GM-CSF were seen at 6 hours post dose in the IV dose groups, which returned to baseline at 24 hours, whereas no dose-dependent increases in these cytokines were observed in the SC dose groups. (**FIGs. 11A and 11B**). These data suggest both IV and SC administration of tAB0050 are highly effective in the Raji lymphoma xenograft model.

[0258] The PK of tAB0050 was also assessed. Blood was drawn and serum collected at 0.5, 2, 4, 24, 74, and 168 hours post single dose. Non-GLP fit-for-purpose methods were used to measure tAB0050 concentrations in mouse serum. Concentrations of tAB0050 after a single dose were only detectable above the LLOQ (6.25 ng/mL) at the 3 and 30 µg/kg IV and 30 µg/kg SC dose levels. Concentrations of tAB0050 at 3 µg/kg IV were below the limit of quantification (BLQ) 24 hours post-dose, whereas tAB0050 was detectable out to 72 hours in all mice in the 30 µg/kg dose groups. (**FIG. 12A**). The PK analysis demonstrated that C<sub>0</sub> values of tAB0050 increased with increasing dose in an approximately dose proportional manner from 3 to 30 µg/kg IV (**FIG. 12B**). Estimated half-life was 1.85 days for the IV administration. C<sub>max</sub> after SC administration was approximately 10-fold lower than C<sub>max</sub> after IV, at 16.7 ng/mL and 126.4 ng/mL, respectively. T<sub>max</sub> at 30 µg/kg SC was approximately 24 hours.

[0259] To evaluate the stability of tAB0050 under relevant physiological conditions, 100 µg/mL tAB0050 was incubated at 37°C in human serum. Samples were collected at 0, 7, and 14 days and stored at -80°C. At the end of the two-week study, all samples were run in the

NFAT-RE T cell activation assay in the presence of Raji target cells. EC50s were calculated at each timepoint.

[0260] As shown in **FIG. 13**, EC50 values ranged from 0.38 ng/mL at Day 0 to 0.52 ng/mL at Day 14. Considering EC50s fall within 2-fold, these data suggest tAB0050 is stable in human serum when incubated at physiological temperatures for two weeks.

**[0261] Example 9: Comparison of Pharmacokinetics, Pharmacodynamics and Tolerability of IV- or SC-Administered tAB0050 in Non-human Primates**

[0262] We hypothesized that SC administration may reduce systemic toxicities commonly associated with IV-delivered T cell engagers due to a reduction in maximal concentration in serum (C<sub>max</sub>), and therefore directly compared tAB0050 at single doses of 0.1 and 1 mg/kg by received a single intravenous (IV) or subcutaneous (SC) injection administration in cynomolgus monkeys (n=2). **FIG. 14A** depicts the study design. Timepoints for PK serum sample collection from each animal were 0.25, 0.5, 1, 2, 6, 24, 48, 144, 312, 480 and 672 hours relative to the dose. The monkeys were also observed for signs of toxicity for up to 29 days.

[0263] Serum tAB0050 dose levels increased in a dose-dependent manner for both routes of administration (**FIG. 14B**). Serum half-lives ranged from 5-7 days, suggesting that half-life extension will support infrequent dosing. Exposure and half-life were similar for both routes of administration. As expected, C<sub>max</sub> values were at least 5-times lower by the SC route than seen for the IV route.

[0264] A single dose of tAB0050 reduced the number of circulating B cells to background levels for the entire duration of the 28-day experiment with no apparent recovery of B cell counts (**FIG. 14C**). T cells also transiently disappeared from the periphery 2 days post-dose but quickly recovered to pre-dose levels by day 8 post infusion, consistent with the observations of other T cell engagers (**FIG. 14D**).

[0265] As expected, administration of tAB0050 induced the transient release of various cytokines and chemokines into peripheral blood (**FIG. 14E**). Following IV infusion of tAB0050, blood levels of TNF $\alpha$ , IL-2, IFN $\gamma$ , and IL-8 were highest at 2 hours post infusion and trended towards baseline by 6 hours, while levels of IL-6 and IL-10 peaked at 6 hours. Notably, the release of the majority of the cytokines was lower after SC delivery compared to

IV infusion, and the kinetics of cytokine release were altered as well. Twenty-four hours post infusion, all cytokine levels were back to baseline irrespective of the route of delivery.

[0266] tAB0050 was well tolerated by cynomolgus monkeys at the 0.1 mg/kg dose level by both IV and SC routes, the 1 mg/kg dose level was tolerated only after SC administration. In contrast, IV administration of 1 mg/kg tAB0050 caused one of two cynomolgus monkeys to experience symptoms of cytokine release, which required euthanasia on Day 1 of study. A dose of 1 mg/kg administered SC exposed the animals to serum concentrations of ~1-5 µg/mL (~15-75 nM) tAB0050 over a time period of 300 hours (12.5 days). This was >10-fold greater than the concentrations of tAB0050 needed for complete target cell lysis in vitro, even for low CD19 expressing cells.

[0267] The serum-concentration-time profiles for individual animals for both dose level groups for IV administration are shown in **FIGs. 15A** and **15B**. The serum-concentration-time profiles for individual animals for both dose level groups for SC administration are shown in **FIGs. 15C** and **15D**. For monkeys administered by IV injections, the C<sub>max</sub> was observed at 0.25 hours, and the t<sub>1/2</sub> ranged from 43.8 to 113 hours. The mean values for clearance were 1.18 and 0.697 mL/hr./kg for 0.1 and 1 mg/kg dose groups, respectively. The mean values for V<sub>ss</sub> were 101 and 98.0 mL/kg for 0.1 and 1 mg/kg dose groups, respectively.

[0268] For monkeys administered by SC injections, the T<sub>max</sub> ranged from 24 to 144 hours, and the t<sub>1/2</sub> ranged from 110 to 167 hours. C<sub>max</sub> after SC administration was observed to be 4-fold lower than IV in the 1 mg/kg dose group.

[0269] CLN-978 (tAB0050) exposures generally increased with dose in female monkeys administered by IV or SC routes. The increases, as indicated by dose-normalized C<sub>max</sub> and AUC<sub>last</sub> values, were dose proportional from 0.1 to 1 mg/kg for both IV and SC administrations. The TK parameters determined for this study are summarized in **FIGs. 16A** and **16B**.

[0270] Parameters evaluated included mortality, cage side observations, detailed clinical observations, body weight, food evaluation, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic analysis, cytokine sample analysis, and flow cytometric analysis.

[0271] There were no treatment-related changes in body weights or urinalysis parameters. One animal treated IV with 1 mg/kg CLN-978 was euthanized on Day 1 due to a moribund condition. All other animals survived until study termination. The moribund animal exhibited

decreased activity, closed eyelids, hunched posture, moderately delayed response time, low blood glucose level, slightly high heart rate, low body temperature, and were cold to touch. This animal also exhibited macroscopic findings of raised foci in the gastrointestinal tract and adhesions in the lung.

**[0272]** After a single IV injection, clinical observations noted included hunched posture, vomitus or vomit-like material (approximately 1 to 2 hours post-dose), and reduced appetite at  $\geq 0.1$  mg/kg. After a single SC injection, clinical observations included vomitus or vomit-like material (approximately 6 to 7 hours post-dose) at  $\geq 0.1$  mg/kg and reduced appetite in animals at 1 mg/kg.

**[0273]** Treatment related changes were noted in hematology, coagulation, and clinical chemistry. Hematology alterations included changes in white blood cell counts including lymphocytes, monocytes, basophils, large unstained cells, and eosinophils of all animals. There was an initial reduction in these blood parameters in all groups on Day 2, followed by a general increase in these parameters at the later timepoints compared to pre-test values. Neutrophil counts fluctuated throughout the study and were increased across groups on Day 29 compared to pre-test values. Coagulation changes included on Day 2, increased fibrinogen across all groups, on Day 8, increased fibrinogen in the remaining IV treated animal at 1 mg/kg and SC animals at 0.1 mg/kg, and on Day 29, increased fibrinogen in IV animals at 0.1 mg/kg and in SC and IV animals at 1 mg/kg.

**[0274]** Clinical chemistry changes included on Day 2 increased alanine aminotransferase and/or aspartate aminotransferase in all groups compared to pre-test values. On Day 29, alkaline phosphatase was increased in a single IV animal at 0.1 mg/kg and in a single SC animal at 1 mg/kg. Total bilirubin and urea nitrogen were elevated across all groups on Day 2 and total bilirubin was elevated in all animals on Day 8. Triglycerides were increased in SC animals at 1 mg/kg CLN-978 on Day 29.

**[0275]** Flow cytometry data showed depletion of B cells in both SC and IV treated animals. Also, transient reductions of peripheral T cells and NK cells occurred at the Day 2 post-dose timepoint in IV and SC treated animals which in general increased on Day 8 and 15 to levels near or above pre-dose levels. Day 29 peripheral lymphocyte values were also reduced compared to pre-dose values for IV treated animals and for one animal treated SC with 1 mg/kg, but to a lesser degree than that seen on Day 2.

[0276] Qualitative analysis of cytokine data showed no change in IL1beta following IV or SC dosing. In general, with IV dosing, for the remainder of cytokines and chemokines (IL1RA, IL2, IL4, IL5, IL6, IL8, MCP1, MIP1 $\beta$ , TNF $\alpha$ ), values increased from pre-dose values beginning at 2 and 6 hours post dosing and were declining by 24 or 672 hours post dosing. SC dosing resulted in essentially no change in IL2, IL4, and IL5 and sporadic transient increases in IL6, IL8, MCP1, MIP1 $\beta$ , and TNF $\alpha$  decreased over time.

[0277] Overall, a single injection of CLN-978 was generally tolerated at 0.1 mg/kg IV or at 0.1 and 1 mg/kg SC. With IV dosing, the death of one animal occurred at 1 mg/kg due to test article-related mortality. In general, treatment related changes following IV dosing included clinical signs of hunched posture, vomiting, reduced appetite and clinical pathology changes at  $\geq 0.1$  mg/kg. A single SC injection was associated with vomiting and reduced appetite at 1 mg/kg, and changes in clinical pathology parameters at  $\geq 0.1$  mg/kg.

**[0278] Example 11: A GLP 4-Week Toxicity Study of CLN-978 by Subcutaneous Injection in Cynomolgus Monkeys with a 4-Week Recovery Period**

[0279] The objectives of this study were to determine the potential toxicity of CLN-978 in cynomolgus monkeys when given by SC injection once a week for 4 weeks and to evaluate the potential reversibility of any findings.

[0280] Cynomolgus monkeys (3-5 sex/group) received once weekly (on Days 1, 8, 15, and 22) SC injections of 0, 0.1, 0.3, and 1 mg/kg CLN-978 for 4 weeks. All main study animals (3 sex/group) were observed through Study Day 29 and a subset of animals (2 sex/group) in the vehicle control and high dose group were held for a 4-week recovery period (through Study Day 57). The study design is shown in Table 7.

**[0281] Table 7: Study design for 4-week toxicity study of CLN-978 by subcutaneous injection in cynomolgus monkeys**

| Group No. | Test Article <sup>a</sup> | Dose Level (mg/kg/day) | Dose Volume <sup>c</sup> (mL/kg) | Dose Concentration (mg/mL) | Main Study   |                | Recovery Study |                |
|-----------|---------------------------|------------------------|----------------------------------|----------------------------|--------------|----------------|----------------|----------------|
|           |                           |                        |                                  |                            | No. of Males | No. of Females | No. of Males   | No. of Females |
| 1         | Vehicle <sup>b</sup>      | 0                      | 0.5                              | 0                          | 3            | 3              | 2              | 2              |
| 2         | CLN-978                   | 0.1                    | 0.5                              | 0.2                        | 3            | 3              | 0              | 0              |
| 3         | CLN-978                   | 0.3                    | 0.5                              | 0.6                        | 3            | 3              | 0              | 0              |
| 4         | CLN-978                   | 1                      | 0.5                              | 2                          | 3            | 3              | 2              | 2              |

No. = Number

<sup>a</sup> Vehicle and CLN-978 were administered subcutaneously into scapular or mid-dorsal sites once weekly (on Days 1, 8, 15, and 22).

<sup>b</sup> Vehicle = 10 mM histidine, 8% sucrose, 0.01% PSSO, pH 6.7.

<sup>c</sup> Based on the most recent body weight measurement.

Note: 0.01% PSSO was specified whereas 0.001% PSSO was the actual concentration.

[0282] Parameters evaluated in this study included mortality, clinical observations (including detailed and post-dose observations), body weights, food evaluation, respiratory rate, ophthalmology, electrocardiology, neurobehavioral examinations, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), bioanalysis and toxicokinetic parameters, anti-drug antibody analysis, cytokine analysis, immunophenotyping and flow cytometry, organ weights, and macroscopic and microscopic examinations.

[0283] There were no CLN-978-related changes in body weight, body weight gains, ophthalmology, electrocardiology, respiratory rate, neurobehavioral parameters, macroscopic pathology, cytokines or urinalysis parameters.

[0284] A single high-dose recovery female was euthanized on Day 31 due to clinical observations that included moderate tremors, moderate dehydration, hunched posture, thinness, liquid feces, and decreased activity. Clinical pathology findings for this animal were similar to those for animals that survived to the scheduled necropsy. This animal had a number of macroscopic and microscopic abnormalities and the cause of moribundity was chronic peritonitis, which most likely represents an opportunistic infection secondary to the CLN-978-induced immunosuppression. CLN-978 related microscopic findings were identical to those identified at terminal sacrifice.

[0285] CLN-978-related clinical observations included hunched posture and decreased activity in a single male at 1 mg/kg/dose. No clinical observations were noted during the 4-week recovery period, indicating full recovery.

[0286] CLN-978-related changes in hematology parameters included minimally to moderately decreased red blood cell count, hemoglobin, and hematocrit, and minimally increased platelet count at  $\geq 0.1$  mg/kg/day, minimally decreased reticulocyte count at  $\geq 0.3$  mg/kg/day, and variable changes in leukocytes (neutrophils, monocytes, basophils, large

unstained cell count and white blood cell count). The neutrophil count increased toward the end of the dosing phase (Days 23 and 29) at  $\geq 0.3$  mg/kg/day. Monocyte count was markedly decreased at 0.1 and 0.3 mg/kg/day at Day 2. The remaining leukocytes were minimal to markedly decreased after administration of the first dose on Day 2, at  $\geq 0.1$  mg/kg/day, followed by an increase at Day 8. Lymphocyte count was also decreased after administration of the fourth dose on Days 23 and 29.

**[0287]** In coagulation and clinical chemistry, CLN-978-related changes were consistent with an acute phase response, including minimally to mildly increased fibrinogen at  $\geq 0.1$  mg/kg/dose, mildly prolonged activated partial thromboplastin time at  $\geq 0.3$  mg/kg/dose at Days 2 and/or 23, and minimally decreased albumin at  $\geq 0.1$  mg/kg/day. Other changes in clinical chemistry were overall minimal to mild and included increased total bilirubin and urea nitrogen at  $\geq 0.1$  mg/kg/day (up to moderate), and creatinine at 0.1 and 1 mg/kg/day, and decreased glucose at 0.1 and 0.3 mg/kg/day, phosphorus at 0.3 and 1 mg/kg/day, and cholesterol, sodium, and globulin (up to moderate) at  $\geq 0.1$  mg/kg/day. The decreased albumin and globulin resulted in decreased total protein, and calcium, and in variable changes in albumin to globulin ratio.

**[0288]** Following a 4-week recovery period, changes in hematology, coagulation, and clinical chemistry were observed at 1 mg/kg/day, indicating lack of recovery. In hematology, these changes included mildly decreased red blood cell count, hemoglobin, and hematocrit, minimally increased neutrophil count, and minimal to moderately decreased lymphocyte count. In coagulation and clinical chemistry, fibrinogen was moderately increased, sodium was minimally decreased, and albumin and globulin were moderately decreased, resulting in decreases in total protein, calcium and variable changes in albumin to globulin ratio.

**[0289]** Immunophenotyping analysis revealed that 4-week CLN-978 treatment (0.1, 0.3 and 1 mg/kg/day) resulted in test article-related depletion of B cells, and transient reductions of peripheral T cells and NK cells at the Day 2 post-dose timepoint. Results suggests that the T cell and NK cell reductions were transient, as these populations recovered to or above pre-dose baseline levels by Day 8 (with exception of T cell, T-helper and NK cell populations in a small number of animals [1-2 per group] at 0.1, 0.3 and 1 mg/kg/dose), and were moderately reduced again on Day 23, following the fourth dose of CLN-978. The peripheral absolute total T cells, T-helper, T-cytotoxic and NK cells for all dosed animals recovered back to their respective pre-dose baseline values by the Day 29 post-dose timepoint. Absolute B cells remained depleted in animals from all the dosing groups in both study cohorts.

**[0290]** Treatment related organ weight changes included decreased splenic weights (absolute, relative to brain and body weight) in males at 0.1 and 1 mg/kg/day and in females at  $\geq 0.1$  mg/kg/day and decreased thymic weight (absolute, relative to brain and body weight) in males and females at  $\geq 0.1$  mg/kg/day. These findings correlated histopathologically with severely decreased lymphoid cellularity of the germinal centers in the splenic white pulp and decreased lymphoid cellularity of the thymic cortex. Thymic weight fully recovered in 1 mg/kg/day males but not females by the recovery sacrifice, whereas the splenic weight decrease persisted in males. The histological change resulting in decreased splenic weight persisted in the 1 mg/kg/day recovery female, however the weight was increased although it was confounded by concurrent pathology. The thymic changes may be secondary to physiologic stress.

**[0291]** Microscopic findings related to CLN-978 administration were identified in all secondary lymphoid tissues evaluated, including spleen; axillary, mandibular and mesenteric lymph nodes; and GALT. The findings consisted of markedly to severely decreased lymphoid cellularity of the germinal centers in  $\geq 0.1$  mg/kg/day males and females and, in the CD20 immunohistochemistry slides, markedly to severely decreased CD20 positive cells (i.e., B cells) in the normally B cell rich regions of the splenic white pulp, lymph node cortices, GALT, spleen, and sternal bone marrow (See e.g., **FIGs. 22A – 22C**). No dose response was apparent with these findings, and they persisted at the same severity as the terminal sacrifice through the recovery period, consistent with no appreciable recovery. These changes are interpreted as a pharmacologic effect of CLN-978.

**[0292]** Additional microscopic findings with a possible relationship to CLN-978 administration included the previously mentioned decreased thymic lymphoid cellularity and increased myeloid to erythroid ratio of the sternal bone marrow. These changes may be secondary to physiologic stress (thymus) and/or a response to the pharmacologically induced immunosuppression of the adaptive immune system (bone marrow).

**[0293]** One 0.3 mg/kg/day male designated for the terminal sacrifice and one 1 mg/kg/day female designated for the recovery sacrifice had microscopic findings throughout their abdominal cavities consistent with chronic bacterial peritonitis, with the worst inflammatory changes localized to the gastrointestinal tract, which is presumably the portal of entry for the bacteria. These changes are interpreted as opportunistic infections secondary to the pharmacologic immunosuppression induced by CLN-978.

[0294] Taken together, the results show that administration of 0.3 mg/kg/day CLN-978 by once weekly SC injection for 4 weeks was tolerated in cynomolgus monkeys. Noted changes included nonadverse CLN-978-related clinical pathology findings, decreased thymic and splenic weights and microscopic findings (severely decreased germinal centers and/or markedly to severely decreased CD20 positive cells) in spleen; axillary, mandibular and mesenteric lymph nodes; and GALT. These microscopic changes are interpreted as a pharmacologic effect of CLN-978. Based on these results, the HNSTD was considered to be 0.3 mg/kg/day.

[0295] **Example 12: Development of Rationale for Starting Dose and Schedule**

[0296] An SP model was developed for CLN-978, using inputs from in vitro binding and in vitro cytotoxicity assays, in vivo murine xenograft models, and cynomolgus monkey PK and toxicology studies, and used to predict the safe starting and efficacious doses in human. Within the overall SP modeling approach, a total of four SP models were developed. The in vitro model was used to capture cytotoxicity data, determine the cross-linking  $k_{on}$  for CLN-978, and establish criteria for starting dose in the clinic based on achieving 50% of the assay's cytotoxicity. The mouse SP model, developed using mouse xenograft data, compared model outputs to the TGI response and was used to define criteria for efficacious dose. The cynomolgus monkey model was developed to capture data from the PK study. Allometric scaling was used to translate the cynomolgus monkey PK parameters to human. The human NHL model was constructed and used to predict the safe starting and efficacious doses of CLN-978 after SC administration. The main output of the model is the projected formation of trimolecular complexes (CD19:CLN-978:CD3, "trimers"). The human model diagram is shown in **FIG. 17**.

[0297] The in vitro cytotoxicity assay involved co-culturing of PBMCs and Raji cells at different E:T ratios with varying concentrations of drug (CLN-978 or blinatumomab).  $k_{on2}$ , the avidity/cross linking parameter, was determined based on the hypothesis that the formation of trimers drives cytotoxicity and that for both blinatumomab and CLN-978, the trimer versus cytotoxicity curves must generally overlap. A range of  $k_{on2}$  values for both molecules was explored. The resulting projected average trimers per T cell ("TpT") over the length of incubation versus % cytotoxicity relationships over the assay concentration range were also explored. Visual examination determined that the best match of the trimer-cytotoxicity curve was when the association rate constant ( $k_{on2}$ ) of both molecules was estimated to be 30 dm<sup>2</sup>/nmol/s. This value was applied to modeling CLN-978 going forward. It was decided that

achieving the average TpT that results in 50% of the observed cytotoxicity in the in vitro assay will be the criteria for the starting dose, hence 60 TpT (48.4% maximum observed cytotoxicity) was initially used to determine the starting dose.

**[0298]** A PK study of CLN-978 in mouse was performed and data from both IV and SC administration were collected. Based on non-compartmental analysis AUC comparison, the SC bioavailability was determined to be 100%, so the bioavailability parameter in the model was fixed to that value. The mouse PK was only used for the mouse model and was not translated to human. The mouse TGI study was conducted with CLN-978 dosed SC weekly at 0.3 µg/kg, 1 µg/kg, and 10 µg/kg. The mouse model was used to simulate the dose-ranging TGI study and project the corresponding TpT. At day 21 the 10 µg/kg dose resulted in the highest TGI and a clear differentiation from the other doses and was therefore chosen as the criteria to define the efficacious dose in the human model. The model also projected the highest average TpT at 10 µg/kg. The resulting average TpT in the peripheral compartment at day 21 was 472, which was adopted as the criteria for efficacious dose.

**[0299]** A dose-ranging PK study was conducted in cynomolgus monkeys with four dosing groups - 0.1 and 1 mg/kg, both IV and SC, n = 2 per dose group. The cynomolgus monkey model was parameterized to include circulating and peripheral T cells and normal B cells and used the reported affinity values. Since CLN-978 will be administered in multiple doses in the clinic, to better translate from the single dose administered in this study and to capture the difference in TMDD after multiple dosing due to B-cell depletion, B-cell depletion was explicitly included in the model. The central and peripheral volumes were fixed to physiological values. Based on internal exposure analysis between the IV and SC doses, the bioavailability was fixed at 100%. Allometric scaling was used to determine the human PK parameters.

**[0300]** A range of doses was simulated with the human NHL model. Dosing frequency was QW SC administration. The criteria for starting and efficacious doses are based on Avg TpT with cancer cells in the tumor at day 28, which corresponds to the end of the fourth dose. At day 28 the trimer formation is close to steady-state, hence day 28 was chosen as the end of the dosing cycle. Based on the preclinical modeling results, the criteria were set at 60 TpT for starting dose and 472 TpT for efficacious dose. Based on the human model simulations, 240 µg is the recommended starting dose and 1850 µg is the projected efficacious dose. PK projections and running average (AUC divided by time point) TpT are presented in **FIGs. 18A and 18B** and exposure results are reported in Table 8.

**[0301] Table 8: Predicted human PK parameters calculated from simulated concentration-time profiles after subcutaneous weekly dosing**

| Proposed Dose                              | Dose (µg)<br>SC | C <sub>max</sub><br>(µg/mL) | AUC <sub>0-7 days</sub><br>(µg·h/mL) | C <sub>max,ss</sub><br>(µg/mL) | AUC <sub>0-7 days,ss</sub><br>(µg·h/mL) | C <sub>max</sub> AR |
|--------------------------------------------|-----------------|-----------------------------|--------------------------------------|--------------------------------|-----------------------------------------|---------------------|
| Starting Dose based<br>on EC <sub>50</sub> | 240             | 0.0143                      | 2.18                                 | 0.0423                         | 17.8                                    | 2.92                |
| Efficacious Dose                           | 1850            | 0.114                       | 17.1                                 | 0.345                          | 140                                     | 3.03                |

C<sub>max</sub> = maximum observed concentration; AUC<sub>0-7 days</sub> = area under the curve from 0-7 days, t = 7 or 28; ss = steady-state;  
AR = accumulation ratio; C<sub>max</sub> AR = C<sub>max,ss</sub> at steady-state / C<sub>max</sub> after single dose

**[0302]** Sensitivity analysis was also performed, and the effect of potential variabilities in the patient population on the starting dose was explored. The number of T cells in the tumor, drug distribution in the tumor, and tumor volume are parameters that may vary among NHL patients. For example, a 3-fold decrease in the CLN-978 tumor distribution half-life (equivalent to a 3-fold faster distribution into the tumor) could suggest lowering the starting dose around three-fold (from 240 µg to 90 µg). Based on the sensitivity analysis and a conservative decision to target the EC20 (7 average trimers per T cell in the tumor) instead of the EC50, 30 µg administered SC QW was defined as the recommended starting dose for the FIH study.

**Example 14: Clinical study of tAB0050 in patients with relapsed or refractory (R/R) B cell Non-Hodgkin Lymphoma (B-NHL)**

**[0303]** This Example describes a phase 1, open-label, multi-center, first-in-human, dose escalation, and dose expansion study of tAB0050 in patients with relapsed or refractory (R/R) B cell Non-Hodgkin Lymphoma (B-NHL).

**[0304]** Adult patients (≥ 18 years of age) with one of the following CD19+ B-cell histologies: diffuse large B-cell lymphoma – de novo or transformed, high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, or marginal zone lymphoma (nodal, extranodal, or mucosa-associated) for whom there is no available approved standard therapy will be recruited. Other key eligibility criteria include: ≥ 2 lines of prior chemotherapy, Eastern Cooperative Oncology Group Performance Status 0-2. Key exclusion criteria include prior treatment with allogeneic hematopoietic stem cell transplantation or any other investigational CD19 x CD3 T cell engager. Prior CAR-T therapy, unconjugated or radio-conjugated CD19 mAb, and CD19 antibody-drug conjugate are allowed.

[0305] The study consists of 2 parts: 1) a dose escalation phase commencing with accelerated titration in single-patient cohorts followed by a conventional 3+3 dose escalation design to identify the recommended phase 2 dose (RP2D) and 2) a dose expansion phase to further characterize the safety and preliminary efficacy of tAB0050 in disease-specific cohorts at the RP2D. **FIG. 19** illustrates the two parts of the study. tAB0050 will be subcutaneously (SC)-administered once weekly (QW) in 28-day cycles until progressive disease, intolerable toxicity, or a maximum of 24 cycles of treatment. Based on emerging data, less frequent administration schedules may be implemented in Cycle 4 and beyond as approved by the Safety Review Committee. Safety, pharmacokinetic (PK), pharmacodynamic, and preliminary efficacy assessments will guide the selection of the dose and schedule for further evaluation. The primary endpoint is safety. Key secondary endpoints include PK, anti-drug antibodies, overall response rate, duration of response, time to response, time to subsequent anti-lymphoma therapy, progression-free survival, and overall survival.

[0306] Up to approximately 90 patients (30 in dose escalation and 60 in dose expansion) will be enrolled at approximately 6 sites in the U.S., reflecting both community and academic centers.

[0307] The study is currently enrolling (NCT05879744).

[0308] The following trial is conducted as described below.

**Table 9. Arms and Interventions**

| Arm                                                                                                                                                                     | Intervention  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| Experimental: Part A Dose Escalation<br>Patients with R/R B-NHL treated with tAB0050 in dose escalation cohorts                                                         | Drug: tAB0050 |
| Experimental: Part B Dose Expansion<br>Patients with R/R DLBCL, R/R FL and other R/R B-NHL treated with tAB0050 at a dose selected from the Part A Dose Escalation arm. | Drug: tAB0050 |

[0309] **Outcome Measures**

[0310] *Primary Outcome Measure:*

1. Safety and tolerability of tAB0050  
Incidence and severity of adverse events (AEs)/adverse events of special interest (AESIs)/serious adverse events (SAEs); incidence of dose interruptions and delays
2. Define dose regimen for tAB0050  
Dose-limiting Toxicities (DLTs)

**[0311]** *Secondary Outcome Measures:*

1. Assess preliminary efficacy of tAB0050 in patients with selective histologies of R/R B-NHL

Overall response rate (ORR), complete response (CR), Duration of response (DOR)

2. Select PK parameters of tAB0050

PK parameters: area under-the-concentration-time curve, maximum concentration, and half-life

3. Immunogenicity of tAB0050 and potential impact on drug exposure

Incidence of anti-drug antibodies to tAB0050

**[0312]** *Eligibility Criteria*

- Ages Eligible for Study: 18 Years and older
- Sexes Eligible for Study: All
- Gender Based: No
- Accepts Healthy Volunteers: No

**[0313]** *Criteria***[0314]** *Inclusion Criteria:*

- Eastern Cooperative Oncology Group (ECOG) PS  $\leq 2$
- Documented diagnosis of one of the below CD19+ B-cell neoplasms according to WHO classification (Swerdlow et al., 2016) or WHO classification 2008:
  - a. Diffuse large B-cell lymphoma (DLBCL) - de novo or transformed
  - b. High-grade B-cell lymphoma
  - c. Primary mediastinal large B-cell lymphoma
  - d. Follicular lymphoma (FL)
  - e. Mantle cell lymphoma
  - f. Marginal zone lymphoma (nodal, extranodal, or mucosa-associated)
- Relapsed, progressive, and/or refractory disease after at least 2 lines of therapy.
- For Part B expansion cohorts:
  - a. Cohort B1: R/R DLBCL that has relapsed after at least 2 prior therapies including a CD20 monoclonal antibody and anthracycline.
  - b. Cohort B2: R/R FL (grade 1-3a) that has relapsed after at least 2 prior therapies including CD20 monoclonal antibody and an alkylating agent.
  - c. Cohort B3: Other R/R B-NHL (DLBCL and FL excepted).

- Measurable disease defined as  $\geq 1$  measurable nodal lesion (long axis  $>1.5$  cm and short axis  $>1.0$  cm) or  $\geq 1$  measurable extra-nodal lesion (long axis  $>1.0$  cm) on computed tomography (CT) scan or magnetic resonance imaging (MRI) AND baseline fluorodeoxyglucose-positron emission tomography (FDG-PET) scan demonstrating positive lesion(s) compatible with CT- or MRI-defined anatomical tumor sites.
- Laboratory parameters including the following:
  - a. Lymphocyte count  $< 5 \times 10^9/L$
  - b. Platelet count  $\geq 75 \times 10^9/L$
  - c. Absolute neutrophil count  $\geq 1.0 \times 10^9/L$ ; growth factor support allowed in cases of documented bone marrow involvement
  - d. Hemoglobin  $\geq 9$  g/dL, with or without transfusion
  - e. Creatinine clearance  $\geq 45$  mL/min
  - f. Total bilirubin  $\leq 1.5 \times$  upper limit of normal (ULN), except patients with confirmed Gilbert's Syndrome
  - g. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)  $\leq 3.0 \times$  ULN (unless attributed to hepatic involvement by lymphoma)

**[0315]** *Exclusion Criteria:*

- Primary CNS lymphoma or known CNS involvement by lymphoma at study screening
- Known past or current malignancy other than the inclusion diagnosis
- Known clinically significant cardiac disease
- Significant central nervous system disease
- Prior organ allograft
- Confirmed history or current autoimmune disorder or other disease requiring ongoing immune suppression
- Active Hepatitis C Virus (HCV), Hepatitis B Virus (HBV), or known Human Immunodeficiency Virus (HIV) infection
- Live virus vaccines within 28 days of the first dose of tAB0050, during treatment, and until the end of last dose of tAB0050
- Known active, clinically significant bacterial, viral, fungal, mycobacterial, parasitic, or other infection, including coronavirus disease of 2019 (COVID-19) infection, at the time of enrollment or within 7 days of the first dose of tAB0050.
- Prior treatment with any of the following:
  - a. Allogeneic HSCT

- b. Autologous HSCT within 30 days prior to the first dose of tAB0050
  - c. Chimeric antigen receptor T cell therapy (CAR-T) within 30 days prior to the first dose of tAB0050
  - d. Any investigational CD19 x CD3 T cell engager (TCE)
  - e. Unconjugated CD19 monoclonal antibody s 4 weeks prior to the first dose tAB0050
  - f. Radio-conjugated or CD19 antibody-drug conjugates 12 weeks prior to the first dose tAB0050
  - g. Investigational or standard of care monoclonal antibodies, chemotherapy, or other investigational agent s 4 weeks or 5 half-lives, whichever is shorter, prior to the first dose of tAB0050
  - h. Radiation therapy (XRT), with the exception of focal treatment for symptom control, s 4 weeks of the first dose of tAB0050
- Woman of child-bearing potential who is pregnant, breast-feeding, or plans to become pregnant
  - Male patients who plan to father a child or donate sperm within 120 days of last study drug administration

#### Clinical Safety and Efficacy

[0316] After the first patient treated at the 30 µg dose level experienced a Grade  $\geq 2$  adverse event, enrollment at that dose level was expanded to three patients as outlined below.

[0317] Patient 1 was a 25-year old male with relapsed T-cell-rich B cell lymphoma. Prior treatment included rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) followed by rituximab maintenance, then rituximab, ifosfamide, carboplatin, and etoposide (R-ICE), and most recently pembrolizumab. The patient received the first dose of CLN-978 (30 µg SC) after pre-medication with dexamethasone, diphenhydramine, famotidine, and acetaminophen. Approximately 10 hours post-dose, the patient developed Grade 1 CRS manifested as fever to 102.9°F (Grade 2) accompanied by dull headache, chills and rigors, for which he was treated with acetaminophen and meperidine. The fever resolved within 2.5 hours without sequelae or recurrence during inpatient monitoring through 48 hours post-dose. Fever to 100.8°F (Grade 1) recurred approximately 50 hours post-dose and again responded to acetaminophen. In both cases, work-up for infection was negative. Other clinically significant adverse events included transient lymphopenia (Grade 4 at nadir 24 hours post-dose) and leukopenia (max Grade 2 on C1D5-C1D7), consistent with the mechanism of action of CLN-978. Patient also experienced related Grade 1 injection site reaction (C1D13-C2D8), Grade 1

intermittent headaches (C1D2-ongoing), and Grade 1 fatigue (C1D2-C1D6, C1D9-C1D13). Other TEAEs included Grade 1 hyperglycemia (C1D2-C1D3), Grade 1 ALT (C1D2-C1D15), Grade 1 decreased creatinine (C1D3-C1D4), and Grade 1 nausea (C1D10-C1D12), all of which were considered unrelated to study treatment. The patient was re-hospitalized for a second administration of CLN-978, which was uneventful. He who discontinued treatment after several doses of CLN-978.

**[0318]** Patient 2 was a 75-year-old male with relapsed follicular lymphoma. Prior treatment included bendamustine and rituximab (BR) followed by rituximab maintenance, R-CHOP, and lenalidomide and rituximab. The patient received the first dose of CLN-978 (30 µg SC) after pre-medication with dexamethasone, diphenhydramine, famotidine, and acetaminophen. Approximately 48 hours post-dose, the patient developed Grade 1 CRS manifested as fever to 103.1°F (Grade 2), for which he received acetaminophen. The fever promptly resolved, and work-up for infection was negative. Other TEAEs included related transient lymphopenia (Grade 4 at nadir 24 hours post-dose), consistent with the mechanism of action of CLN-978, observed beginning C1D2, and Grade 1 pruritus (C1D22-C2D1); and Grade 1 intermittent hypomagnesemia (C1D2-C1D8) and Grade 1 hypocalcemia on C1D4-C1D8, both considered unrelated to study drug. The patient has received 24 weekly doses of CLN-978 in total. Imaging studies obtained after 2 and 4 cycles of therapy demonstrated stable disease; restaging scans after 6 cycles are pending.

**[0319]** Patient 3 was a 68-year-old male with relapsed mantle cell lymphoma. Prior treatment included BR followed by maintenance rituximab and lenalidomide, rituximab, dexamethasone, cytarabine, and cisplatin (R-DHAP), and high-dose chemotherapy and autologous stem cell transplant followed by maintenance rituximab. The patient received the first dose of CLN-978 (30 µg SC) after pre-medication with prednisone, diphenhydramine, famotidine, and acetaminophen. Approximately 26 hours post-dose, the patient experienced a fever to 100.4°F (Grade 1; this was considered unrelated to treatment) accompanied by chills, for which he received an additional dose of acetaminophen. The fever promptly resolved and work-up for infection was negative. Transient lymphopenia (Grade 3 at nadir 24 hours post-dose consistent with the mechanism of action of CLN-978, was observed beginning C1D2. The remainder of the 48-hour inpatient stay was uneventful. At scheduled outpatient follow-up 96 hours post-dose, the patient was noted to be afebrile but ill-appearing, tremoring but fully oriented (immune effector cell encephalopathy [ICE] score 9/10), and clinically hypovolemic with systolic blood pressure in the 90s. He was admitted to the hospital for volume resuscitation

and diagnostic evaluation. Blood pressure promptly normalized after IV fluids, and laboratory testing returned a diagnosis of acute Grade 1 influenza A virus infection (C1D4-C1D6), for which oseltamivir was started and he was discharged from the hospital. A palpable area of mantle cell lymphoma in the right mandible was reported to have resolved from baseline, but there was no clinical or laboratory evidence for tumor lysis or cytokine release syndrome. Tremor (Grade 1; considered unrelated to study treatment) was further treated with corticosteroids, improved after 24 hours, and had resolved by C1D6. Further treatment was deferred for 1 week to allow the patient to complete the 5-day course of oseltamivir and fully recover from influenza. He was readmitted to receive the second dose of CLN-978. Patient also experienced related Grade 2 confusion (C2D14-C2D15) however relatedness with significant usage of corticosteroid cannot be excluded. Other TEAEs included Grade 1 GERD (C1D14-ongoing), Grade 1 intermittent blurred vision (C1D16-ongoing), Grade 2 restlessness (C1D22-ongoing), and Grade 3 vascular access complication (C2D4; patient had a medical history of Grade 3 DVT), all of which were considered unrelated to study treatment. He discontinued treatment after seven doses of CLN-978 due to Grade 2 restlessness and Grade confusion however relatedness with significant usage of corticosteroid cannot be excluded. Imaging studies obtained after 2 cycles of therapy demonstrated complete metabolic response.

**[0320]**     Clinical Pharmacokinetics

**[0321]**     Clinical pharmacokinetic data was limited to patients 2 & 3. These data, provided in **FIG. 20**, show that a preclinical PK model utilized for the B-cell response simulations generally predicts the clinical pharmacokinetic data observed for the two patients administered 30 µg SC on a weekly schedule in the clinical trial.

**[0322]**     Clinical Pharmacodynamics

**[0323]**     Clinical pharmacodynamics data for CLN-978 were generated for each patient. **FIGs. 21A – 21F** are graphs showing B-cell depletion, T-cell activation and cytokine analysis for patients 1, 2 and 3.

**[0324]**     Peripheral blood was tested for the presence of B cells using a quantitative flow cytometry assay (TBNK [T cells, B cells, NK cells] panel). In two out of two B-NHL patients with detectable B cells at baseline (Patients 1 and 3), B-cell depletion occurred within 96 hours at a dose of 30 µg administered subcutaneously once weekly (QW) and remained low, but detectable, for the remainder of timepoints tested (**FIG. 21A**). Patient 2 had low peripheral blood B cells at baseline and remained low through-out the treatment course. Interestingly, in

Patient 1, B-cell lymphocyte counts remained low at the safety follow up visit on Day 149, indicative of persistent peripheral B-cell depletion 90 days after the last dose of CLN-978 was administered on Day 59.

[0325] Peripheral CD3+ cell counts were also assessed using the same TBNK panel. All three patients demonstrated a drop in CD3+ cell counts within 4 days post-treatment, which was likely caused by a recruitment of peripheral T cells to CD19+ tissues (**FIG. 21B**). Rebound from the initial post-treatment drop in CD3+ event was observed, indicative of T cell redistribution to the periphery.

[0326] Further phenotypic characterization of peripheral T cells demonstrated fluctuations in the overall frequencies of CD4+ (**Fig 21C**) and CD8+ (**Fig 21D**) T cells across timepoints tested but a return to baseline frequencies in all 3 patients. T cell activation was examined via the expression of CD69 and PD-1 expression on both CD4 and CD8 T cells (**FIG. 21E**). While there was an increase in the frequency of CD69+ T cells in Patient 3 pre-dose C1D8, expression returned to baseline levels by C1D15. There was large inter-patient variation in PD-1 expression on T cells at baseline between patients but major changes were not observed within patients during the treatment course.

[0327] Patients were also monitored for changes in serum cytokines during the treatment course. Patient 1 showed a transient elevation in levels of IL-6, IL-10, IL-1R $\alpha$  and MCP-1 at 6 hours after the first dose of CLN-978 (C1D1 6 hours), consistent with the clinical presentation of Grade 1 CRS (**FIG. 21F**). Cytokine levels returned to baseline for the remainder of timepoints assessed. Patients 2 and 3 both showed limited changes in cytokine expression relative to baseline, despite the clinical presentation of Grade 1 CRS in Patient 2 at 48 hrs. post-dose. Of note, there was no expression of IL-1 $\beta$ , IFN $\gamma$  or IL-2 at any timepoints tested (data not shown).

[0328] Taken together, these data demonstrate B cell depletion in two out of two B-NHL patients with detectable B cells at baseline, as well as T cell mobilization in the periphery of three patients following treatment with CLN-978.

[0329] The clinical observations for the three patients is further summarized in **FIG. 23**.

[0330] Patient 3 was further analyzed. As shown in **FIG. 24**, a 7.4 x 1.7 cm tumor in the left mandibular ramus and adjacent musculature of patient 3 was palpable on physical exam and visible in the oral cavity at pre-treatment baseline. The tumor was no longer appreciable on physical exam at 96 hours following the first dose of CLN-978. The mass reduced in size

by 66% (sum of the product of diameters (SPD)) and hypermetabolic disease improved (standard uptake value (SUV)) after 7 doses of CLN-978, consistent with partial response to therapy.

#### **INCORPORATION BY REFERENCE**

**[0331]** All publications and patents cited throughout the text of this specification (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety for all purposes. To the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material.

#### **EQUIVALENTS**

**[0332]** The disclosure may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the disclosure described herein. Scope of the disclosure is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

**WHAT IS CLAIMED IS:**

1. A method 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.

2. The method of claim 1, wherein the individual has relapsed following treatment with a CD19-targeting therapy, or is refractory to the CD19-targeting therapy.

3. The method of claim 2, wherein the CD19-targeting therapy comprises blinatumomab.

4. The method of claim 2, wherein the CD19-targeting therapy comprises a CD19 CAR-T cell therapy.

5. The method of any one of claims 1 to 4, wherein 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.

6. The method of any one of claims 1 to 5, wherein 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.

7. The method of any one of claims 1 to 6, wherein 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.
8. The method of any one of claims 1 to 7, wherein the first antigen-binding site comprises the amino acid sequence of SEQ ID NO: 11.
9. The method of any one of claims 1 to 8, wherein 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.
10. The method of any one of claims 1 to 9, wherein 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.
11. The method of any one of claims 1 to 10, wherein 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.
12. The method of any one of claims 1 to 11, wherein the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 22.
13. The method of any one of claims 1 to 12, wherein the multi-specific binding protein further comprises a half-life extension domain.
14. The method of claim 13, wherein the half-life extension domain comprises a third antigen-binding site that binds human serum albumin.
15. The method of claim 13 or claim 14, 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.

16. The method of claim 14 or claim 15, 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.

17. The method of claim 16, wherein 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.

18. The method of claim 16 or claim 17, wherein the VH of the third antigen-binding site comprises the amino acid sequence of SEQ ID NO: 24.

19. The method of any one of claims 14 to 18, 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%, 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.

20. The method of any one of claims 14 to 19, 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 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.

21. The method of any one of claims 1 to 20, wherein 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.
22. The method of any one of claims 1 to 21, wherein the multi-specific binding protein comprises the amino acid sequence of SEQ ID NO: 41.
23. The method of any one of claims 1 to 22, wherein said administration produces B cell depletion in said individual within 96 hours of administration of said multi-specific binding protein.
24. The method of any one of claims 1 to 23, wherein said administration produces a persistent B-cell depletion that is sustained to at least 90 days after administration of said multi-specific binding protein.
25. The method of any one of claims 1 to 24, wherein said administration produces a persistent B-cell depletion that is sustained to at least 90 days after last administration of said multi-specific binding protein.
26. The method of any one of claims 1 to 25, wherein said individual is administered a dose of at least 20µg said multi-specific binding protein.
27. The method of any one of claims 1 to 25, wherein said individual is administered a dose of at least 30µg said multi-specific binding protein.
28. The method of any one of claims 1 to 25, wherein said individual is administered a dose of at least 40µg said multi-specific binding protein.
29. The method of any one of claims 1 to 25, wherein said individual is administered a dose of at least 50µg said multi-specific binding protein.
30. The method of any one of claims 1 to 25, wherein said individual is administered a dose of at least 60µg said multi-specific binding protein.

31. The method of any one of claims 1 to 30, wherein said multi-specific binding protein is administered once per week.
32. The method of any one of claims 1 to 30, wherein said multi-specific binding protein is administered once every two weeks.
33. A method 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.
34. The method of claim 33, wherein the CD19 low expressing cancer is classified as low expressing by flow cytometry.
35. The method of claim 33 or claim 34, wherein the CD19 low expressing cancer is classified by having about 325 to about 17,000 CD19 molecules per cell.
36. The method of claim 33 or claim 34, wherein the CD19 low expressing cancer is classified by having less than about 3000 CD19 molecules per cell.
37. The method of any one of claims 33 to 36, wherein 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.

38. The method of any one of claims 33 to 37, wherein 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.

39. The method of any one of claims 33 to 38, wherein 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.

40. The method of any one of claims 33 to 39, wherein the first antigen-binding site comprises the amino acid sequence of SEQ ID NO: 11.

41. The method of any one of claims 33 to 40, wherein 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.

42. The method of any one of claims 33 to 41, wherein 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.

43. The method of any one of claims 33 to 42, wherein 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.

44. The method of any one of claims 33 to 43, wherein the second antigen-binding site comprises the amino acid sequence of SEQ ID NO: 22.

45. The method of any one of claims 33 to 44, wherein the multi-specific binding protein further comprises a half-life extension domain.

46. The method of claim 45, wherein the half-life extension domain comprises a third antigen-binding site that binds human serum albumin.
47. The method of any one of claims 33 to 46, 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.
48. The method of claim 46 or claim 47, 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.
49. The method of claim 48, wherein 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.
50. The method of claim 48 or claim 49, wherein the VH of the third antigen-binding site comprises the amino acid sequence of SEQ ID NO: 24.
51. The method of any one of claims 48 to 50, 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%, 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.
52. The method of any one of claims 48 to 51, 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 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.

53. The method of any one of claims 33 to 52, wherein 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.

54. The method of any one of claims 33 to 53, wherein the multi-specific binding protein comprises the amino acid sequence of SEQ ID NO: 41.

55. The method of any one of claims 33 to 54, wherein said administration produces B cell depletion in said individual within 96 hours of administration of said multi-specific binding protein.

56. The method of any one of claims 33 to 55, wherein said administration produces a persistent B-cell depletion that is sustained to at least 90 days after administration of said multi-specific binding protein.

57. The method of any one of claims 33 to 56, wherein said 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.

58. The method of any one of claims 33 to 57, wherein said individual is administered a dose of at least about 30µg said multi-specific binding protein.

59. The method of any one of claims 33 to 57, wherein said individual is administered a dose of at least about 40µg said multi-specific binding protein.

60. The method of any one of claims 33 to 57, wherein said individual is administered a dose of at least about 50µg said multi-specific binding protein.

61. The method of any one of claims 33 to 57, wherein said individual is administered a dose of at least 60µg said multi-specific binding protein.

62. The method of any one of claims 33 to 61, wherein said multi-specific binding protein is administered once per week.

63. The method of any one of claims 33 to 61, wherein said multi-specific binding protein is administered once every two weeks.