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(54) Title: TROP2 TARGETING TRISPECIFIC PROTEIN FOR TREATMENT OF CANCER

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Effect of Treatment on Tumor Volume

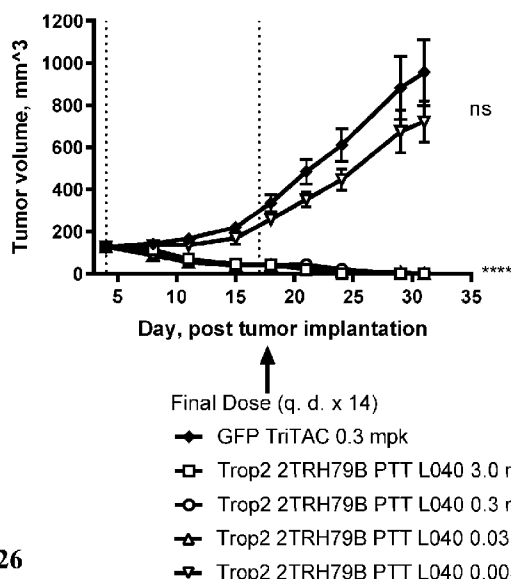


FIG. 26

(57) Abstract: Provided herein are TROP2 binding proteins, pharmaceutical compositions comprising such proteins or fragments thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such TROP2 binding proteins. Also disclosed are methods of using the disclosed TROP2 binding proteins in the prevention, and/or treatment diseases, conditions and disorders.

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TROP2 TARGETING TRISPECIFIC PROTEIN FOR TREATMENT OF CANCER**CROSS-REFERENCE**

[0001] This application claims the benefit of U.S. Provisional Application No. 63/478,640, filed January 5, 2023, and U.S. Provisional Application No. 63/496,159, filed on April 14, 2023, each of which is incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE

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

BACKGROUND OF THE DISCLOSURE

[0003] TROP2 is a protein that in humans is encoded by the TACSTD2 gene. This antigen is a member of a family including at least two type I membrane proteins. It transduces an intracellular calcium signal and acts as a cell surface receptor. Studies have shown that Aberrant TROP2 protein overexpression is associated with several carcinomas, such as colorectal, pancreatic, gastric, oral squamous cell carcinoma, ovarian, and breast cancers. Carcinomas with high TROP2 expression is associated with increased disease recurrence, drug resistance, and is a poor prognostic factor for survival.

[0004] There is a need for a greater choice of treatment options which allows physicians to select the therapeutic with the best side effect profile for an individual patient. The present disclosure provides novel polypeptides and protein therapeutics useful in methods of treatment, particularly for treatment of conditions associated with abnormal expression of TROP2.

SUMMARY OF THE DISCLOSURE

[0005] Provided herein is TROP2 binding domain comprising a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group

consisting of SEQ ID NOS: 172-228. In some embodiments, the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228. In some embodiments, an amino acid sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57. In some embodiments, the TROP2 binding domain is part of a multispecific protein. In some embodiments, the multispecific protein further comprises a CD3 binding domain. In some embodiments, the multispecific protein comprises an active drug format. In some embodiments, the multispecific protein further comprises a bulk serum protein binding domain. In some embodiments, the bulk serum protein comprises a serum albumin protein. In some embodiments, the serum albumin protein comprises a human serum albumin protein. In some embodiments, the bulk serum protein binding domain comprises a sequence that is at least 75% identical the sequence as set forth in SEQ ID NO: 493 or 549. In some embodiments, the CD3 binding domain comprises a sequence that is at least 75% identical the sequence as set forth in SEQ ID NO: 494. In some embodiments, the multispecific protein comprises a sequence that is at least about 75% identical to the sequence as set forth in SEQ ID NOS: 229-264. In some embodiments, the bulk serum protein binding domain is a binding moiety comprising a linker and a masking moiety, wherein the masking moiety is capable of masking or masks the binding of the TROP2 binding domain or the CD3 binding domain, to their respective targets. In some embodiments, the multispecific protein comprises a non-cleavable prodrug format. In some embodiments, the masking moiety comprises a sequence selected from the group consisting of SEQ ID NO: 550, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NO: 550. In some embodiments, the linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-543. In some embodiments, the bulk serum protein binding domain comprises a sequence that is at least 75% identical the sequence selected from the group consisting of SEQ ID NO: 493. In some embodiments, wherein the CD3 binding domain comprises a sequence that is at least 75% identical to the sequence as set forth in SEQ ID NO: 494. In some embodiments, wherein the multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the active drug comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

[0006] Provided herein is a method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering a TROP2 binding domain according to descriptions herein, or a pharmaceutical composition comprising the same, to the subject. In some embodiments, the subject is human.

[0007] Provided here in is a conditionally active TROP2 binding protein that comprises a single polypeptide chain, comprising (a) a binding moiety comprising a non-CDR loop and a cleavable linker; (b) the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228 wherein the binding moiety is capable of masking or masks the binding of the TROP2 binding domain to its target.

[0008] In some embodiments, the TROP2 binding domain comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57.

[0009] Provided herein is a conditionally active TROP2 binding protein comprising a binding moiety (M) which comprises a non-CDR loop, a cleavable linker (L), a first target antigen binding domain (T1), and a second target antigen binding domain (T2), wherein at least one of the first target antigen binding domain (T1) and the second target antigen binding domain (T2) comprises wherein at least one of the first target antigen binding domain (T1) and the second target antigen binding domain (T2) comprises a TROP2 binding domain, wherein the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228, wherein the non-CDR loop is capable of binding to the TROP2 binding domain or the second target antigen binding domain, and wherein the binding moiety is capable of masking or masks the binding of the

TROP2 binding domain or the second target antigen binding domain to its target. In some embodiments, the binding moiety comprises a masking moiety and wherein the masking moiety comprises a sequence selected from the group consisting of SEQ ID NOS: 550 and 558-560, or a sequence comprising one or more substitutions relative to a sequence selected from the group consisting of SEQ ID NOS: 550 and 558-560. In some embodiments, the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543, or a sequence comprising one or more substitutions relative to a sequence selected from the group consisting of SEQ ID NOS: 497-543. In some embodiments, the binding moiety comprises a sequence that is at least 75% identical the sequence selected from the group consisting of SEQ ID NO: 493. In some embodiments, the second target antigen binding domain (T2) comprises a CD3 binding domain. In some embodiments, the CD3 binding domain comprises a sequence that is at least 75% identical to the sequence as set forth in SEQ ID NO: 494.

[0010] Provided herein is a method for treating or ameliorating a proliferative disease or a tumorous disease, comprising administering a conditionally active chimeric antigen receptor according to descriptions herein, or a pharmaceutical composition comprising the same, to the subject. In some embodiments, the subject is human.

[0011] Provided herein is a method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering a conditionally active TROP2 binding protein according to descriptions herein, or a pharmaceutical composition comprising the same, to the subject. In some embodiments, the subject is human.

[0012] In some embodiments, the binding domain is a humanized antibody or an antigen binding fragment thereof. In some embodiments, the binding domain is a single domain antibody, a VHH domain, a scFv, a VH domain, a VL domain, a Fab, a Fab', a non-Ig domain, a ligand, a knottin, or a small molecule entity. In some embodiments, binding domain comprises the single domain antibody. In some embodiments, the binding domain binds to TROP2 with a binding affinity (K_D) of about 0.001 nM to about 500 nM. In some embodiments, the binding domain binds to human TROP2, mouse TROP2, cynomolgus TROP2, or a combination thereof.

[0013] Provided herein is a multispecific protein comprising a TROP2 binding domain, wherein the TROP2 binding domain is according to descriptions herein. In some embodiments, the TROP2 binding domain according to descriptions herein (anti-TROP2 domain), and a CD3 binding domain (anti-CD3 domain). In some embodiments, the anti-TROP2 domain and the anti-CD3 domain are in an anti-TROP2:anti-CD3 orientation. In some embodiments, the anti-TROP2 domain and the anti-CD3 domain are in an anti-CD3: anti-TROP2 orientation. In some embodiments, the TROP2 binding domain according to descriptions herein (anti TROP2 domain), the CD3 binding domain

(anti-CD3 domain), and an albumin binding domain (anti-ALB domain). In some embodiments, the anti-CD3 domain comprises an amino acid as set forth in SEQ ID NO: 494. In some embodiments, the anti-ALB domain comprises an amino acid sequence as set forth in SEQ ID NO: 493. In some embodiments, the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-CD3: anti-ALB: anti-TROP2 orientation. In some embodiments, the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-TROP2: anti-ALB: anti-CD3 orientation. In some embodiments, the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-ALB: anti-TROP2: anti-CD3 orientation. In some embodiments, the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-CD3: anti-TROP2: anti-ALB orientation. In some embodiments, the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-ALB: anti-CD3: anti-TROP2 orientation. In some embodiments, the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-TROP2: anti-CD3: anti-ALB orientation.

[0014] Provided here in is a multivalent protein comprising a sequence as set forth in any one of SEQ ID NOS: 229-264.

[0015] Provided herein is an active drug comprising a sequence as set forth in any one of SEQ ID NOS: 229-264.

[0016] Provided herein is an active drug comprising a sequence as set forth in any one of SEQ ID NOS: 1-57.

[0017] Provided herein is a pharmaceutical composition comprising (i)(a) a TROP2 binding domain described herein; (i)(b) a conditionally active TROP2 binding protein described herein; (i)(c) a multispecific protein described herein; (i)(d) a multivalent protein described herein; or (i)(e) an active drug described herein, and (ii) a pharmaceutically acceptable carrier.

[0018] Provided herein is a process for producing TROP2 binding domain described herein, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding the TROP2 binding domain under conditions allowing the expression of the TROP2 binding domain, and further recovering and purifying the produced protein from the culture.

[0019] Provided herein is a process for producing a multispecific protein according to descriptions herein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the multispecific TROP2 binding protein according to descriptions herein under conditions allowing the expression of the multispecific protein and recovering and purifying the produced protein from the culture.

[0020] Provided herein is a method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering a TROP2 binding domain according to descriptions herein, or a pharmaceutical composition according to descriptions herein, to the subject.

[0021] Provided herein is a method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering the multispecific protein according to descriptions herein, a multivalent protein according to descriptions herein, an active drug according to descriptions herein, or a pharmaceutical composition according to descriptions herein, to the subject. In some embodiments, the subject is human. In some embodiments, the method further comprises administration of an agent in combination with a TROP2 binding domain according to descriptions herein, a multispecific protein according to descriptions herein, a multivalent protein according to descriptions herein, an active drug according to descriptions herein, or a pharmaceutical composition according to descriptions herein. In some embodiments, the TROP2 binding domain selectively binds to tumor cells expressing TROP2. In some embodiments, the tumorous disease comprises a solid tumor disease. In some embodiments, the solid tumor disease is metastatic. In some embodiments, the tumorous disease comprises at least one of: an oral cancer, a colorectal cancer, a head and neck cancer, a prostate cancer, a liver cancer, a cervical cancer, a nasopharyngeal cancer, a thyroid cancer, a non-small cell lung cancer, a small cell lung cancer, a gastric cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a bladder cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma, a nasal NK/T cell lymphoma, a gliomas, a glioblastoma, an osteosarcoma, a pituitary adenoma, or any combination thereof. In some embodiments, the method further comprises administration of an agent in combination with conditionally active TROP2 binding protein according to any one of descriptions herein, or a pharmaceutical composition comprising the same. In some embodiments, the TROP2 binding domain selectively binds to tumor cells expressing TROP2. In some embodiments, the tumorous disease comprises a solid tumor disease. In some embodiments, the solid tumor disease is metastatic. In some embodiments, the tumorous disease is at least one of: a colorectal cancer, an oral cancer, a colorectal cancer, a head and neck cancer, a prostate cancer, a liver cancer, a cervical cancer, a nasopharyngeal cancer, a thyroid cancer, a non-small cell lung cancer, a small cell lung cancer, a gastric cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a bladder cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma, a nasal NK/T cell lymphoma, a gliomas, a glioblastoma, an osteosarcoma, a pituitary adenoma, or any combination thereof.

[0022] Provided herein is a process for producing a conditionally active TROP2 binding protein according to descriptions herein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the conditionally active TROP2 binding protein according to descriptions herein, under conditions allowing the expression of the conditionally active TROP2 binding protein and recovering and purifying the produced protein from the culture.

[0023] Provided herein is a process for producing a multivalent protein according to descriptions herein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the multivalent protein according to descriptions herein, under conditions allowing the expression of the multivalent protein and recovering and purifying the produced protein from the culture.

[0024] Provided herein is a process for producing an active drug according to descriptions herein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the active drug according to descriptions herein, under conditions allowing the expression of the active drug and recovering and purifying the produced drug from the culture.

[0025] Provided herein is a conditionally active TROP2 binding protein, wherein the conditionally active TROP2 binding protein has a greater therapeutic index compared to a TROP2 binding protein that does not comprise the (a) binding moiety but is otherwise identical to the conditionally active TROP2 binding protein. In some embodiments, the conditionally active TROP2 binding protein has a therapeutic index that is at least about 5-fold to about 100-fold greater than that of a TROP2 binding protein that does not comprise the (a) binding moiety but is otherwise identical to the conditionally active TROP2 binding protein. In some embodiments, the protein comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, wherein the protein comprises a sequence that is at least about 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

[0026] Provided herein is a pharmaceutical composition comprising: (i) a conditionally active TROP2 binding protein according to descriptions herein, and (ii) a pharmaceutically acceptable carrier.

[0027] Provided herein is a method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering the conditionally active chimeric antigen receptor according to descriptions herein, or the pharmaceutical composition according to descriptions herein, to the subject.

[0028] Provided herein is a method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering the conditionally active TROP2 binding protein according to descriptions herein, or the pharmaceutical composition according to descriptions herein, to the subject. In some embodiments, the subject is human. In some embodiments, the tumorous disease comprises at least one of: an oral cancer, a colorectal cancer, a head and neck cancer, a prostate cancer, a liver cancer, a cervical cancer, a nasopharyngeal cancer, a thyroid cancer, a non-small cell lung cancer, a small cell lung cancer, a gastric cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a bladder cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma, a nasal NK/T cell lymphoma, a gliomas, a glioblastoma, an osteosarcoma, a pituitary adenoma, or any combination thereof.

[0029] Provided herein is a method of increasing a therapeutic index of TROP2 binding domain, the method comprising conjugating the TROP2 binding domain to a binding moiety comprising a cleavable linker and a non-CDR loop, wherein the non-CDR loop comprises a binding site specific for the TROP2 binding domain, wherein the TROP2 binding domain is masked from binding its target by the binding moiety, wherein the TROP2 binding domain is able to bind its target upon cleavage of the cleavable linker. In some embodiments, the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228. In some embodiments, the TROP2 binding domain conjugated to the binding moiety is part of a conditionally active multispecific protein, wherein the multispecific protein further comprises a CD3 binding domain. In some embodiments, the binding moiety comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NO: 493. In some embodiments, the CD3 binding domain comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NO: 494. In some embodiments, the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-543. In some embodiments, the TROP2 binding domain comprises a sequence that is at

least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228. In some embodiments, the binding moiety comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 493. In some embodiments, the TROP2 binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57. In some embodiments, the TROP2 binding domain comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57.

[0030] Provided herein is a method of increasing a therapeutic index of TROP2 binding protein comprising a first target antigen binding domain and a second target antigen binding domain, wherein at least one of the first and the second target antigen binding domain comprises a TROP2 binding domain, the method comprising conjugating the first or the second target antigen binding domain to a binding moiety comprising a cleavable linker and a non-CDR loop, wherein the non-CDR loop comprises a binding site specific for the first or the second target antigen binding domain, wherein at least one of the first or the second target antigen binding domain is masked from binding its target by the binding moiety, and wherein the first or the second target antigen binding domain that is masked, is able to bind its target upon cleavage of the cleavable linker. In some embodiments, the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a

sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228. In some embodiments, the non-CDR loop comprises a binding site specific for the TROP2 binding domain. In some embodiments, at least one of the first or the second target antigen binding domain comprises a CD3 binding domain. In some embodiments, the non-CDR loop comprises a binding site specific for the CD3 binding domain. In some embodiments, the CD3 binding domain comprises a sequence that is at least about 75% identical to SEQ ID NO: 494. In some embodiments, the binding moiety comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 493. In some embodiments, the TROP2 binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57. In some embodiments, the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-543. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

[0031] Provided herein is a method of increasing a therapeutic index of a TROP2 binding protein comprising a TROP2 binding domain and a CD3 binding domain, the method comprising conjugating CD3 binding domain to a binding moiety comprising a cleavable linker and a non-CDR loop, wherein the non-CDR loop comprises a binding site specific for CD3 binding domain. In some embodiments, the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group

consisting of SEQ ID NOS: 172-228. In some embodiments, the CD3 binding domain comprises a sequence that is at least about 75% identical to SEQ ID NO: 494. In some embodiments, the binding moiety comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 493. In some embodiments, the TROP2 binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57. In some embodiments, the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-543. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

[0032] Provided herein is a TROP2 targeting conditionally active multispecific protein, comprising: a TROP2 binding domain, a CD3 binding domain, an albumin binding domain, wherein the albumin binding domain comprises a non-CDR loops that comprises a binding site specific for the CD3 binding domain and a cleavable linker, wherein the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228. In some embodiments, the albumin binding domain comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 494. In some embodiments, the TROP2 binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57. In some embodiments, the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 496-543, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-543. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group

consisting of SEQ ID NOS: 229-264. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264. In some embodiments, the conditionally active multispecific protein comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

[0033] Provided herein is a pharmaceutical composition comprising a TROP2 targeting conditionally active multispecific protein of descriptions herein. In some embodiments, further comprising a pharmaceutically acceptable carrier.

[0034] Provided herein is a process for producing TROP2 targeting conditionally active multispecific protein of descriptions herein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the TROP2 targeting conditionally active multispecific protein of descriptions herein, under conditions allowing the expression of the TROP2 targeting conditionally active multispecific protein and recovering and purifying the produced protein from the culture.

[0035] Provided herein is a method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering a TROP2 targeting conditionally active multispecific protein of descriptions herein, or a pharmaceutical composition according to descriptions herein, to the subject. In some embodiments, the tumorous disease comprises a solid tumor disease. In some embodiments, the solid tumor disease is metastatic. In some embodiments, the tumorous disease comprises at least one of: an oral cancer, a colorectal cancer, a head and neck cancer, a prostate cancer, a liver cancer, a cervical cancer, a nasopharyngeal cancer, a thyroid cancer, a non-small cell lung cancer, a small cell lung cancer, a gastric cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a bladder cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma, a nasal NK/T cell lymphoma, a gliomas, a glioblastoma, an osteosarcoma, a pituitary adenoma, or any combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0037] **FIG. 1** provides results from a TDCC assay with H292 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 3TRL3, 2TRL92, 3TRL77, 2TRL4, and 2TRL76.

[0038] **FIG. 2** provides results from a TDCC assay with H292 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 3TRL3, 2TRL92, 3TRL77, 2TRL4, and 2TRL76.

[0039] **FIG. 3** provides results from a TDCC assay with H292 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 3TRL56, 3TRL87, 2TRL33, 2TRL1, and 2TRL5.

[0040] **FIG. 4** provides results from a TDCC assay with H292 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL8, 2TRL27, 2TRL46, 2TRL69, and 2TRL94.

[0041] **FIG. 5** provides results from a TDCC assay with H292 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL79, 2TRL81, 2TRL18, 3TRL27, and GFP negative control.

[0042] **FIG. 6** provides results from a TDCC assay with H292 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL28, 3TRL39, 3TRL82, 2TRL68, 2TRL64, and 3TRL53.

[0043] **FIG. 7** provides results from a TDCC assay with H1376 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 3TRL3, 2TRL92, 3TRL77, 2TRL4, and 2TRL76.

[0044] **FIG. 8** provides results from a TDCC assay with H1376 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 3TRL16, 2TRL29, 3TRL58, 2TRL31, and 2TRL70.

[0045] **FIG. 9** provides results from a TDCC assay with H1376 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 3TRL56, 3TRL87, 2TRL33, 2TRL1, and 2TRL5.

[0046] **FIG. 10** provides results from a TDCC assay with H1376 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL8, 2TRL27, 2TRL46, 2TRL69, and 2TRL94.

[0047] **FIG. 11** provides results from a TDCC assay with H1376 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL79, 2TRL81, 2TRL18, 3TRL27, and GFP negative control.

[0048] **FIG. 12** provides results from a TDCC assay with H1376 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL28, 3TRL39, 3TRL82, 2TRL68, 2TRL64, and 3TRL53.

[0049] **FIG. 13** provides results from a TDCC assay with H292 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 3TRL53, 3TRH53, 2TRL76, and 2TRH76.

[0050] **FIG. 14** provides results from a TDCC assay with H292 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL79, 2TRH79, 2TRL81, and 2TRH81.

[0051] **FIG. 15** provides results from a TDCC assay with HT1376 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 3TRL53, 3TRH53, 2TRL76, and 2TRH76.

[0052] **FIG. 16** shows results from a TDCC assay with HT1376 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL79, 2TRH79, 2TRL81, and 2TRH81.

[0053] **FIG. 17** shows results from a TDCC assay with HCC70 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 3TRL53, 3TRH53, 2TRL76, and 2TRH76.

[0054] **FIG. 18** shows results from a TDCC assay with HCC70 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL79, 2TRH79, 2TRL81, and 2TRH81.

[0055] **FIG. 19** shows results from a TDCC assay with HCC70 cells and anti-CD3/anti-TROP2 fusion proteins containing llama anti-TROP2 sequences 2TRL79, 2TRH79, 2TRH79B, and 2TRH79B L040 ProTriTAC.

[0056] **FIG. 20** shows results from a TDCC assay with HPAF-II Cells and Anti-CD3/Anti-TROP2 Fusion Proteins Containing Llama and Humanized Anti-TROP2 Sequences 2TRL79, 2TRH79, 2TRH79B, and 2TRH79B L040 ProTriTAC.

[0057] **FIG. 21** shows results from a TDCC assay with CAL27 Cells and Anti-CD3/Anti-TROP2 Fusion Proteins Containing Humanized Anti-TROP2 Sequences 2TRH79B and 2TRH79B L040 ProTriTAC.

[0058] **FIG. 22** shows results of a admix therapeutic xenograft rodent study with HCC70 cells and anti-CD3/anti-TROP2 fusion protein containing llama anti-TROP2 sequence 2TRL79 ProTriTAC.

[0059] **FIG. 23** shows results of a admix therapeutic xenograft rodent study with HCC70 Cells and anti-CD3/anti-TROP2 fusion protein containing llama anti-TROP2 sequence 2TRL79 ProTriTAC.

[0060] **FIG. 24** shows results of a admix therapeutic xenograft rodent study with HCC70 Cells and anti-CD3/anti-TROP2 fusion protein containing humanized anti-TROP2 sequence 2TRH79 ProTriTAC.

[0061] **FIG. 25** shows results of a admix therapeutic xenograft rodent study with CAL27 Cells and Anti-CD3/anti-TROP2 fusion protein containing humanized anti-TROP2 sequence 2TRH79B ProTriTAC.

[0062] **FIG. 26** shows results of a admix therapeutic xenograft rodent study with HPAF-II Cells and Anti-CD3/anti-TROP2 fusion protein containing humanized anti-TROP2 sequence 2TRH79B ProTriTAC.

[0063] **FIG. 27** shows molar equivalents of prodrug and active drug showed comparably potent anti-tumor activity in HCC70 cells.

[0064] **FIGS. 28A-28B** shows anti-CD3/anti-TROP2 fusion protein containing humanized 2TRH79B binds with comparable affinity to human (FIG.28A) and cynomolgus monkey (cyno) Trop2 (FIG.28B) in the absence or presence of calcium (47-51 nM or 50 nM, respectively).

[0065] **FIG. 29** shows the experimental design of the one-month dose escalation/maximum tolerated dose study.

[0066] **FIG. 30** shows that the anti-CD3/anti-TROP2 fusion protein containing humanized 2TRH79B shows favorable pharmacokinetics, half-life and systemic accumulation.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0067] Described herein are trispecific proteins that target TROP2, pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such proteins thereof. Also provided are methods of using the disclosed TROP2 targeting trispecific proteins in the prevention, and/or treatment of diseases, conditions and disorders. The TROP2 targeting trispecific proteins are capable of specifically binding to TROP2 as well as CD3 and have a half-life extension domain, such as a domain binding to human albumin (ALB).

TROP2 binding proteins

[0068] Described herein are proteins that bind TROP2, pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such proteins thereof. Also provided are methods of using the disclosed TROP2 binding proteins in the prevention, and/or treatment of diseases, conditions and disorders. In some embodiments, the TROP2 binding proteins are part of multispecific (*e.g.*, trispecific) proteins that comprise a TROP2 binding domain as described herein.

[0069] Trophoblast cell surface antigen 2 (Trop2) or Tumor-associated calcium signal transducer 2 (TACSTD2) or epithelial glycoprotein 1 (EGP-1), or pancreatic carcinoma marker protein GA733-1, or GP50, or membrane component 1 surface marker 1 (M1S1) is a widely expressed 35kDa type I transmembrane glycoprotein with four N-linked glycosylation sites, consisting of 323

amino acids encoded by the TACSTD2 gene. It is a Tumor-associated calcium signal transducer (TACSTD) family member and is structurally related to epithelial cell adhesion molecule (EpCAM). Trop-2 comprises a large extracellular domain, a single transmembrane domain, and an intracellular tail. The crystal structure of TROP2 ectodomain revealed a compact subunit composed of three domains: N-terminal (ND), thyroglobulin type-1 (TY), and C-terminal domain (CD). TROP2 extracellular domains can form dimers.

[0070] TROP2 shares similar claudin-interaction capacity with its paralogue EpCAM, and both are implicated in signaling triggered by proteolytic cleavage within the ectodomain. Trop-2 plays an essential role in embryonic development, placental tissue formation, embryo implantation, stem cell proliferation, and organ development. TROP2 is a stem/progenitor cell marker and a low basal expression level of TROP2 is found on the surface of multiple normal epithelial tissues, including skin and oral mucosa. TROP2 overexpression is observed in many types of malignant epithelial tumors such as gastric cancer, thyroid cancer, papillary thyroid cancer, colorectal cancer, lung cancer, e.g., non-small cell lung cancer, e.g., lung adenocarcinoma, breast cancer, e.g., ductal breast cancer, pancreatic cancer, ovarian cancer, prostate cancer, bladder cancer, gallbladder cancer, cervical cancer, uterine serous papillary cancer, uterine and ovarian carcinosarcomas, endometrial cancer, nasopharyngeal cancer, hilar cholangiocarcinoma, oral squamous cell cancer, esophageal squamous cell cancer, head and neck squamous cell cancer, laryngeal squamous cell cancer, liver fluke associated cholangiocarcinoma, lung adenocarcinoma, hepatocellular carcinoma, squamous cell carcinoma of cervix, squamous cell carcinoma of head and neck, squamous cell carcinoma of esophagus. High expression of Trop2 was also found in tumors of non-epithelial origin, such as melanomas, nasal NK/T cell lymphoma, gliomas and glioblastomas, and osteosarcomas. TROP2 overexpression is also linked to pituitary adenomas. Although Trop2 is frequently overexpressed during tumorigenesis, genetic analyses revealed that point mutations and copy number variations in TACSTD2 gene are rather rare in human tumors. TROP2 overexpression is linked to a poor prognosis, for survival and drug resistance.

[0071] Exemplary protein sequences for TROP2 are provided in UniProtKB ID NOS: P09758 and RefSeq NP_002344 (SEQ ID NO:546). In some embodiments, the TROP2 binding proteins of this disclosure binds to a TROP2 protein comprising an amino acid sequence as provided in UniProtKB ID NOS: P09758 or RefSeq NP_002344. In some embodiments, the TROP2 binding proteins of this disclosure binds to a TROP2 protein comprising an amino acid sequence as provided in UniProtKB ID NOS: Q8BGV3 or RefSeq NP_064431. In some embodiments, the TROP2 binding proteins of this disclosure binds to a TROP2 protein comprising an amino acid sequence as provided in Refseq XP_005543292.2. In some embodiments, the TROP2 binding proteins of this

disclosure binds to a TROP2 protein encoded by a nucleic acid as provided in RefSeq NM_002353. In some embodiments, the TROP2 binding proteins of this disclosure binds to a TROP2 protein encoded by a nucleic acid as provided in RefSeq NM_020047. In some embodiments, the TROP2 binding proteins of this disclosure binds to a TROP2 protein comprising an amino acid sequence as set forth in SEQ ID NO:546 or 547.

[0072] MARGPGLAPPPLRLPLLLLVLAAVTGHTAAQDNCTCPTNKMTVCSPDGPGGRCQCRALGSGMAVDCSTLTSKCLLLKARMSAPKNARTLVRPSEHALVDNDGLYDPDCDPEGRFKARQCNQTSVCWCVN SVGVRRTDKGDLSLRCELV RTHHILIDL RHRPTAGAFNHSDLD AELRRLFRERYRLHPKFVAAVHYEQPTIQIELRQNTSQKAAGDVDIGDAAYYFERDIKGESLFQGRGGLDLRVRGEPLQVERTLIYYLDEIPPKFMSKRLTAGLIAVIVVVVVALVAGMAVLVITNRRKSGKYKKVEIKELGELRKEPSL (SEQ ID NO: 546).

[0073] HTAAQDNCTCPTNKMTVCSPDGPGGRCQCRALGSGMAVDCSTLTSKCLLLKARMSAPKNARTLVRPSEHALVDNDGLYDPDCDPEGRFKARQCNQTSVCWCVN SVGVRRTDKGDLSLRCELV RTHHILIDL RHRPTAGAFNHSDLDAELRRLFRERYRLHPKFVAAVHYEQPTIQIELRQNTSQKAAGDVDIGDAAYYFERDIKGESLFQGRGGLDLRVRGEPLQVERTLIYYLDEIPPKFMSKRLTAGLIAVIVVVVVALVAGMAVLVITNRRKSGKYKKVEIKELGELRKEPSL (SEQ ID NO: 547).

[0074] In some embodiments, the TROP2 binding domain binds to an extracellular domain of the mature TROP2 protein. In some embodiments, the TROP2 binding domain binds to a transmembrane domain of the mature TROP2 protein. In some embodiments, the TROP2 binding domain binds to an intracellular tail of the mature TROP2 protein.

[0075] In some embodiments, the TROP2 binding domain binds to a protein comprising a truncated sequence compared to SEQ ID NO: 546 or 547. In some embodiments, the TROP2 binding domain binds to a protein comprising the sequence of SEQ ID NO: 546 or 547. In some embodiments, the TROP2 binding domains disclosed herein recognize full-length TROP2. In certain instances, the TROP2 binding domains disclosed herein recognize an epitope within TROP2, such as, in some cases the TROP2 binding proteins interact with one or more amino acids found within a domain of human TROP2. The epitope to which the antibodies bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within a domain of TROP2. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within a domain of TROP2.

[0076] In some embodiments, the TROP2 binding domains disclosed herein recognize full-length TROP2. In certain instances, the TROP2 binding domains disclosed herein recognize an epitope

within TROP2, such as, in some cases the TROP2 binding proteins interact with one or more amino acids found within a domain of human TROP2. The epitope to which the antibodies bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within a domain of TROP2. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within a domain of TROP2.

[0077] In some embodiments, the TROP2 binding proteins of this disclosure binds to the full length TROP2 protein or to a fragment thereof, such as epitope containing fragments within the full length TROP2 protein, as described above. In some cases, the epitope containing fragment comprises antigenic or immunogenic fragments and derivatives thereof of the TROP2 protein. Epitope containing fragments, including antigenic or immunogenic fragments, are, in some embodiments, 12 amino acids or more, *e.g.*, 20 amino acids or more, 50 or 100 amino acids or more. The TROP2 fragments, in some embodiments, comprises 95% or more of the length of the full protein, 90% or more, 75% or 50% or 25% or 10% or more of the length of the full protein. In some embodiments, the epitope-containing fragments of TROP2 including antigenic or immunogenic fragments are capable of eliciting a relevant immune response in a patient. Derivatives of TROP2 include, in some embodiments, variants on the sequence in which one or more (*e.g.*, 1-20 such as 15 amino acids, or up to 20% such as up to 10% or 5% or 1% by number of amino acids based on the total length of the protein) deletions, insertions or substitutions have been made to the TROP2 sequence provided in SEQ ID NO: 546 or 547.

[0078] In some embodiments, substitutions comprise conservative substitutions. Derivatives and variants of, in some examples, have essentially the same biological function as the protein from which they are derived. For instance, derivatives and variants of TROP2 are, in some cases, comparably antigenic or immunogenic to the protein from which they are derived, have either the ligand-binding activity, or the active receptor-complex forming ability, or preferably both, of the protein from which they are derived, and have the same tissue distribution as TROP2.

[0079] In some embodiments, the TROP2 binding protein specifically binds TROP2 with equivalent or better affinity as that of a reference TROP2 binding protein, and the TROP2 binding protein in such embodiments comprises an affinity matured TROP2 binding molecule, and is derived from the TROP2 binding parental molecule, comprising one or more amino acid mutations (*e.g.*, a stabilizing mutation, a destabilizing mutation) with respect to the TROP2 binding parental molecule. In some embodiments, the affinity matured TROP2 binding molecule has superior stability with respect to selected destabilizing agents, as that of a reference TROP2 binding parental molecule. In some embodiments, the affinity matured TROP2 binding molecule is identified in a

process comprising panning of one or more pre-candidate TROP2 binding molecules derived from one or more TROP2 binding parental molecule, expressed in a phage display library, against a TROP2 protein, such as a human TROP2 protein. The pre-candidate TROP2 binding molecule comprises, in some embodiments, amino acid substitutions in the variable regions, CDRs, or framework residues, relative to a parental molecule.

[0080] As used herein, “Phage display” refers to a technique by which variant polypeptides are displayed as fusion proteins to at least a portion of a coat protein on the surface of phage, *e.g.*, filamentous phage, particles. A utility of phage display lies in the fact that large libraries of randomized protein variants can be rapidly and efficiently selected for those sequences that bind to a target molecule with high affinity. Display of peptide and protein libraries on phage has been used for screening millions of polypeptides for ones with specific binding properties. Polyvalent phage display methods have been used for displaying small random peptides and small proteins **through fusions to either gene III or gene VIII of filamentous phage**. *See e.g.*, Wells and Lowman, *Curr. Opin. Struct. Biol.*, 3:355-362 (1992), and references cited therein. In monovalent phage display, a protein or peptide library is fused to a gene III, or a portion thereof, and expressed at low levels in the presence of wild type gene III protein so that phage particles display one copy or none of the fusion proteins. Avidity effects are reduced relative to polyvalent phage so that selection is on the basis of intrinsic ligand affinity, and phagemid vectors are used, which simplify DNA manipulations. *See e.g.*, Lowman and Wells, *Methods: A companion to Methods in Enzymology*, 3:205-0216 (1991).

[0081] In some embodiments, the panning comprises using varying binding times and concentrations to identify TROP2 binding molecules with increased or decreased on-rates, from pre-candidate TROP2 binding molecules. In some embodiments, the panning comprises using varying wash times to identify TROP2 binding molecules with increased or decreased off-rates, from pre-candidate TROP2 molecules. In some embodiments, the panning comprises using both varying binding times and varying wash times. In some embodiments, one or more stabilizing mutations are combined to increase the stability of the affinity matured TROP2 binding molecule, for example, by shuffling to create a second-stage combinatorial library from such mutants and conducting a second round of panning followed by a binding selection.

[0082] In some embodiments, the affinity matured TROP2 binding molecule comprises an equivalent or better affinity to a TROP2 protein (such as human TROP2 protein) as that of a binding parental molecule, but that has reduced cross reactivity, or in some embodiments, increased cross reactivity, with selected substances, such as ligands, proteins, antigens, or the like, other than the TROP2 epitope for which the TROP2 binding parental molecule is specific, or is designed to be

specific for. Regarding the latter, an affinity matured TROP2 binding molecule, in some embodiments, is more successfully tested in animal models if the affinity matured v binding molecule is reacted with both human TROP2 and the corresponding target of the animal model, *e.g.*, mouse TROP2 or cynomolgus monkey (cyno) TROP2. In some embodiments, the parental TROP2 binding molecule binds to human TROP2 with an affinity of about 500 nM or less, 400 nM or less, 300 nM or less, 200 nM or less, 100 nM or less, 50 nM or less, 10 nM or less, and to cynomolgus TROP2 with an affinity of about 500 nM or less, 400 nM or less, 300 nM or less, 200 nM or less, 100 nM or less, 50 nM or less, 15 nM or less, or 10 nM or less. In some embodiments, the affinity matured TROP2 binding molecule, identified after one round of panning, binds to human TROP2 with an affinity of about 5 nM or less, such as 1 nM or less, and to cynomolgus TROP2 with an affinity of about 7.5 nM or less, such as 1 nM or less. In some embodiments, the affinity matured TROP2 binding molecule, identified after two rounds of panning, binds to human TROP2 with an affinity of about 2.5 nM or less, and to cynomolgus TROP2 with an affinity of about 3.5 nM or less.

[0083] In some embodiments, the TROP2 binding protein comprises an antigen-specific binding domain polypeptide that specifically bind to targets, such as targets on diseased cells, or targets on other cells that support the diseased state, such as targets on stromal cells that support tumor growth or targets on immune cells that support disease-mediated immunosuppression. In some examples, the antigen-specific binding domain includes antibodies, single chain antibodies, Fabs, Fv, T-cell receptor binding domains, ligand binding domains, receptor binding domains, domain antibodies, single domain antibodies, minibodies, nanobodies, peptibodies, or various other antibody mimics (such as AFFIMERS®, affitins, alphabodies, atrimers, CTLA4-based molecules, adnectins, anticalins, Kunitz domain-based proteins, avimers, knottins, fynomers, DARPINS®, affibodies, affilins, monobodies and armadillo repeat protein-based proteins).

[0084] In some embodiments, the TROP2 binding domain is an anti-TROP2 antibody or an antigen binding fragment thereof, or an antibody variant of the TROP2 binding domain or an antigen binding fragment thereof. As used herein, the term “antibody variant” refers to variants and derivatives of an antibody or an antigen binding fragment as described herein. In certain embodiments, amino acid sequence variants of the anti-TROP2 antibodies or antigen binding fragments thereof, as described herein, are contemplated. For example, in certain embodiments amino acid sequence variants of anti-TROP2 antibodies or antigen binding fragments thereof, as described herein, are contemplated to improve the binding affinity and/or other biological properties of the same. Exemplary method for preparing amino acid variants include, but are not limited to, introducing appropriate modifications into the nucleotide sequence encoding the

antibody or antigen binding fragment thereof, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody or antigen binding fragments thereof.

[0085] Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding. In certain embodiments, variants having one or more amino acid substitutions are provided. Sites of interest for substitution mutagenesis include the CDRs and framework regions. Examples of such substitutions are described below. Amino acid substitutions may be introduced into an antibody or antigen binding fragments thereof of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, altered Antibody dependent cellular cytotoxicity (ADCC), or improved T-cell mediated cytotoxicity (TDCC). Both conservative and non-conservative amino acid substitutions are contemplated for preparing the antibody variants.

[0086] In another example of a substitution to create a variant anti-TROP2 antibody or antigen binding fragments thereof, one or more hypervariable region residues of a parent antibody are substituted. In general, variants are then selected based on improvements in desired properties compared to a parent antibody or antigen binding fragments thereof, for example, increased affinity, reduced affinity, reduced immunogenicity, increased pH dependence of binding.

[0087] In some embodiments, the TROP2 binding domain is a single domain antibody (sdAb) such as a heavy chain variable domain (VH), a variable domain (VHH) of a llama derived sdAb, a peptide, a ligand or a small molecule entity specific for TROP2. In some embodiments, the TROP2 binding domain described herein is any domain that binds to TROP2 including but not limited to domains from a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody. In certain embodiments, the TROP2 binding domain is a single-domain antibody. In other embodiments, the TROP2 binding domain is a peptide. In further embodiments, the TROP2 binding domain is a small molecule.

[0088] Generally, it should be noted that the term single domain antibody as used herein in its broadest sense is not limited to a specific biological source or to a specific method of preparation. Single domain antibodies are antibodies whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional 4-chain antibodies, engineered antibodies and single domain scaffolds other than those derived from antibodies. Single domain antibodies may be any of the art, or any future single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse,

human, camel, llama, goat, rabbit, bovine. For example, in some embodiments, the single domain antibodies of the disclosure are obtained: (1) by isolating the VHH domain of a naturally occurring heavy chain antibody; (2) by expression of a nucleotide sequence encoding a naturally occurring VHH domain; (3) by “humanization” of a naturally occurring VHH domain or by expression of a nucleic acid encoding a such humanized VHH domain; (4) by “camelization” of a naturally occurring VH domain from any animal species, and in particular from a species of mammal, such as from a human being, or by expression of a nucleic acid encoding such a camelized VH domain; (5) by “camelization” of a “domain antibody” or “Dab,” or by expression of a nucleic acid encoding such a camelized VH domain; (6) by using synthetic or semi-synthetic techniques for preparing proteins, polypeptides or other amino acid sequences; (7) by preparing a nucleic acid encoding a single domain antibody using techniques for nucleic acid synthesis known in the field, followed by expression of the nucleic acid thus obtained; and/or (8) by any combination of one or more of the foregoing.

[0089] In one embodiment, a single domain antibody corresponds to the VHH domains of naturally occurring heavy chain antibodies directed against TROP2. As further described herein, such VHH sequences can generally be generated or obtained by suitably immunizing a species of Llama with TROP2, (*i.e.*, so as to raise an immune response and/or heavy chain antibodies directed against TROP2), by obtaining a suitable biological sample from said Llama (such as a blood sample, serum sample or sample of B-cells), and by generating VHH sequences directed against TROP2, starting from said sample, using any suitable technique known in the field.

[0090] In another embodiment, such naturally occurring VHH domains against TROP2, are obtained from naïve libraries of Camelid VHH sequences, for example by screening such a library using TROP2, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known in the field. Such libraries and techniques are for example described in WO 99/37681, WO 01/90190, WO 03/025020 and WO 03/035694. Alternatively, improved synthetic or semi-synthetic libraries derived from naïve VHH libraries are used, such as VHH libraries obtained from naïve VHH libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example described in WO 00/43507.

[0091] In a further embodiment, yet another technique for obtaining VHH sequences directed against TROP2, involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain antibodies (*i.e.*, so as to raise an immune response and/or heavy chain antibodies directed against TROP2), obtaining a suitable biological sample from said transgenic mammal (such as a blood sample, serum sample or sample of B-cells), and then generating VHH sequences directed against TROP2, starting from said sample, using any suitable technique known in the field.

For example, for this purpose, the heavy chain antibody-expressing rats or mice and the further methods and techniques described in WO 02/085945 and in WO 04/049794 can be used.

[0092] In some embodiments, an anti-TROP2 single domain antibody of this disclosure comprises a single domain antibody with an amino acid sequence that corresponds to the amino acid sequence of a non-human antibody and/or a naturally occurring VHH domain, *e.g.*, a llama anti-TROP2 antibody, but that has been “humanized,” *i.e.*, by replacing one or more amino acid residues in the amino acid sequence of said non-human anti-TROP2 and/or the naturally occurring VHH sequence (and in particular in the framework sequences) by one or more of the amino acid residues that occur at the corresponding position(s) in a VH domain from a conventional 4-chain antibody from a human being (*e.g.*, as indicated above). This can be performed in a manner known in the field, which will be clear to the skilled person, for example on the basis of the further description herein. Again, it should be noted that such humanized anti-TROP2 single domain antibodies of the disclosure are obtained in any suitable manner known *per se* (*i.e.*, as indicated under points (1)-(8) above) and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VHH domain as a starting material. In some additional embodiments, a single domain anti-TROP2 antibody, as described herein, comprises a single domain antibody with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring VH domain, but that has been “camelized” *i.e.*, by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring VH domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a VHH domain of a heavy chain antibody. Such “camelizing” substitutions are preferably inserted at amino acid positions that form and/or are present at the VH-VL interface, and/or at the so-called Camelidae hallmark residues. *See, e.g.*, WO 94/04678 and Davies and Riechmann (1994 and 1996)). Preferably, the VH sequence that is used as a starting material or starting point for generating or designing the camelized single domain is preferably a VH sequence from a mammal, more preferably the VH sequence of a human being, such as a VH3 sequence. However, it should be noted that such camelized anti-TROP2 single domain antibodies of the disclosure, in certain embodiments, are obtained in any suitable manner known in the field (*i.e.*, as indicated under points (1)-(8) above) and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a non-human anti-TROP2 antibody and/or the naturally occurring VH domain as a starting material. For example, as further described herein, both “humanization” and “camelization” is performed by providing a nucleotide sequence that encodes a naturally occurring VHH domain or VH domain, respectively, and then changing, one or more codons in said nucleotide sequence in such a way that the new nucleotide sequence encodes a

“humanized” or “camelized” single domain antibody, respectively. This nucleic acid can then be expressed, so as to provide a desired anti-TROP2 single domain antibody of the disclosure.

Alternatively, in other embodiments, based on the amino acid sequence of a naturally occurring VHH domain or VH domain, respectively, the amino acid sequence of the desired humanized or camelized anti-TROP2 single domain antibody of the disclosure, respectively, are designed and then synthesized *de novo* using known techniques for peptide synthesis. In some embodiments, based on the amino acid sequence or nucleotide sequence of a naturally occurring VHH domain or VH domain, respectively, a nucleotide sequence encoding the desired humanized or camelized anti-TROP2 single domain antibody of the disclosure, respectively, is designed and then synthesized *de novo* using known techniques for nucleic acid synthesis, after which the nucleic acid thus obtained is expressed in using known expression techniques, so as to provide the desired anti-TROP2 single domain antibody of the disclosure.

[0093] Other suitable methods and techniques for obtaining the anti-TROP2 single domain antibody of the disclosure and/or nucleic acids encoding the same, starting from naturally occurring VH sequences or VHH sequences for example comprises combining one or more parts of one or more naturally occurring VH sequences (such as one or more framework (FR) sequences and/or complementarity determining region (CDR) sequences), one or more parts of one or more naturally occurring VHH sequences (such as one or more FR sequences or CDR sequences), and/or one or more synthetic or semi-synthetic sequences, in a suitable manner, so as to provide an anti-TROP2 single domain antibody of the disclosure or a nucleotide sequence or nucleic acid encoding the same.

[0094] In some embodiments, the TROP2 binding domain is an anti TROP2 specific antibody comprising a heavy chain variable complementarity determining region CDR1, a heavy chain variable CDR2, a heavy chain variable CDR3, a light chain variable CDR1, a light chain variable CDR2, and a light chain variable CDR3. In some embodiments, the TROP2 binding domain comprises any domain that binds to TROP2 including but not limited to domains from a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, or antigen binding fragments such as single domain antibodies (sdAb), Fab, Fab', F(ab)2, and Fv fragments, fragments comprised of one or more CDRs, single-chain antibodies (*e.g.*, single chain Fv fragments (scFv)), disulfide stabilized (dsFv) Fv fragments, heteroconjugate antibodies (*e.g.*, bispecific antibodies), pFv fragments, heavy chain monomers or dimers, light chain monomers or dimers, and dimers consisting of one heavy chain and one light chain. In some embodiments, the TROP2 binding domain is a single domain antibody. In some embodiments, the

anti-TROP2 single domain antibody comprises heavy chain variable complementarity determining regions (CDR), CDR1, CDR2, and CDR3.

[0095] In some embodiments, the TROP2 binding domain is a polypeptide comprising an amino acid sequence that is comprised of four framework regions/sequences (f1-f4) interrupted by three complementarity determining regions/sequences, as represented by the formula: f1-r1-f2-r2-f3-r3-f4, wherein r1, r2, and r3 are complementarity determining regions CDR1, CDR2, and CDR3, respectively, and f1, f2, f3, and f4 are framework residues. The framework residues of the TROP2 binding protein of the present disclosure comprise, for example, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94 amino acid residues, and the complementarity determining regions comprise, for example, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 amino acid residues. In some embodiments, the TROP2 binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57.

[0096] In some embodiments, the binding proteins described herein comprise a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57, subsequences thereof, and variants thereof. In some embodiments, the TROP2 binding protein comprises at least 70%-95% or more homology to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57, subsequences thereof, and variants thereof. In some embodiments, the TROP2 binding protein comprises at least 60%, 61%, 62%, 63%, 63%, 65%, 66%, 67%, 68%, 69%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more homology to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57, subsequences thereof, and variants thereof. In some embodiments, the TROP2 binding protein comprises at least 70%-95% or more identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57, subsequences thereof, and variants thereof. In some embodiments, the TROP2 binding protein comprises at least 60%, 61%, 62%, 63%, 63%, 65%, 66%, 67%, 68%, 69%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57, subsequences thereof, and variants thereof.

[0097] In some embodiments, the CDR1 comprises the amino acid sequence as set forth in any one of SEQ ID NOS: 58-114 or an amino acid sequence comprising one or more substitutions compared to an amino acid sequence selected from the group consisting of SEQ ID NOS: 58-114. In some embodiments, the CDR2 comprises an amino acid sequence as set forth in any one of SEQ ID NOS: 115-171 or an amino acid sequence comprising one or more substitutions compared to an amino acid sequence selected from the group consisting of SEQ ID NOS: 115-171. In some embodiments, the CDR3 comprises an amino acid sequence as set forth in any one of SEQ ID

NOS: 172-228 or an amino acid sequence comprising one or more substitutions compared to an amino acid sequence selected from the group consisting of SEQ ID NOS: 172-228.

[0098] In various embodiments, the TROP2 binding domain of the present disclosure is at least about 60%, about 61%, at least about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 58-114, 115-171, and 172-228.

[0099] In various embodiments, a complementarity determining region of the TROP2 binding domain of the present disclosure is at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NOS: 58-114.

[00100] In various embodiments, a complementarity determining region of the TROP2 binding domain of the present disclosure is at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NOS: 115-171.

[00101] In various embodiments, a complementarity determining region of the TROP2 binding domain of the present disclosure is at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NOS: 172-228.

[00102] In some embodiments, the TROP2 binding domain is cross-reactive with human cynomolgus and mouse TROP2. In some embodiments, the TROP2 binding domain is specific for human TROP2. In certain embodiments, the TROP2 binding domains disclosed herein bind to human TROP2 with a human K_D ($h K_D$). In certain embodiments, the TROP2 binding domains disclosed herein bind to cynomolgus TROP2 with a cyno K_D ($c K_D$). In certain embodiments, the TROP2 binding domains disclosed herein bind to cynomolgus TROP2 with a mouse K_D ($m K_D$). In

certain embodiments, the TROP2 binding domains disclosed herein bind to both cynomolgus TROP2 and a human TROP2, with a cyno K_D (c K_D) and a human K_D (h K_D), respectively. In certain embodiments, the TROP2 binding domains disclosed herein bind to cynomolgus TROP2, mouse TROP2, and a human TROP2, with a cyno K_D (c K_D), mouse K_D (m K_D), and a human K_D (h K_D), respectively. In some embodiments, the TROP2 binding protein binds to human, mouse and cynomolgus TROP2 with comparable binding affinities (*i.e.*, h K_D , m K_D and c K_D values do not differ by more than $\pm 10\%$). In some embodiments, the TROP2 binding domains disclosed herein bind to Trop2 in presence of calcium. In some embodiments, the TROP2 binding domains disclosed herein bind to Trop2 in absence of calcium.

[00103] In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 500 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 450 nM. In some embodiments, h K_D , the m K_D and the c K_D range from about 0.001 nM to about 400 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 350 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 300 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 250 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 200 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 150 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 100 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 80 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 50 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 40 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 200 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 150 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.001 nM to about 100 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.1 nM to about 90 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.2 nM to about 80 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.3 nM to about 70 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.4 nM to about 50 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.5 nM to about 30 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.6 nM to about 10 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.7 nM to about 8 nM. In some embodiments, the h K_D , the m K_D and the c K_D range from about 0.8 nM to about 6 nM. In some embodiments, the h

K_D , the $m K_D$ and the $c K_D$ range from about 0.9 nM to about 4 nM. In some embodiments, the $h K_D$, the $m K_D$ and the $c K_D$ range from about 1 nM to about 2 nM.

[00104] In some embodiments, the $h k_{on}$ (1/Ms) e_5 , $c k_{on}$ (1/Ms) e_5 , $m k_{on}$ (1/Ms) e_5 ranges from about 0.001 to about 100, e.g., about 0.1-1, about 0.5-0.9. In some embodiments, the $h K_{off}$ (1/s), $c K_{off}$ (1/s) and $m K_{off}$ (1/s) ranges from about 1×10^{-2} to 9×10^{-6} , e.g., about 1×10^{-2} to 9×10^{-3} , about 4×10^{-3} to 6×10^{-3} .

[00105] In some embodiments, any of the foregoing TROP2 binding domains (e.g., anti-TROP2 single domain antibodies of SEQ ID NOS: 1-57) are affinity peptide tagged for ease of purification. In some embodiments, the affinity peptide tag is six consecutive histidine residues, also referred to as 6×-His (SEQ ID NO: 496).

[00106] In certain embodiments, the TROP2 binding domains of the present disclosure preferentially bind membrane bound TROP2 over soluble TROP2. Membrane bound TROP2 refers to the presence of TROP2 in or on the cell membrane surface of a cell that expresses TROP2. Soluble TROP2 refers to TROP2 that is no longer on in or on the cell membrane surface of a cell that expresses or expressed TROP2. In certain instances, the soluble TROP2 is present in the blood and/or lymphatic circulation in a subject. In one embodiment, the TROP2 binding domains bind membrane-bound TROP2 at least 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 100-fold, 500-fold, or 1000-fold greater than soluble TROP2. In one embodiment, the TROP2 binding proteins of the present disclosure preferentially bind membrane-bound TROP2 30-fold greater than soluble TROP2. Determining the preferential binding of an antigen binding protein to membrane bound TROP2 over soluble TROP2 can be readily determined using binding assays.

[00107] It is contemplated that in some embodiments the TROP2 binding protein is fairly small and no more than 40 kDa, no more than 30 kDa, no more than 25 kDa, no more than 20 kDa, no more than 15 kDa, or no more than 10 kDa in some embodiments. In certain instances, the TROP2 binding protein is 5 kDa or less if it is a peptide or small molecule entity.

[00108] In other embodiments, the TROP2 binding proteins described herein comprise small molecule entity (SME) binders for TROP2. SME binders are small molecules averaging about 500 to 2000 Da in size and are attached to the TROP2 binding proteins by known methods, such as sortase ligation or conjugation. In these instances, the TROP2 binding protein comprises a domain comprising a sortase recognition sequence, e.g., LPETG (SEQ ID NO: 548). To attach a SME binder to TROP2 binding protein comprising a sortase recognition sequence, the protein is incubated with a sortase and a SME binder whereby the sortase attaches the SME binder to the recognition sequence. In yet other embodiments, the TROP2 binding proteins described herein

comprise a knottin peptide for binding TROP2. Knottins are disulfide-stabilized peptides with a cysteine knot scaffold and have average sizes about 3.5 kDa. Knottins have been contemplated for binding to certain tumor molecules such as TROP2. In further embodiments, the TROP2 binding proteins described herein comprise a natural TROP2 ligand.

[00109] In some embodiments, the TROP2 binding protein comprises more than one domain and are of a single-polypeptide design with flexible linkage of the domains. This allows for facile production and manufacturing of the TROP2 binding proteins as they can be encoded by single cDNA molecule to be easily incorporated into a vector. Further, in some embodiments where the TROP2 binding proteins described herein are a monomeric single polypeptide chain, there are no chain pairing issues or a requirement for dimerization. It is contemplated that, in such embodiments, the TROP2 binding proteins described herein have a reduced tendency to aggregate.

[00110] In the TROP2 binding proteins comprising more than one domain, the domains are linked by one or more internal linker. In certain embodiments, the internal linkers are “short,” *i.e.*, consist of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 amino acid residues. Thus, in certain instances, the internal linkers consist of about 12 or less amino acid residues. In the case of 0 amino acid residues, the internal linker is a peptide bond. In certain embodiments, the internal linkers are “long,” *i.e.*, consist of 15, 20 or 25 amino acid residues. In some embodiments, the internal linkers consist of about 3 to about 15, for example 8, 9 or 10 contiguous amino acid residues. Regarding the amino acid composition of the internal linkers, peptides are selected with properties that confer flexibility to the TROP2 binding proteins, 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 internal linkers suitable for linking the domains in the TROP2 binding proteins include but are not limited to (GS)_n (SEQ ID NO: 514), (GGS)_n (SEQ ID NO: 515), (GGGS)_n (SEQ ID NO: 516), (GGSG)_n (SEQ ID NO: 517), (GGSGG)_n (SEQ ID NO: 518), (GGGGS)_n (SEQ ID NO: 519), (GGGGG)_n (SEQ ID NO: 520), or (GGG)_n (SEQ ID NO: 521), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In one embodiment, the linker is (GGGGSGGGSGGGSGGGGS) (SEQ ID NO: 522), (GGGGSGGGSGGGGS) (SEQ ID NO: 523), or (GGGGSGGGGS) (SEQ ID NO: 524).

[00111] In some cases, where the TROP2 binding protein comprises more than one domain, the domains within the TROP2 binding proteins are conjugated using an enzymatic site-specific conjugation method which involves the use of a mammalian or bacterial transglutaminase enzyme. Microbial transglutaminases (mTGs) are versatile tools in modern research and biotechnology. The availability of large quantities of relatively pure enzymes, ease of use, and lack of regulation by calcium and guanosine-5'-triphosphate (GTP) has propelled mTG to be the main cross-linking enzyme used in both the food industry and biotechnology. Currently, mTGs are used in many

applications to attach proteins and peptides to small molecules, polymers, surfaces, DNA, as well as to other proteins. *See, e.g., Pavel Strp, Veracity of microbial transglutaminase, Bioconjugate Chem.* 25, 5, 855-862.

[00112] In some examples are provided TROP2 binding proteins comprising more than one domain, wherein one of the domains comprises an acceptor glutamine in a constant region, which can then be conjugated to another domain *via* a lysine-based linker (*e.g.*, any primary amine chain which is a substrate for TGase, *e.g.* comprising an alkylamine, oxoamine) wherein the conjugation occurs exclusively on one or more acceptor glutamine residues present in the targeting moiety outside of the antigen combining site (*e.g.*, outside a variable region, in a constant region). Conjugation thus does not occur on a glutamine, *e.g.*, an at least partly surface exposed glutamine, within the variable region. The TROP2 binding protein, in some examples, is formed by reacting one of the domains with a lysine-based linker in the presence of a TGase.

[00113] In some embodiments, where one or more domains within the TROP2 binding proteins are directly joined, a hybrid vector is made where the DNA encoding the directly joined domains are themselves directly ligated to each other. In some embodiments, where linkers are used, a hybrid vector is made where the DNA encoding one domain is ligated to the DNA encoding one end of a linker moiety and the DNA encoding another domain is ligated to the other end of the linker moiety.

[00114] In some embodiments, the TROP2 binding protein is a single chain variable fragments (scFv), single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived single domain antibody. In other embodiments, the TROP2 binding protein is a non-Ig binding domain, *i.e.*, an antibody mimetic, such as anticalins, affilins, affibody molecules, AFFIMERS®, affitins, alphabodies, avimers, DARPINS®, fynomers, kunitz domain peptides, and monobodies. In further embodiments, the TROP2 binding protein is a ligand or peptide that binds to or associates with TROP2. In yet further embodiments, the TROP2 binding protein is a knottin. In yet further embodiments, the binding domain to TROP2 is a small molecular entity.

[00115] In certain embodiments, the TROP2 binding proteins according to the present disclosure may be incorporated into TROP2 targeting trispecific proteins. In some embodiments, the trispecific proteins comprise a CD3 binding domain, a half-life extension domain, and a TROP2 binding domain according to this disclosure. In some embodiments, the TROP2 binding trispecific protein comprises a trispecific antibody.

Multispecific TROP2 targeting proteins, such as TROP2 targeting trispecific proteins (also referred to herein as TROP2 targeting TriTAC proteins or molecules)

[00116] In one aspect is described herein a multispecific or a multivalent protein comprising a TROP2 binding protein according to the present disclosure. In some embodiments, the multispecific protein further comprises a domain which specifically binds to CD3. In some embodiments, the multispecific protein further comprises a domain which specifically binds to human CD3. In some embodiments, the multispecific protein further comprises a domain which specifically binds to CD3-gamma. In some embodiments, the multispecific protein further comprises a domain which specifically binds to CD3-delta. In some embodiments, the multispecific protein further comprises a domain which specifically binds to CD3-epsilon.

[00117] In additional embodiments, the multispecific protein further comprises a domain which specifically binds to the T cell receptor (TCR). In some embodiments, the multispecific protein further comprises a domain which specifically binds the alpha chain of the TCR. In some **embodiments, the multispecific protein further comprises a domain which specifically binds the β chain of the TCR.**

[00118] In certain embodiments, the CD3 binding domain of the multispecific protein exhibits not only potent CD3 binding affinities with human CD3, but also shows excellent cross-reactivity with the respective cynomolgus monkey CD3 proteins. In some instances, the CD3 binding domain of the multispecific proteins are cross-reactive with CD3 from cynomolgus monkey. In certain instances, human:cynomolgous K_D (h K_D : c K_D) ratios for CD3 binding are between 20:1 and 1:2.

[00119] In some embodiments, the CD3 binding domain of the multispecific protein is any domain that binds to CD3 including but not limited to domains from a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, or antigen binding fragments of the CD3 binding antibodies, such as single domain antibodies (sdAb), Fab, F(ab')₂, and Fv fragments, fragments comprised of one or more CDRs, single-chain antibodies (*e.g.*, single chain Fv fragments (scFv)), disulfide stabilized (dsFv) Fv fragments, heteroconjugate antibodies (*e.g.*, bispecific antibodies), pFv fragments, heavy chain monomers or dimers, light chain monomers or dimers, and dimers consisting of one heavy chain and one light chain. In some instances, it is beneficial for the CD3 binding domain to be derived from the same species in which the multispecific protein comprising a single domain serum albumin binding protein described herein will ultimately be used in. For example, for use in humans, it may be beneficial for the CD3 binding domain of the multispecific protein comprising a TROP2 binding protein described herein to comprise human or humanized residues from the antigen binding domain of an antibody or antibody fragment. Exemplary amino acid sequence for the CD3 binding domain of a multispecific

(*e.g.*, trispecific) TROP2 targeting TriTAC protein of this disclosure is provided as SEQ ID NO: 494, or an amino acid sequence that is at least about 75% to 100% identical to SEQ ID NO: 494, such as at least about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to SEQ ID NO: 494.

[00120] In some embodiments, the serum albumin binding domain (also referred to herein as the half-life extension domain) of a multispecific protein comprising a TROP2 binding protein as described herein can be any domain that binds to serum albumin including but not limited to domains from a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody. In some embodiments, the serum albumin binding domain is a single chain variable fragments (scFv), single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived sdAb, or antigen binding fragments of the HSA binding antibodies, such as Fab, F(ab')₂, and Fv fragments, fragments comprised of one or more CDRs, single-chain antibodies (*e.g.*, single chain Fv fragments (scFv)), disulfide stabilized (dsFv) Fv fragments, heteroconjugate antibodies (*e.g.*, bispecific antibodies), pFv fragments, heavy chain monomers or dimers, light chain monomers or dimers, and dimers consisting of one heavy chain and one light chain, peptide, ligand or small molecule entity specific for serum albumin. In certain embodiments, the HSA binding domain is a single-domain antibody. In other embodiments, the serum albumin binding domain is a peptide. In further embodiments, the serum albumin binding domain is a small molecule. It is contemplated that the serum albumin binding domain of the multispecific binding protein comprising a single chain variable fragment CD3 binding protein is fairly small and no more than 25 kDa, no more than 20 kDa, no more than 15 kDa, or no more than 10 kDa in some embodiments. In certain instances, the serum albumin binding is 5 kDa or less if it is a peptide or small molecule entity. Exemplary amino acid sequence for a serum albumin binding domain of a multispecific (*e.g.*, trispecific) TROP2 targeting TriTAC protein of this disclosure is provided as SEQ ID NO: 493, or 566, or an amino acid sequence that is at least about 75% to 100% identical to SEQ ID NO: 493, or 566, such as at least about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to SEQ ID NO: 493 or 549.

[00121] The half-life extension domain of a multispecific binding protein, as described herein, comprising a single chain variable fragment CD3 binding protein provides for altered pharmacodynamics and pharmacokinetics of the single chain variable fragment CD3 binding protein itself. As above, the half-life extension domain extends the elimination half-time. The half-

life extension domain also alters pharmacodynamic properties including alteration of tissue distribution, penetration, and diffusion of the single chain variable fragment CD3 binding protein. In some embodiments, the half-life extension domain provides for improved tissue (including tumor) targeting, tissue distribution, tissue penetration, diffusion within the tissue, and enhanced efficacy as compared with a protein without a half-life extension domain. In one embodiment, therapeutic methods effectively and efficiently utilize a reduced amount of the multispecific binding protein comprising a single chain variable fragment CD3 binding protein, resulting in reduced side effects, such as reduced off-target, such as non-tumor cell cytotoxicity.

[00122] Further, the binding affinity of the half-life extension domain, in some embodiments, is selected so as to target a specific elimination half-time in a particular multispecific binding protein comprising a TROP2 binding protein as described herein. Thus, in some embodiments, the half-life extension domain has a high binding affinity. In other embodiments, the half-life extension domain has a medium binding affinity. In yet other embodiments, the half-life extension domain has a low or marginal binding affinity. Exemplary binding affinities include K_D of 10 nM or less (high), between 10 nM and 100 nM (medium), and greater than 100 nM (low). As above, binding affinities to serum albumin are determined by known methods such as Surface Plasmon Resonance (SPR).

[00123] A TROP2 targeting multispecific protein of this disclosure, in certain embodiments, comprises (A) a first domain which binds to a CD3; (B) a second domain which is a half-life extension domain; and (C) a third domain which is a TROP2 binding protein as described herein. In certain embodiments, the first domain comprises an scFv that specifically binds the CD3. The CD3, for instance, is a human CD3 protein. In certain embodiments, the second domain comprises an sdAb that specifically binds a bulk serum protein. In some instances, the bulk serum protein is albumin, such as, a serum albumin, such as, a human serum albumin. The domains (A), (B), and (C), are, in some embodiments, linked *via* linkers L1 and L2, in any one of the following orientations: H₂N-(A)-L1-(C)-L2-(B)-COOH, H₂N-(B)-L1-(A)-L2-(C)-COOH, H₂N-(C)-L1-(B)-L2-(A)-COOH, H₂N-(C)-L1-(A)-L2-(B)-COOH, H₂N-(A)-L1-(B)-(C)-L2-COOH, or H₂N-(B)-(C)-(A)-COOH.

[00124] A TROP2 targeting multispecific protein of this disclosure, in some embodiments, comprises an amino acid sequence that is at least about 70% to about 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57 and 229-264. In some embodiments, a TROP2 targeting multispecific protein of this disclosure comprises an amino acid sequence that is at least about 70%, at least about 75%, at least about 76%, at least about 77%, about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least

about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, to about 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57 and 229-264.

Conditionally active multispecific TROP2 targeting proteins, such as conditionally active TROP2 targeting trispecific proteins (also referred to herein as TROP2 targeting ProTriTAC or protrispecific proteins or molecules)

[00125] One embodiment of this disclosure provides a conditionally active multispecific protein comprising a TROP2 binding domain as disclosed herein (for example, in some embodiment this disclosure provides a TROP2 targeting protrispecific/ProTriTAC protein comprising a TROP2 binding domain of this disclosure).

[00126] In some embodiments, the conditionally active multispecific protein further comprises a domain which specifically binds to a CD3 and a binding moiety which specifically binds to a bulk serum protein, such as a human serum albumin. In some embodiments, the binding moiety is capable of masking or masks the interaction of the TROP2 binding domain or the CD3 binding domain, to their targets. In some embodiments, a binding moiety of this disclosure comprises a masking moiety and a cleavable linker, such as a protease cleavable linker. Exemplary sequences for masking moiety within a binding moiety having a sequence selected from the group consisting of SEQ ID NOS: 550 and 558-560, or an amino acid sequence comprising one or more substitutions relative to an amino acid sequence selected from the group consisting of SEQ ID NOS: 550 and 558-560. In some embodiments, the binding moiety comprises a modified non-CDR loop sequence and a cleavable linker. In some embodiments, the cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 497-543, or an amino acid sequence that comprises one or substitutions relative to an amino acid sequence selected from the group consisting of SEQ ID NOS: 497-543. In some embodiments, the masking moiety comprises a modified non-CDR loop sequence and a non-cleavable linker. In some embodiments the non-cleavable linker comprises an amino acid sequence as set forth in SEQ ID NO: 544 or 545, or an amino acid sequence comprising one or more substitutions relative to SEQ ID NO: 544 or 545. In some embodiments, a binding moiety comprises an amino acid sequence that is at least about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 549. In some embodiments, a CD3 binding domain of a TROP2 ProTriTAC of this disclosure comprises an amino acid sequence that is at least about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%,

about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to SEQ ID NO: 494.

[00127] In some embodiments, a TROP2 targeting ProTriTAC of this disclosure, comprises, from N-terminal to C-terminal, comprise a binding moiety that is an anti-ALB domain comprising a non-CDR loop with a binding site for a CD3 binding domain (*e.g.*, a CD3 binding domain having the sequence of SEQ ID NO: 549, or at least about 75% identity to the same), a cleavable linker, the CD3 binding domain, and on the C-terminal end the anti-TROP2 binding domain. The TROP2 binding domain of the ProTriTAC, in some embodiments, is at least about 60%, about 61%, at least about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57.

[00128] In some embodiments, a TROP2 targeting ProTriTAC of this disclosure comprises an amino acid sequence that is at least about at least about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57 and 229-264. In some embodiments, a TROP2 targeting ProTriTAC of this disclosure comprises an amino acid sequence as set forth in any one of SEQ ID NOS: 1-57 and 229-264, a pharmaceutical composition comprising the same, and method of using the same for treating a disease, such as a tumorous disease as described herein.

[00129] In some embodiments, a TROP2 targeting ProTriTAC of this disclosure, in a non-cleavable prodrug format, comprises an amino acid sequence that is at least about at least about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 1-57.

[00130] An exemplary sequence for an active TROP2 targeting drug (CT), as described herein, is an amino acid sequence that is at least about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 1-57 and 229-264.

[00131] The binding moiety is capable of synergistically expanding a therapeutic window of a conditionally active TROP2 targeting protrispecific protein, by both steric masking and specific

masking. In some embodiments, the binding moiety combines both steric masking (for example, *via* binding to a bulk serum albumin) and specific masking (for example, *via* non-CDR loops binding to the CDRs of an anti-TROP2 domain or an anti-CD3 scFv domain). For example, the binding moiety masks or is capable of masking binding of the TROP2 binding domain (*e.g.*, hides the TROP2 binding domain and/or prevents premature binding), until activation and the ability of a sequence to “mask” can be tested utilizing an assay where activity with and without the masking sequence (*e.g.*, a sequence selected from the group consisting of SEQ ID NOS: 550 and 558-560, or a sequence comprising one or more substitutions in a sequence having a sequence selected from the group consisting of: SEQ ID NOS: 550 and 558-560) is compared. Briefly, to test masking, ProCAR constructs with and without masking moieties in, for example, CC' loops are made. T cells are infected with lentivirus made from the constructs to generate CAR-T cells, which are subsequently stained with anti-FLAG antibodies and TROP2 -Fc along with fluorescently labeled secondary antibodies and analyzed by flow cytometry. Dot plots of staining are generated and compared. In some instances, the masking moiety is masking peptide/moiety, inserted into one or more non-CDR loops, such that the binding moiety binds to and inhibits the TROP2 antigen binding domain until such time that the construct is delivered to a tumor microenvironment. In some cases, modifying the non-CDR loops within the binding moiety does not affect albumin binding. The protease cleavable linker, in some cases, enables activation of a TROP2 targeting protrispecific protein in a single proteolytic event, thereby allowing more efficient conversion of the protrispecific molecule in tumor microenvironment. Further, tumor-associated proteolytic activation, in some cases, reveals active T cell engager with minimal off-tumor activity after activation. The present disclosure, in some embodiments, provides a half-life extended T cell engager format (ProTriTAC) comprising a TROP2 binding moiety as described herein, which in some cases represents a new and improved approach to engineer conditionally active T cell engagers.

[00132] The half-life of the TROP2 binding domain in a conditionally active protrispecific format is, in some embodiments, extended in systemic circulation by using the binding moiety as described above which acts as a safety switch that keeps the multispecific protein in the pro format in an inert state until it reaches the tumor microenvironment where it is conditionally activated by cleavage of the linker and is able to bind its target antigen(s). The safety switch, in certain instances, provides several advantages: some examples including (i) expanding the therapeutic window of the conditionally active TROP2 targeting protein; (ii) reducing target-mediated drug disposition by maintaining the conditionally active TROP2 targeting protein in systemic circulation; (iii) reducing the concentration of undesirable activated protein in systemic circulation, thereby minimizing the

spread of chemistry, manufacturing, and controls related impurities, *e.g.*, pre-activated drug product, endogenous viruses, host-cell proteins, DNA, leachables, anti-foam, antibiotics, toxins, solvents, heavy metals; (iv) reducing the concentration of undesirable activated proteins in systemic circulation, thereby minimizing the spread of product related impurities, aggregates, breakdown products, product variants due to: oxidation, deamidation, denaturation, loss of C-term Lys in MAbs; (v) preventing aberrant activation in circulation; (vi) reducing the toxicities associated with the leakage of activated species from diseased tissue or other pathophysiological conditions, *e.g.*, tumors, autoimmune diseases, inflammations, viral infections, tissue remodeling events (such as myocardial infarction, skin wound healing), or external injury (such as X-ray, CT scan, UV exposure); and (vii) reducing non-specific binding of the conditionally active TROP2 targeting protein. Furthermore, post-activation, or in other words post breaking of the safety switch, the conditionally active TROP2 targeting protein is separated from the safety switch which provided extended half-life, and thus is cleared from circulation. For instance, if the drug is inadvertently activated outside of a tumor environment or if it leaks out of a tumor environment after activation, it is likely to be cleared rapidly and is less likely to cause damage to normal tissues, thus reducing toxicity.

[00133] In some embodiments, a conditionally active multispecific TROP2 binding protein as described herein has an improved therapeutic index as compared to that of a TROP2 binding protein that is not conditionally active but is, rather, constitutively active. For instance, a TROP2 ProTriTAC, in some embodiments, has an increased therapeutic index than a TROP2 TriTAC. The increase in therapeutic index, in some embodiments, is from at least about 2-fold to about 1000-fold, such as about 4-fold to about 800-fold, about 6-fold to about 800-fold, about 6-fold to about 600-fold, about 10-fold to about 400-fold, about 20-fold to about 200-fold, about 30-fold to about 150-fold, about 50-fold to about 100-fold. The increase in therapeutic index, in some embodiments, is attributed to the conjugation of the TROP2 binding domain to a binding moiety as described above, with the non-CDR loop and the cleavable linker.

[00134] A “therapeutic index” (TI) (also referred to as “therapeutic window”) is, in some embodiments, a comparison of the minimum amount of a therapeutic agent (*e.g.*, a TROP2 TriTAC, a TROP2 ProTriTAC, a TROP2 CAR, a TROP2 ProCAR) that causes the therapeutic effect (*e.g.*, improved survival of a patient with a TROP2 expressing cancer, treated with a therapeutic agent as mentioned above) to minimum tolerated dose. In some instances, the therapeutic index improvement is manifested in terms of an improved EC_{50} of a TROP2 TriTAC compared to a TROP2 ProTriTAC, in T cell mediated killing of cancer cells.

[00135] In some embodiments, the conditionally active TROP2 targeting protein format gives the TROP2 binding domain a significantly longer serum half-life and reduces the likelihood of its undesirable activation in circulation, thereby producing a “biobetter” version.

[00136] A binding moiety as described herein comprises at least one non-CDR loop. In some embodiments, a non-CDR loop provides a binding site for binding of the binding moiety to a TROP2 binding domain of this disclosure. In some cases, the binding moiety masks binding of the TROP2 binding domain to its target antigen, *e.g.*, *via* steric occlusion, *via* specific intermolecular interactions, or a combination of both.

[00137] In some embodiments, a binding moiety as described herein further comprises complementarity determining regions (CDRs), for instance, specific for binding a bulk serum protein (*e.g.*, a human serum albumin). In some instances, a binding moiety of this disclosure is a domain derived from an immunoglobulin molecule (Ig molecule). The Ig may be of any class or subclass (IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, *etc.*). A polypeptide chain of an Ig molecule folds into a series of parallel beta strands linked by loops. In the variable region, three of the loops constitute the “complementarity determining regions” (CDRs) which determine the antigen binding specificity of the molecule. An IgG molecule comprises at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding fragment thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs) which are hypervariable in sequence and/or involved in antigen recognition and/or usually form structurally defined loops, interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[00138] In some embodiments, a binding moiety of this disclosure is a heavy chain only antibody. In some embodiments, the variable domain of a heavy chain only antibody has several beta strands that are arranged in two sheets. The variable domain of a heavy chain only antibody contains three hypervariable loops, or complementarity-determining regions (CDRs) and framework regions FR1, FR2, FR3, and FR4. The three CDRs of the variable domain (CDR1, CDR2, CDR3) cluster at one end of the beta barrel. The CDRs are the loops that connect beta strands B-C, C'-C'', and F-G of the immunoglobulin fold, whereas the bottom loops that connect beta strands AB, CC', C''-D and E-F

of the immunoglobulin fold, and the top loop that connects the D-E strands of the immunoglobulin fold are the non-CDR loops.

[00139] In some embodiments of this disclosure at least some or all of the amino acid sequences of FR1, FR2, FR3, and FR4 are part of the “non-CDR loop” of the binding moieties described herein, such as a binding moiety that is a heavy chain only antibody. In some embodiments of this disclosure, at least some amino acid residues of a constant domain, CH1, CH2, or CH3, are part of the “non-CDR loop” of the binding moieties described herein. Non-CDR loops comprise, in some embodiments, one or more of AB, CD, EF, and DE loops of a C1-set domain of an Ig or an Ig-like molecule; AB, CC', EF, FG, BC, and EC' loops of a C2-set domain of an Ig or an Ig-like molecule; DE, BD, GF, A(A1A2)B, and EF loops of I(Intermediate)-set domain of an Ig or Ig-like molecule.

[00140] Within the variable domain, the CDRs are believed to be responsible for antigen recognition and binding, while the FR residues are considered a scaffold for the CDRs. However, in certain cases, some of the FR residues play an important role in antigen recognition and binding. Framework region residues that affect Ag binding are divided into two categories. The first are FR residues that contact the antigen, thus are part of the binding-site, and some of these residues are close in sequence to the CDRs. Other residues are those that are far from the CDRs in sequence but are in close proximity to it in the 3-D structure of the molecule, *e.g.*, a loop in heavy chain.

[00141] In some embodiments, the non-CDR loop is modified to generate an antigen binding site specific for a bulk serum protein, such as albumin. In some embodiments, the non-CDR loop is modified to generate an antigen binding site specific for a TROP2 binding domain as described herein. In some embodiments, the non-CDR loop is modified to generate an antigen binding site specific for a CD3 binding domain as described herein.

[00142] It is contemplated that various techniques can be used for modifying the non-CDR loop, *e.g.*, site-directed mutagenesis, random mutagenesis, insertion of at least one amino acid that is foreign to the non-CDR loop amino acid sequence, amino acid substitution. An antigen peptide is inserted into a non-CDR loop, in some examples. In some examples, an antigenic peptide is substituted for the non-CDR loop. The modification, to generate an antigen binding site, is in some cases in only one non-CDR loop. In other instances, more than one non-CDR loop are modified. For instance, the modification is in any one of the non-CDR loops are AB, CC', C" D, EF, and D-E. In some cases, the modification is in the DE loop. In other cases, the modifications are in all four of AB, CC', C"D, E-F loops.

[00143] In certain examples, the binding moieties described herein are bound to the TROP2 binding domain *via* their AB, CC', C" D, or EF loop and are bound to a bulk-serum protein, such as albumin, *via* their B-C, C'-C", or F-G loop. In certain examples, the binding moiety is bound to the

TROP2 binding domain *via* its AB, CC', C" D, and EF loop and is bound to a bulk-serum protein, such as albumin, *via* its BC, C'C", and FG loop. In certain examples, the binding moiety is bound to the TROP2 binding domain *via* one or more of AB, CC', C" D, and E-F loop and is bound to a bulk-serum protein, such as albumin, *via* one or more of BC, C'C", and FG loop. In certain examples, the binding moiety is bound to a bulk serum protein, such as albumin, *via* its AB, CC', C" D, or EF loop and is bound to the TROP2 binding domain *via* its BC, C'C", or FG loop. In certain examples, the binding moiety is bound to a bulk serum protein, such as albumin, *via* its AB, CC', C" D, and EF loop and is bound to the TROP2 binding domain *via* its BC, C'C", and FG loop. In certain examples, the binding moiety of the first embodiment is bound to a bulk serum protein, such as albumin, *via* one or more of AB, CC', C" D, and E-F loop and is bound to the TROP2 binding protein, *via* one or more of BC, C'C", and FG loop. In certain examples, the binding moieties described herein are bound to a CD3 binding domain *via* their AB, CC', C" D, or EF loop and are bound to a bulk-serum protein, such as albumin, *via* their B-C, C'-C", or F-G loop. In certain examples, the binding moieties described herein are bound to a bulk serum protein, such as albumin, *via* their AB, CC', C" D, or EF loop and are bound to a CD3 binding domain, *via* their B-C, C'-C", or F-G loop. In certain examples, the binding moieties described herein are bound to a CD3 binding domain *via* their AB, CC', C" D, or EF loop and are bound to a TROP2 binding domain, *via* their B-C, C'-C", or F-G loop. In certain examples, the binding moieties described herein are bound to a TROP2 binding domain *via* their AB, CC', C" D, or EF loop and are bound to a CD3 binding domain, *via* their B-C, C'-C", or F-G loop.

[00144] The bulk serum protein comprises, for example, albumin, fibrinogen, or a globulin. In some embodiments, the binding moieties are engineered scaffolds. Engineered scaffolds comprise, for example, sdAb, a scFv, a Fab, a VHH, a fibronectin type III domain, immunoglobulin-like scaffold (as suggested in Halaby *et al.*, 1999. *Prot Eng* 12(7):563-571), DARPin, cystine knot peptide, lipocalin, three-helix bundle scaffold, protein G-related albumin-binding module, or a DNA or RNA aptamer scaffold.

[00145] In some cases, the binding moieties comprise a binding site for the bulk serum protein. In some embodiments, the CDRs within the binding moieties provide a binding site for the bulk serum protein. The bulk serum protein is, in some examples, a globulin, albumin, transferrin, IgG1, IgG2, IgG4, IgG3, IgA monomer, Factor XIII, Fibrinogen, IgE, or pentameric IgM. In some embodiments, the binding moieties comprise a binding site for an immunoglobulin light chain. In some embodiments, the CDRs provide a binding site for the immunoglobulin light chain. The immunoglobulin light chain is, in some examples, an Igκ free light chain or an Igλ free light chain.

[00146] In additional embodiments, the binding moieties are any kinds of polypeptides. For example, in certain instances the binding moieties are natural peptides, synthetic peptides, or fibronectin scaffolds, or engineered bulk serum proteins. In some examples, the binding moieties comprise any type of binding domain, including but not limited to, domains from a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody. In some embodiments, the binding moiety is a single chain variable fragment (scFv), a soluble TCR fragment, a single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody. In other embodiments, the binding moieties are non-Ig binding domains, *i.e.*, antibody mimetic, such as anticalins, affilins, affibody molecules, AFFIMERS®, affitins, alphabodies, avimers, DARPINS®, fynomers, kunitz domain peptides, or monobodies.

[00147] It is contemplated herein that the binding moiety described herein comprises at least one cleavable linker. In one aspect, the cleavable linker comprises a polypeptide having an amino acid sequence recognized and cleaved in an amino acid sequence-specific manner. The binding moiety described herein, in some cases, comprises a protease cleavable linker recognized and cleaved in an amino acid sequence-specific manner. In some embodiments, the protease cleavable linker is recognized in an amino acid sequence-specific manner by a matrix metalloprotease (MMP), for example a MMP9. In some cases, the protease cleavable linker recognized by a MMP9 comprises a polypeptide having an amino acid sequence PR(S/T)(L/I)(S/T). In some cases, the protease cleavable linker recognized by a MMP9 comprises a polypeptide having an amino acid sequence LEATA. In some cases, the protease cleavable linker is recognized in an amino acid sequence-specific manner by a MMP11.

[00148] Proteases are proteins that cleave proteins, in some cases, in an amino acid sequence-specific manner. Proteases include but are not limited to serine proteases, cysteine proteases, aspartate proteases, threonine proteases, glutamic acid proteases, metalloproteases, asparagine peptide lyases, serum proteases, cathepsins, Cathepsin B, Cathepsin C, Cathepsin D, Cathepsin E, Cathepsin K, Cathepsin L, kallikreins, hK1, hK10, hK15, plasmin, collagenase, Type IV collagenase, stromelysin, Factor Xa, chymotrypsin-like protease, trypsin-like protease, elastase-like protease, subtilisin-like protease, actinidain, bromelain, calpain, caspases, caspase-3, Mir1-CP, papain, HIV-1 protease, HSV protease, CMV protease, chymosin, renin, pepsin, matriptase, legumain, plasmepsin, nepenthesin, metalloexopeptidases, metalloendopeptidases, matrix metalloproteases (MMP), MMP1, MMP2, MMP3, MMP8, MMP9, MMP13, MMP11, MMP14, urokinase plasminogen activator (uPA), enterokinase, prostate-specific antigen (PSA, hK3),

interleukin-1 β converting enzyme, thrombin, FAP (FAP- α), dipeptidyl peptidase, and dipeptidyl peptidase IV (DPPIV/CD26).

Table 1: Exemplary proteases and protease recognition sequences

Protease	Cleavage Domain Sequence	SEQ ID NO:
MMP7	KRALGLPG	551
MMP7	(DE) ₈ RPLALWRS(DR) ₈	552
MMP9	PR(S/T)(L/I)(S/T)	553
MMP9	LEATA	554
MMP11	GGAANLVRGG	555
MMP14	SGRIGFLRTA	556
MMP	PLGLAG	557
MMP	PLGLAX	558
MMP	PLGC(me)AG	559
MMP	ESPAYYTA	560
MMP	RLQLKL	561
MMP	RLQLKAC	562
MMP2, MMP9, MMP14	EP(Cit)G(Hof)YL	563
Urokinase plasminogen activator (uPA)	SGRSA	564
Urokinase plasminogen activator (uPA)	DAFK	565
Urokinase plasminogen activator (uPA)	GGRR	566
Lysosomal Enzyme	GFLG	567
Lysosomal Enzyme	ALAL	568
Lysosomal Enzyme	FK (SEQ IDENTIFIER A569)	
Cathepsin B	NLL (SEQ IDENTIFIER A570)	
Cathepsin D	PIC(Et)FF	571
Cathepsin K	GGPRGLPG	572
Prostate Specific Antigen	HSSKLQ	573
Prostate Specific Antigen	HSSKLQL	574
Prostate Specific Antigen	HSSKLQEDA	575

Protease	Cleavage Domain Sequence	SEQ ID NO:
Herpes Simplex Virus Protease	LVLASSSFGY	576
HIV Protease	GVSQNYPIVG	577
CMV Protease	GVVQASCRLA	578
Thrombin	F(Pip)RS (SEQ IDENTIFIER A579)	
Thrombin	DPRSFL	580
Thrombin	PPRSFL	581
Caspase-3	DEVD	582
Caspase-3	DEVDP	583
Caspase-3	KGSGDVEG	584
Interleukin 1 β converting enzyme	GWEHDG	585
Enterokinase	EDDDDKA	586
FAP	KQEQNPGST	587
Kallikrein 2	GKAFRR	588
Plasmin	DAFK	589
Plasmin	DVLK	590
Plasmin	DAFK	591
TOP	ALLLALL	592

[00149] Proteases are known to be secreted by some diseased cells and tissues, for example tumor or cancer cells, creating a microenvironment that is rich in proteases or a protease-rich microenvironment. In some case, the blood of a subject is rich in proteases. In some cases, cells surrounding the tumor secrete proteases into the tumor microenvironment. Cells surrounding the tumor secreting proteases include but are not limited to the tumor stromal cells, myofibroblasts, blood cells, mast cells, B cells, NK cells, regulatory T cells, macrophages, cytotoxic T lymphocytes, dendritic cells, mesenchymal stem cells, polymorphonuclear cells, and other cells. In some cases, proteases are present in the blood of a subject, for example proteases that target amino acid sequences found in microbial peptides. This feature allows for targeted therapeutics such as antigen binding proteins to have additional specificity because T cells will not be bound by the antigen binding protein except in the protease rich microenvironment of the targeted cells or tissue. Other non-limiting examples of linkers that may be utilized in constructs described herein are provided in the Sequence Listing below.

Integration into chimeric antigen receptors (CAR)

[00150] The TROP2 binding proteins of the present disclosure can, in certain examples, be incorporated into a chimeric antigen receptor (CAR), or to a ProCAR. An engineered immune effector cell, *e.g.*, a T cell or NK cell, can be used to express a CAR that includes a TROP2 binding protein containing, for example, an anti-TROP2 single domain antibody as described herein. In one embodiment, the CAR including the TROP2 binding protein as described herein is connected to a transmembrane domain via a hinge region, and further a costimulatory domain, *e.g.*, a functional signaling domain obtained from OX40, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), or 4-1BB. In some embodiments, the CAR further comprises an amino acid sequence encoding an intracellular signaling domain, such as 4-1BB and/or CD3 zeta. Exemplary sequences for a ProCAR comprising a TROP2 binding domain is provided in SEQ ID NOS:1-57, or sequences that are at least about 75% to 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-57, such as about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100%.

[00151] The conditionally active receptors described herein comprise at least one binding moiety comprising a non-CDR loop. In one aspect, the binding moiety masks binding of the TROP2 binding domain, until activation. The cleavable linker, for example, comprises a protease cleavage site or a pH dependent cleavage site. The cleavable linker, in certain instances, is cleaved only in a tumor microenvironment. Thus, the binding moiety, connected to the cleavable linker, and further bound to the TROP2 binding domain, in some examples, maintains the TROP2 binding domain in an inert state in circulation until the cleavable linker is cleaved off in a tumor microenvironment. In some embodiments, the binding moiety binds to the TROP2 binding domain. In some embodiments, a non-CDR loop provides a binding site for binding of the moiety to the TROP2 binding domain. In some embodiments, the binding moiety masks binding of the TROP2 binding domain to its target antigen, *e.g.*, via steric occlusion, via specific intramolecular interactions, such as interactions within the different domains of the polypeptide comprising the binding moiety. In some embodiments, the binding moiety further comprises complementary determining regions (CDRs).

[00152] In some instances, the binding moiety of a CAR or a proCAR as described herein is a domain derived from an immunoglobulin molecule (Ig molecule), as described above in the section corresponding to conditionally active multispecific TROP2 targeting proteins of this disclosure.

[00153] In some embodiments, the conditionally active receptor comprises a TROP2 binding domain (aTarget1), a cleavable linker, and a binding moiety (aTarget2). The TROP2 binding

domain has specificity for a first target (TROP2), while the binding moiety has specificity for a second target. The binding moiety also has a modified non-CDR loop, which inhibits binding of the TROP2 binding domain to its target. Once cleaved at the cleavable linker, the binding moiety can be released, enabling binding of the TROP2 binding domain.

[00154] In some embodiments, the inactive receptor (Inactive ProCAR) comprises a TROP2 binding domain (Anti-Tumor Target sdAb or scFv) connected to a binding moiety (Anti-Target 2 sdAb) via a linker comprising a protease cleavage site. The binding moiety comprises a masking peptide/moiety, inserted into one or more non-CDR loops, such that the binding moiety binds to and inhibits the TROP2 antigen binding domain. In some embodiments, the binding moiety has specificity for a given target, as described further elsewhere herein. The receptor also comprises a transmembrane domain and an intracellular signaling domain. The receptor is provided in a T-cell (CAR-T). Upon exposure to a tumor environment, the protease cleavage site is cleaved by tumor-associated proteases, thereby activating the receptor, generating the active receptor which does not comprise the binding moiety. The receptor now comprises an active antigen-binding domain. As the receptor is internalized by the cell, new receptors are generated which comprise the binding moiety and are inactive.

[00155] The cleavable linker of the binding moiety, in some embodiments, comprises a protease cleavable site similar to what is described above with respect to the conditionally active multispecific proteins comprising a TROP2 binding domain of this disclosure. For instance, the cleavable linker in some embodiments comprises an amino acid sequence selected from the linker sequences provided in the sequence table.

Transmembrane domain

[00156] The conditionally active chimeric antigen receptors, T-cell receptor fusion proteins, and T-cell receptors of the present disclosure include a transmembrane domain for insertion into a eukaryotic cell membrane. In some embodiments, the transmembrane domain is interposed between the TROP2 binding domain and the intracellular domain. In some embodiments, the transmembrane domain is interposed between the TROP2 binding domain and the costimulatory domain.

[00157] Any transmembrane (TM) domain that provides for insertion of a polypeptide into the cell membrane of a eukaryotic (*e.g.*, mammalian) cell is suitable for use. As one non-limiting example, the TM sequence IYIWAPLAGTCGVLLSLVITLYC (SEQ ID NO: 551) can be used. Additional non-limiting examples of suitable TM sequences include, but are not limited to, a) CD8 beta derived: GLLVAGVLVLLVSLGVAIHLCC (SEQ ID NO: 552); b) CD4 derived: ALIVLGGVAGLLLFIGLGIFFCVRC (SEQ ID NO: 553); c) CD3 zeta derived:

LCYLLDGILFIYGVILTALFLRV (SEQ ID NO: 554); d) CD28 derived:

WVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO: 555); e) CD134 (OX40) derived:

AAILGLGLVLGLLGPLAILLALYLL (SEQ ID NO: 556); and f) CD7 derived:

ALPAALAVISFLLGLGLGVACVLA (SEQ ID NO: 557).

Hinge Region

[00158] In some cases, the conditionally active chimeric antigen receptors, T-cell receptor fusion proteins, and T-cell receptors of the present disclosure comprise a hinge region (also referred to herein as a “spacer”), where the hinge region is interposed between the TROP2 binding domain and the transmembrane domain. In some cases, the hinge region is an immunoglobulin heavy chain hinge region. In some cases, the hinge region is a hinge region polypeptide derived from a receptor (*e.g.*, a CD8-derived hinge region).

[00159] The hinge region can have a length of from about 4 amino acids to about 50 amino acids (aa), *e.g.*, from about 4 aa to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 40 aa, or from about 40 aa to about 50 aa.

[00160] Suitable spacers can be readily selected and can be of any of a number of suitable lengths, such as from 1 amino acid (*e.g.*, Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and can be 1, 2, 3, 4, 5, 6, or 7 amino acids.

[00161] Exemplary spacers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n (SEQ ID NO: 514), (GSGGS)_n (SEQ ID NO: 524) and (GGGS)_n (SEQ ID NO: 516), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers can be used; both Gly and Ser are relatively unstructured, and therefore can serve as a neutral tether between components. Glycine polymers can be used; glycine accesses significantly more phi-psi space than even alanine and is much less restricted than residues with longer side chains (*see*, Scheraga, *Rev.*

Computational Chem. 11173-142 (1992)). Exemplary spacers comprise amino acid sequences including, but not limited to, GGSG (SEQ ID NO: 525), GGSGG (SEQ ID NO: 526), GSGSG (SEQ ID NO: 527), GSGGG (SEQ ID NO: 528), GGGSG (SEQ ID NO: 529), GSSSG (SEQ ID NO: 530), and the like.

[00162] Immunoglobulin hinge region amino acid sequences are known in the art; *see, e.g.*, Tan *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87: 162; and Huck *et al.* (1986) *Nucl. Acids Res.* 14: 1779. As non-limiting examples, an immunoglobulin hinge region can include one of the following amino

acid sequences: DKTHT; CPPC; CPEPKSCDTPPPCPR; (*see, e.g., Glaser et al. (2005) J. Biol. Chem.* 280:41494); ELKTPLGDTTHT; KSCDKTHTCP; KCCVDCP; KYGPPCP; EPKSCDKTHTCPPCP; human IgG1 hinge; ERKCCVECPCP; human IgG2 hinge); ELKTPLGDTTHTCPRCP; human IgG3 hinge; SPNMVPHAHHAQ; human IgG4 hinge); and the like.

[00163] In some embodiments, the hinge region comprises an amino acid sequence of a human IgG1, IgG2, IgG3, or IgG4, hinge region. The hinge region can include one or more amino acid substitutions and/or insertions and/or deletions compared to a wild-type (naturally-occurring) hinge region. For example, His229 of human IgG1 hinge can be substituted with Tyr, so that the hinge region comprises the sequence EPKSCDKTYTCPPCP; *see, e.g., Yan et al. (2012) J. Biol. Chem.* 287:5891.

[00164] In some embodiments, the hinge region comprises an amino acid sequence derived from human CD8; *e.g.,* the hinge region comprises the amino acid sequence:

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:), or a variant thereof.

Conditionally Active Chimeric Antigen Receptors

[00165] In one embodiment, the disclosure provides a conditionally active chimeric antigen receptor (CAR). A CAR generally comprises multiple domains, including a target antigen binding domain, a transmembrane domain, and an intracellular signaling domain. The conditionally active CAR of the present disclosure comprises multiple domains, including a binding moiety, a target antigen binding domain which binds TROP2, a transmembrane domain, and an intracellular signaling domain. In some embodiments, the intracellular signaling domain is a signaling domain of a protein including, but not limited to, ZAP70, CD3 zeta, and 4-1BB.

[00166] In some embodiments, the conditionally active chimeric antigen receptor further comprises a costimulatory domain. In some embodiments, the costimulatory domain is a functional signaling domain of a protein including, but not limited to, OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137), and amino acid sequences thereof having at least one, two, or three modifications but not more than 20, 10, or 5 modifications thereto.

[00167] In some embodiments, the transmembrane domain comprises a transmembrane domain of a protein including, but not limited to, a TCR alpha chain, a TCR beta chain, a TCR zeta chain, a CD3 epsilon TCR subunit, a CD3 gamma TCR subunit, a CD3 delta TCR subunit, CD45, CD4, CDS, CD8, CD9, CD16, CD22, CD33, CD28, CD37, CD64, CD80, CD86, CD134, CD137,

CD154, functional fragments thereof, and amino acid sequences thereof having at least one but not more than 20 modifications thereto.

[00168] In one aspect, the disclosure provides a cell (*e.g.*, T-cell) engineered to express a CAR. In one aspect a cell is transformed with the CAR and the CAR is expressed on the cell surface. In some embodiments, the cell (*e.g.*, T-cell) is transduced with a viral vector encoding a CAR. In some embodiments, the viral vector is a retroviral vector. In some embodiments, the viral vector is a lentiviral vector. In some such embodiments, the cell may stably express the CAR. In another embodiment, the cell (*e.g.*, T cell) is transfected with a nucleic acid, *e.g.*, mRNA, cDNA, DNA, encoding a CAR. In some such embodiments, the cell may transiently express the CAR.

Conditionally Active T-cell Receptor Fusion Proteins

[00169] In one embodiment, the disclosure provides a conditionally active T-cell receptor fusion protein. As used herein, a “T-cell receptor (TCR) fusion protein” or “TFP” includes a recombinant polypeptide derived from the various polypeptides comprising the TCR that is generally capable of i) binding to a surface antigen on target cells and ii) interacting with other polypeptide components of the intact TCR complex, typically when co-located in or on the surface of a T-cell.

[00170] The conditionally active TFP comprises a binding moiety, a TROP2 binding domain, and a T-cell receptor subunit. In some embodiments, the T-cell receptor subunit further comprises at least a portion of a T-cell receptor extracellular domain, a transmembrane domain, and a T-cell receptor intracellular domain. In some embodiments, the transmembrane domain comprises a transmembrane domain of a protein including, but not limited to, a TCR alpha chain, a TCR beta chain, a TCR zeta chain, a CD3 epsilon TCR subunit, a CD3 gamma TCR subunit, a CD3 delta TCR subunit, CD45, CD4, CDS, CD8, CD9, CD16, CD22, CD33, CD28, CD37, CD64, CD80, CD86, CD134, CD137, CD154, functional fragments thereof, or amino acid sequences thereof having at least one, two, or three modifications but not more than 20, 10, or 5 modifications thereto.

[00171] In some embodiments, the T-cell receptor intracellular domain comprises a stimulatory domain. The stimulatory domain may be from T-cell receptor subunit, including but not limited to the beta subunit, alpha subunit, delta subunit, gamma subunit, epsilon subunit, or a combination thereof. In some embodiments, the stimulatory domain comprises an immunoreceptor tyrosine-based activation motif (ITAM) or portion thereof including, but not limited to, CD3 zeta TCR subunit, CD3 epsilon TCR subunit, CD3 gamma TCR subunit, CD3 delta TCR subunit, TCR zeta chain, Fc epsilon receptor 1 chain, Fc epsilon receptor 2 chain, Fc gamma receptor 1 chain, Fc gamma receptor 2a chain, Fc gamma receptor 2b 1 chain, Fc gamma receptor 2b2 chain, Fc gamma receptor 3a chain, Fc gamma receptor 3b chain, Fc beta receptor 1 chain, TYROBP (DAP12), CDS, CD16a, CD16b, CD22, CD23, CD32, CD64, CD79a, CD79b, CD89, CD278, CD66d, functional

fragments thereof, and amino acid sequences thereof having at least one, two, or three modifications but not more than 20, 10, or 5 modifications thereto.

[00172] In some embodiments, the conditionally active TFP further comprises a costimulatory domain. In some embodiments, the costimulatory domain is a functional signaling domain of a protein including, but not limited to, OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137), and amino acid sequences thereof having at least one, two, or three modifications but not more than 20, 10, or 5 modifications thereto.

[00173] In some embodiments, the TROP2 binding domain is connected to the T-cell receptor extracellular domain by a linker sequence. In some instances, the encoded linker sequence comprises (G₄S)_n, wherein n=1 to 4 (SEQ ID NO: 531). In some instances, the encoded linker sequence comprises a long linker (LL) sequence. In some instances, the encoded long linker sequence comprises (G₄S)_n, wherein n=2 to 4 (SEQ ID NO: 532). In some instances, the encoded linker sequence comprises a short linker (SL) sequence. In some instances, the encoded short linker sequence comprises (G₄S)_n, wherein n=1 to 3 (SEQ ID NO: 533).

[00174] In one aspect, the disclosure provides a cell (*e.g.*, T-cell) engineered to express a conditionally active T-cell receptor fusion protein (TFP). In one aspect a cell is transformed with the conditionally active TFP and the conditionally active TFP is expressed on the cell surface. In some embodiments, the cell (*e.g.*, T-cell) is transduced with a viral vector encoding a conditionally active TFP. In some embodiments, the viral vector is a retroviral vector. In some embodiments, the viral vector is a lentiviral vector. In some such embodiments, the cell may stably express the conditionally active TFP. In another embodiment, the cell (*e.g.*, T cell) is transfected with a nucleic acid, *e.g.*, mRNA, cDNA, DNA, encoding a conditionally active TFP. In some such embodiments, the cell may transiently express the conditionally active TFP.

Conditionally Active T-cell Receptors

[00175] In one embodiment, the disclosure provides a conditionally active T-cell receptor. A T-cell receptor generally comprises multiple subunits, including alpha, beta, delta, gamma, epsilon, and zeta subunits. The conditionally active T-cell receptor of the present disclosure comprises a binding moiety. In some embodiments, the binding moiety is attached to a T-cell receptor subunit including, but not limited to, the alpha subunit, beta subunit, or a combination thereof.

[00176] In some embodiments, the binding moiety is capable of masking or masks the binding of the T-cell receptor to its target. In some embodiments, the binding moiety binds to T-cell receptor. In some embodiments, a non-CDR loop provides a binding site for binding of the moiety to the T-cell receptor. In some embodiments, the non-CDR loop provides a binding site specific for T-cell receptor alpha, T-cell receptor beta, or a combination thereof. In some embodiments, the binding

moiety masks binding of the T-cell receptor to its target, *e.g.*, via steric occlusion, via specific intermolecular interactions.

[00177] In one aspect, the disclosure provides a cell (*e.g.*, T-cell) engineered to express a conditionally active T-cell receptor (TCR). In one aspect a cell is transformed with the conditionally active TCR and the conditionally active TCR is expressed on the cell surface. In some embodiments, the cell (*e.g.*, T-cell) is transduced with a viral vector encoding a conditionally active TCR. In some embodiments, the viral vector is a retroviral vector. In some embodiments, the viral vector is a lentiviral vector. In some such embodiments, the cell may stably express the conditionally active TCR. In another embodiment, the cell (*e.g.*, T cell) is transfected with a nucleic acid, *e.g.*, mRNA, cDNA, DNA, encoding a conditionally active TCR. In some such embodiments, the cell may transiently express the conditionally active TCR.

Cells

[00178] In one embodiment, the present disclosure provides a cell comprising the chimeric antigen receptor or the conditionally active chimeric antigen receptor, conditionally active T-cell receptor fusion protein, or conditionally active T-cell receptor of the present disclosure. The cell may be a mammalian cell.

[00179] Suitable mammalian cells include primary cells and immortalized cell lines. Suitable mammalian cell lines include human cell lines, non-human primate cell lines, rodent (*e.g.*, mouse, rat) cell lines, and the like. Suitable mammalian cell lines include, but are not limited to, HeLa cells (*e.g.*, American Type Culture Collection (ATCC) No. CCL-2), CHO cells (*e.g.*, ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (*e.g.*, ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (*e.g.*, ATCC No. CRL-1658), Huh-7 cells, BHK cells (*e.g.*, ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCL1.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, HuT-78, Jurkat, HL-60, NK cell lines (*e.g.*, NKL, NK92, and YTS), and the like.

[00180] In some instances, the cell is not an immortalized cell line, but is instead a cell (*e.g.*, a primary cell) obtained from an individual. For example, in some cases, the cell is an immune cell obtained from an individual. As an example, the cell is a T lymphocyte obtained from an individual. As another example, the cell is a cytotoxic cell obtained from an individual. As another example, the cell is a stem cell or progenitor cell obtained from an individual.

[00181] In recent studies, CAR constructs have been used to direct natural killer (NK) cell activity, reviewed by Hermanson & Kaufman (2015, *Front Immunol* 6:195) and Carlsten & Childs (2015, *Front Immunol* 6:266). Similar to T cells, NK cells can be transfected with CAR expression constructs and used to induce an immune response. Because NK cells do not require HLA

matching, they can be used as allogeneic effector cells (Harmanson & Kaufman, 2015). Also, peripheral blood NK cells (PB-NK), of use for therapy, may be isolated from donors by a simple blood draw. The CAR constructs of use may contain similar elements to those used to make CAR-T cells.

[00182] As such, in some embodiments, the present disclosure provides a cell comprising an NK cell comprising the chimeric antigen receptor, the conditionally active chimeric antigen receptor, conditionally active T-cell receptor fusion protein, or conditionally active T-cell receptor of the present disclosure.

[00183] As discussed above in the context of a conditionally active TROP2 binding protein (*e.g.*, a TROP2 ProTriTAC), in some embodiments, a conditionally active chimeric antigen receptor as described herein has an improved therapeutic index as compared to that of a chimeric antigen receptor comprising the same TROP2 binding domain as the conditionally active variant but is constitutively active instead of being conditionally active. For instance, a TROP2 ProCAR, in some embodiments, has an increased therapeutic index than a TROP2 CAR. The increased, in some embodiments, is from at least about 2-fold to about 1000-fold, such as about 4-fold to about 800-fold, about 6-fold to about 800-fold, about 6-fold to about 600-fold, about 10-fold to about 400-fold, about 20-fold to about 200-fold, about 30-fold to about 150-fold, about 50-fold to about 100-fold. The increase in therapeutic index, in some embodiments, is attributed to the conjugation of the TROP2 binding domain to a binding moiety as described above, with the non-CDR loop and the cleavable linker.

Methods of Generating a Cell Comprising a Conditionally Active Receptor

[00184] The present disclosure provides a method of generating a cell comprising a conditionally active chimeric antigen receptor, T-cell receptor fusion protein, or T-cell receptor. The method generally involves genetically modifying a mammalian cell with an expression vector, or an RNA (*e.g.*, in vitro transcribed RNA), comprising nucleotide sequences encoding a conditionally active chimeric antigen receptor, T-cell receptor fusion protein, or T-cell receptor of the present disclosure. The genetic modification can be carried out *in vivo*, *in vitro*, or *ex vivo*. The cell can be, for example, an immune cell (*e.g.*, a T lymphocyte or NK cell), a stem cell, or a progenitor cell.

[00185] In some cases, the genetic modification is carried out *ex vivo*. For example, a T lymphocyte, a stem cell, or an NK cell is obtained from an individual; and the cell obtained from the individual is genetically modified to express a conditionally active chimeric antigen receptor, T-cell receptor fusion protein, or T-cell receptor of the present disclosure.

Sources of T-Cells

[00186] In some embodiments, a source of T-cells is obtained from a subject. The term “subject,” as used throughout this disclosure, is intended to include living organisms in which an immune response can be elicited (*e.g.*, mammals). Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof. T-cells can be obtained from a number of sources, including, but not limited to, allogenic T cells (*e.g.*, allogeneic donor-derived CAR T cells), natural killer cells (*e.g.*, donor derived natural killer cells), peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present disclosure, any number of T-cell lines available in the art, may be used. In certain embodiments of the present disclosure, T-cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation. In one embodiment, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T-cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis are washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment of the disclosure, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. Initial activation steps in the absence of calcium can lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated “flow-through” centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[00187] In one embodiment, T-cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T-cells, such as CD3⁺, CD28⁺, CD4⁺, CD8⁺, CD45RA⁺, and CD45RO⁺ T-cells, can be further isolated by positive or negative selection techniques. For example, in one embodiment, T-cells are isolated by incubation with anti-CD3/anti-CD28 (*e.g.*, 3×28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the

desired T-cells. In one embodiment, the time period is about 30 minutes. In a further embodiment, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further embodiment, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another embodiment, the time period is 10 to 24 hours. In one embodiment, the incubation time period is 24 hours. Longer incubation times may be used to isolate T-cells in any situation where there are few T-cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immunocompromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8⁺ T-cells. Thus, by simply shortening or lengthening the time T-cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T-cells (as described further herein), subpopulations of T-cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T-cells can be preferentially selected for or against at culture initiation or at other desired time points. Multiple rounds of selection can also be used in the context of this disclosure. In certain embodiments, it may be desirable to perform the selection procedure and use the “unselected” cells in the activation and expansion process. “Unselected” cells can also be subjected to further rounds of selection.

[00188] Enrichment of a T-cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4⁺ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain embodiments, it may be desirable to enrich for or positively select for regulatory T-cells which typically express CD4⁺, CD25⁺, CD62Lhi, GITR⁺, and FoxP3⁺. Alternatively, in certain embodiments, T regulatory cells are depleted by anti-CD25 conjugated beads or other similar method of selection.

[00189] In one embodiment, a T-cell population can be selected that expresses one or more of IFN- γ , TNF α , IL-17A, IL-2, IL-3, IL-4, GM-CSF, IL-10, IL-13, granzyme B, and perforin, or other appropriate molecules, *e.g.*, other cytokines. Methods for screening for cell expression can be determined, *e.g.*, by the methods described in PCT Publication No. WO 2013/126712.

[00190] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (*e.g.*, particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which beads and cells are

mixed together (*e.g.*, increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T-cells, or from samples where there are many tumor cells present (*e.g.*, leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of CD8⁺ T-cells that normally have weaker CD28 expression.

[00191] In another embodiment, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T-cells and surface (*e.g.*, particles such as beads), interactions between the particles and cells are minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, CD4⁺ T-cells express higher levels of CD28 and are more efficiently captured than CD8⁺ T-cells in dilute concentrations. In one embodiment, the concentration of cells used is 5×10^6 /ml. In other embodiments, the concentration used can be from about 1×10^5 /ml to 1×10^6 /ml, and any integer value in between. In other embodiments, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10° C. or at room temperature.

[00192] T-cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80° C. at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20° C. or in

liquid nitrogen. In certain embodiments, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present disclosure.

[00193] Also contemplated in the context of the disclosure is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T-cells, isolated and frozen for later use in T-cell therapy for any number of diseases or conditions that would benefit from T-cell therapy, such as those described herein. In one embodiment a blood sample or an apheresis is taken from a generally healthy subject. In certain embodiments, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain embodiments, the T-cells may be expanded, frozen, and used at a later time. In certain embodiments, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further embodiment, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation.

[00194] In a further embodiment of the present disclosure, T-cells are obtained from a patient directly following treatment that leaves the subject with functional T-cells. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T-cells obtained may be optimal or improved for their ability to expand *ex vivo*. Likewise, following *ex vivo* manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and *in vivo* expansion. Thus, it is contemplated within the context of the present disclosure to collect blood cells, including T-cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in certain embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored,

especially during a defined window of time following therapy. Illustrative cell types include T-cells, B cells, dendritic cells, and other cells of the immune system.

Activation and Expansion of T-Cells

[00195] T-cells may be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005 A1.

[00196] Generally, the T-cells of the disclosure may be expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a costimulatory molecule on the surface of the T-cells. In particular, T-cell populations may be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T-cells, a ligand that binds the accessory molecule is used. For example, a population of T-cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T-cells. To stimulate proliferation of either CD4⁺ T-cells or CD8⁺ T-cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diacclone, Besancon, France) can be used as can other methods commonly known in the art (Berg *et al.*, *Transplant Proc.* 30(8):3975-3977, 1998; Haanen *et al.*, *J. Exp. Med.* 190(9):1319-1328, 1999; Garland *et al.*, *J. Immunol. Meth.* 227(1-2):53-63, 1999).

[00197] In certain embodiments, the primary stimulatory signal and the costimulatory signal for the T-cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (*i.e.*, in “cis” formation) or to separate surfaces (*i.e.*, in “trans” formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one embodiment, the agent providing the costimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In one embodiment, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos. US 20040101519 A1 and US 20060034810 A1 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T-cells in the present disclosure.

[00198] In one embodiment, the two agents are immobilized on beads, either on the same bead, *i.e.*, “cis,” or to separate beads, *i.e.*, “trans.” By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an antigen binding fragment thereof and the agent providing the costimulatory signal is an anti-CD28 antibody or antigen binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one embodiment, a 1:1 ratio of each antibody bound to the beads for CD4⁺ T-cell expansion and T-cell growth is used. In certain embodiments of the present disclosure, a ratio of anti CD3:CD28 antibodies bound to the beads is used such that an increase in T-cell expansion is observed as compared to the expansion observed using a ratio of 1:1. In one particular embodiment an increase of from about 1-fold to about 3-fold is observed as compared to the expansion observed using a ratio of 1:1. In one embodiment, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all integer values there between. In one embodiment of the present disclosure, more anti-CD28 antibody is bound to the particles than anti-CD3 antibody, *i.e.*, the ratio of CD3:CD28 is less than one. In certain embodiments of the disclosure, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1. In one particular embodiment, a 1:100 CD3:CD28 ratio of antibody bound to beads is used. In one embodiment, a 1:75 CD3:CD28 ratio of antibody bound to beads is used. In a further embodiment, a 1:50 CD3:CD28 ratio of antibody bound to beads is used. In one embodiment, a 1:30 CD3:CD28 ratio of antibody bound to beads is used. In one embodiment, a 1:10 CD3:CD28 ratio of antibody bound to beads is used. In one embodiment, a 1:3 CD3:CD28 ratio of antibody bound to the beads is used. In yet one embodiment, a 3:1 CD3:CD28 ratio of antibody bound to the beads is used.

[00199] Ratios of particles to cells from 1:500 to 500:1 and any integer values in between may be used to stimulate T-cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain embodiments the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further embodiments the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T-cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T-cells that result in T-cell stimulation can vary as noted above, however certain values include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 15:1 with one preferred ratio being at least 1:1 particles per T-cell. In one embodiment, a ratio of particles to cells of 1:1 or less is used. In one particular embodiment, a particle: cell ratio is 1:5. In further embodiments, the ratio of particles to cells can be varied depending on the day of stimulation. For example, in one embodiment, the ratio of particles to cells

is from 1:1 to 10:1 on the first day and additional particles are added to the cells every day or every other day thereafter for up to 10 days, at final ratios of from 1:1 to 1:10 (based on cell counts on the day of addition). In one particular embodiment, the ratio of particles to cells is 1:1 on the first day of stimulation and adjusted to 1:5 on the third and fifth days of stimulation. In one embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:5 on the third and fifth days of stimulation. In one embodiment, the ratio of particles to cells is 2:1 on the first day of stimulation and adjusted to 1:10 on the third and fifth days of stimulation. In one embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:10 on the third and fifth days of stimulation. One of skill in the art will appreciate that a variety of other ratios may be suitable for use in the present disclosure. In particular, ratios will vary depending on particle size and on cell size and type.

[00200] In further embodiments of the present disclosure, the cells, such as T-cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further embodiment, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

[00201] By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3×28 beads) to contact the T-cells. In one embodiment the cells (for example, 10^4 to 10^9 T-cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, for example PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. For example, the target cell may be very rare in the sample and comprise only 0.01% of the sample or the entire sample (i.e., 100%) may comprise the target cell of interest. Accordingly, any cell number is within the context of the present disclosure. In certain embodiments, it may be desirable to significantly decrease the volume in which particles and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one embodiment, a concentration of about 2 billion cells/ml is used. In one embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly

express target antigens of interest, such as CD28-negative T-cells. Such populations of cells may have therapeutic value and would be desirable to obtain in certain embodiments. For example, using high concentration of cells allows more efficient selection of CD8⁺ T-cells that normally have weaker CD28 expression.

[00202] In one embodiment of the present disclosure, the mixture may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In one embodiment, the mixture may be cultured for 21 days. In one embodiment of the disclosure the beads and the T-cells are cultured together for about eight days. In one embodiment, the beads and T-cells are cultured together for 2-3 days. Several cycles of stimulation may also be desired such that culture time of T-cells can be 60 days or more. Conditions appropriate for T-cell culture include an appropriate media (*e.g.*, Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (*e.g.*, fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGF β , and TNF- α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α -MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T-cells. Antibiotics, *e.g.*, penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (*e.g.*, 37° C.) and atmosphere (*e.g.*, air plus 5% CO₂).

[00203] T-cells that have been exposed to varied stimulation times may exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T-cell population (TH, CD4⁺) that is greater than the cytotoxic or suppressor T-cell population (TC, CD8⁺). *Ex vivo* expansion of T-cells by stimulating CD3 and CD28 receptors produces a population of T-cells that prior to about days 8-9 consists predominately of TH cells, while after about days 8-9, the population of T-cells comprises an increasingly greater population of TC cells.

TROP2 Binding Protein Modifications

[00204] The TROP2 binding proteins described herein, including TROP2 binding domains (*e.g.*, a TROP2 binding sdAb of this disclosure) and TROP2 targeting multispecific proteins (*e.g.*, a TROP2 targeting trispecific or protrispecific protein as described herein) encompass derivatives or

analogs in which (i) an amino acid is substituted with an amino acid residue that is not one encoded by the genetic code, (ii) the mature polypeptide is fused with another compound such as polyethylene glycol, or (iii) additional amino acids are fused to the protein, such as a leader or secretory sequence or an amino acid sequence for purification of the protein.

[00205] Typical modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

[00206] Modifications are made anywhere in the TROP2 binding proteins described herein, including the peptide backbone, the amino acid side chains, and the amino or carboxyl termini. Certain common peptide modifications that are useful for modification of the TROP2 binding proteins include glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, and ADP-ribosylation.

[00207] In some embodiments, derivatives of the TROP2 binding proteins as described herein comprise immunoreactive modulator derivatives and antigen binding molecules comprising one or more modifications.

[00208] In some embodiments, the TROP2 binding proteins of the disclosure are monovalent or multivalent bivalent, trivalent, *etc.*). As used herein, the term “valency” refers to the number of potential target binding sites associated with an antibody. Each target binding site specifically binds one target molecule or specific position or locus on a target molecule. When an antibody is monovalent, each binding site of the molecule will specifically bind to a single antigen position or epitope. When an antibody comprises more than one target binding site (multivalent), each target binding site may specifically bind the same or different molecules (*e.g.*, may bind to different ligands or different antigens, or different epitopes or positions on the same antigen).

[00209] In some embodiments, the TROP2 binding proteins as set forth above are fused to an Fc region from any species, including but not limited to, human immunoglobulin, such as human IgG1, a human IgG2, a human IgG3, human IgG4, to generate Fc-fusion TROP2 binding proteins. In some embodiments, the Fc-fusion TROP2 binding proteins of this disclosure have extended half-

life compared to an otherwise identical TROP2 binding protein. In some embodiments, the Fc-fusion TROP2 binding proteins of this disclosure contain *inter alia* one or more additional amino acid residue substitutions, mutations and/or modifications, *e.g.*, in the Fc region, which result in a binding protein with preferred characteristics including, but not limited to, altered pharmacokinetics, extended serum half-life, *etc.*

[00210] In some embodiments, such Fc-fused TROP2 binding proteins provide extended half-lives in a mammal, such as in a human, of greater than 5 days, greater than 10 days, greater than 15 days, greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-life, in some cases, results in a higher serum titer which thus reduces the frequency of the administration of the TROP2 binding proteins and/or reduces the concentration of the antibodies to be administered. Binding to human FcRn *in vivo* and serum half-life of human FcRn high affinity binding polypeptides is assayed, in some examples, in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides with a variant Fc region are administered.

[00211] The TROP2 binding proteins, in some cases, are differentially modified during or after production, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, *etc.* Any of numerous chemical modifications are carried out by techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, *etc.*

[00212] Various post-translational modifications of the TROP2 binding proteins also encompassed by the disclosure include, for example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends, attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. Moreover, the TROP2 binding proteins are, in some cases, modified with a detectable label, such as an enzymatic, fluorescent, radioisotopic or affinity label to allow for detection and isolation of the modulator.

Polynucleotides Encoding TROP2 Binding Proteins

[00213] Also provided, in some embodiments, are polynucleotide molecules encoding TROP2 binding proteins described herein. In some embodiments, the polynucleotide molecules are provided as a DNA construct. In other embodiments, the polynucleotide molecules are provided as a messenger RNA transcript.

[00214] The polynucleotide molecules are constructed by known methods such as by combining the genes encoding a single domain TROP2 binding protein or gene encoding various domains of TROP2 binding proteins comprising more than one domain. In some embodiments, the gene encoding the domains are either separated by peptide linkers or, in other embodiments, directly linked by a peptide bond, into a single genetic construct operably linked to a suitable promoter, and optionally a suitable transcription terminator, and expressing it in bacteria or other appropriate expression system such as, for example CHO cells. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. The promoter is selected such that it drives the expression of the polynucleotide in the respective host cell.

[00215] In some embodiments, the polynucleotide coding for a TROP2 binding protein as described herein is inserted into a vector, preferably an expression vector, which represents a further embodiment. This recombinant vector can be constructed according to known methods. Vectors of particular interest include plasmids, phagemids, phage derivatives, virii (*e.g.*, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, lentiviruses, and the like), and cosmids.

[00216] A variety of expression vector/host systems may be utilized to contain and express the polynucleotide encoding the polypeptide of the described TROP2 binding protein. Examples of expression vectors for expression in *E. coli* are pSKK (Le Gall *et al.*, *J Immunol Methods*. (2004) 285(1):111-27) or pcDNA5 (Invitrogen) for expression in mammalian cells.

Thus, the TROP2 binding proteins as described herein, in some embodiments, are produced by introducing a vector encoding the protein as described above into a host cell and culturing said host cell under conditions whereby the protein domains are expressed, may be isolated and, optionally, further purified.

Pharmaceutical Compositions

[00217] Also provided, in some embodiments, are pharmaceutical compositions comprising an anti-TROP2 binding protein described herein, a vector comprising the polynucleotide encoding the polypeptide of the TROP2 binding proteins or a host cell transformed by this vector and at least one pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" includes, but is not limited to, any carrier that does not interfere with the effectiveness of the biological activity of the ingredients and that is not toxic to the patient to whom it is administered. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at

a suitable dose. Preferably, the compositions are sterile. These compositions may also contain adjuvants such as preservative, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents. A further embodiment provides one or more of the above described TROP2 binding proteins packaged in lyophilized form or packaged in an aqueous medium.

[00218] In some embodiments of the pharmaceutical compositions, the TROP2 binding proteins described herein are encapsulated in nanoparticles. In some embodiments, the nanoparticles are fullerenes, liquid crystals, liposome, quantum dots, superparamagnetic nanoparticles, dendrimers, or nanorods. In other embodiments of the pharmaceutical compositions, the TROP2 binding protein is attached to liposomes. In some instances, the TROP2 binding proteins are conjugated to the surface of liposomes. In some instances, the TROP2 binding proteins are encapsulated within the shell of a liposome. In some instances, the liposome is a cationic liposome.

[00219] The TROP2 binding proteins described herein are contemplated for use as a medicament. Administration is effected by different ways, *e.g.* by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. In some embodiments, the route of administration depends on the kind of therapy and the kind of compound contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. Dosages for any one patient depends on many factors, including the patient's size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind of therapy, general health and other drugs being administered concurrently. An "effective dose" refers to amounts of the active ingredient that are sufficient to affect the course and the severity of the disease, leading to the reduction or remission of such pathology and may be determined using known methods.

[00220] In some embodiments, the TROP2 binding proteins of this disclosure are administered at a dosage of up to 10 mg/kg at a frequency of once a week. In some cases, the dosage ranges from about 1 ng/kg to about 10 mg/kg, for example about 1 ng/kg to about 70 ng/kg. In some embodiments, the dose is from about 1 ng/kg to about 10 ng/kg, about 5 ng/kg to about 15 ng/kg, about 12 ng/kg to about 20 ng/kg, about 18 ng/kg to about 30 ng/kg, about 25 ng/kg to about 50 ng/kg, about 35 ng/kg to about 60 ng/kg, about 45 ng/kg to about 70 ng/kg, about 65 ng/kg to about 85 ng/kg, about 80 ng/kg to about 1 µg/kg, about 0.5 µg/kg to about 5 µg/kg, about 2 µg/kg to about 10 µg/kg, about 7 µg/kg to about 15 µg/kg, about 12 µg/kg to about 25 µg/kg, about 20 µg/kg to about 50 µg/kg, about 20 µg/kg to about 60 µg/kg, about 35 µg/kg to about 70 µg/kg, about 45 µg/kg to about 80 µg/kg, about 65 µg/kg to about 90 µg/kg, about 85 µg/kg to about 0.1 mg/kg, about 0.095 mg/kg to about 10 mg/kg, about 20 µg/kg to 540 µg/kg. In some cases, the

dosage is about 0.1 mg/kg to about 0.2 mg/kg; about 0.25 mg/kg to about 0.5 mg/kg, about 0.45 mg/kg to about 1 mg/kg, about 0.75 mg/kg to about 3 mg/kg, about 2.5 mg/kg to about 4 mg/kg, about 3.5 mg/kg to about 5 mg/kg, about 4.5 mg/kg to about 6 mg/kg, about 5.5 mg/kg to about 7 mg/kg, about 6.5 mg/kg to about 8 mg/kg, about 7.5 mg/kg to about 9 mg/kg, or about 8.5 mg/kg to about 10 mg/kg. In some embodiments, the TROP2 binding proteins of this disclosure are administered at a dosage of 1 ng/kg, 2 ng/kg, 5ng/kg, 10ng/kg, 20ng/kg, 30 ng/kg, 40ng/kg, 50 ng/kg, 60 ng/kg, 60 ng/kg, 70 ng/kg, 80 ng/kg, 90 ng/kg, 100 ng/kg, 200 ng/kg, 300 ng/kg, 400 ng/kg, 500 ng/kg, 600 ng/kg, 700 ng/kg, 800 ng/kg, 900ng/kg, 1µg/kg, 2 µg/kg, 5 µg/kg, 10 µg/kg, 12 µg/kg, 15 µg/kg, 20 µg/kg, 22.5 µg/kg, 25 µg/kg, 30 µg/kg, 40 µg/kg, 50 µg/kg, 60 µg/kg, 70 µg/kg, 80 µg/kg, 90 µg/kg, 100 µg/kg, 130 µg/kg, 150 µg/kg, 180 µg/kg, 200 µg/kg, 225 µg/kg, 250 µg/kg, 280 µg/kg, 300 µg/kg, 350 µg/kg, 370 µg/kg, 400 µg/kg, 430 µg/kg, 460 µg/kg, 500 µg/kg, 540 µg/kg, 590 µg/kg, 600 µg/kg, 630 µg/kg, 670 µg/kg, 700 µg/kg, 730 µg/kg, 780 µg/kg, 800 µg/kg, 840 µg/kg, 900 µg/kg, 950 µg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, 4.5 mg/kg, 5 mg/kg, 5.5 mg/kg, 6 mg/kg, 6.5 mg/kg, 7 mg/kg, 7.5 mg/kg, 8 mg/kg, 8.5 mg/kg, 9 mg/kg, 9.5 mg/kg, or 10 mg/kg once every week. The frequency of administration, in some embodiments, is about less than daily, every other day, less than once a day, twice a week, weekly, once in 7 days, once in two weeks, once in three weeks, once in four weeks, or once a month. In some cases, the frequency of administration is weekly. In some cases, the frequency of administration is weekly and the dosage is up to 10 mg/kg. In some cases, duration of administration is from about 1 day to about 4 weeks or longer.

Methods of treatments and Tumor growth reduction properties

[00221] Also provided in certain embodiments are methods of treating a condition associated with malignant cells expressing TROP2 in a subject comprising administering to a subject in need thereof an effective amount of a TROP2 binding domains or multispecific proteins (including conditionally active multispecific proteins) comprising a TROP2 binding domain of this disclosure, or a CAR or a ProCAR comprising a TROP2 binding protein as described herein, or a pharmaceutical composition comprising the same. In some embodiments, the condition is a cancer.

[00222] In another aspect, the disclosure provides a method of inhibiting tumor growth or progression in a subject who has malignant cells expressing TROP2, comprising administering to the subject in need thereof an effective amount of a TROP2 binding domains or multispecific proteins comprising a TROP2 binding domain of this disclosure, or a CAR comprising a TROP2 binding protein as described herein, or a pharmaceutical composition comprising the same. In another aspect, the disclosure provides a method of inhibiting metastasis of malignant cells expressing TROP2 in a subject, comprising administering to the subject in need thereof an effective

amount of a TROP2 binding domains or multispecific proteins comprising a TROP2 binding domain of this disclosure, or a pharmaceutical composition comprising the same. In another aspect, the disclosure provides a method of inducing tumor regression in a subject who has malignant cells expressing TROP2, comprising administering to the subject in need thereof an effective amount of a TROP2 binding domains or multispecific proteins comprising a TROP2 binding domain of this disclosure, or a pharmaceutical composition comprising the same. In some embodiments, the methods as described herein further comprise administering an effective amount of a second therapeutic agent. In some embodiments, the second therapeutic agent is a biotherapeutic agent, for example, an antibody. In some embodiments, the second therapeutic agent is a cytokine, TNF α (Tumor Necrosis Factor alpha), a PAP (phosphatidic acid phosphatase) inhibitor, an oncolytic virus, a kinase inhibitor, an IDO (Indoleamine-pyrrole 2,3-dioxygenase) inhibitor, a glutaminase GLS1 inhibitor, a CAR (Chimeric Antigen Receptor)-T cell or T cell therapy, a TLR (Toll-Like Receptor) Agonist (e.g., TLR3, TLR4, TLR5, TLR7, TLR9), or a tumor vaccine.

[00223] In certain embodiments, the TROP2 binding proteins of the disclosure reduce the growth of tumor cells *in vivo* when administered to a subject who has tumor cells that express TROP2. Measurement of the reduction of the growth of tumor cells can be determined by multiple different methodologies well known in the art. Nonlimiting examples include direct measurement of tumor dimension, measurement of excised tumor mass and comparison to control subjects, measurement via imaging techniques (e.g., CT or MRI) that may or may not use isotopes or luminescent molecules (e.g., luciferase) for enhanced analysis, and the like. In specific embodiments, administration of the TROP2 binding proteins of the disclosure results in a reduction of *in vivo* growth of tumor cells as compared to a control antigen binding agent by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, with an about 100% reduction in tumor growth indicating a complete response and disappearance of the tumor. In further embodiments, administration of the TROP2 binding proteins of the disclosure results in a reduction of *in vivo* growth of tumor cells as compared to a control antigen binding agent by about 50-100%, about 75-100% or about 90-100%. In further embodiments, administration of the TROP2 binding proteins of the disclosure results in a reduction of *in vivo* growth of tumor cells as compared to a control antigen binding agent by about 50-60%, about 60-70%, about 70-80%, about 80-90%, or about 90-100%. In some embodiments, administration of the TROP2 binding proteins of the disclosure results in complete reduction of tumor cell growth, e.g., *in vivo* tumor cell growth within 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 15 days, 20 days, 25 days, 30 days of first administration. In some embodiments, the reduction of growth of tumor cells, e.g., in-

vivo tumor cell growth lasts longer than 1 hour, 2 hours, 5 hours, 10 hours, 23 hours, 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 20 days, 30 days, 35 days, 40 days, 45 days, 50 days, or longer.

[00224] In some embodiments, an active drug and a pro-drug containing identical TROP2 binding proteins of the disclosure reduce the growth of tumor cells comparably *in vivo* when administered to a subject who has tumor cells that express TROP2 in molar equivalent amount.

[00225] In some embodiments, the TROP2 binding proteins of the present disclosure are administered to treat a neoplastic condition. Neoplastic conditions, in some embodiments, are benign or malignant; solid tumors or other blood neoplasia; and, in some embodiments, are selected from the group including, but not limited to: adrenal gland tumors, AIDS-associated cancers, alveolar soft part sarcoma, astrocytic tumors, autonomic ganglia tumors, bladder cancer (squamous cell carcinoma and transitional cell carcinoma), blastocoelic disorders, bone cancer (adamantinoma, aneurismal bone cysts, osteochondroma, osteosarcoma), brain and spinal cord cancers, metastatic brain tumors, breast cancer including triple negative breast cancer, carotid body tumors, cervical cancer, chondrosarcoma, chordoma, chromophobe renal cell carcinoma, clear cell carcinoma, colon cancer, colorectal cancer, cutaneous benign fibrous histiocytomas, desmoplastic small round cell tumors, ependymomas, epithelial disorders, Ewing's tumors, extraskeletal myxoid chondrosarcoma, fibrogenesis imperfecta ossium, fibrous dysplasia of the bone, gallbladder and bile duct cancers, gastric cancer, gastrointestinal, gestational trophoblastic disease, germ cell tumors, glandular disorders, head and neck cancers, hypothalamic, intestinal cancer, islet cell tumors, Kaposi's Sarcoma, kidney cancer (nephroblastoma, papillary renal cell carcinoma), leukemias, lipoma/benign lipomatous tumors, liposarcoma/malignant lipomatous tumors, liver cancer (hepatoblastoma, hepatocellular carcinoma), lymphomas, lung cancers (small cell carcinoma, adenocarcinoma, squamous cell carcinoma, large cell carcinoma etc.), macrophagal disorders, medulloblastoma, melanoma, meningiomas, multiple endocrine neoplasia, multiple myeloma, myelodysplastic syndrome, neuroblastoma, neuroendocrine tumors, ovarian cancer, pancreatic cancers, papillary thyroid carcinomas, parathyroid tumors, pediatric cancers, peripheral nerve sheath tumors, phaeochromocytoma, pituitary tumors, prostate cancer, posterior uveal melanoma, rare hematologic disorders, renal metastatic cancer, rhabdoid tumor, rhabdomyosarcoma, sarcomas, skin cancer, soft-tissue sarcomas, squamous cell cancer, stomach cancer, stromal disorders, synovial sarcoma, testicular cancer, thymic carcinoma, thymoma, thyroid metastatic cancer, and uterine cancers (carcinoma of the cervix, endometrial carcinoma, and leiomyoma).

[00226] In certain embodiments the TROP2 binding proteins of the present disclosure are used as a front-line therapy and administered to subjects who have not previously been treated for the cancerous condition. In other embodiments the TROP2 binding proteins of the present disclosure

are used to treat subjects that have previously been treated (with a TROP2 binding protein of this disclosure or with other anti-cancer agent) and have relapsed or determined to be refractory to the previous treatment. In some embodiments the TROP2 binding proteins of the present disclosure are used to treat subjects that have recurrent tumors.

[00227] In some embodiments, a TROP2 binding protein as described herein, including a multispecific protein, a CAR or a ProCAR as described herein, is administered to treat a cancer with widespread TROP2 expression and prevalence, including, but not limited to, colorectal, prostate, neuroendocrine, thyroid, lung (both non-small cell lung and small cell lung cancers), gastric, ovarian, endometrial, pancreatic, biliary tract and gallbladder cancer, esophageal, breast, an adenocarcinoma, or any combination thereof.

[00228] In some aspects, the TROP2 binding proteins of the present disclosure are administered to treat a proliferative disorder comprising a solid tumor including, but not limited to, adrenal, liver, kidney, bladder, breast, gastric, ovarian, cervical, uterine, esophageal, colorectal, prostate, pancreatic, lung (both small cell and non-small cell), thyroid, carcinomas, sarcomas, glioblastomas, various head and neck tumors, or any combination thereof.

[00229] In some embodiments, the TROP2 binding proteins of the present disclosure are administered to a subject suffering from melanoma. In some embodiments, the TROP2 binding proteins of the present disclosure are used to diagnose, monitor, treat or prevent melanoma. The term “melanoma,” as used herein, includes all types of melanoma including, but not limited to, primary melanoma, malignant melanoma, cutaneous melanoma, extracutaneous melanoma, superficial spreading melanoma, polypoid melanoma, melanocarcinomas, melanoepitheliomas, melanomasarcomas, melanoma *in situ*, nodular malignant melanoma, lentigo maligna melanoma, lentiginous melanoma, lentiginous malignant melanoma, mucosal lentiginous melanoma, mucosal melanoma, acral lentiginous melanoma, soft tissue melanoma, ocular melanoma, invasive melanoma, familial atypical mole and melanoma (FAM-M) syndrome, desmoplastic malignant melanoma, uveal melanoma, or any combination thereof.

[00230] In some embodiments, possible indications for administration of the TROP2 binding proteins of this disclosure or pharmaceutical compositions comprising the same are tumorous diseases especially epithelial cancers/carcinomas such as breast cancer, colon cancer, prostate cancer, head and neck cancer, skin cancer, cancers of the genito-urinary tract, *e.g.*, ovarian cancer, endometrial cancer, cervix cancer and kidney cancer, lung cancer, gastric cancer, cancer of the small intestine, liver cancer, pancreas cancer, gall bladder cancer, cancers of the bile duct, esophagus cancer, cancer of the salivary glands and cancer of the thyroid gland. In some embodiments, the administration of the TROP2 binding proteins of this disclosure or

pharmaceutical compositions comprising the same is indicated for minimal residual disease, such as early solid tumor, advanced solid tumor or metastatic solid tumor, which is characterized by the local and non-local reoccurrence of the tumor caused by the survival of single cells, or any combination thereof.

[00231] In selected aspects a TROP2 binding proteins of the disclosure is incorporated into a chimeric antigen receptors (CAR) and the TROP2 CAR is administered in a CAR based therapy effective at treating a cancer, such as: epithelial cancers/carcinomas such as breast cancer, colon cancer, prostate cancer, head and neck cancer, skin cancer, cancers of the genito-urinary tract, *e.g.*, ovarian cancer, endometrial cancer, cervix cancer and kidney cancer, lung cancer, gastric cancer, cancer of the small intestine, liver cancer, pancreas cancer, gall bladder cancer, cancers of the bile duct, esophagus cancer, cancer of the salivatory glands and cancer of the thyroid gland, small cell lung cancer, non-small cell lung cancer (*e.g.*, squamous cell non-small cell lung cancer or squamous cell small cell lung cancer), large cell neuroendocrine carcinoma (LCNEC), or any combination thereof.

[00232] A chimeric antigen receptor is generally an artificially constructed hybrid protein or polypeptide containing or comprising an antigen binding domain of an antibody linked to a signaling domain (*e.g.*, T-cell signaling or T-cell activation domains). In some embodiments, CARs comprising the TROP2 binding protein of the present disclosure have the ability to redirect the specificity and reactivity of sensitized lymphocytes (*e.g.*, T-cells) toward TROP2 positive target cells in a non-MHC-restricted manner by exploiting the antigen-binding properties of antibodies or antigen binding fragments thereof. The non-MHC-restricted antigen recognition gives T-cells expressing TROP2 CARs the ability to recognize tumorigenic TROP2 independent of antigen processing, thus bypassing a major mechanism of tumor escape. Moreover, when expressed in T-cells, CARs advantageously do not dimerize with endogenous T cell receptor (TCR) alpha and beta chains.

[00233] In some embodiments the disclosed TROP2 binding proteins are administered to refractory patients (*i.e.*, those whose disease recurs during or shortly after completing a course of initial therapy); sensitive patients (*i.e.*, those whose relapse is longer than 2-3 months after primary therapy); or patients exhibiting resistance to a platinum based agent (*e.g.*, carboplatin, cisplatin, oxaliplatin) and/or a taxane (*e.g.*, docetaxel, paclitaxel, larotaxel or cabazitaxel). In another embodiment the disclosed TROP2 CAR treatments are effective at treating ovarian cancer, including ovarian-serous carcinoma and ovarian-papillary serous carcinoma.

[00234] In another embodiment the TROP2 binding proteins of the disclosure, the TROP2 CAR, or the TROP2 sensitized lymphocytes, or any combination thereof are used in maintenance therapy

to reduce or eliminate the chance of tumor recurrence following the initial presentation of the disease. In some cases, the disorder has been treated and the initial tumor mass eliminated, reduced or otherwise ameliorated so the patient is asymptomatic or in remission. At such time the subject is administered pharmaceutically effective amounts of the disclosed the TROP2 binding proteins of the disclosure, the TROP2 CAR, or the TROP2 sensitized lymphocytes, or any combination thereof one or more times regardless of if there is little or no indication of disease using standard diagnostic procedures. In some embodiments, the TROP2 binding proteins of the disclosure, the TROP2 CAR, or the TROP2 sensitized lymphocytes, or any combination thereof is administered on a regular schedule over a period of time, such as weekly, every two weeks, monthly, every six weeks, every two months, every three months every six months or annually, for example, to reduce the potential of disease recurrence. Moreover, such treatments are in some embodiments continued for a period of weeks, months, years or even indefinitely depending on the patient response and clinical and diagnostic parameters.

[00235] In yet another embodiment, the TROP2 binding proteins of the disclosure, the TROP2CAR, or the TROP2 sensitized lymphocytes, or any combination thereof are used to prophylactically or as an adjuvant therapy to prevent or reduce the possibility of tumor metastasis following a debulking procedure. As used in the present disclosure a “debulking procedure,” is means any procedure, technique or method that eliminates, reduces, treats or ameliorates a tumor or tumor proliferation. Exemplary debulking procedures include, but are not limited to, surgery, radiation treatments (*i.e.*, beam radiation), chemotherapy, immunotherapy or ablation. In some embodiments, at appropriate times, the TROP2 binding proteins of the disclosure, the TROP2 CAR, or the TROP2 sensitized lymphocytes, or any combination thereof are administered as suggested by clinical, diagnostic or theranostic procedures to reduce tumor metastasis. In some embodiments, the dosing regimen is accompanied by appropriate diagnostic or monitoring techniques that allow it to be modified.

[00236] Yet other embodiments of the disclosure comprise administering the TROP2 binding protein of the disclosure, the TROP2 CAR, or the TROP2 sensitized lymphocytes, or any combination thereof to subjects that are asymptomatic but at risk of developing a proliferative disorder. That is, in some embodiments, the TROP2 binding protein of the disclosure, the TROP2 CAR, or the TROP2-sensitized lymphocytes, or any combination thereof are used in preventative sense and given to patients that have been examined or tested and have one or more noted risk factors (*e.g.*, genomic indications, family history, *in vivo* or *in vitro* test results, etc.) but have not developed neoplasia. In such cases those skilled in the art would be able to determine an effective dosing regimen through empirical observation or through accepted clinical practices.

[00237] In some embodiments of the methods described herein, the TROP2 binding proteins, or compositions as described herein are administered in combination with an agent for treatment of the particular disease, disorder or condition, also referred to herein as an additional therapeutic agent. Such agents include but are not limited to, therapies involving antibodies, small molecules (*e.g.*, **chemotherapeutics**), **hormones (steroidal, peptide, and the like)**, **radiotherapies (γ -rays, X-rays, and/or the directed delivery of radioisotopes, microwaves, UV radiation and the like)**, gene therapies (*e.g.*, antisense, retroviral therapy and the like) and other immunotherapies. In some embodiments, a TROP2 binding protein as described herein is administered in combination with anti-diarrheal agents, anti-emetic agents, analgesics, opioids and/or non-steroidal anti-inflammatory agents. In some embodiments, a TROP2 binding protein as described herein is administered in combination with anti-cancer agents. Nonlimiting examples of anti-cancer agents that can be used in the various embodiments of the disclosure, including pharmaceutical compositions and dosage forms and kits of the disclosure, include: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropiramine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; interleukin II (including recombinant interleukin II, or rIL2), interferon alpha-2a; interferon alpha-2b; interferon alpha-n1; interferon alpha-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedopa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane;

mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; pipsulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safinol; safinol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; tricycline phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinylicinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinzolidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride. Other examples of anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; broprimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatin; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydridemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl

spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol;
 duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur;
 epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole;
 etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride;
 flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex;
 formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix;
 gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene
 bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofofosine; ilomastat;
 imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-I receptor
 inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol,
4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide
F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin;
 letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone;
 leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide;
 lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine;
 losoxantrone; HMG-CoA reductase inhibitor (such as but not limited to, Lovastatin, Pravastatin,
 Fluvastatin, Statin, Simvastatin, and Atorvastatin); loxoribine; lurtotecan; lutetium texaphyrin;
 lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocil; maspin; matrilysin
 inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase;
 metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double
 stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast
 growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human
 chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol;
 multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard
 anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline;
 N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin;
 nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide;
 nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide;
 okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer;
 ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel
 derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin;
 pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole;
 perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors;
 picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen

activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; tricinibine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; Vitaxin®; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer. Additional anti-cancer drugs are 5-fluorouracil and leucovorin. These two agents are particularly useful when used in methods employing thalidomide and a topoisomerase inhibitor. In some embodiments, the TROP2 binding protein of the present disclosure is used in combination with gemcitabine. In some embodiments, the TROP2 binding protein as described herein is administered before, during, or after surgery.

Methods of detection of TROP2 expression and diagnosis of TROP2 associated cancer

[00238] According to another embodiment of the disclosure, kits for detecting expression of TROP2 *in vitro* or *in vivo* are provided. The kits include the foregoing TROP2 binding protein (e.g., a TROP2 binding protein containing a labeled anti-TROP2 single domain antibody or antigen binding fragments thereof), and one or more compounds for detecting the label. In some

embodiments, the label is selected from the group consisting of a fluorescent label, an enzyme label, a radioactive label, a nuclear magnetic resonance active label, a luminescent label, and a chromophore label.

[00239] In some cases, TROP2 expression is detected in a biological sample. The sample can be any sample, including, but not limited to, tissue from biopsies, autopsies and pathology specimens. Biological samples also include sections of tissues, for example, frozen sections taken for histological purposes. Biological samples further include body fluids, such as blood, serum, plasma, sputum, spinal fluid or urine. A biological sample is typically obtained from a mammal, such as a human or non-human primate.

[00240] In one embodiment, provided is a method of determining if a subject has cancer by contacting a sample from the subject with an anti-TROP2 single domain antibody as disclosed herein; and detecting binding of the single domain antibody to the sample. An increase in binding of the antibody to the sample as compared to binding of the antibody to a control sample identifies the subject as having cancer.

[00241] In another embodiment, provided is a method of confirming a diagnosis of cancer in a subject by contacting a sample from a subject diagnosed with cancer with an anti-TROP2 single domain antibody as disclosed herein; and detecting binding of the antibody to the sample. An increase in binding of the antibody to the sample as compared to binding of the antibody to a control sample confirms the diagnosis of cancer in the subject.

[00242] In some examples of the disclosed methods, the TROP2 single domain antibody is directly labeled. In some examples, the methods further include contacting a second antibody that specifically binds the anti-TROP2 single domain antibody with the sample; and detecting the binding of the second antibody. An increase in binding of the second antibody to the sample as compared to binding of the second antibody to a control sample detects cancer in the subject or confirms the diagnosis of cancer in the subject. In some cases, the cancer is a neuroendocrine cancer, prostate cancer, lung cancer, stomach cancer, squamous cell carcinoma, pancreatic cancer, cholangiocarcinoma, triple negative breast cancer or ovarian cancer (such as epithelial ovarian carcinoma), or any other type of cancer that expresses TROP2. In some examples, the control sample is a sample from a subject without cancer. In particular examples, the sample is a blood or tissue sample.

[00243] In some cases, the antibody that binds (for example specifically binds) TROP2 is directly labeled with a detectable label. In another embodiment, the antibody that binds (for example, specifically binds) TROP2 (the first antibody) is unlabeled and a second antibody or other molecule that can bind the antibody that specifically binds TROP2 is labeled. A second antibody is chosen

such that it is able to specifically bind the specific species and class of the first antibody. For example, if the first antibody is a llama IgG, then the secondary antibody may be an anti-llama-IgG. Other molecules that can bind to antibodies include, without limitation, Protein A and Protein G, both of which are available commercially. Suitable labels for the antibody or secondary antibody are described above, and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, magnetic agents and radioactive materials. Non-limiting examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase. Non-limiting examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin. Non-limiting examples of suitable fluorescent materials include umbelliferon, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, or phycoerythrin. A non-limiting exemplary luminescent material is luminol; a non-limiting exemplary magnetic agent is gadolinium, and non-limiting exemplary radioactive labels include ^{125}I , ^{131}I , ^{35}S or ^3H .

[00244] In an alternative embodiment, TROP2 can be assayed in a biological sample by a competition immunoassay utilizing TROP2 standards labeled with a detectable substance and an unlabeled antibody that specifically binds TROP2. In this assay, the biological sample, the labeled TROP2 standards and the antibody that specifically bind TROP2 are combined and the amount of labeled TROP2 standard bound to the unlabeled antibody is determined. The amount of TROP2 in the biological sample is inversely proportional to the amount of labeled TROP2 standard bound to the antibody that specifically binds TROP2.

[00245] The immunoassays and method disclosed herein can be used for a number of purposes. In one embodiment, the antibody that specifically binds TROP2 may be used to detect the production of TROP2 in cells in cell culture. In another embodiment, the antibody can be used to detect the amount of TROP2 in a biological sample, such as a tissue sample, or a blood or serum sample. In some examples, the TROP2 is cell-surface TROP2. In other examples, the TROP2 is soluble TROP2 (*e.g.*, TROP2 in a cell culture supernatant or soluble TROP2 in a body fluid sample, such as a blood or serum sample).

[00246] In one embodiment, a kit is provided for detecting TROP2 in a biological sample, such as a blood sample or tissue sample. For example, to confirm a cancer diagnosis in a subject, a biopsy can be performed to obtain a tissue sample for histological examination. Alternatively, a blood sample can be obtained to detect the presence of soluble TROP2 protein or fragment. Kits for detecting a polypeptide will typically comprise a single domain antibody, according to the present disclosure, that specifically binds TROP2. In some embodiments, an antibody fragment, such as an

scFv fragment, a VH domain, or a Fab is included in the kit. In a further embodiment, the antibody is labeled (for example, with a fluorescent, radioactive, or an enzymatic label).

[00247] In one embodiment, a kit includes instructional materials disclosing means of use of an antibody that binds TROP2. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk), may be visual (such as video files), or provided through an electronic network, for example, over the internet, World Wide Web, an intranet, or other network. The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means of detecting a label (such as enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

[00248] In one embodiment, the diagnostic kit comprises an immunoassay. Although the details of the immunoassays may vary with the particular format employed, the method of detecting TROP2 in a biological sample generally includes the steps of contacting the biological sample with an antibody which specifically reacts, under immunologically reactive conditions, to a TROP2 polypeptide. The antibody is allowed to specifically bind under immunologically reactive conditions to form an immune complex, and the presence of the immune complex (bound antibody) is detected directly or indirectly.

[00249] Methods of determining the presence or absence of a cell surface marker are well known in the art. For example, the antibodies can be conjugated to other compounds including, but not limited to, enzymes, magnetic beads, colloidal magnetic beads, haptens, fluorochromes, metal compounds, radioactive compounds or drugs. The antibodies can also be utilized in immunoassays such as but not limited to radioimmunoassays (RIAs), ELISA, or immunohistochemical assays. The antibodies can also be used for fluorescence activated cell sorting (FACS). FACS employs a plurality of color channels, low angle and obtuse light-scattering detection channels, and impedance channels, among other more sophisticated levels of detection, to separate or sort cells. *See* U.S. Patent No. 5, 061,620). Any of the single domain antibodies that bind TROP2, as disclosed herein, can be used in these assays. Thus, the antibodies can be used in a conventional immunoassay, including, without limitation, an ELISA, an RIA, FACS, tissue immunohistochemistry, Western blot or immunoprecipitation.

Certain definitions

[00250] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms “a”, “an” and “the” are intended to

include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.”

[00251] The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *e.g.*, the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term “about” should be assumed to mean an acceptable error range for the particular value.

[00252] The terms “individual,” “patient,” or “subject” are used interchangeably. None of the terms require or are limited to situation characterized by the supervision (*e.g.*, constant or intermittent) of a health care worker (*e.g.*, a doctor, a registered nurse, a nurse practitioner, a physician’s assistant, an orderly, or a hospice worker).

[00253] An “antibody” typically refers to a Y-shaped tetrameric protein comprising two heavy (H) and two light (L) polypeptide chains held together by covalent disulfide bonds and non-covalent interactions. Human light chains comprise a variable domain (VL) and a constant domain (CL) wherein the constant domain may be readily classified as kappa or lambda based on amino acid sequence and gene loci. Each heavy chain comprises one variable domain (VH) and a constant region, which in the case of IgG, IgA, and IgD, comprises three domains termed CH1, CH2, and CH3 (IgM and IgE have a fourth domain, CH4). In IgG, IgA, and IgD classes the CH1 and CH2 domains are separated by a flexible hinge region, which is a proline and cysteine rich segment of variable length (generally from about 10 to about 60 amino acids in IgG). The variable domains in both the light and heavy chains are joined to the constant domains by a “J” region of about 12 or more amino acids and the heavy chain also has a “D” region of about 10 additional amino acids. Each class of antibody further comprises inter-chain and intra-chain disulfide bonds formed by paired cysteine residues. There are two types of native disulfide bridges or bonds in immunoglobulin molecules: interchain and intrachain disulfide bonds. The location and number of interchain disulfide bonds vary according to the immunoglobulin class and species. Interchain disulfide bonds are located on the surface of the immunoglobulin, are accessible to solvent and are usually relatively easily reduced. In the human IgG1 isotype there are four interchain disulfide bonds, one from each heavy chain to the light chain and two between the heavy chains. The interchain disulfide bonds are not required for chain association. As is well known the cysteine rich IgG1 hinge region of the heavy chain has generally been held to consist of three parts: an upper

hinge, a core hinge, and a lower hinge. Those skilled in the art will appreciate that that the IgG1 hinge region contain the cysteines in the heavy chain that comprise the interchain disulfide bonds (two heavy/heavy, two heavy/light), which provide structural flexibility that facilitates Fab movements. The interchain disulfide bond between the light and heavy chain of IgG1 are formed between C214 of the kappa or lambda light chain and C220 in the upper hinge region of the heavy chain. The interchain disulfide bonds between the heavy chains are at positions C226 and C229 (all numbered per the EU index according to Kabat, *et al.*, *infra*.)

[00254] As used herein the term “antibody” includes polyclonal antibodies, multiclonal antibodies, monoclonal antibodies, chimeric antibodies, deimmunized, humanized and primatized antibodies, CDR grafted antibodies, human antibodies, recombinantly produced antibodies, intrabodies, multispecific antibodies, bispecific antibodies, monovalent antibodies (*e.g.*, a monovalent IgG), multivalent antibodies, anti-idiotypic antibodies, synthetic antibodies, including muteins and variants thereof, immunospecific antibody fragments such as: hcIgG, a V-NAR, Fv, Fd, Fab, F(ab')₂, F(ab'), Fab2, Fab3 fragments, single-chain fragments (*e.g.*, di-scFv, scFv, scFvFc, scFv-zipper, scFab), disulfide-linked Fvs (sdFv), a Fd fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies such as nanobodies or single variable domain antibodies comprising merely one variable domain such as sdAb (VH, VL, or VHH domains), “r IgG” (“half antibody”), diabodies, single chain diabodies, tandem diabodies (Tandab's), tandem di-scFv, tandem tri-scFv, “minibodies” are in some instances exemplified by a structure which is as follows: (VH-VL-CH3)₂, (scFv-CH3)₂, ((scFv)₂-CH3+CH3), ((scFv)₂-CH3) or (scFv-CH3-scFv)₂, multibodies such as triabodies or tetrabodies; and derivatives thereof including Fc fusions and other modifications, and any other immunoreactive molecule so long as it comprises a domain having a binding site for preferential association or binding with a TROP2-protein. Moreover, unless dictated otherwise by contextual constraints the term further comprises all classes of antibodies (*i.e.*, IgA, IgD, IgE, IgG, and IgM) and all subclasses (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2). Heavy-chain constant domains that correspond to the different classes of antibodies are typically denoted by the corresponding lower-case Greek letter alpha, delta, epsilon, gamma, and mu, respectively. Light chains of the antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains. In some embodiments, the TROP2 binding proteins comprise a heavy chain only antibody, such as a VH or a VHH domain. In some cases, the TROP2 binding proteins comprise a heavy chain only antibody that is an engineered human VH domain. In some examples, the engineered human VH domain is produced by panning of phage display libraries. In some embodiments, the TROP2 binding proteins comprise a VHH. The term “VHH,” as used herein,

refers to single chain antibody binding domain devoid of light chain. In some cases, a VHH is derived from an antibody of the type that can be found in Camelidae or cartilaginous fish which are naturally devoid of light chains or to a synthetic and non-immunized VHH which can be constructed accordingly. Each heavy chain comprises a variable region encoded by V-, D- and J exons. A VHH, in some cases, is a natural VHH, such as a Camelid-derived VHH, or a recombinant protein comprising a heavy chain variable domain. In some embodiments, the VHH is derived from a species selected from the group consisting of camels, llamas, vicunas, guanacos, and cartilaginous fish (such as, but not limited to, sharks). In another embodiment, the VHH is derived from an alpaca (such as, but not limited to, a Huacaya Alpaca or a Suri alpaca).

[00255] As used herein, “Variable region” or “variable domain” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity. The assignment of amino acids to each domain, framework region and CDR is, in some embodiments, in accordance with one of the numbering schemes provided by Kabat *et al.* (1991) Sequences of Proteins of Immunological Interest (5th Ed.), US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242; Chothia *et al.*, 1987, PMID: 3681981; Chothia *et al.*, 1989, PMID: 2687698; MacCallum *et al.*, 1996, PMID: 8876650; or Dubel, Ed. (2007) Handbook of Therapeutic Antibodies, 3rd Ed., Wiley-VCH Verlag GmbH and Co or AbM (Oxford Molecular/MSI Pharmacopeia) unless otherwise noted. In some embodiments of this disclosure, the TROP2 binding proteins comprise heavy chain only antibodies, such as VH or VHH domains, and comprise three CDRs. Such heavy chain only antibodies, in some embodiments, bind TROP2 as a monomer with no dependency on dimerization with a VL (light chain variable) region for optimal binding affinity.

[00256] “Variable domain residue numbering as in Kabat” or “amino acid position numbering as in Kabat,” and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.*, residues 82a, 82b, and 82c, *etc.* according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence. It is not intended that CDRs of the present disclosure necessarily correspond to the Kabat numbering convention.

[00257] The term “Framework” or “FR” residues (or regions) refer to variable domain residues other than the CDR or hypervariable region residues as herein defined. A “human consensus framework” is a framework which represents the most commonly occurring amino acid residue in a selection of human immunoglobulin VL or VH framework sequences.

[00258] The term “epitope,” as used herein, refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[00259] As used herein, the term “percent (%) amino acid sequence identity” with respect to a sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software programs such as EMBOSS MATCHER, EMBOSS WATER, EMBOSS STRETCHER, EMBOSS NEEDLE, EMBOSS LALIGN, BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those

skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[00260] As used herein, “elimination half-time” is used in its ordinary sense, as is described in Goodman and Gillman's *The Pharmaceutical Basis of Therapeutics* 21-25 (Alfred Goodman Gilman, Louis S. Goodman, and Alfred Gilman, eds., 6th ed. 1980). Briefly, the term is meant to encompass a quantitative measure of the time course of drug elimination. The elimination of most drugs is exponential (*i.e.*, follows first-order kinetics), since drug concentrations usually do not approach those required for saturation of the elimination process. The rate of an exponential process may be expressed by its rate constant, k , which expresses the fractional change per unit of time, or by its half-time, $t_{1/2}$ the time required for 50% completion of the process. The units of these two constants are time⁻¹ and time, respectively. A first-order rate constant and the half-time of the reaction are simply related ($k \times t_{1/2} = 0.693$) and may be interchanged accordingly. Since first-order elimination kinetics dictates that a constant fraction of drug is lost per unit time, a plot of the log of drug concentration versus time is linear at all times following the initial distribution phase (*i.e.*, after drug absorption and distribution are complete). The half-time for drug elimination can be accurately determined from such a graph.

[00261] As used herein, the term “binding affinity” refers to the affinity of the proteins described in the disclosure to their binding targets and is expressed numerically using “ K_D ” values. If two or more proteins are indicated to have comparable binding affinities towards their binding targets, then the K_D values for binding of the respective proteins towards their binding targets, are within ± 2 -fold of each other. If two or more proteins are indicated to have comparable binding affinities towards single binding target, then the K_D values for binding of the respective proteins towards said single binding target, are within ± 2 -fold of each other. If a protein is indicated to bind two or more targets with comparable binding affinities, then the K_D values for binding of said protein to the two or more targets are within ± 2 -fold of each other. In general, a higher K_D value corresponds to a weaker binding. In some embodiments, the “ K_D ” is measured by a radiolabeled antigen binding assay (RIA) or surface plasmon resonance assays using a BIACORE™-2000 or a BIACORE™-3000 (BIAcore, Inc., Piscataway, N.J.). In certain embodiments, an “on-rate” or “rate of association” or “association rate” or “kon” and an “off-rate” or “rate of dissociation” or “dissociation rate” or “koff” are also determined with the surface plasmon resonance technique using a BIACORE™-2000 or a BIACORE™-3000 (BIAcore, Inc., Piscataway, N.J.). In additional embodiments, the “ K_D ”, “kon”, and “koff” are measured using the OCTET® Systems (Pall Life Sciences). In an exemplary method for measuring binding affinity using the OCTET® Systems, the

ligand, *e.g.*, biotinylated human or cynomolgus TROP2, is immobilized on the OCTET® streptavidin capillary sensor tip surface which streptavidin tips are then activated according to manufacturer's instructions using about 20-50 µg/ml human or cynomolgus TROP2 protein. A solution of PBS/Casein is also introduced as a blocking agent. For association kinetic measurements, TROP2 binding protein variants are introduced at a concentration ranging from about 10 ng/mL to about 100 µg/mL, about 50 ng/mL to about 5 µg/mL, or about 2 ng/mL to about 20 µg/mL. In some embodiments, the TROP2 binding single domain proteins are used at a concentration ranging from about 2 ng/mL to about 20 µg/mL. Complete dissociation is observed in case of the negative control, assay buffer without the binding proteins. The kinetic parameters of the binding reactions are then determined using an appropriate tool, *e.g.*, ForteBio software.

[00262] As used herein, in some embodiments, “treatment” or “treating” or “treated” refers to therapeutic treatment wherein the object is to slow (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results. For the purposes described herein, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; stabilization (*i.e.*, not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. In other embodiments, “treatment” or “treating” or “treated” refers to prophylactic measures, wherein the object is to delay onset of or reduce severity of an undesired physiological condition, disorder or disease, such as, for example is a person who is predisposed to a disease (*e.g.*, an individual who carries a genetic marker for a disease such as breast cancer).

[00263] A “TriTAC,” a “TROP2 targeting TriTAC,” or a “TROP2 targeting trispecific protein,” as used herein refers to a trispecific binding protein that is not conditionally activated, and comprises a binding moiety that is specific for a bulk serum protein, a first target antigen binding domain, and a second target antigen binding domain, wherein at least one of the first target antigen binding domain and the second target antigen binding domain comprises a TROP2 binding protein as described herein, and at least one of the first target antigen binding domain and the second target antigen binding domain comprises a domain that binds a CD3, such as a human CD3.

[00264] A “ProTriTAC,” or a “TROP2 targeting protrispecific protein,” as used herein refers to a trispecific binding protein that is conditionally activated, and comprises (i) a cleavable linker (*e.g.*,

comprising an amino acid sequence as set forth in SEQ ID NOS: 497-543)), (ii) a binding moiety that is specific for a bulk serum protein and also comprises a masking moiety (*e.g.*, comprising an amino acid sequence as set forth in SEQ ID NO:549) which prohibits the binding of a first target antigen binding domain or a second target antigen binding domain to its target, wherein at least one of the first target antigen binding domain and the second target antigen binding domain comprises a TROP2 binding protein as described herein. The ProTriTAC proteins of this disclosure are, in some cases, activated from a masked state to an active state by cleavage of the cleavable linker, for example, in a protease rich environment, such as in a tumor microenvironment, to form an active drug. An active drug, as provided herein, in some instances, comprises a TROP2 binding domain of the disclosure and a CD3 binding domain of the disclosure. An example of an active drug is provided in SEQ ID NOS: 229-264, or an amino acid sequence that is at least about 75% to 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 229-264 such as about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 229-264.

[00265] A “non-cleavable prodrug,” as used herein refers to a ProTriTAC as described above where the cleavable linker is replaced by a non-cleavable linker (*e.g.*, a linker as in SEQ ID NO: 696). An example of an active drug is provided in SEQ ID NOS: 229-264, or an amino acid sequence that is at least about 75% to 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 229-264, such as about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 229-264.

[00266] In a non-beta-sandwich scaffold (*e.g.*, a DARPIN®, an AFFIMER®, an affibody), the “non-CDR loops” refer to an area that is (1) amenable for sequence randomization to allow engineered specificities to a second antigen, and (2) distal to the primary specificity determining region(s) typically used on the scaffold to allow simultaneous engagement of the scaffold to both antigens without steric interference. For this purpose, the primary specificity determining region(s) can be defined using the framework established in the Skrllec 2015 publication (*Trends in Biotechnol.*, 33:408-418). An excerpt of the framework is listed below:

Table 2	
Scaffold	Primary specificity determining region(s)
Affibody	13 residues in two helices
AFFIMER®	12-36 residues

Table 2	
Scaffold	Primary specificity determining region(s)
Anticalin	Four loops (up to 24 aa)
Avimer	11 residues
Centyrin	13 residues
DARPIN®	7 residues in each n-repeat, or 8 residues in each n-repeat
Fynomer	6 residues in the RT- and n-Src-loop
Kunitz domain	1-2 loops

[00267] “Chimeric antigen receptor” or “CAR” or “CARs”, as used herein, refers to engineered receptors which provide antigen specificity to cells (for example T cells). CARs comprise multiple domains, for example, at least one target antigen binding domain, a transmembrane domain, one or more co-stimulatory domains, and an intracellular signaling domain. Each domain may be connected by a linker. A “ProCAR,” as used herein, refers to a conditionally activatable CAR, comprising a TROP2 binding domain of this disclosure.

EXAMPLES

[00268] The application may be better understood by reference to the following non-limiting examples, which are provided as exemplary embodiments of the application. The following examples are presented in order to more fully illustrate embodiments and should in no way be construed, however, as limiting the broad scope of the application.

Example 1: Screening of phage display library for identification of TROP2 binding domains

[00269] Llamas were immunized with purified TROP2 dimer protein expressed in 293 cells. A phage display library for expression of heavy variable antibody domains was constructed from circulating B cells isolated from the immunized llamas (van der Linden *et al.* 2000. *J Immunol Methods* 240: 185–195). Phage clones were screened for binding to TROP2 dimer by expressing llama anti-TROP2 proteins in E coli, preparing periplasmic extracts, and performing colorimetric ELISAs. 52 unique heavy chain only sequences were identified (SEQ ID NOS: 1 to 52) that produced a signal in the ELISA screening relative to the control with human or cynomolgus monkey TROP2 protein (Table 3). The CDR1, CDR2, and CDR3 sequences for these heavy variable domains are, respectively, SEQ ID NOS: 58 to 109, 115 to 166, and 172 to 223.

Table 3: Binding of Llama Anti-TROP2 Antibodies to Human or Cynomolgus Monkey TROP2 in an ELISA Assay

Sequence name	ELISA huTROP2	ELISA cyTROP2	huTROP2/control	cyTROP2/control
2TRL2	1.0	1.8	6.0	11.1
2TRL4	2.0	4.1	15.5	32.1

Sequence name	ELISA huTROP2	ELISA cyTROP2	huTROP2/control	cyTROP2/control
2TRL11	1.5	2.6	7.3	12.2
2TRL12	1.3	1.9	8.9	13.5
2TRL20	1.2	2.3	7.3	14.1
2TRL22	2.6	3.6	3.2	4.6
2TRL29	1.7	2.7	4.9	7.6
2TRL31	1.5	2.6	9.3	15.7
2TRL33	2.1	3.5	6.3	10.7
2TRL37	1.5	2.5	4.4	7.5
2TRL50	1.4	2.1	3.3	5.0
2TRL55	1.7	2.3	8.7	11.5
2TRL61	0.5	1.0	5.0	10.7
2TRL70	1.2	2.1	4.7	8.4
2TRL74	1.4	2.4	6.0	10.6
2TRL76	3.1	5.4	21.3	37.8
2TRL78	3.7	4.9	4.6	6.1
2TRL84	1.5	2.1	7.5	10.9
2TRL89	0.8	1.3	6.9	10.9
2TRL92	0.8	1.3	3.4	5.3
3TRL3	1.1	2.1	11.4	22.3
3TRL9	0.8	1.4	6.3	10.5
3TRL10	1.7	2.7	7.4	12.1
3TRL16	1.3	2.5	13.1	24.7
3TRL24	1.6	3.0	10.3	18.9
3TRL25	0.7	1.5	9.2	19.5
3TRL29	3.0	5.0	16.5	27.3
3TRL56	1.5	2.5	9.7	16.6
3TRL58	2.8	4.5	12.3	19.3
3TRL68	1.4	2.4	6.0	10.3
3TRL73	3.2	4.2	3.9	5.3
3TRL77	1.6	2.8	10.3	18.4
3TRL78	1.9	2.9	4.8	7.3
3TRL87	1.2	2.9	8.3	20.7
2TRL1	3.6	5.0	21.3	29.5
2TRL5	2.8	4.1	12.1	18.1
2TRL8	3.7	5.1	4.5	6.2
2TRL10	3.9	6.0	9.3	14.1
2TRL27	3.8	5.0	3.8	5.0
2TRL46	4.0	5.0	25.4	31.5
2TRL69	0.8	1.5	8.8	15.4
2TRL94	4.0	4.9	15.4	19.0
2TRL79	1.5	2.1	4.5	6.0
2TRL81	1.8	2.5	5.0	6.8
2TRL18	2.0	4.3	16.1	34.1
3TRL27	0.6	1.1	4.4	8.2

Sequence name	ELISA huTROP2	ELISA cyTROP2	huTROP2/control	cyTROP2/control
2TRL28	0.5	1.4	4.8	14.0
2TRL39	0.7	2.1	5.8	17.8
3TRL82	2.3	4.0	6.6	11.5
2TRL68	0.4	1.1	4.2	10.7
2TRL64	1.9	3.7	6.7	13.1
3TRL53	1.9	2.5	3.5	4.5

[00270] The numerical values in Table 1 represent the absorbance readings for the colorimetric ELISA.

Example 2: Incorporation of TROP2 Binding heavy chain only single domain antibodies into fusion proteins and T cell dependent cellular cytotoxicity assays

[00271] The anti-TROP2 antibody sequences were cloned into DNA constructs for expression of recombinant fusion proteins (SEQ ID NOS: 229 to 258). The coding sequences of the fusion proteins contained a signal peptide for secreted cell expression, a stub to mimic the cleaved version of a conditionally-active T cell engager (SEQ ID NO: 495), humanized anti-CD3 antibody scFv fragment (SEQ ID NO: 494), one of the anti-TROP2 antibody variable domains (SEQ ID NOS: 1 to 52), and a repeat of six histidine sequences (SEQ ID NO: 496). A linker sequence was inserted at the junctions between antibody domains (SEQ ID NO: 497). These anti-CD3/anti-TROP2 fusion protein constructs were transfected into Expi293 cells (Life Technologies). The amount fusion protein in the conditioned media from the transfected Expi293 cells was quantitated using by using an Octet instrument with either Protein A or anti-6xHis tips using a fusion protein of similar molecular weight to the anti-CD3/anti-TROP2 proteins as a standard.

[00272] The conditioned media were tested in a T-cell dependent cellular cytotoxicity assay (TDCC) (Nazarian AA, Archibeque IL, Nguyen YH, Wang P, Sinclair AM, Powers DA. 2015. *J Biomol Screen.* 20:519-27). In this assay, target cells, either luciferase labelled H292 cells or luciferase labelled HT1376 cells, which both express TROP2, were combined with purified human T cells and a titration of anti-CD3/anti-TROP2 fusion protein. If a fusion protein directs T cells to kill the target cells, the signal in a luciferase assay performed at 48 hours after starting the experiment should decrease. FIGS. 1 to 12 have graphs of TDCC viability results. EC50 values from the TDCC assay are listed in Table 4. The most potent molecule had an EC50 value of 37.4 pM with H292 cells and 152 pM with H1376 cells. A negative control for the TDCC assays was an anti-GFP/anti-albumin/anti-CD3 protein, and this protein did not direct the T cells to kill the H292 cells except for slight activity at the highest concentration tested (e.g., FIGS. 5 and 11).

Table 4: EC50 Values for Redirected T Cell Killing of H292 Cells by anti-CD3/anti-TROP2 Fusion Proteins Containing Llama Anti-TROP2 Sequences.

TROP2 Binder	H292 EC50 (pM)	H1376 EC50 (pM)
3TRL3	618	1800
2TRL92	1430	2620
3TRL77	676	2000
2TRL4	353	965
2TRL76	37.4	152
3TRL16	5070	4070
3TRL29	438	996
3TRL58	1960	3640
2TRL31	1350	2740
2TRL70	n/a	n/a
3TRL56	1720	2110
3TRL87	6470	7410
2TRL33	287	714
2TRL1	1650	8150
2TRL5	n/a	n/a
2TRL8	4070	8490
2TRL27	6730	n/a
2TRL46	6800	n/a
2TRL69	n/a	n/a
2TRL94	2780	n/a
2TRL79	59.8	209
2TRL81	208	624
2TRL18	7450	n/a
3TRL27	8750	n/a
2TRL28	n/a	n/a
2TRL39	5330	n/a
3TRL82	n/a	n/a
2TRL68	n/a	n/a
2TRL64	n/a	n/a
3TRL53	94.3	362

[00273] n/a: insufficient activity to calculate an EC50 using the protein concentrations tested.

Example 3: Humanization of TROP2 binding antibodies and T cell dependent cellular cytotoxicity assays

[00274] Four of the llama anti-TROP2 antibody sequences (SEQ ID NOs: 16, 41, 43, and 52) were humanized by grafting their CDR sequences onto human germline sequences, while retaining some llama framework sequences to ensure the antibodies did not lose activity (SEQ ID NOS: 53 to 57). These humanized sequences were cloned into expression constructs for expression as anti-CD3/anti-TROP2 fusion proteins (SEQ IDS 259 to 263) in Expi293 cells, along with their parental constructs, as before. The amount of anti-CD3/anti-TROP2 fusion proteins in the conditioned medium was quantitated as before. Conditioned medium containing the anti-CD3/anti-TROP2 fusion proteins was used in a TDCC assay as before only using the TROP2-expressing, luciferase

labeled cell lines H292, HT1376, or HCC70. The results of the TDCC assay are plotted in FIGS. 13 to 18, and the EC50 values for directed T cell killing are listed in Table 3. Potent directed T cell killing was observed with both the llama and humanized TROP2 antibodies.

[00275] The humanized binder 2TRH79B was used to make a fusion protein containing an anti-ALB domain containing a non-CDR loop mask that blocks CD3 binding connected to the anti-CD3 and anti-TROP2 2TRH79B binder by a cleavable linker (SEQ ID NO: 498). The 2TRH79B binder along with its parental constructs were purified and quantitated. The anti-CD3/anti-TROP2 fusion proteins were used in a TDCC assay using three different luciferase labeled TROP2-expressing cell lines HCC70, HPAF-II, or CAL27. The results of the TDCC assay are plotted in FIGS. 19-21, and the EC50 values for directed T cell killing are listed in Table 5.

[00276] *Table 5: EC50 Values for Redirected T Cell Killing of H292, HT1376, or HCC70 Cells by anti-CD3/anti-Trop2 Fusion Proteins Containing Llama or Humanized Anti-Trop2 Sequences.*

TROP2 Binder	H292 EC50 (pM)	HT1376 EC50 (pM)	HCC70 EC50 (pM)
3TRL53	48.7	20.5	57.7
3TRH53	108.6	53.4	119.1
2TRL76	8.8	4.6	10.1
2TRH76	217	150.8	328.8
2TRL79	9.7	13.3	3.2
2TRH79	23.5	37.5	10.1
2TRL81	8.3	14	2.7
2TRH81	15.3	27.1	4.4

[00277] The humanized binder 2TRH79B is a sequence variant of the 2TRH79 binder. A DNA construct was produced that contains an anti-ALB domain sequence containing a non-CDR loop mask that blocks CD3 binding connected by a cleavable linker (L040) to the anti-CD3 and anti-TROP2 2TRH79B binder sequences (SEQ ID NO: 498). This fusion construct was transfected into Expi293 cells and conditioned media was collected days later. The anti-ALB:anti-CD3:antiTROP2 2TRH79B fusion protein was purified by protein A chromatography using standard binding and elution conditions. Also transfected into Expi293 cells for protein expression were DNA constructs containing an anti-CD3 domain joined by a GGGGSGGGS linker to one of three anti-TROP2 sequences: llama anti-TROP2 binder sequence 2TRL79 and the humanized anti-TROP2 binder sequences 2TRH79 and 2TRH79B. Five days after transfection, the condition media were harvested, and the anti-CD3:anti-TROP2 sequences were purified by immobilized metal affinity chromatography (IMAC) using standard methods. The anti-ALB:anti-CD3:antiTROP2 2TRH79B fusion protein and the anti-CD3:anti-TROP2 sequences were tested in a TDCC assay using three

different luciferase labeled TROP2-expressing cell lines, HCC70, HPAF-II, or CAL27 in the presence of 15 mg/ml human serum albumin. The results of the TDCC assay are plotted in FIGS. 19-21, and the EC50 values for directed T cell killing are listed in Table 4. Amongst the anti-CD3:anti-TROP2 proteins with humanized anti-TROP2 domains, the 2TRH79B protein was more potent and was only 2 to 3 fold less potent than the protein with a llama anti-TROP2 domain. Comparing the EC50 values (Table 6) of the anti-CD3:anti-TROP2 2TRH79B and the anti-ALB:anti-CD3:anti-TROP2 proteins, one can see that the anti-ALB:anti-CD3:anti-TROP protein (2TRH79B L040 ProTriTAC) kills with 36 to 173 fold reduced potency compared to the anti-CD3:anti-TROP2 protein (2TRH79B), demonstrating that the presence of the anti-ALB domain, with its non-CDR loop mask that binds to the anti-CD3 domain, reduces TDCC activity. It is possible that this difference could be even greater if the cell lines used in this assay have protease activity that partially activated the anti-ALB:anti-CD3:anti-TROP protein.

Table 6: EC50 Values for Redirected T Cell Killing of HCC70, HPAF-II, or CAL27 Cells by anti-CD3/anti-Trop2 Fusion Proteins Containing Llama or Humanized Anti-Trop2 Sequences.

TROP2 Binder	HCC70 EC50 (pM)	HPAF-II EC50 (pM)	CAL27 EC50 (pM)
2TRL79	5.7	4.5	nd
2TRH79	25.9	14.8	nd
2TRH79B	16.2	9.7	11.3
2TRH79B L040 ProTriTAC	1112	1681	409.6

[00278] nd = no data.

Example 4: Demonstration of improved tolerability in mouse, conferred by exemplary anti-CD3/anti-TROP2 fusion proteins

[00279] All animal experiments were conducted according to the protocol approved by Institutional Animal Care and Use Committee of Harpoon Therapeutics (protocol number HAR-001-2019). Animals were purchased from The Jackson Laboratory then housed in a pathogen free animal facility located at Harpoon Therapeutics in accordance with IACUC guidelines. All studies were performed in NSG™ (NOD-SCID IL2Rgammanull) female mice 6, 7, or 11 weeks of age with n = 5-10 mice per group. For each experiment, mice were age matched.

[00280] An admixture of Trop2-expressing human tumor cells, either HCC70 (10E6), CAL27 (5E6) or HPAF II (10E6) and activated and expanded human T cells (5E6, 2.5E6, or 5E6, respectively) at an E:T ratio of 1:2 was implanted subcutaneously on the right flank of NSG™ mice (Day 0). For HCC70 xenografts experiment (exp) 1 and 2, treatment followed 7-day post implant (Day 7) once tumors were established (average of 184 mm³ (HCC70 exp 1) or 155 mm³ (HCC70 exp 2). For CAL27 xenografts, treatment began once tumors were established (average of 120

mm³) on Day 4. For HPAF II xenografts, treatment began once tumors were established (average of 128 mm³) on Day 4. Mice were administered a repeat intraperitoneal dose (qdx14) of the negative control, non-Trop2 targeting anti-GFP TriTAC, anti-Trop2 2TRL79 (llama binder) ProTriTAC Linker 040, anti-Trop2 2TRH79 (humanized binder) ProTriTAC Linker 040, or anti-Trop2 2TRH79B (humanized binder) ProTriTAC Linker 040. Tumor growth was monitored at least twice weekly as indicated. Average tumor volume shown was calculated from measurements taken on the final day of each xenograft model study. Statistics represent RM one-way ANOVA with Dunnett post-hoc test, all groups compared to the negative control, anti-GFP TriTAC. The results of each Admix Therapeutic Xenograft Rodent Study are plotted in FIGS. 22-26 and statistics for each xenograft model study are reported in Table 7.

Table 7: P Values from Multiple Rodent Xenograft Studies with anti-CD3/anti-Trop2 Fusion Proteins Containing Human Anti-TROP2 Sequences 2TRL79, 2TRH79, and 2TRH79B L040 ProTriTACs

Xenograft	Test Article	Dose (mg/kg)	Average Tumor Volume (mm ³)	P Value	
HCC70 ¹ (breast)	α Trop2 2TRL79 ProTriTAC	3	0	****	< 0.0001
		0.3	0	****	< 0.0001
		0.03	0	****	< 0.0001
	α GFP TriTAC	0.3	681	n/a	n/a
HCC70 ² (breast)	α Trop2 2TRL79 ProTriTAC	0.1	0	****	< 0.0001
		0.01	14	****	< 0.0001
		0.001	417	ns	ns
	α Trop2 2TRH79 ProTriTAC	1	0	****	< 0.0001
		0.1	0	****	< 0.0001
		0.01	439	ns	ns
		0.001	503	ns	ns
	α GFP TriTAC	0.3	489	n/a	n/a
CAL27 (head and neck)	α Trop2 2TRH79B ProTriTAC	3	0	****	< 0.0001
		0.3	0	****	< 0.0001
		0.03	224	*	0.0215
		0.003	391	ns	ns
	α GFP TriTAC	0.3	426	n/a	n/a
HPAF-II (pancreas)	α Trop2 2TRH79B ProTriTAC	3	0	****	< 0.0001
		0.3	0	****	< 0.0001
		0.03	0	****	< 0.0001
		0.003	ns	ns	ns
	α GFP TriTAC	0.3	956	n/a	n/a

[00281] HCC70 experiment 1 2 HCC70 experiment 2 α : anti; E:T; effector to target cell ratio; n/a: not applicable; ns: not statistically significant ($P > 0.05$); and P: probability value.

Example 5: Comparable, potent anti-tumor activity achieved by molar equivalents of prodrug and active drug

[00282] All animal experiments were conducted according to the protocol approved by Institutional Animal Care and Use Committee of Harpoon Therapeutics (protocol number HAR-001-2019). Animals were purchased from The Jackson Laboratory then housed in a pathogen free animal facility located at Harpoon Therapeutics in accordance with IACUC guidelines. All studies were performed in NSG™ (NOD-SCID IL2R γ manull) female mice 6, 7, or 11 weeks of age with n = 5-10 mice per group. For each experiment, mice were age matched.

An admixture of Trop2-expressing HCC70 (10E6) cells and activated and expanded human T cells (5E6) at an E:T ratio of (2.5 million:5 million) was implanted subcutaneously on the right flank of NSG™ mice (Day 0). For HCC70 xenografts experiment (exp) 1 and 2, treatment followed 7-day post implant (Day 7) once tumors were established (average of 184 mm³ (HCC70 exp 1) or 155 mm³ (HCC70 exp 2). Mice were administered a repeat intraperitoneal dose (qdx14) of the control dose (300 μg/kg), Prodrug (30 μg/kg, 300 μg/kg, or 3000 μg/kg) or the active drug (22.5 μg/kg or 225 μg/kg) respectively. Tumor growth was monitored at least twice weekly as indicated. Average tumor volume shown was calculated from measurements taken on the final day of each xenograft model study. Statistics represent RM one-way ANOVA with Dunnett post-hoc test, all groups compared to the negative control, anti-GFP TriTAC. The results of the Admix Therapeutic Xenograft Rodent Study are plotted in FIG. 27. Results showed molar equivalents of prodrug and active drug showed comparable potent anti-tumor activity (FIG.27).

Example 6: Determination of Binding Activity and Species Cross-Reactivity of TroP2

ProTriTAC Linker 40 molecule As Assessed by Biolayer Interferometry

[00283] Biolayer Interferometry (BLI) is a well-established analytical method used to determine binding kinetics of specific biomolecular interactions. One molecule, the target ligand, is tagged or modified to enable specific capture and presentation to a second molecule, the analyte, in solution. In some uses, the ligand might be expressed as a fusion protein with histidine repeats, an antibody Fc constant domain, or conjugated with a small molecule such as biotin. During the loading phase, the ligand is captured on a glass fiber biosensor tip chemically derivatized with agents capable of binding with high affinity to the tagged ligand i.e., metal chelating nitrilotriacetic acid, an anti-Fc monoclonal antibody, or streptavidin by the previous examples. Using an instrument such as the Octet RED96 (Sartorius), white light is projected down the biosensor tip and reflected from two surfaces, a reference layer and a biocompatible surface with immobilized ligand. The distance of reflection to the reference layer is constant while the distance of reflection from the surface immobilized ligand varies upon association or dissociation of analyte, leading to interference of light

waves resulting in changes in amplitude measured over time. Binding sensorgrams generated from serial dilutions of known analyte concentrations are then fit globally to a one-to-one binding model. This global fit determines the association rate constant (k_{on}) and dissociation rate constant (k_{off}) used to calculate a binding dissociation constant (K_D). Because the streptavidin-biotin interaction is known to be among the highest biomolecular affinities identified, and to minimize confounding contributions of other ligand/surface chemistries, all ligands in this study were biotinylated.

[00284] To better characterize and to help identify relevant toxicological species, the binding kinetics and affinity of a TroP2 ProTriTAC Linker 40 molecule (2TRH79B, were evaluated for tumor-associated calcium signal transducer 2 (Trop2). Biotinylated derivatives of target ligands from human and non-human primate (NHP) species (cyno) were prepared. As such binding of 2TRH79B and 2TRH79 to biotinylated target ligands were evaluated by Biolayer Interferometry (BLI) in the absence or presence of calcium. FIGS.28A-28B demonstrated 2TRH79B binds with comparable affinity to human (FIG.28A) and cynomolgus monkey (cyno) Trop2 (FIG.28B) in the absence or presence of calcium (47-51 nM or 50 nM, respectively). Table 8 summarizes the findings from the binding kinetics study.

Table 8: Binding Kinetics Values of anti-CD3/anti-Trop2 Fusion Proteins Containing Human Anti-TROP2 Sequences 2TRH79B, L040 ProTriTAC With Human and Cyno Trop2

	Human TroP2	Cyno TroP2
k_{on} (1/Ms) e5	0.9	0.8
K_{off} (1/s)	4.1×10^{-3}	5.7×10^{-3}
K_D (nM)	48	73

[00285] Additionally, the binding kinetics and affinity of a second TroP2 ProTriTAC Linker 40 molecule (2TRH79), a related construct was evaluated for human and cyno Trop2 in the absence or presence of calcium by BLI, and the results were similar. 2TRH79 bound with comparable affinity to human and cyno Trop2 in the absence or presence of calcium (168-186 nM or 122-179 nM, respectively) (data not shown).

Example 7: One-month dose escalation/maximum tolerated dose study

[00286] In this dose escalation/maximum tolerated dose study, TroP2 ProTriTAC Linker 40 molecule (2TRH79B) is used for intravenous (slow bolus) injection in Cynomolgus Monkeys.

[00287] The primary objectives of this Good Laboratory Practice (GLP) compliant, escalating repeat dose study were to evaluate the potential toxicity and systemic exposure of TroP2 ProTriTAC, when administered by intravenous (iv) slow bolus injections on Days 1, 8, 15, and 22 at 20, 60, 180, and 540 $\mu\text{g/kg}$, respectively, to cynomolgus monkeys. **FIG. 29** shows the experimental design. In addition, the toxicokinetic (TK) characteristics of TroP2 ProTriTAC were determined. Study

endpoints included: mortality, clinical observations (daily cage side, post-dose, and weekly detailed), body weights, qualitative food consumption, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), bioanalytical and TK parameters, cytokine analysis, organ weights, and macroscopic and microscopic examinations.

[00288] TroP2 ProTriTAC was well-tolerated up to 540 µg/kg, the highest dose tested. PK was assessed by capturing with a biotinylated anti-idiotypic Ab recognizing the αCD3 domain and detecting with a sulfotagged anti-idiotypic antibody raised against the αALB domain. **FIG. 30** demonstrates that the TroP2 ProTriTAC shows favorable pharmacokinetics, half-life and systemic accumulation. The hematological changes consisted of minimally to moderately decreased neutrophils at all dose levels, as well as transient decreases in lymphocytes and basophils in week 3 of the study. There were no notable findings in plasma cytokines (IFNγ, IL-1β, IL-2, IL-6, IL-10, IL-8, TNF-α) throughout the study, no notable findings from coagulation, clinical chemistry, or urinalysis parameters and no notable histopathological findings based on organ weight and gross examination.

SEQUENCE TABLE

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
1	TROP2 antibody variable domain	2TRL2	QVQLQESGGGLAQAGDSLRLSCAASRRTFSSRV MGWFRQAPGKEREFVAGINWSSGTYYVDSVK GRFAISTDDAKNTVYLQMNNLKPEDTAVYYCAA GAPRTWTGGYDYWGQGTQVTVSS
2	TROP2 antibody variable domain	2TRL4	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGVNWGSDATYYADSV KGRFTISTDNAKNTVYLQMDSLKPEDTAVYYCA AGAPRKWTDGYDYWGQGTQVTVSS
3	TROP2 antibody variable domain	2TRL11	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK GRFTISTDNAKNTVYLQMNSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
4	TROP2 antibody variable domain	2TRL12	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK GRFTISTDNAKNTVYLQMNSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
5	TROP2 antibody variable domain	2TRL20	QVQLQESGGGLVQAGDSLRLSCAASGRSFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK GRFTISTDNAKNTVYLQMNSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
6	TROP2 antibody variable domain	2TRL22	QVQLQESGGGLVQAGDSLRLSCAVSGRTFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK GRFTISTDNAKNTVYLQMNSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
7	TROP2 antibody variable domain	2TRL29	QVQLQESGGGVVQAGGSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK GRFTISTDNAKNTVYQLQMNSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
8	TROP2 antibody variable domain	2TRL31	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK GRFTISTDNAKNTVYQLQMNSLKPEDTAVYYCAA GAPRKWTGGYDYWGQGTQVTVSS
9	TROP2 antibody variable domain	2TRL33	QVQLQESGGGLVQAGDSLRLSCAASGRSFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK GRFTISTDNANTVYQLQMNRLKPEDTAVYYCAAG APRKWTDGYAYWGQGTQVTVSS
10	TROP2 antibody variable domain	2TRL37	QVQLQESGGGLVQAGGSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK GRFTISTDNAKNTVYQLQMNSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
11	TROP2 antibody variable domain	2TRL50	QVQLQESGGGLVQAGGSLRLSCAASRRTFSSRV MGWFRQAPGKEREFVAGINWSSTGTYTVDSVK GRFAISTDDAKNTVYQLQMNNLKPEDTAVYYCAA GAPRTWTGGYDYWGQGTQVTVSS
12	TROP2 antibody variable domain	2TRL55	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGINWSTEVTNYADSVK GRFTISTDNAKNTVYQLQMNSLKPEDAAVYYCAA GAPRTWTRGYDYWGQGTQVTVSS
13	TROP2 antibody variable domain	2TRL61	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGANWGS DATYYADSV KGRFTISTDNAKNTVYQLQMDSLKPEDTAVYYCA AGAPRKWTDGYDYWGQGTQVTVSS
14	TROP2 antibody variable domain	2TRL70	QVQLQESGGGLVQAGGSLRLSCAASVRTFSSRV MGWFRQAPGKEREFVAGINWVSGSTYYTDSVK GRFTFSRDNAKNTVYQLQMNSLKPEDTAVYYCAA GAPRTWTDGYDYWGQGTQVTVSS
15	TROP2 antibody variable domain	2TRL74	QVQLQESGGGLVQAGDSLRLSCTASGRSFSSRV MGWFRQAPGKEREFVAGINWSTEVTNYADSVK GRFTISTDNAKNTVYQLQMNSLKPEDAAVYYCAA GAPRTWTRGYDYWGQGTQVTVSS
16	TROP2 antibody variable domain	2TRL76	QVQLQESGGGLVQAGDSLRLSCAASGLTFSSRV MGWFRQYPGKEREFVAGINTGSDATYYADSVK GRFTISTDNAKTISTDNAKNTVYQLQMNSLKPEDT AVYYCAAGAPRKWTDGYDYWGQGTQVTVSS
17	TROP2 antibody variable domain	2TRL78	QVQLQESGGGLAQPGGSLRLSCVASGRTFSSRV MGWFRQAPGKEREFVAGVNWGS DATYYADSV KGRFTISTDNAKNTVYQLQMDSLKPEDTAVYYCA AGAPRKWTDGYDYWGQGTQVTVSS
18	TROP2 antibody variable domain	2TRL84	QVQLQESGGGLAQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
			GRFTISTDNAKNTVYLQMNSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
19	TROP2 antibody variable domain	2TRL89	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSRV MGWLRQAPGKEREVAGINWSSDATYYADSVK GRFTISTDNAKNTVYLQMNSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
20	TROP2 antibody variable domain	2TRL92	QVQLQESGGGLVQAGGSLRLSCAASRRTFSSRV VGWFRQAPGKEREVAGINWSSTGTYYVDSVK RFAISTDDAKNTVYLQMNNLKPEDTAVYYCAAG APPTWTDGYDYWGQGTQVTVSS
21	TROP2 antibody variable domain	3TRL3	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREVGGINWTTDATSYAESVK GRFTISTDNAKNTVYLQMNSLKPEDTAVYYCAA GAPRWTDGYEYWGQGTQVTVSS
22	TROP2 antibody variable domain	3TRL9	QVQLQESGGGLAQAGDSLRLTCAASRRTFSSRV MGWFRQAPGKEREVAGINWSSTGTYYVDSVK GRFAISTDDAKNTVYLQMNNLKPEDTAVYYCAA GAPRTWTGGYDYWGQGTQVTVSS
23	TROP2 antibody variable domain	3TRL10	QVQLQESGGGSVQAGGSLRLSCAASRRTFSSRV MGWFRQAPGKEREVAGINWSSTGTYYVDSVK GRFAISTDDAKNTVYLQMNNLKPEDTAVYYCAA GAPRTWTGGYDYWGQGTQVTVSS
24	TROP2 antibody variable domain	3TRL16	QVQLQESGGGLVQPGESLRLSCAASGRTFSSRV MGWFRQAPGKEREVAGINWSSDATYYADSV GRFTISTDNAKNTVYLQMNSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
25	TROP2 antibody variable domain	3TRL24	QVQLQESGGGLVQAGDSLRLSCAASGRSFSSRV MGWFRQAPGKEREVAGINWSSDATYYADSVK GRFTISTDNAKNTVYLQMNSLKPEDTAVYSCAA GAPRKWTDGYDYWGQGTQVTVSS
26	TROP2 antibody variable domain	3TRL25	QVQLQESGGGLAQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREVAGVNWGSDATYYADSV KGRFTISTDNAKNTVYLQMDSLKPEDTAVYYCA AGAPRKWTDGYDYQGQGTQVTVSS
27	TROP2 antibody variable domain	3TRL29	QVQLQESGGGLVQAGGSLRLSCAASGRTFSSRV MGWFRQAPGKEREVAGVNWGSDATYYADSV KGRFTISTDNAKNTVYLQMDSLKPEDTAVYYCA AGAPRKWTDGYDYWGQGTQVTVSS
28	TROP2 antibody variable domain	3TRL56	QVQLQESGGGLVQTGGSLRLSCAASRRTFSSRV MGWFRQAPGKEREVAGINWSSTGTYYVDSVK GRFAISTDDAKNTVYLQMNNLKPEDTAVYYCAA GAPRTWTGGYDYWGQGTQVTVSS
29	TROP2 antibody variable domain	3TRL58	QVQLQESGGGLVQPGGSLRLSCALSGRTFSSRV MGWFRQAPGKEREVAGVNWGSDATYYADSV KGRFTISTDNAKNTVYLQMDSLKPEDTAVYYCA AGAPRKWTDGYDYWGQGTQVTVSS

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
30	TROP2 antibody variable domain	3TRL68	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSRV MGWFRQAPGKEREFVAGINWSSDVAYYADSVK GRFTISTDNAKNTVYLMNLSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
31	TROP2 antibody variable domain	3TRL73	QVQLQESGGGLVQAGGSLRLSCAASGRSFSSRV MGWFRQAPGKEREFVAGINWSSDATYYADSVK GRFTISTDNAKNTVYLMNLSLKPEDTAVYYCAA GAPRKWTDGYDYWGQGTQVTVSS
32	TROP2 antibody variable domain	3TRL77	QVQLQESGGGLVQPGGSLRLSCAASRRTFSSRVV GWFRQAPGKEREFVAGINWSSTGTYTVDSVKGR FAISTDDAKNTVYLMNNLSLKPEDTAVYYCAAG APPTWTDTRYDYWGQGTQVTVSS
33	TROP2 antibody variable domain	3TRL78	QVQLQESGGGLVQPGGSLRLSCAASRRTFSSRV MGWFRQAPGKEREFVAGINWSSTGTYTVDSVK GRFAISTDDAKNTVYLMNNLSLKPEDTAVYYCAA GAPRTWTGGYDYWGQGTQVTVSS
34	TROP2 antibody variable domain	3TRL87	QVQLQESGGGLVQAGDSLRLSCAASGRSFSSRV MGWFRQAPGKEREFVAGINWSTEVTNYADSVK GRFTISTDNAKNTVYLMNLSLKPEDAAVYYCAA GAPRTWTRGYDYWGQGTQVTVSS
35	TROP2 antibody variable domain	2TRL1	QVQLQESGGGLVQPGGSLRLSCAASGSFTRISAM AWYRQAPGNQRELVASIIDGTTNYAESVKGRFT ISRDNKNTVYLMNNLSLKPEDTAVYYCNVGAR MHWGPTRDYWGQGTQVTVSS
36	TROP2 antibody variable domain	2TRL5	QVQLQESGGGLVQPGGSLRLSCAASGSFFRISAM AWYRQAPGNQRELVASIISDGSANYAESVNGRF TISRDNKNTVYLMNNLSLKPEDTADYYCNVGA RMHWGPTRDYWGQGTQVTVSS
37	TROP2 antibody variable domain	2TRL8	QVQLQESGGGLVQPGGSLRLSCAASGSFTRISAM AWYRQAPGNQRELVASIISDGSTNYAESVKGRFT ISRDNKNTVYLMNNLSLKPEDTAVYSCNVGAR MYWGPTRDYWGQGTQVTVSS
38	TROP2 antibody variable domain	2TRL10	QVQLQESGGGLVQPGGSLRLSCAASGSFTRISAM AWYRQAPGNQRELVASIISDGSTNYAESVKGRFT ISRDNKNTVYLMNNLSLKPEDTAVYYCNVGAR MYWGPTRDYWGQGTQVTVSS
39	TROP2 antibody variable domain	2TRL27	QVQLQESGGGLVQPGGSLRLSCAASGSFFRISAM AWYRQAPGNQRELVASIISDGSTNYVESVKGRFT ISRDNKNTVYLMNNLSLKPEDTAVYYCNVGAR MHWGPTRDYWGQGTQVTVSS
40	TROP2 antibody variable domain	2TRL46	QVQLQESGGGLVQPGGSLRLSCAASGSFTRISAM AWYRQAPGNQRELVASIADGSSNYAESVKGRF TISRDNKNTVYLMNNLSLKPEDTAGYYCNVGA RMHWGPTRDYWGQGTQVTVSS
41	TROP2 antibody variable domain	2TRL69	QVQLQESGGGLVQPGGSLRLSCAASGSFFRISAM AWYRQAPGNQRELVASIISDGSTNYVESVRGRFT

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
			ISRDNAKNTVYLQMNNLKPEDTAVYYCNVGAR MHQGPTRDYWGQGTQVTVSS
42	TROP2 antibody variable domain	2TRL94	QVQLQESGGGLVQPGGSLRLSCAASGSFTRISAM AWYRQAPGNQRELVASIIIDGSTNYAESVKGRFT ISRDNAKNTVYLQMNNLKPEDTAVYYCNVGAR MHWGPTRDYWGQGTQVTVSS
43	TROP2 antibody variable domain	2TRL79	QVQLQESGGGLVQAGGSLRLSCAASGRTFGSYT TGWFRQAPGKEREFVAALSWIGSSTIYPDSVKG RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAG PGITPNPRTYDYWGQGTQVTVSS
44	TROP2 antibody variable domain	2TRL81	QVQLQESGGGLVQAGGSLRLSCAASGRTIGSYTT GWFRQAPGKEREFVAALSWIGSSTIYPDSVKG FTISRDNAKNTVYLQMNSLKPEDTAVYYCAAGP GITPNPRTYDYWGQGTQVTVSS
45	TROP2 antibody variable domain	2TRL18	QVQLQESGGGLVQAGGSLRLSCEVSGGTFSNLG MGWVRQAPGKERELIAGITKSGSSVYYADSVKG RFTISRDNAKNTVFLQMNSLKPADTSVYYCGASP LIGSSWLTDNRNKFYWGQGTQVTVSS
46	TROP2 antibody variable domain	3TRL27	QVQLQESGGGLVQAGGSLRLSCVVSOGGTFSNLA MGWVRQAPGKEREFVVAISKSGSSVYYADSVKG RFTISRDNAKNTVFLQMNSLKPADTSVYYCGASP LIGSSWLTDNRNKFYWGQGTQVTVSS
47	TROP2 antibody variable domain	2TRL28	QVQLQESGGGLAQAGDSLRLSCAASGRTFSSYA MGWFRQAPGKEREFVAISWSDSSTIYADSVKG RFTISTDNAKNTVYLQMNSLKPEDTAVYYCAAT AAYCSGYGCDRARVYDYWGQGTQVTVSS
48	TROP2 antibody variable domain	2TRL39	QVQLQESGGGLVQAGDSLRLSCAASERTFRQYA MGWFRQAPGKEREFVATISWGGRTYYADSVK GRFTISRDNANTVYLQMNSLKPEDTAVYYCAA SNYYCAGYGCYDRASLFDYWGQGTQVTVSS
49	TROP2 antibody variable domain	3TRL82	QVQLQESGGGLVQPGGSLRLSCAASGSIFGINAM GWYRQPGKQRDLVAVTTSAGMTSYADSVKGR FTISRDNAKNTVYLQMNSLKPEDTAVYYCNLRS PYITALNYWGQGTQVTVSS
50	TROP2 antibody variable domain	2TRL68	QVQLQESGGGLVQAGGSLRLSCAASGLTFSNHD MAWFRQAPGKEREFIAAISWGGNTDYVDFVK RFTSSRDNAKNTVYLQMNSLKPEDTALYFCAAV RVAGRSYEHYWGQGTQVTVSS
51	TROP2 antibody variable domain	2TRL64	QVQLQESGGGLVQPGDSLRLSCVASRRTFNMGW FRQAPGKEREFVAAIKESGGDAYYADSVKGRFFI SRDNAKNMVYLQMNNLKPEDTAVYSCAADPRL IIPSGNSYWYWGQGTQVTVSS
52	TROP2 antibody variable domain	3TRL53	QVQLQESGGGLVQAGDSLRLSCAASGRTFSSYA MGWFRQAPGKEREFVAITRSGGSTLYADSVKG RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAS GTGWPLEYEHYDYWGQGTQVTVSS

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
53	TROP2 antibody variable domain	2TRH76	EVQLLESGGGLVQPGGSLRLSCAASGLTFSSRVM GWFRQYPGKEREVAGINTGSDATYYADSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCAAGA PRKWTDGYDYWGQGLTVTVSS
54	TROP2 antibody variable domain	2TRH79	EVQLLESGGGLVQPGGSLRLSCAASGRTFGSYTT GWFRQAPGKEREVFAALSWIGSSTIYPDSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCAAGP GITPNPRTRTYDYWGQGLTVTVSS
55	TROP2 antibody variable domain	2TRH79B	EVQLLESGGGLVQPGGSLRLSCAASGRTFGSYTT GWFRQAPGKEREVFAALSWIGSSTIYPDSVKGR FTISRDNSKNTVYLQMNSLRAEDTAVYYCAAGP GITPNPRTRTYDYWGQGLTVTVSS
56	TROP2 antibody variable domain	2TRH81	EVQLLESGGGLVQPGGSLRLSCAASGRTIGSYTT GWFRQAPGKEREVFAALSWIGSSTIYPDSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCAAGP GITPNPRTRTYDYWGQGLTVTVSS
57	TROP2 antibody variable domain	3TRH53	EVQLLESGGGLVQPGGSLRLSCAASGRTFSSYA MGWFRQAPGKEREVFAAITRSGGSTLYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAS GTGWPLEY EY EYDYWGQGLTVTVSS
58	CDR1	2TRL2	RRTFSSR
59	CDR1	2TRL4	GRTFSSR
60	CDR1	2TRL11	GRTFSSR
61	CDR1	2TRL12	GRTFSSR
62	CDR1	2TRL20	GRSFSSR
63	CDR1	2TRL22	GRTFSSR
64	CDR1	2TRL29	GRTFSSR
65	CDR1	2TRL31	GRTFSSR
66	CDR1	2TRL33	GRSFSSR
67	CDR1	2TRL37	GRTFSSR
68	CDR1	2TRL50	RRTFSSR
69	CDR1	2TRL55	GRTFSSR
70	CDR1	2TRL61	GRTFSSR
71	CDR1	2TRL70	VRTFSSR
72	CDR1	2TRL74	GRSFSSR
73	CDR1	2TRL76	GLTFSSR
74	CDR1	2TRL78	GRTFSSR
75	CDR1	2TRL84	GRTFSSR
76	CDR1	2TRL89	GRTFSSR
77	CDR1	2TRL92	RRTFSSR
78	CDR1	3TRL3	GRTFSSR
79	CDR1	3TRL9	RRTFSSR
80	CDR1	3TRL10	RRTFSSR
81	CDR1	3TRL16	GRTFSSR
82	CDR1	3TRL24	GRSFSSR

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
83	CDR1	3TRL25	GRTFSSR
84	CDR1	3TRL29	GRTFSSR
85	CDR1	3TRL56	RRTFSSR
86	CDR1	3TRL58	GRTFSSR
87	CDR1	3TRL68	GRTFSSR
88	CDR1	3TRL73	GRSFSSR
89	CDR1	3TRL77	RRTFSSR
90	CDR1	3TRL78	RRTFSSR
91	CDR1	3TRL87	GRSFSSR
92	CDR1	2TRL1	GSFTRIS
93	CDR1	2TRL5	GSFFRIS
94	CDR1	2TRL8	GSFTRIS
95	CDR1	2TRL10	GSFTRIS
96	CDR1	2TRL27	GSFFRIS
97	CDR1	2TRL46	GSFTRIS
98	CDR1	2TRL69	GSFFRIS
99	CDR1	2TRL94	GSFTRIS
100	CDR1	2TRL79	GRTFGSY
101	CDR1	2TRL81	GRTIGSY
102	CDR1	2TRL18	GGTFSNL
103	CDR1	3TRL27	GGTFSNL
104	CDR1	2TRL28	GRTFSSY
105	CDR1	2TRL39	ERTFRXY
106	CDR1	3TRL82	GSIFGIN
107	CDR1	2TRL68	GLTFSNH
108	CDR1	2TRL64	RRTFN
109	CDR1	3TRL53	GRTFSSY
110	CDR1	2TRH76	GRTFSSR
111	CDR1	2TRH79	GRTFGSY
112	CDR1	2TRH79B	GRTFGSY
113	CDR1	2TRH81	GRTIGSY
114	CDR1	3TRH53	GRTFSSY
115	CDR2	2TRL2	NWSSTG
116	CDR2	2TRL4	NWGSDA
117	CDR2	2TRL11	NWSSDA
118	CDR2	2TRL12	NWSSDA
119	CDR2	2TRL20	NWSSDA
120	CDR2	2TRL22	NWSSDA
121	CDR2	2TRL29	NWSSDA
122	CDR2	2TRL31	NWSSDA
123	CDR2	2TRL33	NWSSDA
124	CDR2	2TRL37	NWSSDA
125	CDR2	2TRL50	NWSSTG
126	CDR2	2TRL55	NWSTEV

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
127	CDR2	2TRL61	NWGSDA
128	CDR2	2TRL70	NWVSGS
129	CDR2	2TRL74	NWSTEV
130	CDR2	2TRL76	NTGSDA
131	CDR2	2TRL78	NWGSDA
132	CDR2	2TRL84	NWSSDA
133	CDR2	2TRL89	NWSSDA
134	CDR2	2TRL92	NWSSTG
135	CDR2	3TRL3	NWTTDA
136	CDR2	3TRL9	NWSSTG
137	CDR2	3TRL10	NWSSTG
138	CDR2	3TRL16	NWSSDA
139	CDR2	3TRL24	NWSSDA
140	CDR2	3TRL25	NWGSDA
141	CDR2	3TRL29	NWGSDA
142	CDR2	3TRL56	NWSSTG
143	CDR2	3TRL58	NWGSDA
144	CDR2	3TRL68	NWSSDV
145	CDR2	3TRL73	NWSSDA
146	CDR2	3TRL77	NWSSTG
147	CDR2	3TRL78	NWSSTG
148	CDR2	3TRL87	NWSTEV
149	CDR2	2TRL1	IIDGT
150	CDR2	2TRL5	ISDGS
151	CDR2	2TRL8	ISDGS
152	CDR2	2TRL10	ISDGS
153	CDR2	2TRL27	ISDGS
154	CDR2	2TRL46	IADGS
155	CDR2	2TRL69	ISDGS
156	CDR2	2TRL94	IIDGS
157	CDR2	2TRL79	SWIGSS
158	CDR2	2TRL81	SWIGSS
159	CDR2	2TRL18	TKSGSS
160	CDR2	3TRL27	SKSGSS
161	CDR2	2TRL28	SWSDSS
162	CDR2	2TRL39	SWGGR
163	CDR2	3TRL82	TSAGM
164	CDR2	2TRL68	SWGGRN
165	CDR2	2TRL64	KESGGD
166	CDR2	3TRL53	TRSGGS
167	CDR2	2TRH76	NTGSDA
168	CDR2	2TRH79	SWIGSS
169	CDR2	2TRH79B	SWIGSS
170	CDR2	2TRH81	SWIGSS

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
171	CDR2	3TRH53	TRSGGS
172	CDR3	2TRL2	GAPRTWTGGYDY
173	CDR3	2TRL4	GAPRKWTDGYDY
174	CDR3	2TRL11	GAPRKWTDGYDY
175	CDR3	2TRL12	GAPRKWTDGYDY
176	CDR3	2TRL20	GAPRKWTDGYDY
177	CDR3	2TRL22	GAPRKWTDGYDY
178	CDR3	2TRL29	GAPRKWTDGYDY
179	CDR3	2TRL31	GAPRKWTGGYDY
180	CDR3	2TRL33	GAPRKWTDGYAY
181	CDR3	2TRL37	GAPRKWTDGYDY
182	CDR3	2TRL50	GAPRTWTGGYDY
183	CDR3	2TRL55	GAPRTWTRGYDY
184	CDR3	2TRL61	GAPRKWTDGYDY
185	CDR3	2TRL70	GAPRTWTDGYDY
186	CDR3	2TRL74	GAPRTWTRGYDY
187	CDR3	2TRL76	GAPRKWTDGYDY
188	CDR3	2TRL78	GAPRKWTDGYDY
189	CDR3	2TRL84	GAPRKWTDGYDY
190	CDR3	2TRL89	GAPRKWTDGYDY
191	CDR3	2TRL92	GAPPTWTDGYDY
192	CDR3	3TRL3	GAPPRWTDGYEY
193	CDR3	3TRL9	GAPRTWTGGYDY
194	CDR3	3TRL10	GAPRTWTGGYDY
195	CDR3	3TRL16	GAPRKWTDGYDY
196	CDR3	3TRL24	GAPRKWTDGYDY
197	CDR3	3TRL25	GAPRKWTDGYDY
198	CDR3	3TRL29	GAPRKWTDGYDY
199	CDR3	3TRL56	GAPRTWTGGYDY
200	CDR3	3TRL58	GAPRKWTDGYDY
201	CDR3	3TRL68	GAPRKWTDGYDY
202	CDR3	3TRL73	GAPRKWTDGYDY
203	CDR3	3TRL77	GAPPTWTDYDY
204	CDR3	3TRL78	GAPRTWTGGYDY
205	CDR3	3TRL87	GAPRTWTRGYDY
206	CDR3	2TRL1	GARMHWGPTRDY
207	CDR3	2TRL5	GARMHWGPTRDY
208	CDR3	2TRL8	GARMYWGPTRDY
209	CDR3	2TRL10	GARMYWGPTRDY
210	CDR3	2TRL27	GARMHWGPTRDY
211	CDR3	2TRL46	GARMHWGPTRDY
212	CDR3	2TRL69	GARMHXGPTRDY
213	CDR3	2TRL94	GARMHWGPTRDY
214	CDR3	2TRL79	GPGITPNPRTRTYDY

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
215	CDR3	2TRL81	GPGITPNPRTRTYDY
216	CDR3	2TRL18	SPLIGSSWLTDNRKFGY
217	CDR3	3TRL27	SPLIGSSWLTDNRKFGY
218	CDR3	2TRL28	TAYYCSGYGCYDRARVYDY
219	CDR3	2TRL39	SNYYCAGYGCYDRASLFDY
220	CDR3	3TRL82	RSPYITALNY
221	CDR3	2TRL68	VRVAGRSYEHY
222	CDR3	2TRL64	DPRLIIPSGNSYWY
223	CDR3	3TRL53	SGTGWPLEYEYDY
224	CDR3	2TRH76	GAPRKWTDGYDY
225	CDR3	2TRH79	GPGITPNPRTRTYDY
226	CDR3	2TRH79B	GPGITPNPRTRTYDY
227	CDR3	2TRH81	GPGITPNPRTRTYDY
228	CDR3	3TRH53	SGTGWPLEYEYDY
229	anti-CD3/anti-TROP2 fusion protein	3TRL3	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGLTVTVSSGGGSG GGSQVQLQESGGGLVQAGDSLRLSCAASGRFTS SRVMGWFRQAPGKEREVGGINWTTDATSYAES VKGRFTISTDNAKDTVYLMNSLKPEDTAVYYC AAGAPPRWTDGYEYWGQGTQVTVSSHHHHHH*
230	anti-CD3/anti-TROP2 fusion protein	2TRL92	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGLTVTVSSGGGSG GGSQVQLQESGGGLVQAGGSLRLSCAASRRTFS SRVVGWFRQAPGKEREVAGINWSSTGTYYVDS VKGRFAISTDDAKNTVYLMNNLKPEDTAVYYC AAGAPPTWTDGYDYWGQGTQVTVSSHHHHHH*
231	anti-CD3/anti-TROP2 fusion protein	3TRL77	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
			RHANFGNSYISYWAYWGQGT LVT VSSGGGGSG GGSQVQLQESGGGLVQPGGSLRLSCAASRRTFSS RVVGWFRQAPGKEREFVAGINWSSGTYYVDSV KGRFAISTDDAKNTVY LQMNNLKPEDTAVYYCA AGAPPTWTD RYDYWGQGTQVT VSSHHHHH*
232	anti-CD3/anti-TROP2 fusion protein	2TRL4	SGGGGGGSQT VVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTL SGVQPEDEAEYYCTL WYSNRWVF GGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYN NYATYYADQV KDRFTISRDDSKNTAY LQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGT LVT VSSGGGGSG GGSQVQLQESGGGLVQAGDSLRLSCAASGR TFS SRVMGWFRQAPGKEREFVAGVNWGSDATYYA DSVKGRFTISTDNAKNTVY LQMDSLKPEDTAVY YCAAGAPRKWTDGYDYWGQGTQVT VSSHHHH HH*
233	anti-CD3/anti-TROP2 fusion protein	2TRL76	SGGGGGGSQT VVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTL SGVQPEDEAEYYCTL WYSNRWVF GGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYN NYATYYADQV KDRFTISRDDSKNTAY LQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGT LVT VSSGGGGSG GGSQVQLQESGGGLVQAGDSLRLSCAASGLTFSS RVMGWFRQYPGKEREFVAGINTGSDATYYADS VKGRFTISTDNAKTISTDNAKNTVY LQMNSLKPE DTAVYYCAAGAPRKWTDGYDYWGQGTQVT V SHHHHHH*
234	anti-CD3/anti-TROP2 fusion protein	3TRL16	SGGGGGGSQT VVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTL SGVQPEDEAEYYCTL WYSNRWVF GGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYN NYATYYADQV KDRFTISRDDSKNTAY LQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGT LVT VSSGGGGSG GGSQVQLQESGGGLVQPGESLRLSCAASGR TFS RVMGWFRQAPGKEREFVAGINWSSDATYYADS VEGRFTISTDNAKNTVY LQMNSLKPEDTAVYYC AAGAPRKWTDGYDYWGQGTQVT VSSHHHHHH *

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
235	anti-CD3/anti-TROP2 fusion protein	3TRL29	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGLTVTVSSGGGSG GGSQVQLQESGGGLVQAGGSLRLSCAASGRFES SRVMGWFRQAPGKEREVAGVNWGSDATYYA DSVKGRFTISTDNAKNTVYLLQMDSLKPEDTAVY YCAAGAPRKWTDGYDYWGQGTQVTVSSHHHH HH*
236	anti-CD3/anti-TROP2 fusion protein	3TRL58	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGLTVTVSSGGGSG GGSQVQLQESGGGLVQPGGSLRLSCALSGRTFSS RVMGWFRQAPGKEREVAGVNWGSDATYYAD SVKGRFTISTDNAKNTVYLLQMDSLKPEDTAVYY CAAGAPRKWTDGYDYWGQGTQVTVSSHHHHH H*
237	anti-CD3/anti-TROP2 fusion protein	2TRL31	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGLTVTVSSGGGSG GGSQVQLQESGGGLVQAGDSLRLSCAASGRFES SRVMGWFRQAPGKEREVAGINWSSDATYYAD SVKGRFTISTDNAKNTVYLLQMNSLKPEDTAVYY CAAGAPRKWTGGYDYWGQGTQVTVSSHHHHH H*
238	anti-CD3/anti-TROP2 fusion protein	2TRL70	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
			RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQAGGSLRLSCAASVRTFS SRVMGWFRQAPGKEREFVAGINWVSGSTYYTDS VKGRFTFSRDNAKNTVYLMNSLKPEDTAVYYC AAGAPRTWTDGYDYWGQGTQVTVSSHHHHHH*
239	anti-CD3/anti-TROP2 fusion protein	3TRL56	SGGGGGGSQTVVTQEPLSLTVSPGGTVTLTCASST GAVTSGNYPNWWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQAGGSLRLSCAASRRTFSS RVMGWFRQAPGKEREFVAGINWSSTGTYYVDS VKGRFAISTDDAKNTVYLMNNLKPEDTAVYYC AAGAPRTWTGGYDYWGQGTQVTVSSHHHHHH*
240	anti-CD3/anti-TROP2 fusion protein	3TRL87	SGGGGGGSQTVVTQEPLSLTVSPGGTVTLTCASST GAVTSGNYPNWWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQAGDSLRLSCAASGRSFS SRVMGWFRQAPGKEREFVAGINWSTEVTNYADS VKGRFTISTDNAKNTVYLMNSLKPEDAAYYYC AAGAPRTWTRGYDYWGQGTQVTVSSHHHHHH*
241	anti-CD3/anti-TROP2 fusion protein	2TRL33	SGGGGGGSQTVVTQEPLSLTVSPGGTVTLTCASST GAVTSGNYPNWWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQAGDSLRLSCAASGRSFS SRVMGWFRQAPGKEREFVAGINWSSDATYYAD SVKGRFTISTDNANTVYLMNRLKPEDTAVYYC AAGAPRKWTDGYAYWGQGTQVTVSSHHHHHH *
242	anti-CD3/anti-TROP2 fusion protein	2TRL1	SGGGGGGSQTVVTQEPLSLTVSPGGTVTLTCASST GAVTSGNYPNWWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGKLTVLGGGGSGGGGSGGGGS

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
			EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGLTVTVSSGGGGSG GGSQVQLQESGGGLVQPGGSLRLSCAASGSFTRI SAMAWYRQAPGNQRELVASIIDGTTNYAESVK GRFTISRDNKNTVYLQMNNLKPEDTAVYYCNV GARMHWGPTRDYWGQGTQVTVSSHHHHHH*
243	anti-CD3/anti-TROP2 fusion protein	2TRL5	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGLTVTVSSGGGGSG GGSQVQLQESGGGLVQPGGSLRLSCAASGSFFRI SAMAWYRQAPGNQRELVASIISDGSANYAESVN GRFTISRDNKNTVYLQMNNLKPEDTADYYCNV GARMHWGPTRDYWGQGTQVTVSSHHHHHH*
244	anti-CD3/anti-TROP2 fusion protein	2TRL8	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGLTVTVSSGGGGSG GGSQVQLQESGGGLVQPGGSLRLSCAASGSFTRI SAMAWYRQAPGNQRELVASIISDGSTNYAESVK GRFTISRDNKNTVYLQMNNLKPEDTAVYSCN VGARMYWGPTRDYWGQGTQVTVSSHHHHHH*
245	anti-CD3/anti-TROP2 fusion protein	2TRL27	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGLTVTVSSGGGGSG GGSQVQLQESGGGLVQPGGSLRLSCAASGSFFRI SAMAWYRQAPGNQRELVASIISDGSTNYVESVK GRFTISRDNKNTVYLQMNNLKPEDTAVYYCNV GARMHWGPTRDYWGQGTQVTVSSHHHHHH*

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
246	anti-CD3/anti-TROP2 fusion protein	2TRL46	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFVGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQPGGSLRLSCAASGSFTRI SAMAWYRQAPGNQRELVASIADGSSNYAESVK GRFTISRDNKNTVYLMNNLKPEDTAGYYCNV GARMHWGPTRDYGQGTQVTVSSHHHHHH*
247	anti-CD3/anti-TROP2 fusion protein	2TRL69	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFVGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQPGGSLRLSCAASGSFFRI SAMAWYRQAPGNQRELVASIISDGSTNYVESVR GRFTISRDNKNTVYLMNNLKPEDTAVYYCNV GARMHQGPTRDYGQGTQVTVSSHHHHHH*
248	anti-CD3/anti-TROP2 fusion protein	2TRL94	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFVGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQPGGSLRLSCAASGSFTRI SAMAWYRQAPGNQRELVASIIDGSTNYAESVK GRFTISRDNKNTVYLMNNLKPEDTAVYYCN VGARMHWGPTRDYGQGTQVTVSSHHHHHH*
249	anti-CD3/anti-TROP2 fusion protein	2TRL79	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFVGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQAGGSLRLSCAASGRFTG SYTTGWFRQAPGKEREFVAALSWIGSSTYYPDSV

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
			KGRFTISRDNKNTVYLMNSLKPEDTAVYYCA AGPGITPNPRTRTYDYWGQGTQVTVSSHHHHHH *
250	anti-CD3/anti-TROP2 fusion protein	2TRL81	SGGGGGGSQTVVTQEPLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTQVTVSSGGGGSG GGSQVQLQESGGGLVQAGGSLRLSCAASGRTIGS YTTGWFRQAPGKEREFVAALSWIGSSTYYPDVS KGRFTISRDNKNTVYLMNSLKPEDTAVYYCA AGPGITPNPRTRTYDYWGQGTQVTVSSHHHHHH *
251	anti-CD3/anti-TROP2 fusion protein	2TRL18	SGGGGGGSQTVVTQEPLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTQVTVSSGGGGSG GGSQVQLQESGGGLVQAGGSLRLSCEVSGGTFS NLGMGWVRQAPGKERELIAGITKSGSSVYYADS VKGRFTISRDNKNTVFLQMNSLKPADTSVYYC GASPLIGSSWLTDRNKFGYWGQGTQVTVSSHHH HHH*
252	anti-CD3/anti-TROP2 fusion protein	3TRL27	SGGGGGGSQTVVTQEPLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTQVTVSSGGGGSG GGSQVQLQESGGGLVQAGGSLRLSCVVSAGGTFS NLAMGWVRQAPGKEREFVVAISKSGSSVYYADS VKGRFTISRDNKNTVFLQMNSLKPADTSVYYC GASPLIGSSWLTDRNKFGYWGQGTQVTVSSHHH HHH*
253	anti-CD3/anti-TROP2 fusion protein	2TRL28	SGGGGGGSQTVVTQEPLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS

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			EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLAQAGDSLRLSCAASGRFTS SYAMGWFRQAPGKREFVAAISWSDSSSTYYADS VKGRFTISTDNAKNTVYLQMNSLKPEDTAVYYC AATAYYCSGYGCYDRARVYDYWGQGTQVTVSS HHHHHH*
254	anti-CD3/anti-TROP2 fusion protein	2TRL39	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQAGDSLRLSCAASERTFR QYAMGWFRQAPGKREFVATISWSSGRTYYAD SVKGRFTISRDNAAENTVYLQMNSLKPEDTAVYY CAASNYYCAGYGCYDRASLFDYWGQGTQVTVS SHHHHHH*
255	anti-CD3/anti-TROP2 fusion protein	3TRL82	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQPGGSLRLSCAASGSIFGI NAMGWYRQGPQKQKQDLVAVTTSAGMTSYADS VKGRFTISRDNAAKNTVYLQMNSLKPEDTAVYYC NLRSPYITALNYWGQGTQVTVSSHHHHHHH*
256	anti-CD3/anti-TROP2 fusion protein	2TRL68	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGGSG GGSQVQLQESGGGLVQAGGSLRLSCAASGLTFS NHDMAWFRQAPGKREFIAAISWGGNTDYVDF VKGRFTSSRDNAKNTVYLQMNSLKPEDTALYFC AAVRVAGRSYEHYWGQGTQVTVSSHHHHHHH*

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
257	anti-CD3/anti-TROP2 fusion protein	2TRL64	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGSG GGSQVQLQESGGGLVQPGDSLRLSCVASRRTFN MGWFRQAPGKEREFVAAIKESGGDAYYADSVK GRFFISRDNAMVMVYLQMNNLKPEDTAVYSCAA DPRLIIPSGNSYWYWGQGTQVTVSSHHHHHH*
258	anti-CD3/anti-TROP2 fusion protein	3TRL53	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGSG GGSQVQLQESGGGLVQAGDSLRLSCAASGRFTS SYAMGWFRQAPGKEREFVAAITRSGGSTLYADS VKGRFTISRDNAMNTVYLQMNSLKPEDTAVYYC AASGTGWPLEY EY EYDYWGQGTQVTVSSHHHH HH*
259	anti-CD3/anti-TROP2 fusion protein	2TRH76	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGSG GGSEVQLLES GGGLVQPGGSLRLSCAASGLTFSS RVMGWFRQYPGKEREFVAGINTGSDATYYADS VKGRFTISRDNAMNTLYLQMNSLRAEDTAVYYC AAGAPRKWTDGYDYWGQGTLLTVVSSHHHHHH*
260	anti-CD3/anti-TROP2 fusion protein	2TRH79	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQQKPGQAPRGLIGGTKFLVPG TPARFSGSLLGGKAALTLSGVQPEDEAEYYCTL WYSNRWVFGGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGTLLTVSSGGGSG GGSEVQLLES GGGLVQPGGSLRLSCAASGRFTFS

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
			YTTGWFRQAPGKEREFAALSWIGSSTYYPDSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA AGPGITPNPRTRTYDYWGQGT LTVSSH HHHHHH *
261	anti-CD3/anti-TROP2 fusion protein	2TRH79B	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQKPGQAPRGLIGG TKFLVPG TPARFSGSLLGGKAALTL SGVQPEDEAEYYCTL WYSNRWVF GGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLS CAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGT LTVSSGGGSG GGSEVQLLES GGGLVQPGGSLRLSCAASGRTFGS YTTGWFRQAPGKEREFAALSWIGSSTYYPDSV KGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCA AGPGITPNPRTRTYDYWGQGT LTVSSH HHHHHH *
262	anti-CD3/anti-TROP2 fusion protein	2TRH81	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQKPGQAPRGLIGG TKFLVPG TPARFSGSLLGGKAALTL SGVQPEDEAEYYCTL WYSNRWVF GGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLS CAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGT LTVSSGGGSG GGSEVQLLES GGGLVQPGGSLRLSCAASGR TIGS YTTGWFRQAPGKEREFAALSWIGSSTYYPDSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA AGPGITPNPRTRTYDYWGQGT LTVSSH HHHHHH *
263	anti-CD3/anti-TROP2 fusion protein	3TRH53	SGGGGGGSQTVVTQEPSLTVSPGGTVTLTCASST GAVTSGNYPNWVQKPGQAPRGLIGG TKFLVPG TPARFSGSLLGGKAALTL SGVQPEDEAEYYCTL WYSNRWVF GGGTKLTVLGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLS CAASGFTFNKYAI NWVRQAPGKGLEWVARIRSKYNNYATYYADQV KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHANFGNSYISYWAYWGQGT LTVSSGGGSG GGSEVQLLES GGGLVQPGGSLRLSCAASGR TFS YAMGWFRQAPGKEREFAAITRSGGSTLYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA ASGTGWPLEY EYDYWGQGT LTVSSH HHHHHH H*
264	cleavable anti-ALB/anti-	2TRH79B	EVQLVESGGGLVQPGNSLRLSCAASGFTFSKFG MSWVRQGGGGGLDGNEEPGGLEWVSSISGSGR DTLYADSVKGRFTISRDN AKTTLYLQMNSLRPED

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	CD3/anti-TROP2 fusion protein		TAVYYCTIGGSLSVSSQGTLTVSSGGGGPQAST GRSGGGGGGSQTVVTQEPSLTVSPGGTVTLTCAS STGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLV PGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCT LWYSNRWVFGGGTKLTVLGGGGSGGGSGGGG SEVQLVESGGGLVQPGGSLKLSCAASGFTFNKY AINWVRQAPGKGLEWVARIRSKYNNYATYYAD QVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYY CVRHANFGNSYISYWAYWGQGTTLTVSSGGGG SGGGSEVQLLES GGGLVQPGGSLTLSCAASGRTF GSYTTGWFRQAPGKEREFVAALSWIGSSTYYPDS VKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYC AAGPGITPNPRTRTYDYWGQGTTLTVSSHHHHH H*
265	framework 1	2TRL2	QVQLQESGGGLAQAGDSLRLSCAAS
266	framework 1	2TRL4	QVQLQESGGGLVQAGDSLRLSCAAS
267	framework 1	2TRL11	QVQLQESGGGLVQAGDSLRLSCAAS
268	framework 1	2TRL12	QVQLQESGGGLVQAGDSLRLSCAAS
269	framework 1	2TRL20	QVQLQESGGGLVQAGDSLRLSCAAS
270	framework 1	2TRL22	QVQLQESGGGLVQAGDSLRLSCAVS
271	framework 1	2TRL29	QVQLQESGGGVVQAGGSLRLSCAAS
272	framework 1	2TRL31	QVQLQESGGGLVQAGDSLRLSCAAS
273	framework 1	2TRL33	QVQLQESGGGLVQAGDSLRLSCAAS
274	framework 1	2TRL37	QVQLQESGGGLVQAGGSLRLSCAAS
275	framework 1	2TRL50	QVQLQESGGGLVQAGGSLRLSCAAS
276	framework 1	2TRL55	QVQLQESGGGLVQAGDSLRLSCAAS
277	framework 1	2TRL61	QVQLQESGGGLVQAGDSLRLSCAAS
278	framework 1	2TRL70	QVQLQESGGGLVQAGGSLRLSCAAS
279	framework 1	2TRL74	QVQLQESGGGLVQAGDSLRLSCTAS
280	framework 1	2TRL76	QVQLQESGGGLVQAGDSLRLSCAAS
281	framework 1	2TRL78	QVQLQESGGGLAQPGGSLRLSCVAS
282	framework 1	2TRL84	QVQLQESGGGLAQAGDSLRLSCAAS
283	framework 1	2TRL89	QVQLQESGGGLVQAGDSLRLSCAAS
284	framework 1	2TRL92	QVQLQESGGGLVQAGGSLRLSCAAS
285	framework 1	3TRL3	QVQLQESGGGLVQAGDSLRLSCAAS
286	framework 1	3TRL9	QVQLQESGGGLAQAGDSLRLTCAAS
287	framework 1	3TRL10	QVQLQESGGGSVQAGGSLRLSCAAS
288	framework 1	3TRL16	QVQLQESGGGLVQPGESLRLSCAAS
289	framework 1	3TRL24	QVQLQESGGGLVQAGDSLRLSCAAS
290	framework 1	3TRL25	QVQLQESGGGLAQAGDSLRLSCAAS
291	framework 1	3TRL29	QVQLQESGGGLVQAGGSLRLSCAAS
292	framework 1	3TRL56	QVQLQESGGGLVQTGGSLRLSCAAS
293	framework 1	3TRL58	QVQLQESGGGLVQPGGSLRLSCALS
294	framework 1	3TRL68	QVQLQESGGGLVQAGDSLRLSCAAS

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
295	framework 1	3TRL73	QVQLQESGGGLVQAGGSLRLSCAAS
296	framework 1	3TRL77	QVQLQESGGGLVQPGGSLRLSCAAS
297	framework 1	3TRL78	QVQLQESGGGLVQPGGSLRLSCAAS
298	framework 1	3TRL87	QVQLQESGGGLVQAGDSLRLSCAAS
299	framework 1	2TRL1	QVQLQESGGGLVQPGGSLRLSCAAS
300	framework 1	2TRL5	QVQLQESGGGLVQPGGSLRLSCAAS
301	framework 1	2TRL8	QVQLQESGGGLVQPGGSLRLSCAAS
302	framework 1	2TRL10	QVQLQESGGGLVQPGGSLRLSCAAS
303	framework 1	2TRL27	QVQLQESGGGLVQPGGSLRLSCAAS
304	framework 1	2TRL46	QVQLQESGGGLVQPGGSLRLSCAAS
305	framework 1	2TRL69	QVQLQESGGGLVQPGGSLRLSCAAS
306	framework 1	2TRL94	QVQLQESGGGLVQPGGSLRLSCAAS
307	framework 1	2TRL79	QVQLQESGGGLVQAGGSLRLSCAAS
308	framework 1	2TRL81	QVQLQESGGGLVQAGGSLRLSCAAS
309	framework 1	2TRL18	QVQLQESGGGLVQAGGSLRLSCEVS
310	framework 1	3TRL27	QVQLQESGGGLVQAGGSLRLSCVVS
311	framework 1	2TRL28	QVQLQESGGGLAQAGDSLRLSCAAS
312	framework 1	2TRL39	QVQLQESGGGLVQAGDSLRLSCAAS
313	framework 1	3TRL82	QVQLQESGGGLVQPGGSLRLSCAAS
314	framework 1	2TRL68	QVQLQESGGGLVQAGGSLRLSCAAS
315	framework 1	2TRL64	QVQLQESGGGLVQPGDSLRLSCVAS
316	framework 1	3TRL53	QVQLQESGGGLVQAGDSLRLSCAAS
317	framework 1	2TRH76	EVQLLESGGGLVQPGGSLRLSCAAS
318	framework 1	2TRH79	EVQLLESGGGLVQPGGSLRLSCAAS
319	framework 1	2TRH79B	EVQLLESGGGLVQPGGSLRLSCAAS
320	framework 1	2TRH81	EVQLLESGGGLVQPGGSLRLSCAAS
321	framework 1	3TRH53	EVQLLESGGGLVQPGGSLRLSCAAS
322	framework 2	2TRL2	VMGWFRQAPGKEREVAGI
323	framework 2	2TRL4	VMGWFRQAPGKEREVAGV
324	framework 2	2TRL11	VMGWFRQAPGKEREVAGI
325	framework 2	2TRL12	VMGWFRQAPGKEREVAGI
326	framework 2	2TRL20	VMGWFRQAPGKEREVAGI
327	framework 2	2TRL22	VMGWFRQAPGKEREVAGI
328	framework 2	2TRL29	VMGWFRQAPGKEREVAGI
329	framework 2	2TRL31	VMGWFRQAPGKEREVAGI
330	framework 2	2TRL33	VMGWFRQAPGKEREVAGI
331	framework 2	2TRL37	VMGWFRQAPGKEREVAGI
332	framework 2	2TRL50	VMGWFRQAPGKEREVAGI
333	framework 2	2TRL55	VMGWFRQAPGKEREVAGI
334	framework 2	2TRL61	VMGWFRQAPGKEREVAGA
335	framework 2	2TRL70	VMGWFRQAPGKEREVAGI
336	framework 2	2TRL74	VMGWFRQAPGKEREVAGI
337	framework 2	2TRL76	VMGWFRQYPGKEREVAGI
338	framework 2	2TRL78	VMGWFRQAPGKEREVAGV

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
339	framework 2	2TRL84	VMGWFRQAPGKEREVAGI
340	framework 2	2TRL89	VMGWLRQAPGKEREVAGI
341	framework 2	2TRL92	VVGWFRQAPGKEREVAGI
342	framework 2	3TRL3	VMGWFRQAPGKEREVGGI
343	framework 2	3TRL9	VMGWFRQAPGKEREVAGI
344	framework 2	3TRL10	VMGWFRQAPGKEREVAGI
345	framework 2	3TRL16	VMGWFRQAPGKEREVAGI
346	framework 2	3TRL24	VMGWFRQAPGKEREVAGI
347	framework 2	3TRL25	VMGWFRQAPGKEREVAGV
348	framework 2	3TRL29	VMGWFRQAPGKEREVAGV
349	framework 2	3TRL56	VMGWFRQAPGKEREVAGI
350	framework 2	3TRL58	VMGWFRQAPGKEREVAGV
351	framework 2	3TRL68	VMGWFRQAPGKEREVAGI
352	framework 2	3TRL73	VMGWFRQAPGKEREVAGI
353	framework 2	3TRL77	VVGWFRQAPGKEREVAGI
354	framework 2	3TRL78	VMGWFRQAPGKEREVAGI
355	framework 2	3TRL87	VMGWFRQAPGKEREVAGI
356	framework 2	2TRL1	AMAWYRQAPGNQRELVASI
357	framework 2	2TRL5	AMAWYRQAPGNQRELVASI
358	framework 2	2TRL8	AMAWYRQAPGNQRELVASI
359	framework 2	2TRL10	AMAWYRQAPGNQRELVASI
360	framework 2	2TRL27	AMAWYRQAPGNQRELVASI
361	framework 2	2TRL46	AMAWYRQAPGNQRELVASI
362	framework 2	2TRL69	AMAWYRQAPGNQRELVASI
363	framework 2	2TRL94	AMAWYRQAPGNQRELVASI
364	framework 2	2TRL79	TTGWFRQAPGKEREVAAAL
365	framework 2	2TRL81	TTGWFRQAPGKEREVAAAL
366	framework 2	2TRL18	GMGWVRQAPGKERELIAGI
367	framework 2	3TRL27	AMGWVRQAPGKEREVVAI
368	framework 2	2TRL28	AMGWFRQAPGKEREVAAI
369	framework 2	2TRL39	AMGWFRQAPGKEREVATI
370	framework 2	3TRL82	AMGWYRQGPQKQRDVAVT
371	framework 2	2TRL68	DMAWFRQAPGKEREVIAAI
372	framework 2	2TRL64	MGWFRQAPGKEREVAAI
373	framework 2	3TRL53	AMGWFRQAPGKEREVAAI
374	framework 2	2TRH76	VMGWFRQYYPGKEREVAGI
375	framework 2	2TRH79	TTGWFRQAPGKEREVAAAL
376	framework 2	2TRH79B	TTGWFRQAPGKEREVAAAL
377	framework 2	2TRH81	TTGWFRQAPGKEREVAAAL
378	framework 2	3TRH53	AMGWFRQAPGKEREVAAI
379	framework 3	2TRL2	TYVVDVSVKGRFAISTDDAKNTVYLQMNNLKPED TAVYYCAA
380	framework 3	2TRL4	TYVADSVKGRFTISTDNAKNTVYLQMDSLKPED TAVYYCAA

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
381	framework 3	2TRL11	TYYADSVKGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA
382	framework 3	2TRL12	TYYADSVKGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA
383	framework 3	2TRL20	TYYADSVKGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA
384	framework 3	2TRL22	TYYADSVKGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA
385	framework 3	2TRL29	TYYADSVKGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA
386	framework 3	2TRL31	TYYADSVKGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA
387	framework 3	2TRL33	TYYADSVKGRFTISTDNANTVYLQMNRLKPEDT AVYYCAA
388	framework 3	2TRL37	TYYADSVKGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA
389	framework 3	2TRL50	TYYVDSVKGRFAISTDDAKNTVYLQMNNLKPED TAVYYCAA
390	framework 3	2TRL55	TNYADSVKGRFTISTDNAKNTVYLQMNSLKPED AAVYYCAA
391	framework 3	2TRL61	TYYADSVKGRFTISTDNAKNTVYLQMDSLKPED TAVYYCAA
392	framework 3	2TRL70	TYYTDSVKGRFTFSRDNANTVYLQMNSLKPED TAVYYCAA
393	framework 3	2TRL74	TNYADSVKGRFTISTDNAKNTVYLQMNSLKPED AAVYYCAA
394	framework 3	2TRL76	TYYADSVKGRFTISTDNAKTISTDNAKNTVYLQ MNSLKPEDTAVYYCAA
395	framework 3	2TRL78	TYYADSVKGRFTISTDNAKNTVYLQMDSLKPED TAVYYCAA
396	framework 3	2TRL84	TYYADSVKGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA
397	framework 3	2TRL89	TYYADSVKGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA
398	framework 3	2TRL92	TYYVDSVKGRFAISTDDAKNTVYLQMNNLKPED TAVYYCAA
399	framework 3	3TRL3	TSYAESVKGRFTISTDNAKDTVYLQMNSLKPEDT AVYYCAA
400	framework 3	3TRL9	TYYVDSVKGRFAISTDDAKNTVYLQMNNLKPED TAVYYCAA
401	framework 3	3TRL10	TYYVDSVKGRFAISTDDAKNTVYLQMNNLKPED TAVYYCAA
402	framework 3	3TRL16	TYYADSVEGRFTISTDNAKNTVYLQMNSLKPED TAVYYCAA

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
403	framework 3	3TRL24	TYYADSVKGRFTISTDNAKNTVYLMNSLKPED TAVYSCAA
404	framework 3	3TRL25	TYYADSVKGRFTISTDNAKNTVYLMDSLKPED TAVYYCAA
405	framework 3	3TRL29	TYYADSVKGRFTISTDNAKNTVYLMDSLKPED TAVYYCAA
406	framework 3	3TRL56	TYYVDSVKGRFAISTDDAKNTVYLMNNLKPED TAVYYCAA
407	framework 3	3TRL58	TYYADSVKGRFTISTDNAKNTVYLMDSLKPED TAVYYCAA
408	framework 3	3TRL68	AYYADSVKGRFTISTDNAKNTVYLMNSLKPED TAVYYCAA
409	framework 3	3TRL73	TYYADSVKGRFTISTDNAKNTVYLMNSLKPED TAVYYCAA
410	framework 3	3TRL77	TYYVDSVKGRFAISTDDAKNTVYLMNNLKPED TAVYYCAA
411	framework 3	3TRL78	TYYVDSVKGRFAISTDDAKNTVYLMNNLKPED TAVYYCAA
412	framework 3	3TRL87	TNYADSVKGRFTISTDNAKNTVYLMNSLKPED AAVYYCAA
413	framework 3	2TRL1	TNYAESVKGRFTISRDNKNTVYLMNNLKPED TAVYYCNV
414	framework 3	2TRL5	ANYAESVNGRFTISRDNKNTVYLMNNLKPED TADYYCNV
415	framework 3	2TRL8	TNYAESVKGRFTISRDNKNTVYLMNNLKPED TAVYSCNV
416	framework 3	2TRL10	TNYAESVKGRFTISRDNKNTAYLMNNLKPED TAVYYCNV
417	framework 3	2TRL27	TNYVESVKGRFTISRDNKNTVYLMNNLKPED TAVYYCNV
418	framework 3	2TRL46	SNYAESVKGRFTISRDNKNTVYLMNNLKPED TAGYYCNV
419	framework 3	2TRL69	TNYVESVRGRFTISRDNKNTVYLMNNLKPED TAVYYCNV
420	framework 3	2TRL94	TNYAESVKGRFTISRDNKNTVYLMNNLKPED TAVYYCNV
421	framework 3	2TRL79	TYYPDSVKGRFTISRDNKNTVYLMNSLKPED TAVYYCAA
422	framework 3	2TRL81	TYYPDSVKGRFTISRDNKNTVYLMNSLKPED TAVYYCAA
423	framework 3	2TRL18	VYYADSVKGRFTISRDNKNTVFLQMNSLKPAD TSVYYCGA
424	framework 3	3TRL27	VYYADSVKGRFTISRDNKNTVFLQMNSLKPAD TSVYYCGA

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
425	framework 3	2TRL28	TYYADSVKGRFTISTDNAKNTVYLMNSLKPED TAVYYCAA
426	framework 3	2TRL39	TYYADSVKGRFTISRDNAAENTVYLMNSLKPED TAVYYCAA
427	framework 3	3TRL82	TSYADSVKGRFTISRDNAAKNTVYLMNSLKPED TAVYYCNL
428	framework 3	2TRL68	TDYVDFVKGRFTSSRDNAKNTVYLMNSLKPED TALYFCAA
429	framework 3	2TRL64	AYYADSVKGRFFISRDNAAKNTVYLMNSLKPED DTAVYSCAA
430	framework 3	3TRL53	TLYADSVKGRFTISRDNAAKNTVYLMNSLKPED TAVYYCAA
431	framework 3	2TRH76	TYYADSVKGRFTISRDNASKNTLYLMNSLRAED TAVYYCAA
432	framework 3	2TRH79	TYYPDSVKGRFTISRDNASKNTLYLMNSLRAEDT AVYYCAA
433	framework 3	2TRH79B	TYYPDSVKGRFTISRDNASKNTVYLMNSLRAED TAVYYCAA
434	framework 3	2TRH81	TYYPDSVKGRFTISRDNASKNTLYLMNSLRAEDT AVYYCAA
435	framework 3	3TRH53	TLYADSVKGRFTISRDNASKNTLYLMNSLRAEDT AVYYCAA
436	framework 4	2TRL2	WGQGTQVTVSS
437	framework 4	2TRL4	WGQGTQVTVSS
438	framework 4	2TRL11	WGQGTQVTVSS
439	framework 4	2TRL12	WGQGTQVTVSS
440	framework 4	2TRL20	WGQGTQVTVSS
441	framework 4	2TRL22	WGQGTQVTVSS
442	framework 4	2TRL29	WGQGTQVTVSS
443	framework 4	2TRL31	WGQGTQVTVSS
444	framework 4	2TRL33	WGQGTQVTVSS
445	framework 4	2TRL37	WGQGTQVTVSS
446	framework 4	2TRL50	WGQGTQVTVSS
447	framework 4	2TRL55	WGQGTQVTVSS
448	framework 4	2TRL61	WGQGTQVTVSS
449	framework 4	2TRL70	WGQGTQVTVSS
450	framework 4	2TRL74	WGQGTQVTVSS
451	framework 4	2TRL76	WGQGTQVTVSS
452	framework 4	2TRL78	WGQGTQVTVSS
453	framework 4	2TRL84	WGQGTQVTVSS
454	framework 4	2TRL89	WGQGTQVTVSS
455	framework 4	2TRL92	WGQGTQVTVSS
456	framework 4	3TRL3	WGQGTQVTVSS
457	framework 4	3TRL9	WGQGTQVTVSS

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
458	framework 4	3TRL10	WGQGTQVTVSS
459	framework 4	3TRL16	WGQGTQVTVSS
460	framework 4	3TRL24	WGQGTQVTVSS
461	framework 4	3TRL25	XGQGTQVTVSS
462	framework 4	3TRL29	WGQGTQVTVSS
463	framework 4	3TRL56	WGQGTQVTVSS
464	framework 4	3TRL58	WGQGTQVTVSS
465	framework 4	3TRL68	WGQGTQVTVSS
466	framework 4	3TRL73	WGQGTQVTVSS
467	framework 4	3TRL77	WGQGTQVTVSS
468	framework 4	3TRL78	WGQGTQVTVSS
469	framework 4	3TRL87	WGQGTQVTVSS
470	framework 4	2TRL1	WGQGTQVTVSS
471	framework 4	2TRL5	WGQGTQVTVSS
472	framework 4	2TRL8	WGQGTQVTVSS
473	framework 4	2TRL10	WGQGTQVTVSS
474	framework 4	2TRL27	WGQGTQVTVSS
475	framework 4	2TRL46	WGQGTQVTVSS
476	framework 4	2TRL69	WGQGTQVTVSS
477	framework 4	2TRL94	WGQGTQVTVSS
478	framework 4	2TRL79	WGQGTQVTVSS
479	framework 4	2TRL81	WGQGTQVTVSS
480	framework 4	2TRL18	WGQGTQVTVSS
481	framework 4	3TRL27	WGQGTQVTVSS
482	framework 4	2TRL28	WGQGTQVTVSS
483	framework 4	2TRL39	WGQGTQVTVSS
484	framework 4	3TRL82	WGQGTQVTVSS
485	framework 4	2TRL68	WGQGTQVTVSS
486	framework 4	2TRL64	WGQGTQVTVSS
487	framework 4	3TRL53	WGQGTQVTVSS
488	framework 4	2TRH76	WGQGTSLTVSS
489	framework 4	2TRH79	WGQGTSLTVSS
490	framework 4	2TRH79B	WGQGTSLTVSS
491	framework 4	2TRH81	WGQGTSLTVSS
492	framework 4	3TRH53	WGQGTSLTVSS
493	sdAb	Anti-albumin	EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFG MSWVRQAPGKGLEWVSSISGSGRDTLYADSVKG RFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGG SLSVSSQGTLTVSS
494	scFv	Anti-CD3	QTVVTQEPSLTVSPGGTVTLTCASSTGAVTSGNY PNWVQQKPGQAPRGLIGGTKFLVPGTPARFSGSL LGGKAALTLSGVQPEDEAEYYCTLWYSNRWVF GGGTKLTVLGGGSGGGGSGGGGSEVQLVESG GGLVQPGGSLKLSCAASGFTFNKYAINWVRQAP

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
			GKGLEWVARIRSKYNNYATYYADQVKDRFTISR DDSKNTAYLQMNNLKTEDTAVYYCVRHANFGN SYISYWAYWGQGLVTVSS
495	stub	L040 stub	SGGGGGGS
496	tag	HHHHHH	HHHHHH
497	linker	G4SG3S	GGGSGGGGS
498	cleavable linker	L040 Full Linker Sequence v2	GGGGPQASTGRSGGGGGGS
499		protease cleavable linker 4	GGGGPLGLQAGVVGGGGT
500		protease cleavable linker 5	GGGGPLNLQAGVVGGGGT
501		protease cleavable linker 6	GGGGPAGLQAGVVGGGGT
502		protease cleavable linker 7	GGGGPANLQAGVVGGGGT
503		protease cleavable linker 8	GGGGEYSGLSDGGGGGT
504		protease cleavable linker 9	GGGGKPAEFTVDGGGGT
505		protease cleavable linker 11	GGGGGDFLAEGGGGT
506		protease cleavable linker 12	GGGGAGPLIVGGGGT
507		protease cleavable linker 13	GGGGLRELHLGGGGT
508		L276	PQASTGGSGG
509		protease cleavable linker 77	GGGGLRELHLGGGGT
510		L276 full sequence v1	GGGGPQASTGGSGGGGGGT
511		L276 full sequence v2	GGGGPQASTGGSGGGGGGS
512		L077 full sequence v1	GGGGLRELHLGGGGS

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
513		L077 full sequence v2	GGGGLRELHLGGGGT
514		Linker	(GS) _n wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10
515		Linker	(GSS) _n wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10
516		Linker	(GGGS) _n wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10
517		Linker	(GGSG) _n wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10
518		Linker	(GGSGG) _n wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10
519		Linker	(GGGGS) _n wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10
520		Linker	(GGGGG) _n wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10
521		Linker	(GGG) _n wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10
522		Linker	(GGGSGGGGSGGGGSGGGGS)
523		Linker	(GGGSGGGGSGGGGS)
524		Linker	(GSGGS) _n
525		Linker	GGSG
526		Linker	GGSGG
527		Linker	GSGSG
528		Linker	GSGGG
529		Linker	GGGSG
530		Linker	GSSSG
531		Linker	(G ₄ S) _n , wherein n=1 to 4
532		Linker	(G ₄ S) _n , wherein n=2 to 4
533		Linker	(G ₄ S) _n , wherein n=1 to 3
534	Exemplary cleavable linker sequence		KPLGLQARVV
535	Exemplary cleavable linker sequence		PQASTGRSGG
536	Exemplary cleavable linker sequence		PQGSTGRAAG
537	Exemplary cleavable linker sequence		PPASSGRAGG
538	Exemplary cleavable linker sequence		PIPVQGRAH
539	Exemplary linker sequence	L040	GGGGPQASTGRSGGGGGG
540	Exemplary linker sequence	L041	GGGGPQGSTGRAAGGGGGG
541	Exemplary linker sequence	L043	GGGGPQGSTARSAAGGGGGG
542	Exemplary linker sequence	L001	GGGGKPLGLQARVVGGGG

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
543	Exemplary linker sequence		GGGGT
544	Linker	NCLV	GGGSGGGSGGVVGGGG
545	non-cleavable linker	NCLV	GGGSGGGSGGVVGGGGT
546	Wild type human TROP2 protein		MARGPGLAPPPLRLPLLLLVLAAVTGHTAAQDN CTCPTNKMTVCSPDGPGRQCRCALGSGMAVD CSTLTSKCLLLKARMSAPKNARTLVRPSEHALVD NDGLYDPDCDPEGRFKARQCNQTSVCWCVNSV GVRRTDKGDLRLCDELVRTHHILIDLRHRPTAG AFNHSDLDALRRLFRERYRLHPKFVAAVHYEQ PTIQIELRQNTSQKAAGDVDIGDAAYYFERDIKG ESLFQGRGGGLDLRVRGEPLQVERTLIYYLDEIPPK FSMKRLTAGLIAVIVVVVVALVAGMAVLVITNR RKSGKYKKVEIKELGELRKEPS
547	Mature TROP2 protein		HTAAQDNCTCPTNKMTVCSPDGPGRQCRCAL GSGMAVDCSTLTSKCLLLKARMSAPKNARTLVR PSEHALVDNDGLYDPDCDPEGRFKARQCNQTSV CWCVNSVGVRRTDKGDLRLCDELVRTHHILID LRHRPTAGAFNHSDLDALRRLFRERYRLHPKFV AAVHYEQPTIQIELRQNTSQKAAGDVDIGDAAY YFERDIKGESLFQGRGGGLDLRVRGEPLQVERTLI YYLDEIPPKFSMKRLTAGLIAVIVVVVVALVAGM AVLVITNRRKSGKYKKVEIKELGELRKEPSL
548		sortase recognition sequence	LPETG
549		Anti-albumin with non-CDR loop mask	EVQLVESGGGLVQPGNSLRLSAASGFTFSKFG MSWVRQGGGGGLDGNEEPGGLEWVSSISGSGR DTLYADSVKGRFTISRDNAKTTLYLQMNSLRPED TAVYYCTIGGSLSVSSQGTLVTVSS
550	Exemplary masking moiety		GGGGGLDGNEEPGG
551	Transmembrane sequence		IYIWAPLAGTCGVLLLSLVITLYC
552	Transmembrane sequence	CD8 beta derived	GLLVAGVLVLLVSLGVAIHLCC
553	Transmembrane sequence	CD4 derived	ALIVLGGVAGLLFIGLGIFFCVRC
554	Transmembrane sequence	CD3 zeta derived	LCYLLDGILFIYGVILTALFLRV
555	Transmembrane sequence	CD28 derived	WVLVVVGGVLACYSLLVTVAFIIFWV

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
556	Transmembrane sequence	CD134 (OX40) derived	AAILGLGLVLGLLGPLAILLALYLL
557	Transmembrane sequence	CD7 derived	ALPAALAVISFLLGLGLGVACVLA
558	Exemplary masking moiety		APGKG
559	Exemplary masking moiety		GGQDGNEEMGGG
560	Exemplary masking moiety		GGQDGNEEGG
561	Exemplary masking moiety		GGGGQDGNEEMGGGGG
562	Exemplary masking moiety		APFGSEM
563	Exemplary masking moiety		AWNGPYE
564	Exemplary masking moiety		AQDNGDTKTG
565	Exemplary masking moiety		AEAKETQG
566	Exemplary masking moiety		ATRREQVEG
567	Exemplary masking moiety		AQAPSQP
568	Exemplary masking moiety		ATRSRTRNDG
569	Exemplary masking moiety		ADVDPDGLG
570	Exemplary masking moiety		AADISDPGG
571	Exemplary masking moiety		ALSVDPSG
572	Exemplary masking moiety		ARLSVDPG
573	Exemplary masking moiety		AVEAADRG
574	Exemplary masking moiety		GGPDGNEEMGGG
575	Exemplary masking moiety		GGFDGNEEMGGG
576	Exemplary masking moiety		GGGDGNEEMGGG
577	Exemplary masking moiety		GGEMDGEGQNGG

SEQ ID NO.	Sequence descriptor	Seq. name	Amino acid sequence
578	Exemplary masking moiety		GGGGGPDGNEEPGG
579	Exemplary masking moiety		GGGGSLDGNEEPGG
580	Exemplary masking moiety		GGGGALDGNEEPGG
581	Exemplary masking moiety		GGGALDGNEEPGG
582	Exemplary masking moiety		GGGGGPDGNEEPGGG
583	Exemplary masking moiety		GGSGALDGNEEPGG
584	Exemplary masking moiety		GGGSLDGNEEPGG
585	Exemplary masking moiety		GGSGGPDGNEEPGG
586	Exemplary masking moiety		GGVRDGPDPGNEEPGG
587	Exemplary masking moiety		GGSGGPDGNEEPGGGG
588	Exemplary masking moiety		GGGRGPDGNEEPGG
589	Exemplary masking moiety		GGSGGLDGNEEPGG
590	Exemplary masking moiety		GGGVGPDGNEEPGG
591	Exemplary masking moiety		GGGEGPDGNEEPGG
592	Exemplary masking moiety		GGGVALDGNEEPGG
593	Exemplary masking moiety		GGGRALDGNEEPGG
594	Exemplary masking moiety		GGYAGLDGNEEPGG
595	Exemplary masking moiety		GGAGGPDGNEEPGG
596	Exemplary masking moiety		GGRGGPDGNEEPGG
597	Exemplary masking moiety		GGGGPDGNEEPGGGG
598	Exemplary masking moiety		GGGEALDGNEEPGG
599	Exemplary masking moiety		GGDASLDGNEEPGG
560	Exemplary masking moiety		GGRDAPDGNEEGG

[00289] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A TROP2 binding domain comprising a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228.

2. The TROP2 binding domain of claim 1, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228.

3. The TROP2 binding domain of claim 1 or 2, comprising an amino acid sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57.

4. The TROP2 binding domain of any one of claims 1-3, wherein the TROP2 binding domain is part of a multispecific protein.

5. The TROP2 binding domain of claim 4, wherein the multispecific protein further comprises a CD3 binding domain.

6. The TROP2 binding domain of claim 5, wherein the multispecific protein comprises an active drug format.

7. The TROP2 binding domain of any one of claims 4-6, wherein the multispecific protein further comprises a bulk serum protein binding domain.

8. The TROP2 binding domain of claim 7, wherein the bulk serum protein comprises a serum albumin protein.

9. The TROP2 binding domain of claim 8, wherein the serum albumin protein comprises a human serum albumin protein.

10. The TROP2 binding domain of any one of claims 7-9, wherein the bulk serum protein binding domain comprises a sequence that is at least 75% identical to SEQ ID NO: 493 or 549.

11. The TROP2 binding domain of any one of claims 5-9, wherein the CD3 binding domain comprises a sequence that is at least 75% identical to the SEQ ID NO: 494.
12. The TROP2 binding domain of any one of claims 4-11, wherein the multispecific protein comprises a sequence that is at least about 75% identical to the sequence as set forth in SEQ ID NOS: 229-264.
13. The TROP2 binding domain of any one of claims 7-9, wherein the bulk serum protein binding domain is a binding moiety comprising a linker and a masking moiety, wherein the masking moiety masks the binding of the TROP2 binding domain or the CD3 binding domain, to their respective targets.
14. The TROP2 binding domain of any one of claims 7-9 and 13, wherein the multispecific protein comprises a non-cleavable prodrug format.
15. The TROP2 binding domain of claim 13 or 14, wherein the masking moiety comprises a sequence selected from the group consisting of SEQ ID NOS: 550 and 558-560, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NO: 550 and 558-560.
16. The TROP2 binding domain of any one of claims 13-15, wherein the linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-545 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-545.
17. The TROP2 binding domain of any one of claims 13-16, wherein the bulk serum protein binding domain comprises a sequence that is at least 75% identical to SEQ ID NO: 493 or 549.
18. The TROP2 binding domain of any one of claims 5-9 and 13-17, wherein the CD3 binding domain comprises a sequence that is at least 75% identical to the sequence as set forth in SEQ ID NO: 494.
19. The TROP2 binding domain of any one of claims 7-9 and 13-18, wherein the multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.
20. The TROP2 binding domain of any one of claims 7-9 and 13-19, wherein the multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.
21. The TROP2 binding domain of any one of claims 7-9 and 13-20, wherein the multispecific protein comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

22. The TROP2 binding domain of claim 6, wherein the active drug comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

23. The TROP2 binding domain of any one of claims 1-3, wherein the TROP2 binding domain is part of a chimeric antigen receptor (a CAR) or a conditionally activatable chimeric antigen receptor (a ProCAR), wherein the CAR further comprises at least one of a transmembrane domain, a costimulatory domain, and an intracellular signaling domain.

24. The TROP2 binding domain of claim 23, wherein the TROP2 binding domain is part of the ProCAR and the ProCAR further comprises (a) a binding moiety comprising a non-CDR loop and a cleavable linker; (b) a transmembrane domain; and (c) an intracellular signaling domain; wherein the binding moiety masks the binding of the TROP2 binding domain to its target.

25. The TROP2 binding domain of claim 24, wherein the binding moiety further comprises one or more complementarity determining regions (CDRs).

26. The TROP2 binding domain of claim 25, wherein the non-CDR loop provides a binding site specific for a bulk serum protein.

27. The TROP2 binding domain of claim 26, wherein the bulk serum protein comprises at least one of: a serum albumin, a transferrin, an IgG1, an IgG2, an IgG4, an IgG3, an IgA monomer, a Factor XIII, a fibrinogen, or a pentameric IgM.

28. The TROP2 binding domain of claim 27, wherein the bulk serum protein comprises the serum albumin.

29. The TROP2 binding domain of claim 28, wherein the serum albumin is a human serum albumin.

30. The TROP2 binding domain of any one of claims 23-29, wherein the ProCAR further comprising a costimulatory domain, wherein the costimulatory domain is a functional signaling domain of a protein selected from the group consisting of OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137), and amino acid sequences thereof having at least one but not more than 20 modifications thereto.

31. The TROP2 binding domain of claim 30, wherein the at least one, but not more than 20, modifications thereto comprise a modification of an amino acid that mediates cell signaling or a modification of an amino acid that is phosphorylated in response to a ligand binding to the encoded T-cell receptor fusion protein.

32. The TROP2 binding domain of any one of claims 23-31, wherein the CAR or the ProCAR further comprises the transmembrane domain and the transmembrane domain is a protein selected from the group consisting of a TCR alpha chain, a TCR beta chain, a TCR zeta chain, a

CD3 epsilon TCR subunit, a CD3 gamma TCR subunit, a CD3 delta TCR subunit, CD45, CD4, CDS, CD8, CD9, CD16, CD22, CD33, CD28, CD37, CD64, CD80, CD86, CD134, CD137, CD154, functional fragments thereof, and amino acid sequences thereof having at least one but not more than 20 modifications thereto.

33. The TROP2 binding domain of any of claims 23-32, wherein the CAR or the ProCAR further comprises the intracellular signaling domain and the intracellular signaling domain is derived from a CD3 epsilon, a CD3 gamma, a CD3 delta, a CD3 alpha, a CD3 beta, or a combination thereof.

34. The TROP2 binding domain of claim 33, wherein the intracellular signaling domain is derived from the CD3 epsilon.

35. A method for treating or ameliorating a proliferative disease or a tumorous disease, comprising administering a TROP2 binding domain according to any one of claims 1-34, or a pharmaceutical composition comprising the same, to a subject in need thereof.

36. The method of claim 35, wherein the subject is human.

37. A conditionally active TROP2 binding protein comprising a binding moiety (M) which comprises a non-CDR loop, a cleavable linker (L), a first target antigen binding domain (T1), and a second target antigen binding domain (T2), wherein at least one of the first target antigen binding domain (T1) and the second target antigen binding domain (T2) comprises a TROP2 binding domain, wherein the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228, wherein the non-CDR loop is capable of binding to the TROP2 binding domain or the second target antigen binding domain, and wherein the binding moiety is capable of masking or masks the binding of the TROP2 binding domain or the second target antigen binding domain to its target.

38. The conditionally active TROP2 binding protein of claim 37, wherein the binding moiety comprises a masking moiety and wherein the masking moiety comprises a sequence selected from the group consisting of SEQ ID NO: 550 and 558-560, or a sequence comprising one

or more substitutions relative to a sequence selected from the group consisting of SEQ ID NO: 550 and 558-560.

39. The conditionally active TROP2 binding protein of claim 37 or 38, wherein the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543, or a sequence comprising one or more substitutions relative to a sequence selected from the group consisting of SEQ ID NOS: 497-543.

40. The conditionally active TROP2 binding protein of any one of claims 37-39, wherein the binding moiety comprises a sequence that is at least 75% identical to SEQ ID NO: 493.

41. The conditionally active TROP2 binding protein of any one of claims 37-40, wherein the second target antigen binding domain (T2) comprises a CD3 binding domain.

42. The conditionally active TROP2 binding protein of claim 41, wherein the CD3 binding domain comprises a sequence that is at least 75% identical to SEQ ID NO: 494.

43. A method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering a conditionally active TROP2 binding protein according to any of claims 37-42, or a pharmaceutical composition comprising the same, to the subject.

44. The method of claim 43, wherein the subject is human.

45. A method of providing anti-tumor immunity to a subject in need thereof, comprising administering a conditionally active TROP2 binding protein according to any one of claims 37-42, or a pharmaceutical composition comprising the same, to the subject.

46. The method of claim 45, wherein the subject is human.

47. The TROP2 binding domain of any one of claims 1-34, wherein the binding domain is a humanized antibody or an antigen binding fragment thereof.

48. The TROP2 binding domain of any one of claims 1-34 and 47, wherein the binding domain is a single domain antibody, a VHH domain, a scFv, a VH domain, a VL domain, a Fab, a F(ab')₂, a Fab', a non-Ig domain, a ligand, a knottin, or a small molecule entity.

49. The TROP2 binding domain of claim 48, wherein the binding domain comprises the single domain antibody.

50. The TROP2 binding domain of any one of claims 1-3 and 47-49, wherein the binding domain binds to TROP2 with a binding affinity (K_D) of about 0.001 nM to about 500 nM.

51. The TROP2 binding domain of any one of claims 1-3 and 47-50, wherein the binding domain binds to human TROP2, mouse TROP2, cynomolgus TROP2, or a combination thereof.

52. A multispecific protein comprising a TROP2 binding domain, wherein the TROP2 binding domain is according to any one of claims 1-3 and 47-51.

53. The multispecific protein of claim 52, wherein the TROP2 binding domain further comprises a CD3 binding domain (anti-CD3 domain).

54. The multispecific protein of claim 53, wherein the anti-TROP2 domain and the anti-CD3 domain are in an anti-TROP2:anti-CD3 orientation.

55. The multispecific protein of claim 53, wherein the anti-TROP2 domain and the anti-CD3 domain are in an anti-CD3:anti-TROP2 orientation.

56. The multispecific protein of any one of claims 52-55, comprising the TROP2 binding domain according to any one of claims 1-3 and 47-51 (anti-TROP2 domain), the CD3 binding domain (anti-CD3 domain), and an albumin binding domain (anti-ALB domain).

57. The multispecific protein of any one of claims 52-56, wherein the anti-CD3 domain comprises an amino acid as set forth in SEQ ID NO: 494.

58. The multispecific protein of any one of claims 56-57, wherein the anti-ALB domain comprises an amino acid sequence as set forth in SEQ ID NO: 493.

59. The multispecific protein of any one of claims 56-58, wherein the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-CD3: anti-ALB: anti-TROP2 orientation.

60. The multispecific protein of any one of claims 56-58, wherein the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-TROP2: anti-ALB: anti-CD3 orientation.

61. The multispecific protein of any one of claims 56-58, wherein the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-ALB: anti-TROP2: anti-CD3 orientation.

62. The multispecific protein of any one of claims 56-58, wherein the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-CD3: anti-TROP2: anti-ALB orientation.

63. The multispecific protein of any one of claims 56-58, wherein the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-ALB: anti-CD3: anti-TROP2 orientation.

64. The multispecific protein of any one of claims 56-58, wherein the anti-TROP2 domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-TROP2: anti-CD3: anti-ALB orientation.

65. A multivalent protein comprising a sequence as set forth in any one of SEQ ID NOS: 229-264.
66. An active drug comprising a sequence as set forth in any one of SEQ ID NOS: 229-264.
67. An active drug comprising a sequence as set forth in any one of SEQ ID NOS: 1-57.
68. A pharmaceutical composition comprising: (i)(a) a TROP2 binding domain according to any one of claims 1-34 and 47-51; (i)(b) a conditionally active TROP2 binding protein according to any one of claims 37-42; (i)(c) a multispecific protein according to any one of claims 52-64; (i)(d) a multivalent protein according to claim 65; or (i)(e) an active drug according to claim 66 or 67, and (ii) a pharmaceutically acceptable carrier.
69. A process for producing a TROP2 binding domain, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding the TROP2 binding domain according to any one of claims 1-3 and 47-51, under conditions allowing the expression of the TROP2 binding domain and recovering and purifying the produced protein from the culture.
70. A process for producing a multispecific protein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the multispecific TROP2 binding protein according to any one of claims 52-64 under conditions allowing the expression of the multispecific protein and recovering and purifying the produced protein from the culture.
71. A method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering a TROP2 binding domain according to any one of claims 1-34 and 47-51, or a pharmaceutical composition according to claim 68, to the subject.
72. A method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering the multispecific protein according to any one of claims 52-64, a multivalent protein according to claim 65, an active drug according to claim 66 or 67, or a pharmaceutical composition according to claim 68, to the subject.
73. The method of claim 71 or 72, wherein the subject is human.
74. The method of claim 73, wherein the method further comprises administration of an agent, wherein the agent is biotherapeutic agent, a cytokine, a PAP (phosphatidic acid phosphatase) inhibitor, an oncolytic virus, a kinase inhibitor, an IDO (Indoleamine-pyrrole 2,3-dioxygenase) inhibitor, a glutaminase GLS1 inhibitor, a CAR (Chimeric Antigen Receptor)-T cell or T cell therapy, a TLR (Toll-Like Receptor) Agonist, or a tumor vaccine.

75. The method of any one of claims 71-74, wherein the TROP2 binding domain selectively binds to tumor cells expressing TROP2.

76. The method of any one of claims 71-75, wherein the tumorous disease comprises a solid tumor disease.

77. The method of claim 76, wherein the solid tumor disease is metastatic.

78. The method of any one of claims 71-77, wherein the tumorous disease comprises at least one of: an oral cancer, a colorectal cancer, a head and neck cancer, a prostate cancer, a liver cancer, a cervical cancer, a nasopharyngeal cancer, a thyroid cancer, a non-small cell lung cancer, a small cell lung cancer, a gastric cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a bladder cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma, a nasal NK/T cell lymphoma, a gliomas, a glioblastoma, an osteosarcoma, a pituitary adenoma, or any combination thereof.

79. The method of any one of claims 43-46, wherein the method further comprises administration of an agent, wherein the agent is biotherapeutic agent, a cytokine, a PAP (phosphatidic acid phosphatase) inhibitor, an oncolytic virus, a kinase inhibitor, an IDO (Indoleamine-pyrrole 2,3-dioxygenase) inhibitor, a glutaminase GLS1 inhibitor, a CAR (Chimeric Antigen Receptor)-T cell or T cell therapy, a TLR (Toll-Like Receptor) Agonist, or a tumor vaccine.

80. The method of claim 79, wherein the TROP2 binding domain selectively binds to tumor cells expressing TROP2.

81. The method of claim 79 or 80, wherein the tumorous disease comprises a solid tumor disease.

82. The method of claim 81, wherein the solid tumor disease is metastatic.

83. The method of any one of claims 79-82, wherein the tumorous disease is at least one of: a colorectal cancer, an oral cancer, a colorectal cancer, a head and neck cancer, a prostate cancer, a liver cancer, a cervical cancer, a nasopharyngeal cancer, a thyroid cancer, a non-small cell lung cancer, a small cell lung cancer, a gastric cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a bladder cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma, a nasal NK/T cell lymphoma, a gliomas, a glioblastoma, an osteosarcoma, a pituitary adenoma, or any combination thereof.

84. A process for producing a conditionally active TROP2 binding protein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the conditionally active TROP2 binding protein

according to any one of claims 37-42, under conditions allowing the expression of the conditionally active TROP2 binding protein and recovering and purifying the produced protein from the culture.

85. A process for producing a multivalent protein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the multivalent protein according to claim 65, under conditions allowing the expression of the multivalent protein and recovering and purifying the produced protein from the culture.

86. A process for producing an active drug, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the active drug according to claim 66 or 67, under conditions allowing the expression of the active drug and recovering and purifying the produced drug from the culture.

87. A cell comprising a CAR according to any one of claims 23 and 30-33.

88. A cell comprising a ProCAR according to any one of claims 23-33.

89. The cell of claim 87 or 88, wherein the cell is a T cell or an NK cell.

90. A method of preparing a CAR or a ProCAR, comprising transfecting a cell according to any one of claims 87-89, with a vector or an RNA comprising a nucleotide sequence encoding the CAR or the ProCAR.

91. The conditionally active TROP2 binding protein of any one of claims 37-42, wherein the protein comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

92. The conditionally active TROP2 binding protein of any one of claims 37-42, wherein the protein comprises a sequence that is at least about 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

93. The conditionally active TROP2 binding protein of any one of claims 37-42, wherein the protein comprises a sequence that is at least about 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

94. The conditionally active TROP2 binding protein of any one of claims 37-42 and 91-93, wherein the conditionally active TROP2 binding protein has a greater therapeutic index compared to a TROP2 binding protein that does not comprise the binding moiety (M) or the cleavable linker (L) but is otherwise identical to the conditionally active TROP2 binding protein.

95. The conditionally active TROP2 binding protein of claim 94, wherein the conditionally active TROP2 binding protein has a therapeutic index that is at least about 5-fold to about 100-fold greater than that of a TROP2 binding protein that does not comprise the binding

moiety (M) or the cleavable linker (L) but is otherwise identical to the conditionally active TROP2 binding protein.

96. A pharmaceutical composition comprising: (i)(a) a conditionally active TROP2 binding protein according to any one of claims 91-95, and (ii) a pharmaceutically acceptable carrier.

97. A method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering the pharmaceutical composition according to claim 96 to the subject.

98. A method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering the conditionally active TROP2 binding protein according to claim 94 or 95, or the pharmaceutical composition according to claim 96, to the subject.

99. The method of claim 97 or 98, wherein the subject is human.

100. The method of any one of claims 97-99, wherein the tumorous disease comprises at least one of: an oral cancer, a colorectal cancer, a head and neck cancer, a prostate cancer, a liver cancer, a cervical cancer, a nasopharyngeal cancer, a thyroid cancer, a non-small cell lung cancer, a small cell lung cancer, a gastric cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a bladder cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma, a nasal NK/T cell lymphoma, a gliomas, a glioblastoma, a osteosarcoma, a pituitary adenoma, or any combination thereof.

101. A method of increasing a therapeutic index of a TROP2 binding domain, the method comprising conjugating the TROP2 binding domain to a binding moiety comprising a cleavable linker and a non-CDR loop,

- wherein the non-CDR loop comprises a binding site specific for the TROP2 binding domain,

- wherein the TROP2 binding domain is masked from binding its target by the binding moiety, and

- wherein the TROP2 binding domain binds its target upon cleavage of the cleavable linker.

102. The method of claim 101, wherein the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOS:

115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228.

103. The method of claim 101 or 102, wherein the TROP2 binding domain conjugated to the binding moiety is part of a conditionally active multispecific protein, wherein the conditionally active multispecific protein further comprises a CD3 binding domain.

104. The method of any one of claims 101-103, wherein the binding moiety comprises a sequence that is at least about 75% identical to SEQ ID NO: 493.

105. The method of claim 103 or 104, wherein the CD3 binding domain comprises a sequence that is at least about 75% identical to SEQ ID NO: 494.

106. The method of any one of claims 101-105, wherein the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-543.

107. The method of claim any one of claims 101-106, wherein the TROP2 binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57.

108. The method of any one of claims 101-107, wherein the conditionally active multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

109. The method of any one of claims 101-107, wherein the conditionally active multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

110. The method of any one of claims 101-107, wherein the conditionally active multispecific protein comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

111. The method of any one of claims 101-110, wherein the TROP2 binding domain conjugated to the binding moiety is part of a conditionally active chimeric antigen receptor, wherein the conditionally active chimeric antigen receptor further comprises at least one of: a transmembrane domain, an intracellular signaling domain, and a costimulatory domain.

112. The method of claim 111, wherein the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence

comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228.

113. The method of claim 111 or 112, wherein the binding moiety comprises a sequence that is at least about 75% identical to SEQ ID NO: 493.

114. The method of any one of claims 111-113, wherein the TROP2 binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57.

115. The method of any one of claims 111-113, wherein the TROP2 binding domain comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57.

116. A method of increasing a therapeutic index of a TROP2 binding protein comprising a first target antigen binding domain and a second target antigen binding domain, wherein at least one of the first and the second target antigen binding domain comprises a TROP2 binding domain, the method comprising conjugating the first or the second target antigen binding domain to a binding moiety comprising a cleavable linker and a non-CDR loop,

- wherein the non-CDR loop comprises a binding site specific for the first or the second target antigen binding domain,
- wherein at least one of the first or the second target antigen binding domain is masked from binding its target by the binding moiety, and
- wherein the first or the second target antigen binding domain that is masked, binds its target upon cleavage of the cleavable linker.

117. The method of claim 116, wherein the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228.

118. The method of claim 116 or 117, wherein the non-CDR loop comprises a binding site specific for the TROP2 binding domain.

119. The method of claim 116 or 117, wherein at least one of the first or the second target antigen binding domain comprises a CD3 binding domain.

120. The method of claim 119, wherein the non-CDR loop comprises a binding site specific for the CD3 binding domain.

121. The method of claim 120, wherein the CD3 binding domain comprises a sequence that is at least about 75% identical to SEQ ID NO: 494.

122. The method of any one of claims 116-121, wherein the binding moiety comprises a sequence that is at least about 75% identical to SEQ ID NO: 493.

123. The method of claim any one of claims 116-122, wherein the TROP2 binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57.

124. The method of any one of claims 116-123, wherein the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-543.

125. The method of any one of claims 116-124, wherein the conditionally active multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

126. The method of any one of claims 116-124, wherein the conditionally active multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

127. The method of any one of claims 116-124, wherein the conditionally active multispecific protein comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

128. A method of increasing a therapeutic index of a TROP2 binding protein comprising a TROP2 binding domain and a CD3 binding domain, the method comprising conjugating CD3 binding domain to a binding moiety comprising a cleavable linker and a non-CDR loop, wherein the non-CDR loop comprises a binding site specific for CD3 binding domain.

129. The method of claim 128, wherein the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID

NOS: 58-114; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228.

130. The method of claim 128 or 129, wherein the CD3 binding domain comprises a sequence that is at least about 75% identical to SEQ ID NO: 494.

131. The method of any one of claims 128-130, wherein the binding moiety comprises a sequence that is at least about 75% identical to SEQ ID NO: 493.

132. The method of claim any one of claims 128-131, wherein the TROP2 binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57.

133. The method of any one of claims 128-132, wherein the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-543.

134. The method of any one of claims 128-133, wherein the TROP2 binding domain is part of a conditionally active multispecific protein and the conditionally active multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

135. The method of any one of claims 128-133, wherein the conditionally active multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

136. The method of any one of claims 128-133, wherein the conditionally active multispecific protein comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

137. A TROP2 targeting conditionally active multispecific protein, comprising: a TROP2 binding domain, a CD3 binding domain, an albumin binding domain, wherein the albumin binding domain comprises a non-CDR loops that comprises a binding site specific for the CD3 binding domain and a cleavable linker, wherein the TROP2 binding domain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID NOS: 58-114, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 58-114; the CDR2 comprises a sequence selected from the group consisting of SEQ ID NOS: 115-171 or a sequence

comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 115-171; and the CDR3 comprises a sequence selected from the group consisting of SEQ ID NOS: 172-228 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 172-228.

138. The TROP2 targeting conditionally active multispecific protein of claim 137, wherein the albumin binding domain comprises a sequence that is at least about 75% identical SEQ ID NO: 493.

139. The TROP2 targeting conditionally active multispecific protein of claim 137 or 138, wherein the TROP2 binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-57.

140. The TROP2 targeting conditionally active multispecific protein of any one of claims 137-139, wherein the cleavable linker comprises a sequence selected from the group consisting of SEQ ID NOS: 497-543, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID NOS: 497-543.

141. The TROP2 targeting conditionally active multispecific protein of any one of claims 137-140, wherein the conditionally active multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

142. The TROP2 targeting conditionally active multispecific protein of any one of claims 137-140, wherein the conditionally active multispecific protein comprises a sequence that is at least 85% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

143. The TROP2 targeting conditionally active multispecific protein of any one of claims 137-140, wherein the conditionally active multispecific protein comprises a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 229-264.

144. A pharmaceutical composition comprising a TROP2 targeting conditionally active multispecific protein of any one of claims 137-143.

145. The pharmaceutical composition of claim 144, further comprising a pharmaceutically acceptable carrier.

146. A process for producing TROP2 targeting conditionally active multispecific protein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the TROP2 targeting conditionally active multispecific protein of any one of claims 137-143, under conditions allowing the expression of the TROP2 targeting conditionally active multispecific protein, and recovering and purifying the produced protein from the culture.

147. A method for treating or ameliorating a proliferative disease or a tumorous disease in a subject in need thereof, comprising administering a TROP2 targeting conditionally active multispecific protein of any one of claims 137-143, or a pharmaceutical composition according to claim 144 or 145, to the subject.

148. The method of claim 147, wherein the tumorous disease comprises a solid tumor disease.

149. The method of claim 148, wherein the solid tumor disease is metastatic.

150. The method of any one of claims 147-149, wherein the tumorous disease comprises at least one of: an oral cancer, a colorectal cancer, a head and neck cancer, a prostate cancer, a liver cancer, a cervical cancer, a nasopharyngeal cancer, a thyroid cancer, a non-small cell lung cancer, a small cell lung cancer, a gastric cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a bladder cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma, a nasal NK/T cell lymphoma, a gliomas, a glioblastoma, an osteosarcoma, a pituitary adenoma, or any combination thereof.

1/18

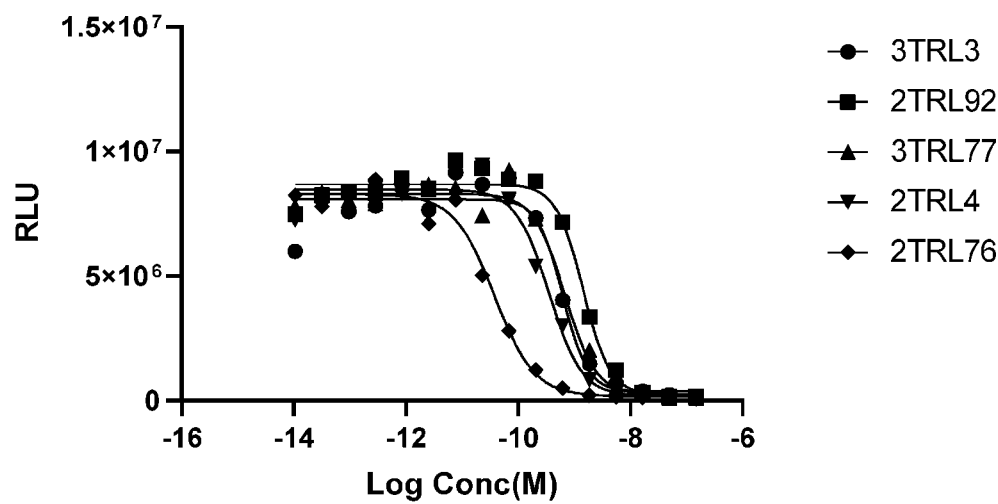


FIG. 1

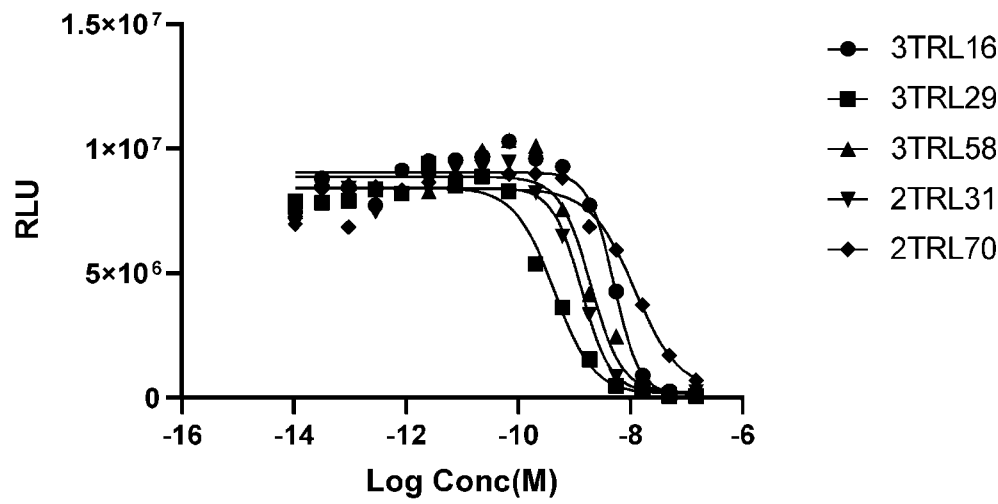


FIG. 2

2/18

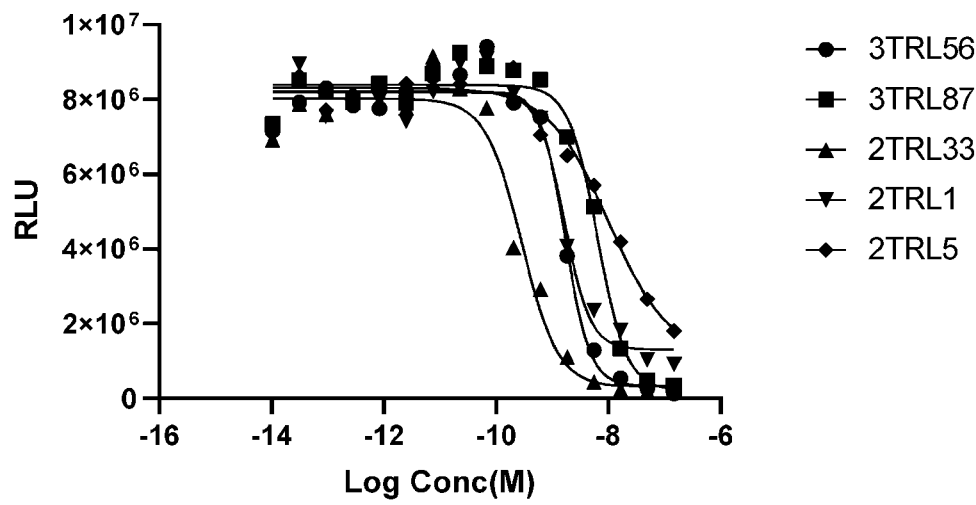


FIG. 3

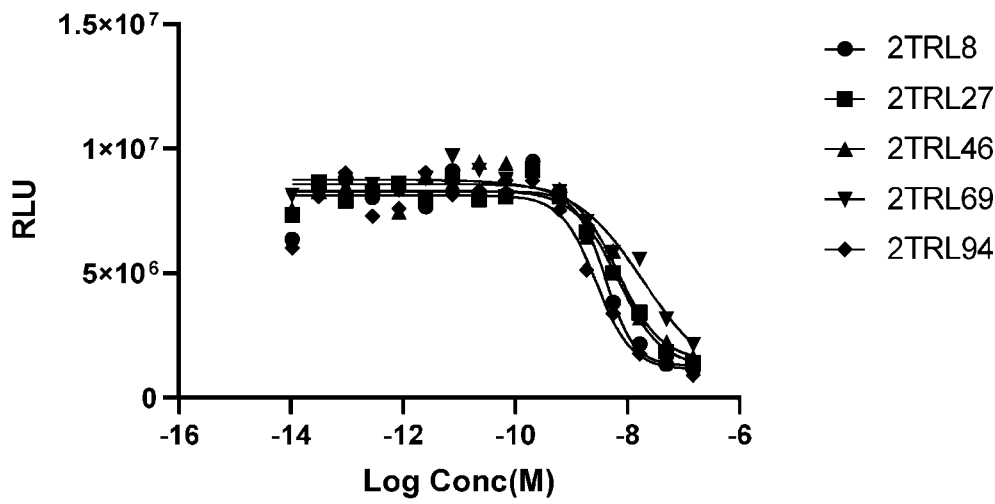


FIG. 4

3/18

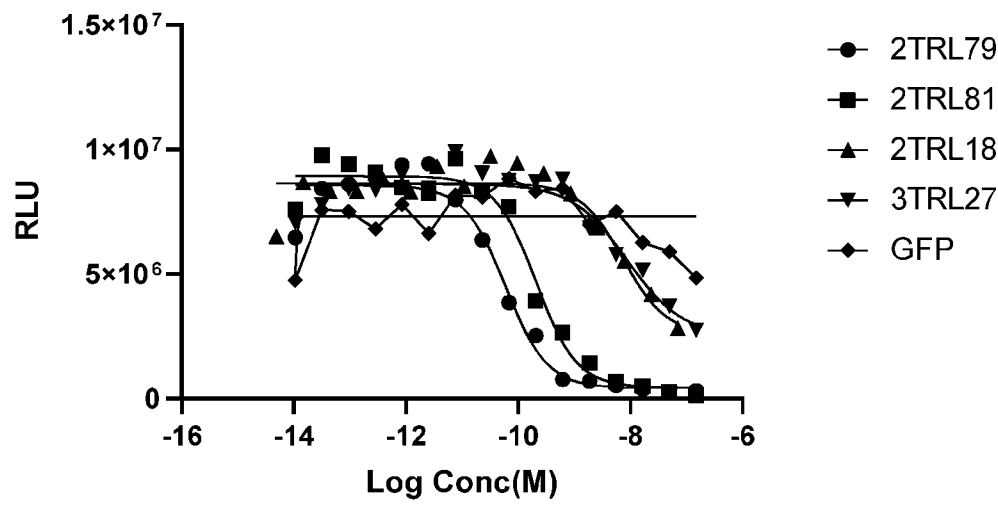


FIG. 5

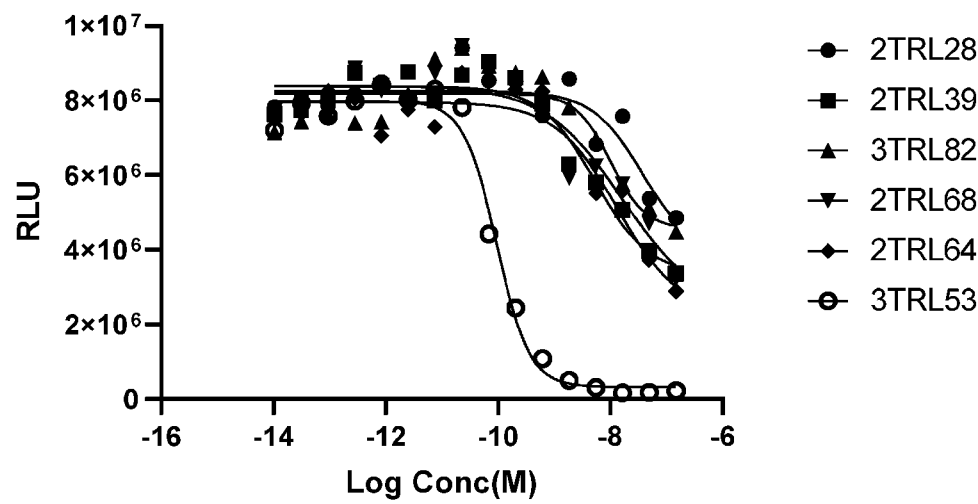


FIG. 6

4/18

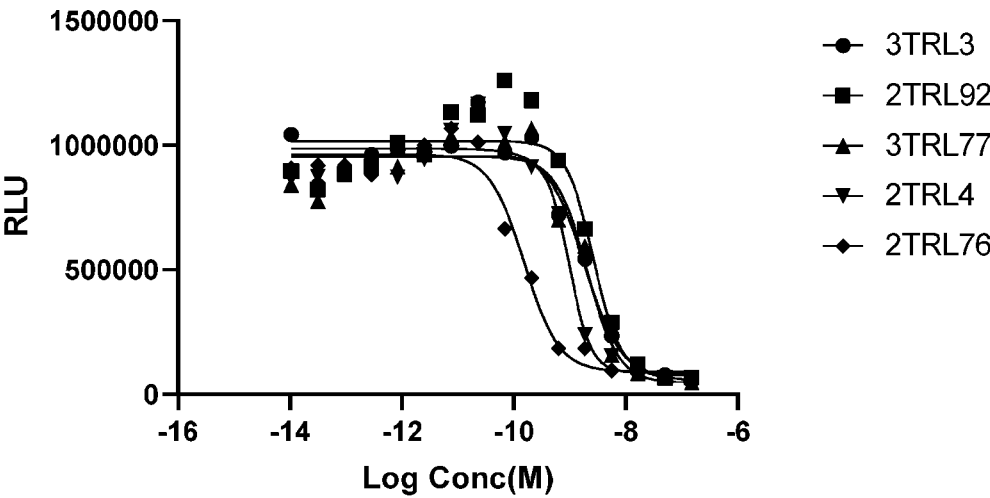


FIG. 7

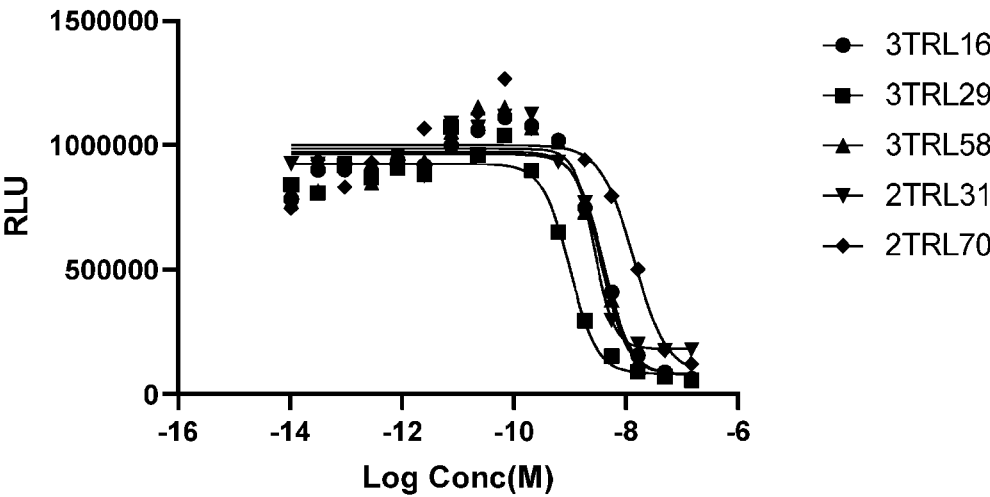


FIG. 8

5/18

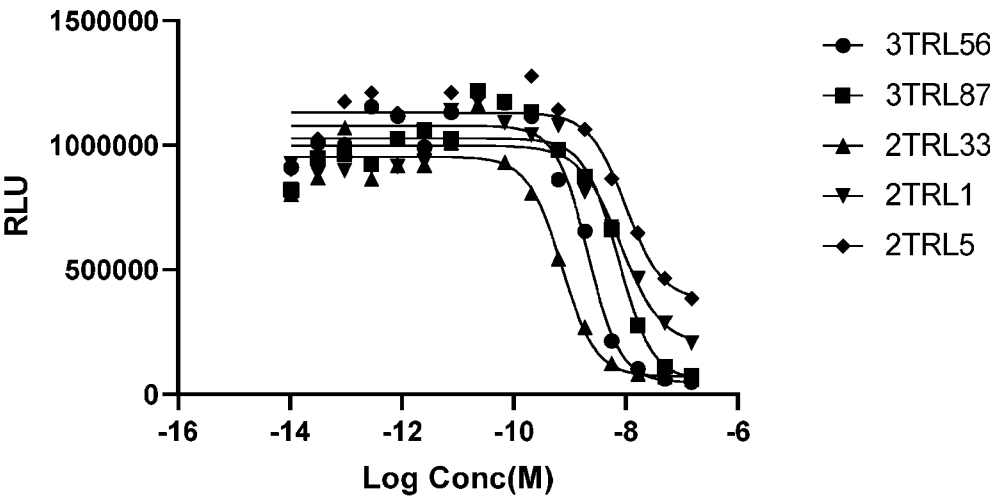


FIG. 9

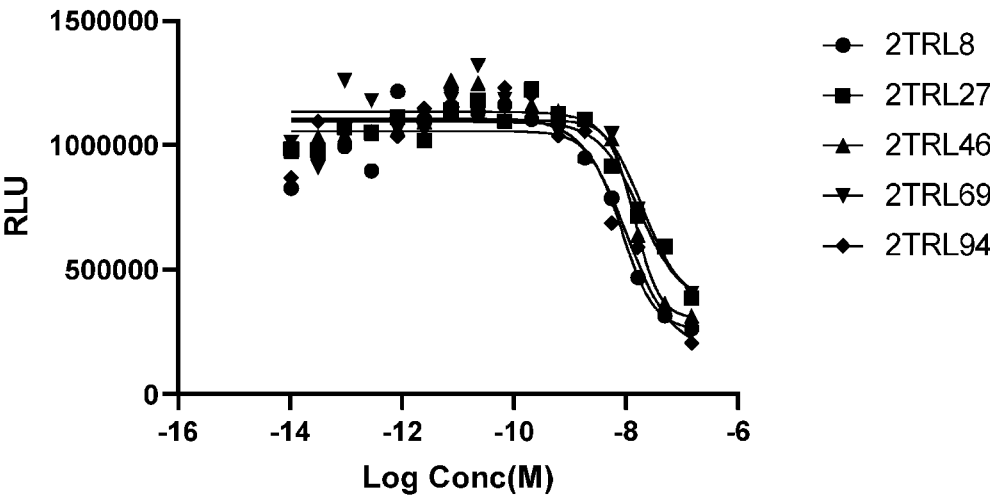


FIG. 10

6/18

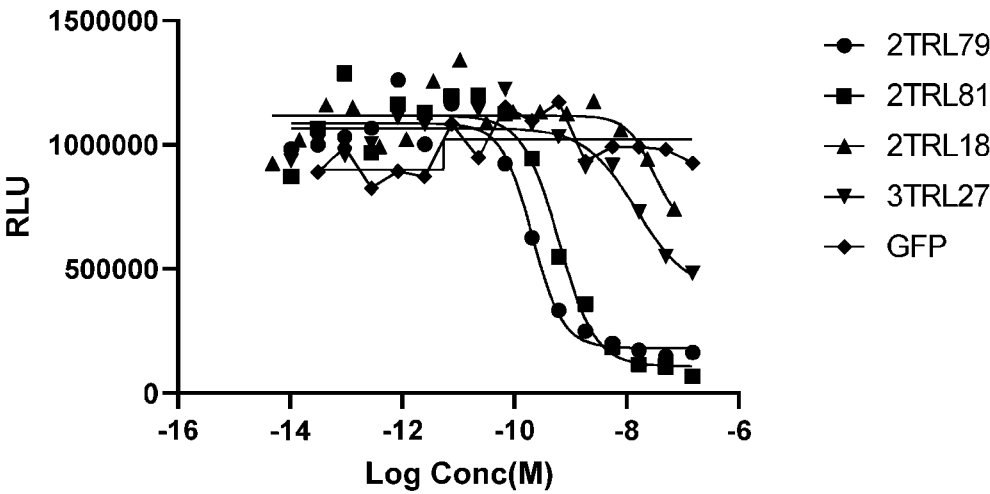


FIG. 11

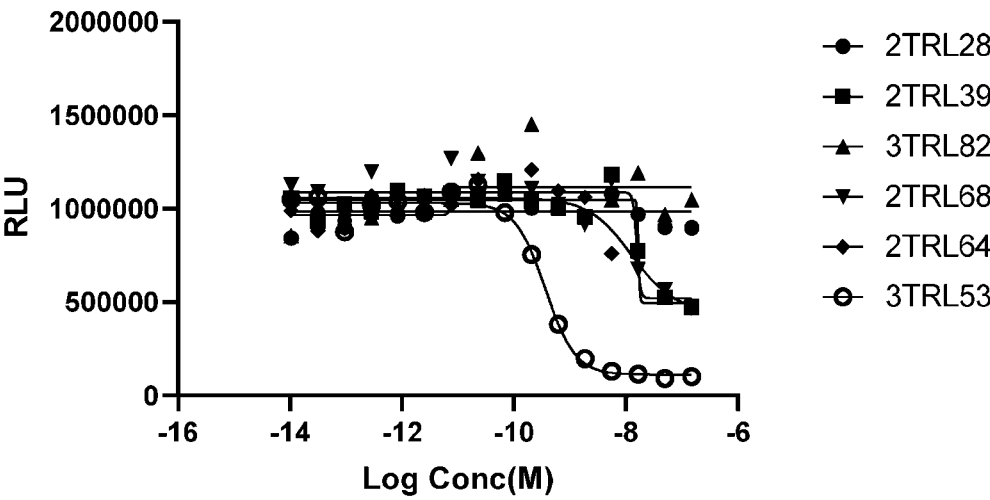


FIG. 12

7/18

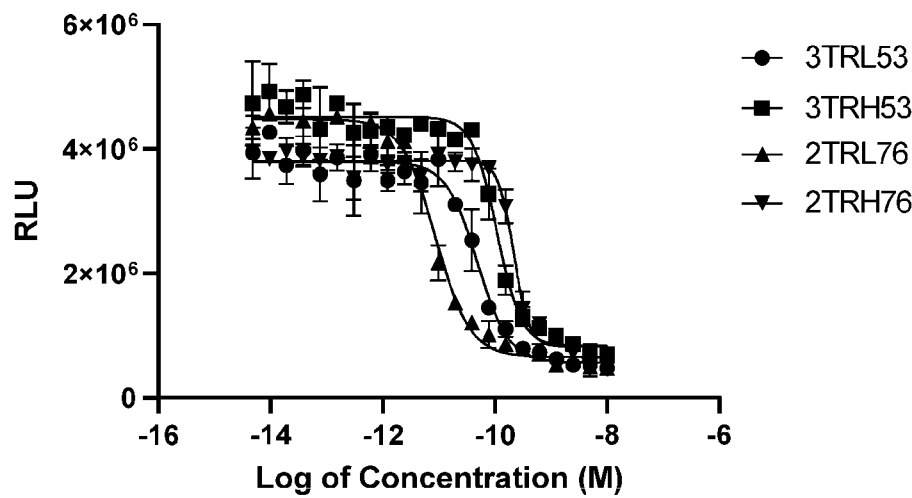


FIG. 13

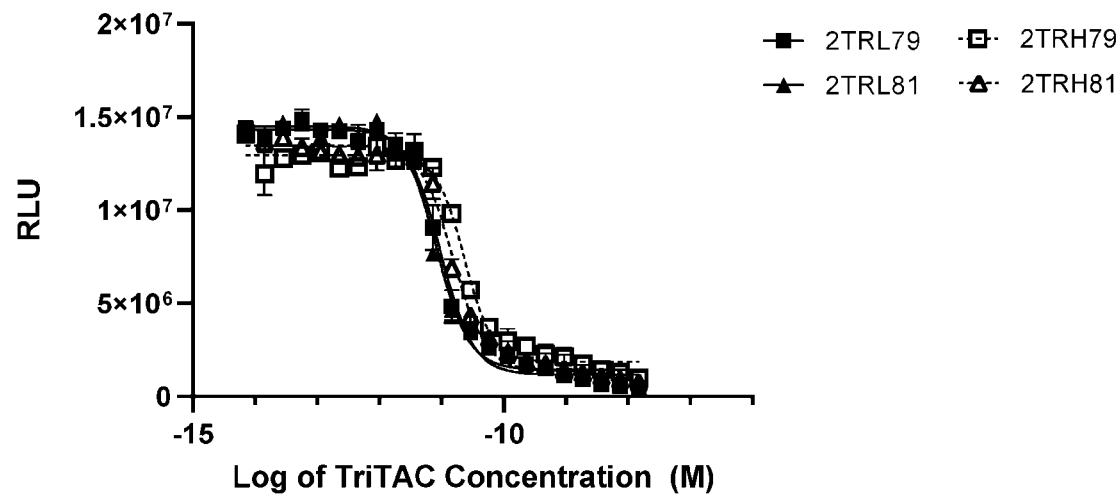


FIG. 14

8/18

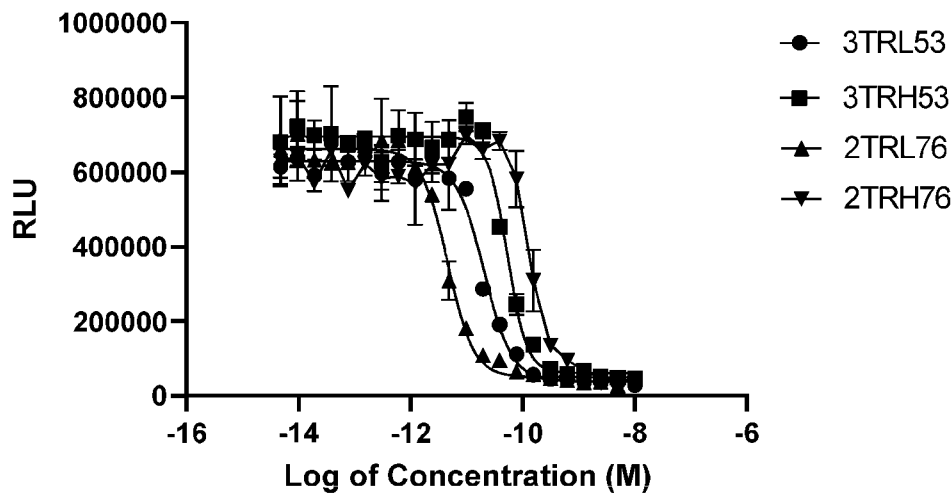


FIG. 15

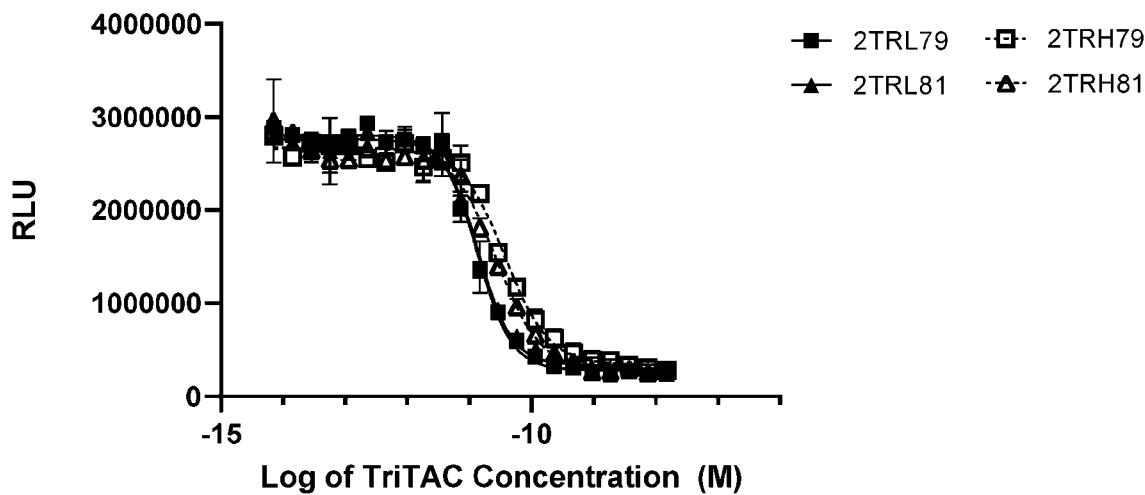


FIG. 16

9/18

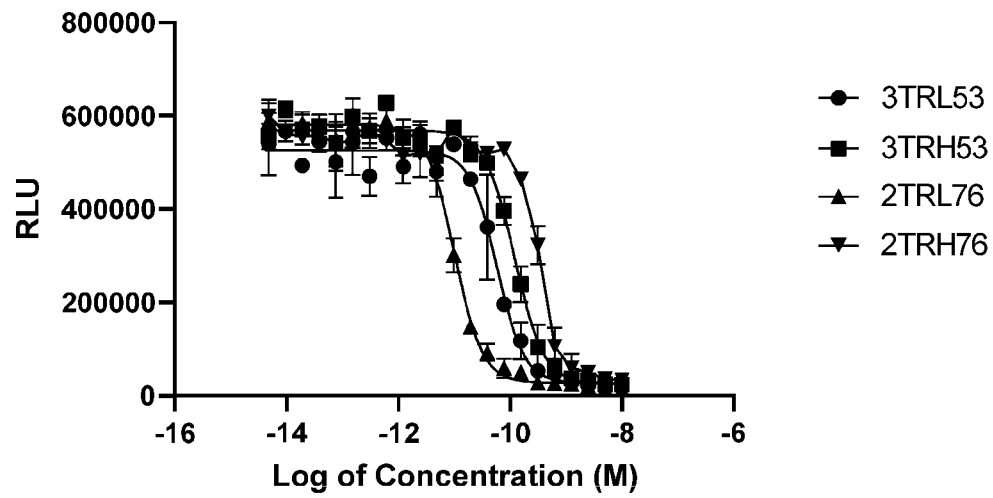


FIG. 17

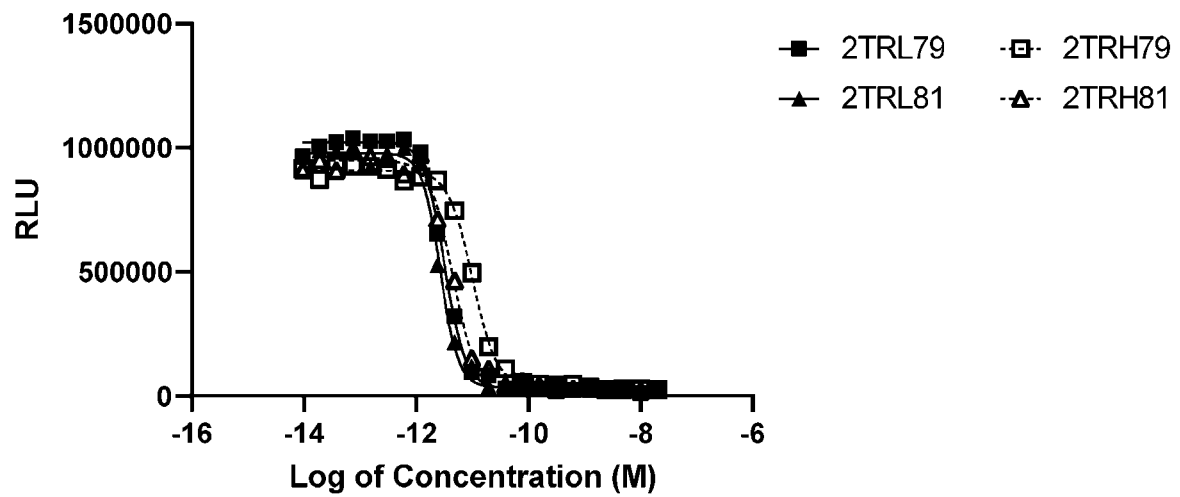


FIG. 18

10/18

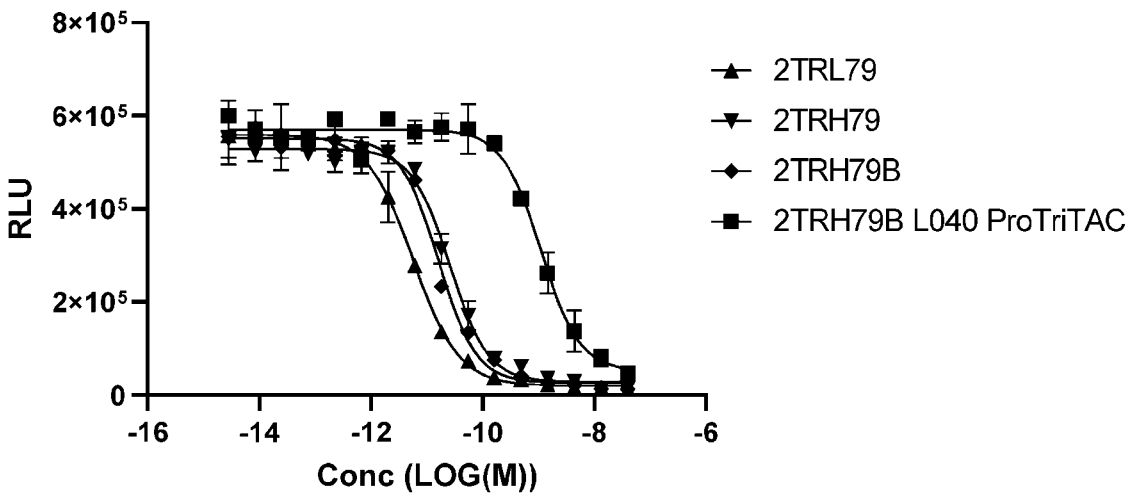


FIG. 19

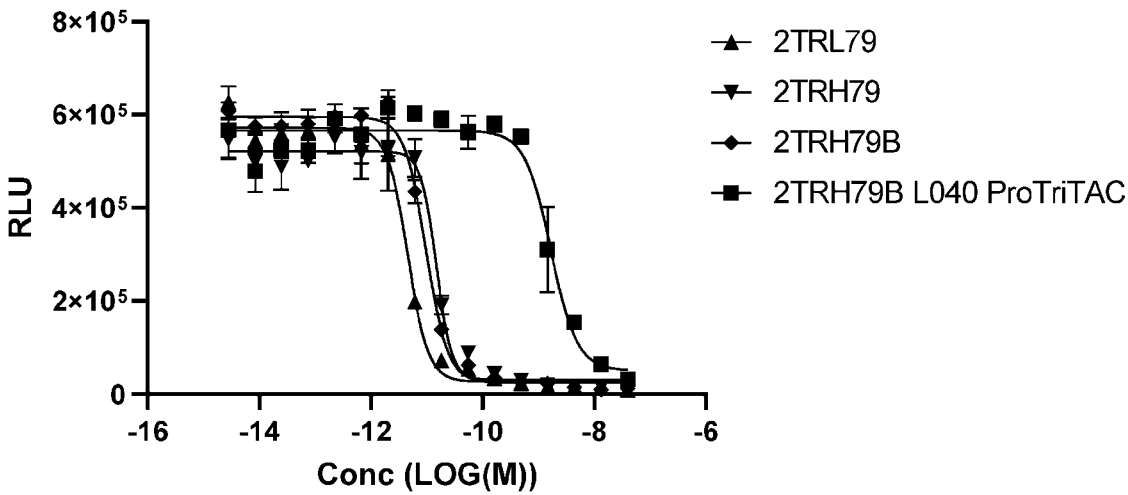


FIG. 20

11/18

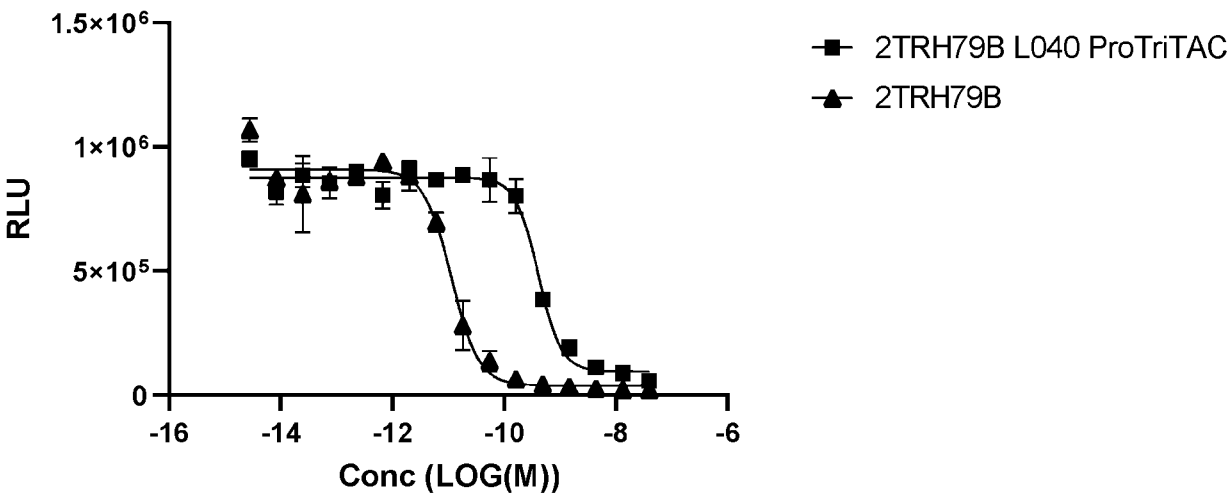


FIG. 21

Admix Therapeutic Xenograft HCC70-002 in NSG Mice
ProTriTACs Trop2 2TRL79
Effect of Treatment on Tumor Volume

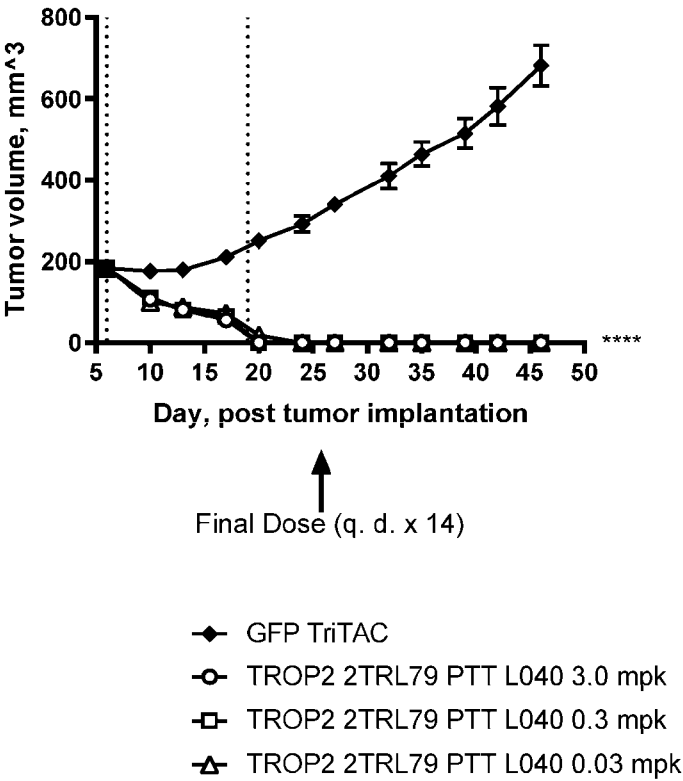
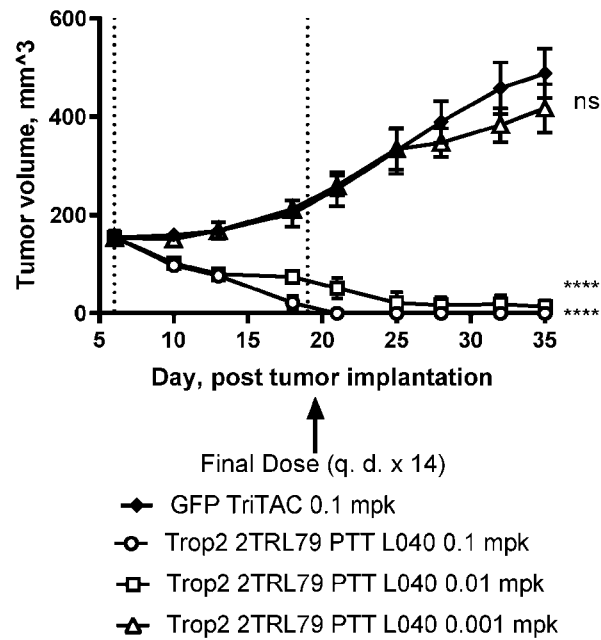


FIG. 22

12/18

Admix Therapeutic Xenograft HCC70-003 in NSG Mice
ProTriTAC Trop2 2TRL79
Effect of Treatment on Tumor Volume

**FIG. 23**

13/18

Admix Therapeutic Xenograft HCC70-003 in NSG Mice
ProTriTAC Trop2 2TRH79
Effect of Treatment on Tumor Volume

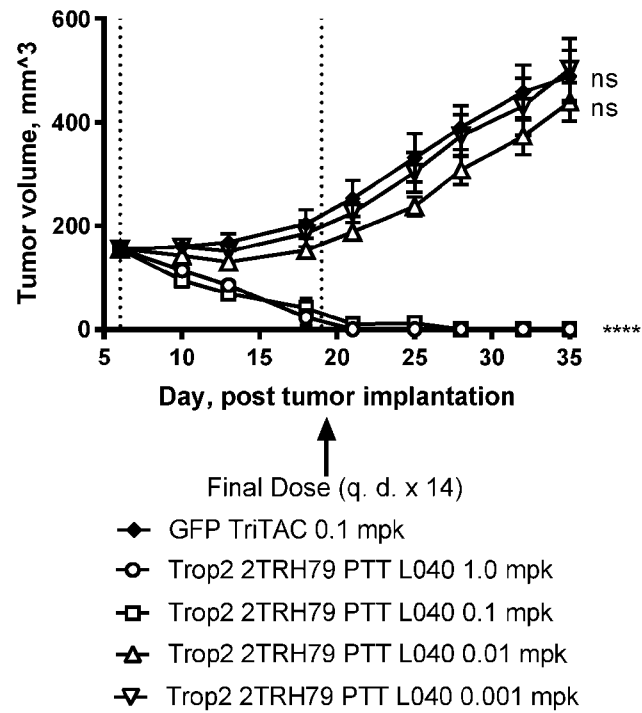
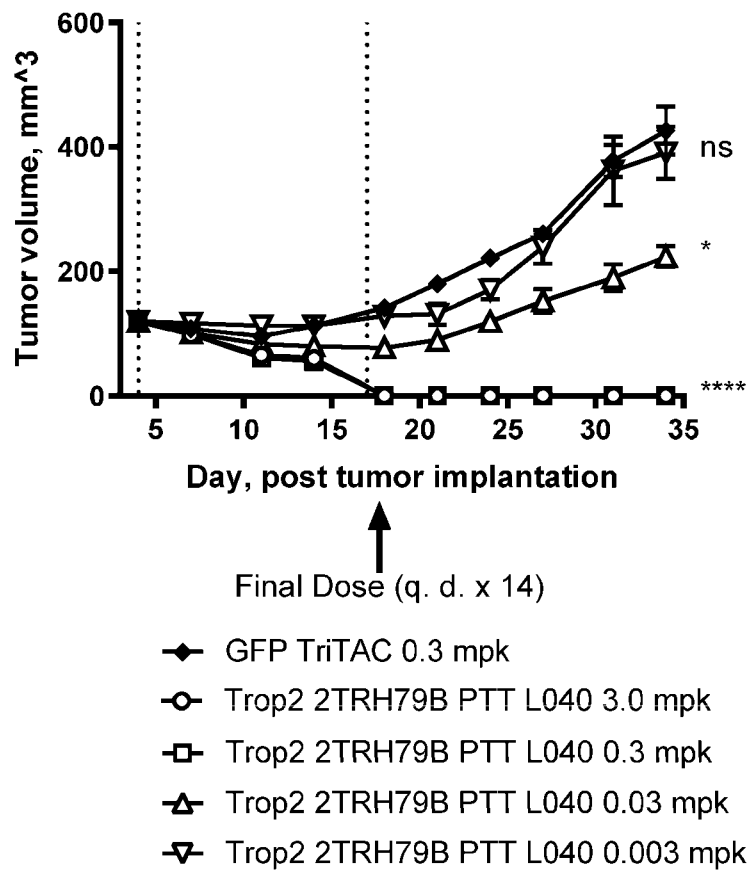


FIG. 24

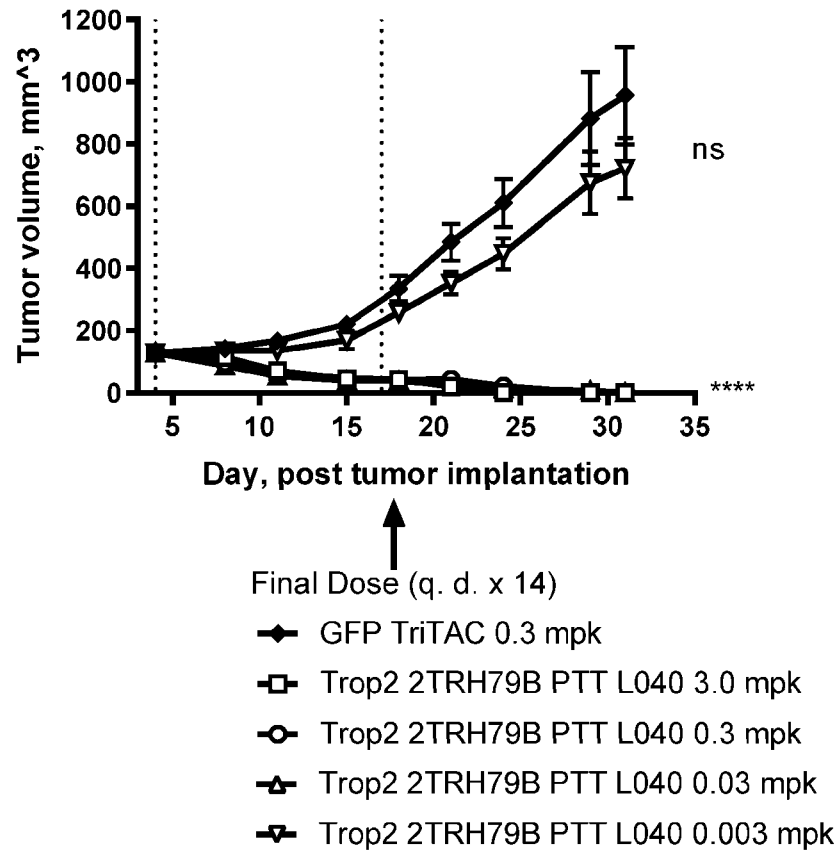
14/18

Admix Therapeutic Xenograft Cal27-002 in NSG Mice
ProTriTAC Trop2 2TRH79B
Effect of Treatment on Tumor Volume

**FIG. 25**

15/18

Admix Therapeutic Xenograft HPAFII-012 in NSG Mice
ProTriTAC Trop2 2TRH79B
Effect of Treatment on Tumor Volume

**FIG. 26**

16/18

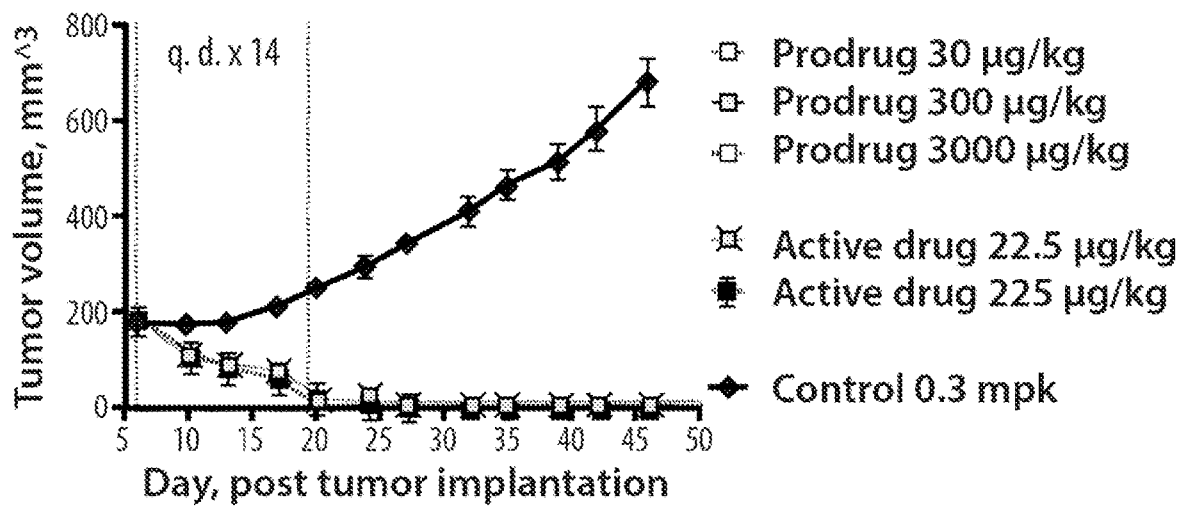
HCC70
(Breast carcinoma)

FIG. 27

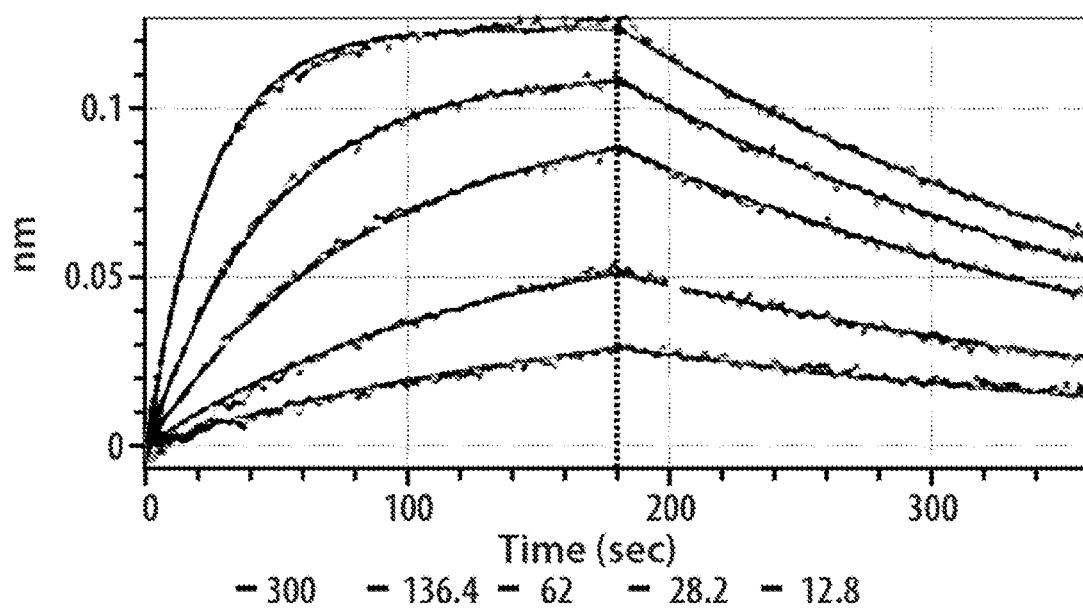


FIG. 28A

17/18

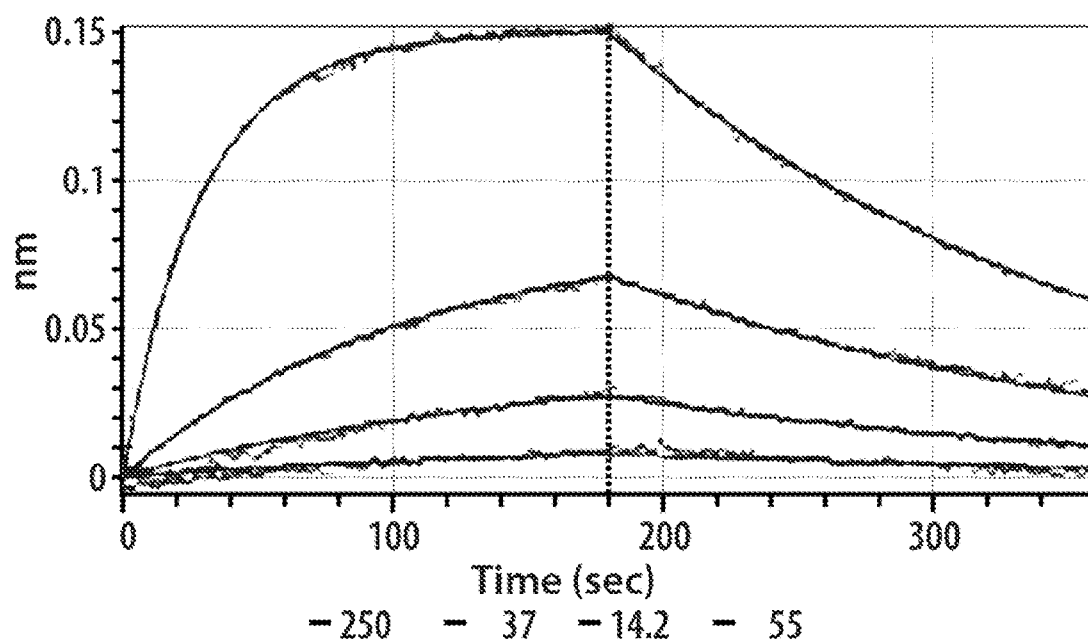


FIG. 28B

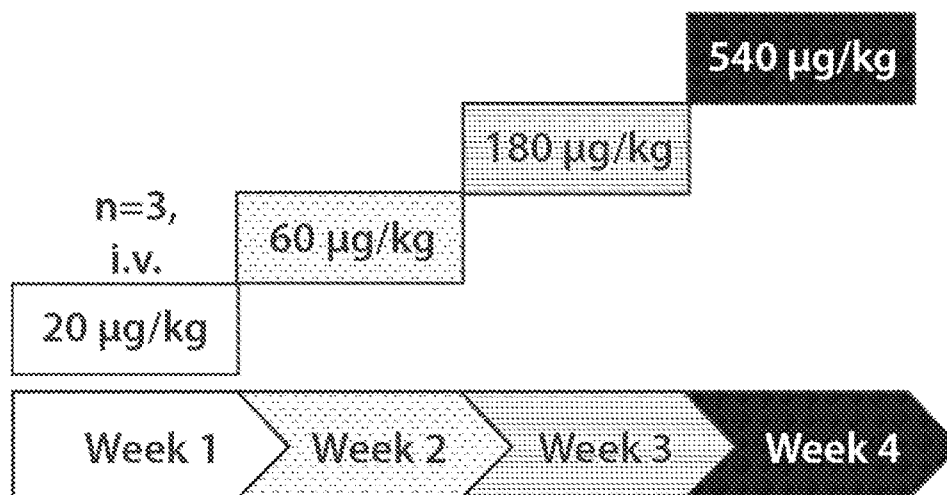


FIG. 29

18/18

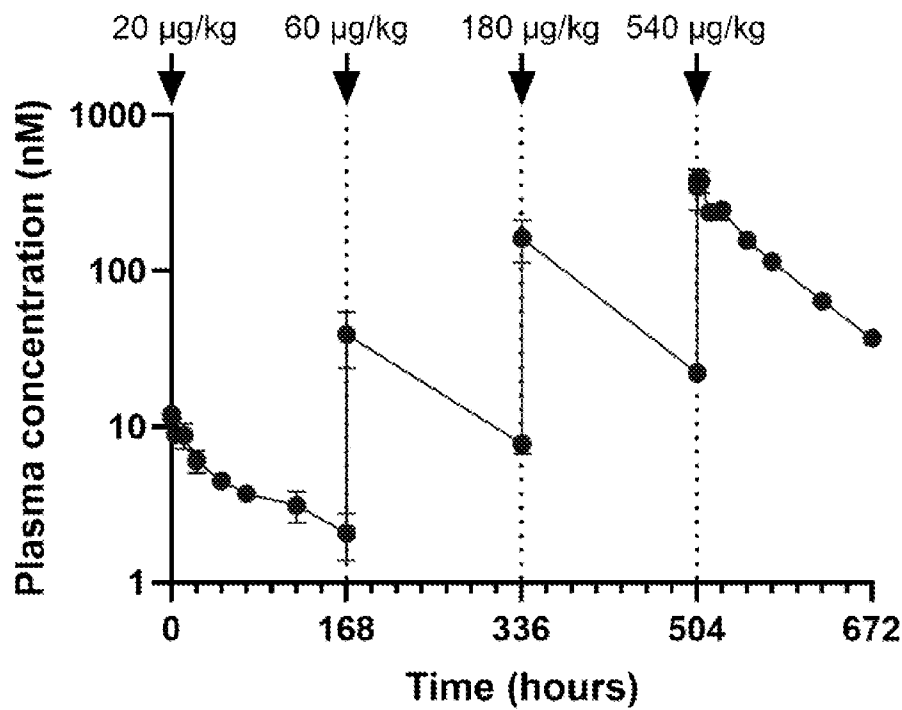


Fig. 30