

provides novel polypeptides and protein therapeutics useful in methods of treatment, particularly for treatment of conditions associated with abnormal expression of EpCAM.

SUMMARY OF THE DISCLOSURE

[0005a] In one aspect, provided herein is an multispecific protein comprising (a) an EpCAM binding domain, (b) a CD3 ϵ binding domain, and (c) a bulk serum protein binding domain that binds to a human serum albumin protein, wherein the multispecific protein comprises an amino acid sequence of any one of SEQ ID NOS: 495, 498-502, 569-570, 572, 573, 575, 576, 577, and 578.

[0005b] In another aspect, provided herein is a pharmaceutical composition comprising (i) a multispecific protein as described herein, and (ii) a pharmaceutically acceptable carrier.

[0005c] In another aspect, provided herein is a use of the multispecific protein as described herein in the manufacture of a medicament for treating a cancer in a subject in need thereof.

[0005d] In another aspect, provided herein is a process for production of a multispecific protein as described herein, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the multispecific protein under conditions allowing the expression of the multispecific protein, recovering and purifying the produced multispecific protein from the culture.

[0005e] In another aspect, provided herein is a multispecific protein as described herein for use in therapy.

[0006] One example provides an EpCAM 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. 39-76, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 39-76; the CDR2 comprising a sequence selected from the group consisting of SEQ ID Nos. 77-114 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 77-114; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID Nos. 115-152 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 115-152. In some examples, the CDR1 comprises a sequence selected from the group consisting of SEQ ID Nos. 39-76; the CDR2 comprising a sequence selected from the group consisting of SEQ ID Nos. 77-114; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID Nos. 115-152. In some examples, the EpCAM binding domain comprises an

amino acid sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-38, 207-209, and 496-497.

[0007] In some examples, the EpCAM binding domain is part of a multispecific protein. In some examples, the multispecific protein further comprises a CD3 binding domain. In some examples, the multispecific protein comprises an active drug format. In some examples, the multispecific protein further comprises a bulk serum protein binding domain. In some examples, the bulk serum protein comprises a serum albumin protein. In some examples, the serum albumin protein comprises a human serum albumin protein. In some examples, the bulk serum protein binding domain comprises a sequence that is at least 75% identical the sequence as set forth in SEQ ID No. 378. In some examples, the CD3 binding domain comprises a sequence that is at least 75% identical the sequence as set forth in SEQ ID No. 379. In some examples, the multispecific protein comprises a sequence that is at least about 75% identical to the sequence as set forth in SEQ ID No. 492. In some examples, 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 the binding of the EpCAM binding domain or the CD3 binding domain, to their respective targets. In some examples, the multispecific protein comprises a non-cleavable prodrug format. In some examples, the masking moiety comprises

a sequence selected from the group consisting of: SEQ ID Nos. 380-424, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of: SEQ ID Nos. 380-424. In some examples, the linker comprises a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-507, and 581, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-507, and 581. In some examples, the bulk serum protein binding domain comprises a sequence that is at least 75% identical to the sequence selected from the group consisting of SEQ ID Nos. 472-473 and 482-483. In some examples, the CD3 binding domain comprises a sequence that is at least 75% identical to the sequence as set forth in SEQ ID No. 474.

[0008] In some examples, the multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 495, 498-502, 569-570, 572, 573, 575, 576, 577, and 578. In some examples, the multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 495 and 502. In some examples, the multispecific protein comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 498-501, 569-570, 572, 573, 575, 576, 577, and 578. In some examples, the active drug comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 153-179, 180-206, 210-212, 494, 571, and 574.

[0009] In some examples, the EpCAM 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. In some examples, the EpCAM 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 is capable of masking the binding of the EpCAM binding domain to its target. In some examples, the binding moiety further comprises one or more complementarity determining regions (CDRs). In some examples, the CDR loop provides a binding site specific for a bulk serum protein. In some examples, 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, a pentameric IgM. In some examples, the bulk serum protein comprises the serum albumin. In some examples, the serum albumin is a human serum albumin. In some examples, 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. In some examples, the at least one but not more than 20 modifications thereto comprises 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. In some examples, the transmembrane domain comprises a transmembrane domain of 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. In some examples, the intracellular signaling domain is derived from CD3 epsilon CD3 gamma, CD3 delta, CD3 alpha, CD3 beta, or a combination thereof. In some examples, the EpCAM binding domain is part of the ProCAR, and wherein the ProCAR comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 485-491.

[0010] One example provides a method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of an EpCAM binding domain according to this disclosure, or a pharmaceutical composition comprising the same, to a subject in need thereof. In some examples, the subject is human.

[0011] One example provides a conditionally active chimeric antigen receptor (ProCAR) that comprises a single polypeptide chain, comprising (a) a binding moiety comprising a non-CDR loop and a cleavable linker; (b) an EpCAM binding domain, wherein the EpCAM 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. 39-76, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 39-76; the CDR2 comprising a sequence selected from the group consisting of SEQ ID Nos. 77-114 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 77-114; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID Nos. 115-152 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 115-152; (c) a transmembrane domain; and (d) an intracellular signaling domain; wherein the binding moiety is capable of masking the binding of the EpCAM binding domain to its target. In some examples, the EpCAM binding domain comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-38, 207-209, and 496-497.

[0012] One example provides a conditionally active EpCAM 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 an EpCAM binding domain, wherein the EpCAM 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. 39-76, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 39-76; the CDR2 comprising a sequence selected from the group consisting of SEQ ID Nos. 77-114 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 77-114; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID Nos. 115-152 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 115-152, wherein the non-CDR loop is capable of binding to the EpCAM binding domain or the second target antigen binding domain, and wherein the binding moiety is capable of masking the binding of the EpCAM binding domain or the second target antigen binding domain to its target. In some examples, the binding moiety comprises a masking moiety and wherein the masking moiety comprises a sequence selected from the group consisting of SEQ ID Nos. 380-424, or a sequence comprising one or more substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 380-424. In some examples, the linker comprises a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581, or a sequence comprising one or more substitutions relative to a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581. In some examples, the binding moiety comprises a sequence that is at least 75% identical the sequence selected from the group consisting of SEQ ID Nos. 472-473 and 482-483. In some examples, the second target antigen binding domain (T2) comprises a CD3 binding domain. In some examples, the CD3 binding domain comprises a sequence that is at least 75% identical to the sequence as set forth in SEQ ID No. 474.

[0013] One example provides a method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of a conditionally active chimeric antigen receptor according to this disclosure, or a pharmaceutical composition comprising the same, to a subject in need thereof. In some examples, the subject is human. One example provides a method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of a conditionally active EpCAM binding protein according to this disclosure, or a pharmaceutical composition comprising the same, to a subject in need thereof. In some examples, the subject is human.

[0014] In some examples, the binding domain is a humanized antibody or an antigen binding fragment thereof. In some examples, 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 examples, the binding domain comprises the single domain antibody. In some examples, the binding domain binds to EpCAM with a binding affinity (K_d) of about 0.001 nM to about 500 nM. In some examples, the binding domain binds to human EpCAM, mouse EpCAM, cynomolgus EpCAM, or a combination thereof.

One example provides a multispecific protein comprising an EpCAM binding domain, wherein the EpCAM binding domain is according to this disclosure. In some examples, the multispecific protein comprises the EpCAM binding domain according to this disclosure (anti-EpCAM domain), and a CD3 binding domain (anti-CD3 domain). In some examples, the anti-EpCAM domain and the anti-CD3 domain are in an anti-EpCAM:anti-CD3 orientation. In some examples, the anti-EpCAM domain and the anti-CD3 domain are in an anti-CD3: anti-EpCAM orientation. In some examples, the EpCAM binding domain according to any one of claims 1-3 and 49-53 (anti-EpCAM domain), the CD3 binding domain (anti-CD3 domain), and an albumin binding domain (anti-ALB domain). In some examples, the anti-CD3 domain comprises an amino acid as set forth in SEQ ID No. 379 or SEQ ID No. 474. In some examples, the anti-ALB domain comprises an amino acid sequence as set forth in SEQ ID No. 375, SEQ ID No. 472, SEQ ID No. 473, SEQ ID No. 482, or SEQ ID No. 483.

In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-CD3: anti-ALB: anti-EpCAM orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-EpCAM: anti-ALB: anti-CD3 orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-ALB: anti-EpCAM: anti-CD3 orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-CD3: anti-EpCAM: anti-ALB orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-ALB: anti-CD3: anti-EpCAM orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-EpCAM: anti-CD3: anti-ALB orientation.

[0015] One example provides a multivalent protein comprising a sequence as set forth in any one of SEQ ID Nos. 495, 498-502, 569-570, 572, 573, 575, 576, 577, and 578. One example provides an active drug comprising a sequence as set forth in any one of SEQ ID Nos. 494, 571, and 574. One example provides a multivalent protein comprising a sequence as set forth in any one of SEQ ID Nos. 485-491.

[0016] One example provides a pharmaceutical composition comprising (i)(a) an EpCAM binding domain according to this disclosure; (i)(b) a conditionally active chimeric antigen receptor according to this disclosure; (i)(c) a conditionally active EpCAM binding protein according to this disclosure; (i)(d) a multispecific protein according to this disclosure; (i)(e) a

multivalent protein according to this disclosure; or (i)(f) an active drug according to this disclosure, and (ii) a pharmaceutically acceptable carrier.

[0017] One example provides a process for the production of an EpCAM binding domain according to this disclosure, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding the EpCAM binding domain according to this disclosure, under conditions allowing the expression of the EpCAM binding domain and recovering and purifying the produced protein from the culture.

[0018] One example provides a process for the production of a multispecific protein according to this disclosure, 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 EpCAM binding protein according to this disclosure under conditions allowing the expression of the multispecific protein and recovering and purifying the produced protein from the culture.

[0019] One example provides a method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of an EpCAM binding domain according to this disclosure, or a pharmaceutical composition comprising the same, to a subject in need thereof. One example provides a method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of the multispecific protein according to this disclosure, a multivalent protein according to this disclosure, an active drug according to this disclosure, or a pharmaceutical composition according to this disclosure, to a subject in need thereof. In some examples, the subject is human. In some examples, the method further comprises administration of an agent in combination with an EpCAM binding domain according to this disclosure, a multispecific protein according to this disclosure, a multivalent protein according to this disclosure, an active drug according to this disclosure, or a pharmaceutical composition according to this disclosure. In some examples, the EpCAM binding domain selectively binds to tumor cells expressing EpCAM. In some examples, the tumorous disease comprises a solid tumor disease. In some examples, the solid tumor disease is metastatic. In some examples, the tumorous disease comprises at least one of: a colorectal cancer, a prostate cancer, a neuroendocrine 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 biliary track cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma. In some examples, the method further comprises administration of an agent in combination with conditionally active chimeric antigen receptor according to this disclosure, a conditionally active EpCAM binding protein according to this disclosure, or a pharmaceutical composition comprising the same. In some examples, the EpCAM binding domain selectively binds to tumor cells expressing EpCAM. In some examples, the tumorous disease comprises a solid tumor

disease. In some examples, the solid tumor disease is metastatic. In some examples, the tumorous disease is at least one of: a colorectal cancer, a prostate cancer, a neuroendocrine 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 biliary track cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma.

[0020] One example provides a process for the production of a conditionally active chimeric antigen receptor according to this disclosure, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding the conditionally active chimeric antigen receptor according to this disclosure, under conditions allowing the expression of the conditionally active chimeric antigen receptor and recovering and purifying the produced protein from the culture. One example provides a process for the production of a conditionally active EpCAM binding protein according to this disclosure, 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 EpCAM binding protein according to this disclosure, under conditions allowing the expression of the conditionally active EpCAM binding protein and recovering and purifying the produced protein from the culture. One example provides a process for the production of a multivalent protein according to this disclosure, 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 this disclosure, under conditions allowing the expression of the multivalent protein and recovering and purifying the produced protein from the culture. One example provides a process for the production of an active drug according to this disclosure, 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 this disclosure, under conditions allowing the expression of the active drug and recovering and purifying the produced drug from the culture.

[0021] One example provides a cell comprising a CAR according to this disclosure. One example provides a cell comprising a ProCAR according to this disclosure. In some examples, the cell is a T cell or an NK cell. One example provides a method comprising transfecting a cell according to this disclosure, with a vector or an RNA comprising a nucleotide sequence encoding the CAR or the ProCAR.

[0022] One example provides a conditionally active chimeric antigen receptor that has a greater therapeutic index compared to a chimeric antigen receptor (CAR) that does not comprise the (a) binding moiety but is otherwise identical to the conditionally active chimeric antigen receptor. In some examples, the conditionally active chimeric antigen receptor has a therapeutic index that is

at least about 5-fold to about 100-fold greater than that of a chimeric antigen receptor (CAR) that does not comprise the (a) binding moiety but is otherwise identical to the conditionally active chimeric antigen receptor. In some examples, the protein comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 498-501, 569-570, 572, 573, 575, 576, 577, and 578. In some examples, the protein comprises a sequence that is at least about 75% identical to the sequence of SEQ ID No. 576. In some examples, the protein comprises a sequence as set forth in SEQ ID No. 576. In some examples, the conditionally active EpCAM binding protein has a greater therapeutic index compared to an EpCAM binding protein that does not comprise the binding moiety (M) or the cleavable linker (L) but is otherwise identical to the conditionally active EpCAM binding protein. In some examples, the conditionally active EpCAM binding protein has a therapeutic index that is at least about 5-fold to about 100-fold greater than that of an EpCAM binding protein that does not comprise the binding moiety (M) or the cleavable linker (L) but is otherwise identical to the conditionally active EpCAM binding protein.

[0023] One example provides a pharmaceutical composition comprising: (i)(a) a conditionally active chimeric antigen receptor according to this disclosure; or (i)(b) a conditionally active EpCAM binding protein according to this disclosure, and (ii) a pharmaceutically acceptable carrier.

One example provides a method of for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of the conditionally active chimeric antigen receptor according to this disclosure, or a pharmaceutical composition comprising the same, to a subject in need thereof.

[0024] One example provides a method of for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of the conditionally active EpCAM binding protein according to this disclosure, or a pharmaceutical composition comprising the same, to a subject in need thereof. In some examples, the subject is human. In some examples, the tumorous disease comprises at least one of: a colorectal cancer, a prostate cancer, a neuroendocrine 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 biliary track cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma.

[0025] One example provides a method of increasing a therapeutic index of an EpCAM binding domain, the method comprising conjugating the EpCAM 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 EpCAM binding domain,

- wherein the EpCAM binding domain is masked from binding its target by the binding moiety,
- wherein the EpCAM binding domain is able to bind its target upon cleavage of the cleavable linker.

[0026] In some examples, the EpCAM 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. 39-76, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 39-76; the CDR2 comprising a sequence selected from the group consisting of SEQ ID Nos. 77-114 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 77-114; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID Nos. 115-152 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 115-152. In some examples, the EpCAM 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 examples, 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. 472-473 and 482-483. In some examples, 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. 379 and 474. In some examples, the cleavable linker comprises a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581.

[0027] In some examples, the EpCAM binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-38, 207-209, and 496-497. In some examples, 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. 498-501, 569-570, 572, 573, 575, 576, 577, and 578. In some examples, the conditionally active multispecific protein comprises a sequence that is at least 75% identical to SEQ ID No. 576. In some examples, the conditionally active multispecific protein comprises a sequence as set forth in SEQ ID No. 576. In some examples, the EpCAM 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. In some examples, the EpCAM 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. 39-76, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 39-76; the CDR2 comprising a sequence selected from the group consisting of SEQ ID Nos. 77-114 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 77-114; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID Nos. 115-152 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 115-152. In some examples, 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. 472-473 and 482-483. In some examples, the EpCAM binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-38, 207-209, and 496-497. In some examples, the conditionally active chimeric antigen receptor comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 485-491.

[0028] One example provides a method of increasing a therapeutic index of an EpCAM 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 an EpCAM 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.

[0029] In some examples, the EpCAM 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. 39-76, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 39-76; the CDR2 comprising a sequence selected from the group consisting of SEQ ID Nos. 77-114 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 77-114; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID Nos. 115-152 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 115-152. In some examples, the non-CDR loop comprises a

binding site specific for the EpCAM binding domain. In some examples, at least one of the first or the second target antigen binding domain comprises a CD3 binding domain. In some examples, the non-CDR loop comprises a binding site specific for the CD3 binding domain. In some examples, the CD3 binding domain comprises a sequence that is at least about 75% identical to SEQ ID No. 474. In some examples, 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. 472-473 and 482-483. In some examples, the EpCAM binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-38, 207-209, and 496-497. In some examples, the cleavable linker comprises a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581. In some examples, 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. 498-501, 569-570, 572, 573, 575, 576, 577, and 578. In some examples, the conditionally active multispecific protein comprises a sequence that is at least 75% identical to SEQ ID No. 576. In some examples, the conditionally active multispecific protein comprises a as set forth in SEQ ID No. 576.

[0030] One example provides a method of increasing a therapeutic index of an EpCAM binding protein comprising an EpCAM 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 examples, the EpCAM 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. 39-76, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 39-76; the CDR2 comprising a sequence selected from the group consisting of SEQ ID Nos. 77-114 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 77-114; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID Nos. 115-152 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 115-152. In some examples, the CD3 binding domain comprises a sequence that is at least about 75% identical to SEQ ID No. 474. In some examples, 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. 472-473 and 482-483. In some examples, the EpCAM binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-38, 207-209, and 496-497. In some

examples, the cleavable linker comprises a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581. In some examples, 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. 498-501, 569-570, 572, 573, 575, 576, 577, and 578. In some examples, the conditionally active multispecific protein comprises a sequence that is at least 75% identical to SEQ ID No. 576. In some examples, the conditionally active multispecific protein comprises a as set forth in SEQ ID No. 576.

[0031] One example provides an EpCAM targeting conditionally active multispecific protein, comprising: an EpCAM 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 EpCAM 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. 39-76, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 39-76; the CDR2 comprising a sequence selected from the group consisting of SEQ ID Nos. 77-114 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 77-114; and the CDR3 comprising a sequence selected from the group consisting of SEQ ID Nos. 115-152 or a sequence comprising one or more substitutions in a sequence selected from the group consisting of SEQ ID Nos. 115-152. In some examples, 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. 472-473 and 482-483. In some examples, the the EpCAM binding domain comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-38, 207-209, and 496-497. In some examples, the cleavable linker comprises a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581, or a sequence comprising one or more substitutions in a sequence selected from the group consisting of: SEQ ID Nos. 425-471, 503-506, and 581. In some examples, 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. 498-501, 569-570, 572, 573, 575, 576, 577, and 578. In some examples, the conditionally active multispecific protein comprises a sequence that is at least 75% identical to SEQ ID No. 576. In some examples, the conditionally active multispecific protein comprises a as set forth in SEQ ID No. 576.

[0032] One example provides a pharmaceutical composition comprising an EpCAM targeting conditionally active multispecific protein of this disclosure. In some examples, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier. One example provides a process for the production of EpCAM targeting conditionally active multispecific protein of this disclosure, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the domains of the EpCAM targeting conditionally active multispecific protein this disclosure, under conditions allowing the expression of the EpCAM targeting conditionally active multispecific protein and recovering and purifying the produced protein from the culture.

[0033] One example provides a method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of an of EpCAM targeting conditionally active multispecific protein of this disclosure, or a pharmaceutical composition according to claim 149 or 150, to a subject in need thereof. In some examples, the tumorous disease comprises a solid tumor disease. In some examples, the solid tumor disease is metastatic. In some examples, the tumorous disease comprises at least one of: a colorectal cancer, a prostate cancer, a neuroendocrine 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 biliary track cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, an adenocarcinoma.

[0034] One example provides an EpCAM binding domain comprising a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-38. In some examples, the EpCAM binding domain comprises a CDR1, a CDR2, and a CDR3. In some examples, the CDR1 comprises a sequence selected from the group consisting of SEQ ID Nos. 39-76, or one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 39-76. In some examples, the CDR2 comprises a sequence selected from the group consisting of SEQ ID Nos. 77-114, or one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 77-114. In some examples, the CDR3 comprises a sequence selected from the group consisting of SEQ ID Nos. 115-152, or one or more substitutions relative to a sequence selected from SEQ ID Nos. 115-152. In some examples, the EpCAM binding domain comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-38. In some examples, the EpCAM binding domain comprises a sequence selected from the group consisting of SEQ ID Nos. 1-38. In some examples, the EpCAM binding domain is a humanized antibody or an antigen binding fragment thereof. In some examples, the EpCAM 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 examples, the EpCAM

binding domain comprises the single domain antibody. In some examples, the binding domain binds to EpCAM with a binding affinity (K_d) of about 0.001 nM to about 500 nM. In some examples, the EpCAM binding domain binds to human EpCAM, mouse EpCAM, cynomolgus EpCAM, or a combination thereof.

[0035] One example provides a multispecific protein comprising an EpCAM binding domain, wherein the EpCAM binding domain is according to this disclosure. In some examples, the multispecific protein comprises the EpCAM binding domain according to this disclosure (anti-EpCAM domain), and a CD3 binding domain (anti-CD3 domain). In some examples, the anti-EpCAM domain and the anti-CD3 domain are in an anti-EpCAM:anti-CD3 orientation. In some examples, the anti-EpCAM domain and the anti-CD3 domain are in an anti-CD3: anti-EpCAM orientation. In some examples, the multispecific protein comprises an EpCAM binding domain according to this disclosure (anti-EpCAM domain), the CD3 binding domain (anti-CD3 domain), and an albumin binding domain (anti-ALB domain). In some examples, the anti-CD3 domain comprises an amino acid as set forth in SEQ ID No. 379 or SEQ ID No. 474. In some examples, the anti-ALB domain comprises an amino acid sequence as set forth in SEQ ID No. 375, SEQ ID No. 472, SEQ ID No. 473, SEQ ID No. 482, or SEQ ID No. 483. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-CD3: anti-ALB: anti-EpCAM orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-EpCAM: anti-ALB: anti-CD3 orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-ALB: anti-EpCAM: anti-CD3 orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-ALB: anti-EpCAM: anti-CD3 orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-CD3: anti-EpCAM: anti-ALB orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-ALB: anti-CD3: anti-EpCAM orientation. In some examples, the anti-EpCAM domain, the anti-CD3 domain, and the anti-ALB domain are in an anti-EpCAM: anti-CD3: anti-ALB orientation.

[0036] One example provides a multivalent protein comprising a sequence as set forth in any one of SEQ ID Nos. 485-491. One example provides a pharmaceutical composition comprising (i)(a) an EpCAM binding domain according to this disclosure, (i)(b) a multispecific protein according to this disclosure, or (i)(c) a multivalent protein according to this disclosure; and (ii) a pharmaceutically acceptable carrier.

[0037] One example a process for the production of an EpCAM binding domain according to this disclosure, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding the EpCAM binding domain according to this

disclosure under conditions allowing the expression of the EpCAM binding domain and recovering and purifying the produced protein from the culture. One example provides a process for the production of a multispecific protein according to any this disclosure, 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 EpCAM binding protein according to this disclosure under conditions allowing the expression of the multispecific protein and recovering and purifying the produced protein from the culture.

[0038] One example provides a method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of an EpCAM binding domain according to this disclosure, or a pharmaceutical composition according to this disclosure, to a subject in need thereof.

[0039] One example provides a method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of the multispecific protein according to this disclosure, a multivalent protein according to this disclosure, or a pharmaceutical composition according to this disclosure, to a subject in need thereof. In some examples, the subject is human. In some examples, the method further comprises administration of an agent in combination with an EpCAM binding domain according to this disclosure, a multispecific protein according to this disclosure, a multivalent protein according to this disclosure, or a pharmaceutical composition according to this disclosure. In some examples, the EpCAM binding domain selectively binds to tumor cells expressing EpCAM. In some examples, the tumorous disease comprises a solid tumor disease. In some examples, the solid tumor disease is metastatic.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0041] FIG 1 provides results from a representative T cell dependency cellular cytotoxicity assay with NCI-H508 cells using exemplary fusion proteins of this disclosure containing an anti-EpCAM domain as described herein and an anti-CD3 domain.

[0042] FIG. 2 provides results from a representative T cell dependency cellular cytotoxicity assay using exemplary fusion proteins of this disclosure containing an anti-EpCAM domain as described herein and an anti-CD3 domain.

[0043] FIG. 3 provides results from a representative T cell dependency cellular cytotoxicity assay using exemplary fusion proteins of this disclosure containing an anti-EpCAM domain as described herein and an anti-CD3 domain.

[0044] FIG. 4 provides results from a representative T cell dependency cellular cytotoxicity assay using exemplary fusion proteins of this disclosure containing an anti-EpCAM domain as described herein and an anti-CD3 domain.

[0045] FIG. 5 provides results from a representative T cell dependency cellular cytotoxicity assay using exemplary fusion proteins of this disclosure containing a humanized anti-EpCAM domain as described herein and an anti-CD3 domain.

[0046] FIG. 6 illustrate exemplary ProCAR constructs. One exemplary construct includes an anti-human EpCAM sdAb, a FLAG epitope, a CD8 hinge/transmembrane domain, a 4-1BB intracellular domain, and a CD3 zeta intracellular domain (SEQ ID NO: 485). One exemplary

CLAIMS

WHAT IS CLAIMED IS:

1. A multispecific protein comprising (a) an EpCAM binding domain, (b) a CD3 ϵ binding domain, and (c) a bulk serum protein binding domain that binds to a human serum albumin protein, wherein the multispecific protein comprises an amino acid sequence of any one of SEQ ID NOS: 495, 498-502, 569-570, 572, 573, 575, 576, 577, and 578.
2. The multispecific protein of claim 1, wherein the bulk serum protein binding domain is a binding moiety comprising a linker and a masking moiety, and wherein the masking moiety is capable of masking the binding of the EpCAM binding domain or the CD3 ϵ binding domain, to their respective targets.
3. The multispecific protein of claim 1, wherein the EpCAM binding domain comprises an amino acid sequence of any one of SEQ ID NOS: 1-38, 207-209, and 496-497.
4. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 495.
5. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 498.
6. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 499.
7. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 500.
8. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 501.
9. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 502.
10. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 569.
11. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 570.
12. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 572.
13. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 573.
14. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 575.

15. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 576.
16. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 577.
17. The multispecific protein according to claim 1, wherein the multispecific protein comprises the amino acid sequence of SEQ ID NO: 578.
18. A pharmaceutical composition comprising (i) a multispecific protein according to any one of claims 1-17, and (ii) a pharmaceutically acceptable carrier.
19. Use of the multispecific protein according to any one of claims 1-17 in the manufacture of a medicament for treating a cancer in a subject in need thereof.
20. The use according to claim 19, wherein the subject is human.
21. The use according to claim 19, wherein the cancer comprises a solid tumor.
22. The use according to claim 21, wherein the solid tumor is metastatic.
23. The use according to claim 19, wherein the cancer comprises a colorectal cancer, a prostate cancer, a neuroendocrine 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 biliary track cancer, a gall bladder cancer, an esophageal cancer, a breast cancer, or an adenocarcinoma.
24. A process for production of a multispecific protein according to any one of claims 1-17, said process comprising culturing a host transformed or transfected with a vector comprising one or more nucleic acid sequences encoding the multispecific protein under conditions allowing the expression of the multispecific protein, recovering and purifying the produced multispecific protein from the culture.
25. The multispecific protein of any one of claims 1 to 17 for use in therapy.