

MACROPHAGE SPECIFIC ENGAGER COMPOSITIONS AND METHODS OF USE THEREOF

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/860,055, filed on June 11, 2019, and U.S. Provisional Application No. 62/908,978, filed on October 1, 2019, each of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0001.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 7, 2020, is named 56371-703_601_SL.txt and is 87,312 bytes in size.

BACKGROUND

[0002] Cellular immunotherapy is a promising new technology for fighting difficult to treat diseases, such as cancer, persistent infections and diseases that are refractory to other forms of treatment. Macrophages represent the dominant cell type present in a tumor or an infection site and possess several strategic advantages such that they can be potentially utilized to treat the disease most effectively. As natural sentinels of the immune system, these cells can sense and eliminate aberrant and non-healthy cell types, including cancer cells. However, potential use of macrophages for immunotherapy has not been fully explored. Newer avenues are sought for using these cell types towards development of improved therapeutics, including but not limited to T cell malignancies.

SUMMARY

[0002a] In one aspect, provided herein is a composition comprising a therapeutic agent, wherein the therapeutic agent comprises: (a) a first binding domain, wherein the first binding domain is a binding domain of a first antibody that specifically interacts with an antigen on a target cell, and (b) a second binding domain, wherein the second binding domain is a binding domain of a second antibody that specifically interacts with a myeloid cell; wherein the first and second binding domains are operably linked by one or more linkers.

[0002b] In another aspect, provided herein is a pharmaceutical composition comprising the composition as described herein.

[0002c] In another aspect, provided herein is the use of the pharmaceutical composition as described herein in the manufacture of a medicament for the treatment of cancer.

[0002d] In yet another aspect, provided herein is the pharmaceutical composition as described herein for use in therapy.

[0003] The present disclosure relates to new compositions and methods that initiate a target cell destruction pathway through phagocytosis. This application is based on an unexpected finding that when a phagocytic receptor is triggered with at least a second concurrent or subsequent activation signal in addition to binding to its classical ligand, the second or additional signal(s) can lead to efficient destruction of a target cell by phagocytosis. Presented herein are chimeric receptors, and chimeric receptor-binding extracellular elements designed for enhancing phagocytosis of a cell, such as a myeloid cell, or a monocyte or macrophage. Careful design and/or manipulation of the at least second concurrent or subsequent signal is useful for successful activation of a chimeric phagocytic receptor such as those described herein, such that the target cell is effectively destroyed thereafter. For example, the first signal (signal 1) can be mediated via phagocytosis/tethering receptors and the second signal (signal 2) can be mediated by danger signals such as pathogen-associated molecular patterns (DAMPs), or cytokines that trigger nuclear factor- κ B (NF- κ B)-mediated upregulation of inflammatory genes. As described in the following section, triggering phagocytosis alone may be insufficient to activate monocytes or macrophages in the context of harnessing the phagocytic ability of monocytes or macrophages to kill cancer cells, and to drive an effective anti-tumor response.

[0004] One of the specific advantages of the ~~invention~~disclosures described here is that the compositions for effective cellular immunotherapy disclosed herein are cost-effective and efficient.

[0005] In some [aspectexample](#), provided herein are new chimeric cell surface binding elements or “engagers” that bind to an extracellular portion of a chimeric phagocytic receptor, and bind additionally to at least a cell surface component on a target cell such as a cancer cell.

[0006] In one [embodimentexample](#), the new chimeric engagers can bind to an extracellular portion of a chimeric phagocytic receptor, and additionally bind to one or more cell surface components, at least one of which is on a target cancer cell. Accordingly, an engager may be a bi-specific monocyte or macrophage engager (BiME) and have two binding portions, wherein one binding portion binds to an extracellular portion of a chimeric phagocytic receptor, and the other binds to the cell surface component on a target cell. Likewise, an engager may be a trispecific monocyte or macrophage engager (TriME) and have three binding portions, wherein one binding portion binds to an extracellular portion of a chimeric phagocytic receptor, another binding portion binds to the cell surface component on a target cell and the third binding portion binds to the cell surface component on the phagocytic cell.

[0007] In one [aspectexample](#), the engager is a synthetic protein or a peptide, a conjugated protein or conjugated peptide. Provided herein is a composition comprising: a first therapeutic agent, wherein the therapeutic agent comprises: (a) a first binding domain, wherein the first binding domain is a first antibody or functional fragment thereof that specifically interacts with an antigen of a target cell, and (b) a second binding domain, wherein the second binding domain is a second antibody or functional fragment thereof that specifically interacts with a myeloid cell; wherein, (i) the first therapeutic agent is coupled to a first component, wherein the first component is an additional therapeutic agent or a third binding domain, or (ii) the composition comprises an additional therapeutic agent.

[0008] In one [aspectexample](#), provided herein is a composition comprising: a therapeutic agent, wherein the therapeutic agent is an engager that comprises: (a) a first binding domain that specifically interacts with an antigen of a target cell, (b) a second binding domain that specifically interacts with a myeloid cell, and (c) a third binding domain that specifically interacts with the myeloid cell.

[0009] In one [aspectexample](#), provided herein is a composition comprising: a therapeutic agent, wherein the therapeutic agent is an engager that comprises: (a) a first binding domain that specifically interacts with an antigen of a target cell, (b) a second binding domain, wherein the second binding domain: (i) specifically interacts with a myeloid cell (e.g., a monocyte or macrophage, or a dendritic cell) and promotes phagocytosis activity of the myeloid cell, or, (ii) specifically interacts with a myeloid cell and promotes inflammatory signaling of the myeloid cell, or (iii) specifically interacts with a myeloid cell or an adhesion molecule and promotes adhesion of the myeloid cell to the target cell, and (c) a third binding domain, wherein the third binding domain (i) specifically interacts with the myeloid cell and promotes phagocytic activity of the myeloid cell, or, (ii) specifically interacts with the myeloid cell and promotes inflammatory signaling of the myeloid cell, or, (iii) specifically interacts with the myeloid cell and promotes adhesion of the myeloid cell to the target cell, or, (iv) specifically interacts with the myeloid cell

and inhibits anti-phagocytic activity of the myeloid cell mediated by the target cell, or (v) specifically interacts with the myeloid cell and inhibits anti-inflammatory activity of the myeloid cell mediated by the target cell.

[0010] In some [embodimentexamples](#), the myeloid cell is a monocyte or macrophage cell.

[0011] In some [embodimentexamples](#), the target cell is a cancer cell.

[0012] In some [embodimentexamples](#), the second binding domain that specifically interacts with a myeloid cell interacts with a phagocytic or tethering receptor of the myeloid cell or monocyte or macrophage cell.

[0013] In some [embodimentexamples](#), the third binding domain that specifically interacts with a myeloid cell interacts with an extracellular region of a first phagocytic or tethering receptor of the myeloid cell or monocyte or macrophage cell.

[0014] The composition of any one of the preceding claims wherein any one of binding domains of the therapeutic agent comprises the binding domain of an antibody, a functional fragment of an antibody, a variable domain thereof, a V_H domain, a V_L domain, a VNAR domain, a V_{HH} domain, a single chain variable fragment (scFv), an Fab, a single-domain antibody (sdAb), a nanobody, a bispecific antibody, a diabody, or a functional fragment or a combination thereof.

[0015] In some [embodimentexamples](#), the therapeutic agent is a recombinant protein or more than one recombinant proteins.

[0016] In some [embodimentexamples](#), the therapeutic agent comprises recombinant proteins comprising one or more fusion proteins.

[0017] In some [embodimentexamples](#), the therapeutic agent is a recombinant protein comprising an antibody, a functional fragment of an antibody, a variable domain thereof, a V_H domain, a V_L domain, a VNAR domain, a V_{HH} domain, a single chain variable fragment (scFv), an Fab, a single-domain antibody (sdAb), a nanobody, a bispecific antibody, a diabody, or a functional fragment or a combination thereof. In some [embodimentexamples](#), the therapeutic agent is a recombinant protein or more than one recombinant proteins, each comprising multiple binding fragments, each binding fragment constituting a functional fragment of an antibody, a variable domain thereof, a V_H domain, a V_L domain, a VNAR domain, a V_{HH} domain, a single chain variable fragment (scFv), an Fab, a single-domain antibody (sdAb), a nanobody, a bispecific antibody, a diabody, or a functional fragment or a combination thereof.

[0018] In some [embodimentexamples](#), the therapeutic agent is a recombinant protein (the engager) comprising multiple binding domains, each having individual binding specificities, that are each linked together by linkers (e.g., peptide linkers) that exhibit complementary binding with each other. For example, one binding domain of the recombinant protein is fused with the first of a pair of linker peptides, and the other binding domain is fused with the second of the pair of linker peptides, wherein, the pair of linker peptides exhibit complementary binding with each other, wherein the pair of linker peptides comprise: (a) leucine zipper domains that exhibit complementary binding with each other; for example, leucine zippers in

naturally occurring protein-protein interactions, such as the zipper sequences within the binding regions of c-Fos and c-Jun proteins, (b) synthetic peptides designed to specifically bind to each other via designed affinities, such as synthetic clasps.

[0019] In some [embodimentexamples](#), the therapeutic agent is a recombinant protein comprising multiple binding fragments configured to facilitate accelerated association with each other by means of leucine zipper peptide pairs comprised in the recombinant proteins.

[0020] In some [embodimentexamples](#), the therapeutic agent is a recombinant protein comprising multiple binding fragments configured to facilitate accelerated association with each other by means of c-Fos/c-Jun binding domains in the peptide pairs comprised within the recombinant proteins.

[0021] In some [embodimentexamples](#), the therapeutic agent is a recombinant protein comprising multiple binding fragments configured to facilitate accelerated association with each other by means of synthetic clasps.

[0022] In some [embodimentexamples](#), the antigen on the target cell to which the first binding domain binds, is a cancer antigen or a pathogenic antigen on the target cell or an autoimmune antigen.

[0023] In some [embodimentexamples](#), the antigen on the target cell to which the first binding domain binds, is a viral antigen

[0024] In some [embodimentexamples](#), the antigen on the target cell to which the first binding domain binds is a T-lymphocyte antigen.

[0025] In some [embodimentexamples](#), the antigen on the target cell to which the first binding domain binds is an extracellular antigen.

[0026] In some [embodimentexamples](#), the antigen on the target cell to which the first binding domain binds is an intracellular antigen.

[0027] In some [embodimentexamples](#), the antigen on the target cell to which the first binding domain binds is selected from the group consisting of Thymidine Kinase (TK1), Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT), Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1), Mucin-1, Mucin-16 (MUC16), MUC1, Epidermal Growth Factor Receptor vIII (EGFRvIII), Mesothelin, Human Epidermal Growth Factor Receptor 2 (HER2), Mesothelin, EBNA-1, LEMD1, Phosphatidyl Serine, Carcinoembryonic Antigen (CEA), B-Cell Maturation Antigen (BCMA), Glypican 3 (GPC3), Follicular Stimulating Hormone receptor, Fibroblast Activation Protein (FAP), Erythropoietin-Producing Hepatocellular Carcinoma A2 (EphA2), EphB2, a Natural Killer Group 2D (NKG2D) ligand, Disialoganglioside 2 (GD2), CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD24, CD30, CD33, CD38, CD44v6, CD45, CD56CD79b, CD97, CD117, CD123, CD133, CD138, CD171, CD179a, CD213A2, CD248, CD276, PSCA, CS-1, CLECL1, GD3, PSMA, FLT3, TAG72, EPCAM, IL-1, an integrin receptor, PRSS21, VEGFR2, PDGFR-beta, SSEA-4, EGFR, NCAM, prostase, PAP, ELF2M, GM3, TEM7R, CLDN6, TSHR, GPRC5D, ALK, IGLL1 and combinations thereof.

- [0028] In some [embodimentexamples](#), the antigen on the target cell to which the first binding domain binds is selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CCR4, CD8, CD30, CD45, CD56.
- [0029] In some [embodimentexamples](#), the antigen on the target cell to which the first binding domain binds is an ovarian cancer antigen or a T lymphoma antigen.
- [0030] In some [embodimentexamples](#), the antigen on the target cell to which the first binding domain binds is an integrin receptor.
- [0031] In some [embodimentexamples](#), the second binding domain or the third binding domain binds to an integrin receptor.
- [0032] In some [embodimentexamples](#), the second binding domain or the third binding domain binds to an integrin receptor selected from the group consisting of $\alpha 1$, $\alpha 2$, αIIb , $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 7$, $\alpha 8$, $\alpha 9$, $\alpha 10$, $\alpha 11$, αD , αE , αL , αM , αV , αX , $\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$, $\beta 5$, $\beta 6$, $\beta 7$, and $\beta 8$.
- [0033] In some [embodimentexamples](#), the therapeutic agent binds to a phagocytic or tethering receptor that comprises a phagocytosis activation domain.
- [0034] In some [embodimentexamples](#), the therapeutic agent binds to a receptor or a protein selected from the group consisting the receptors listed in **Table 2A** and **Table 2B**, or a fragment thereof.
- [0035] In some [embodimentexamples](#), the therapeutic agent binds to a phagocytic receptor selected from the group consisting of lectin, dectin 1, CD206, scavenger receptor A1 (SRA1), MARCO, CD36, CD163, MSR1, SCARA3, COLEC12, SCARA5, SCARB1, SCARB2, CD68, OLR1, SCARF1, SCARF2, CXCL16, STAB1, STAB2, SRCRB4D, SSC5D, CD205, CD207, CD209, RAGE, CD14, CD64, F4/80, CCR2, CX3CR1, CSF1R, Tie2, HuCRIg(L), CD64, CD32a, CD16a, CD89, Fc-alpha receptor I, CR1, CD35, CR3, CR4, Tim-1, Tim-4 and CD169.
- [0036] In some [embodimentexamples](#), the therapeutic agent binds to a receptor comprising an intracellular signaling domain that comprises a pro-inflammatory signaling domain.
- [0037] In some [embodimentexamples](#), the first therapeutic agent comprises a polypeptide that is less than 1000 amino acids or 1000 nm in length or 1000 nm.
- [0038] In some [embodimentexamples](#), the first therapeutic agent comprises a polypeptide that is less than 500 amino acids or 500 nm in length.
- [0039] In some [embodimentexamples](#), the first therapeutic agent comprises a polypeptide that is 200-1000 amino acids or 200-1000 nm in length.
- [0040] In some [embodimentexamples](#), engagement of the binding domains of the first therapeutic agent contacts the cancer cell to the myeloid cell.
- [0041] In some [embodimentexamples](#), the second binding domain specifically interacts with a myeloid cell and promotes phagocytosis activity of the myeloid cell.

- [0042] In some [embodimentexamples](#), the second binding domain specifically interacts with a myeloid cell and promotes inflammatory signaling of the myeloid cell.
- [0043] In some [embodimentexamples](#), the second binding domain specifically interacts with a myeloid cell or an adhesion molecule and promotes adhesion of the myeloid cell to the target cell.
- [0044] In some [embodimentexamples](#), the second binding domain specifically interacts with a myeloid cell and inhibits anti-phagocytic activity of the myeloid cell mediated by the target cell.
- [0045] In some [embodimentexamples](#), the second binding domain specifically interacts with a myeloid cell and inhibits anti-inflammatory activity of the myeloid cell mediated by the target cell.
- [0046] In some [embodimentexamples](#), the second and/or the third binding domain promotes phagocytic activity of the myeloid cell.
- [0047] In some [embodimentexamples](#), the second and/or the third binding domain promotes inflammatory signaling of the myeloid cell.
- [0048] In some [embodimentexamples](#), the second and/or the third binding domain specifically interacts with a myeloid cell or an adhesion molecule and promotes adhesion of the myeloid cell to the target cell.
- [0049] In some [embodimentexamples](#), the second and/or the third binding domain inhibits anti-phagocytic activity of the myeloid cell mediated by the target cell.
- [0050] In some [embodimentexamples](#), the second and/or the third binding domain inhibits anti-inflammatory activity of the myeloid cell mediated by the target cell.
- [0051] In some [embodimentexamples](#), the therapeutic agent comprises a therapeutic polypeptide.
- [0052] In some [embodimentexamples](#), the therapeutic agent comprises a recombinant nucleic acid encoding the therapeutic polypeptide.
- [0053] In some [embodimentexamples](#), the third binding domain or the additional therapeutic agent comprises a CD47 antagonist, a CD47 blocker, an antibody, a chimeric CD47 receptor, a sialidase, a cytokine, a proinflammatory gene, a procaspase, or an anti-cancer agent.
- [0054] In some [embodimentexamples](#), the first binding domain, the second binding domain and the third binding domain bind to distinct non-identical target antigens.
- [0055] In some [embodimentexamples](#), the first binding domain, the second binding domain or the third binding domain is a ligand binding domain.
- [0056] In some [embodimentexamples](#), the first, the second or the third binding domains are operably linked by one or more linkers.
- [0057] In some [embodimentexamples](#), the linker is a polypeptide. In some [embodimentexamples](#), the linker is a functional peptide. In some [embodimentexamples](#), the linker is a ligand for a receptor. In some [embodimentexamples](#), the linker is a ligand for a monocyte or macrophage receptor. In some [embodimentexamples](#), the linker activates the receptor. In some [embodimentexamples](#), the linker inhibits the receptor. In some [embodimentexamples](#), the linker is a

ligand for a M2 monocyte or macrophage. In some [embodimentexamples](#), the linker is a ligand for a TLR receptor. In some [embodimentexamples](#), the linker activates the TLR receptor.

[0058] In some [embodimentexamples](#), the first, the second and /or the third binding domains are associated with a mask that binds to the binding domain.

[0059] In some [embodimentexamples](#), the mask is an inhibitor that inhibits the interaction of binding domain to its target when the mask remains associated with the respective binding domain.

[0060] In some [embodimentexamples](#), the mask is associated with the binding domain via a peptide linker.

[0061] In some [embodimentexamples](#), the peptide linker comprises a cleavable moiety.

[0062] In some [embodimentexamples](#), the cleavable moiety is cleaved by a protein or an enzyme selectively abundant in the site of the cancer or tumor.

[0063] In some [embodimentexamples](#), the third binding domain that specifically interacts with an extracellular region of a second receptor of the monocyte or macrophage activates the monocyte or macrophage.

[0064] In some [embodimentexamples](#), upon binding of the therapeutic agent to the myeloid cell, the killing or phagocytosis activity of the myeloid cell is increased by at least 10%, or 20%, or 30%, or 40 %, or 50%, or 60%, or 70% or 90% or 100% compared to a myeloid cell not bound by the therapeutic agent, as measured by a particle uptake assay.

[0065] In some [embodimentexamples](#), engagement of the binding domains of first therapeutic agent triggers phagocytosis of the cancer cell by the myeloid cell.

[0066] In some [embodimentexamples](#), engagement of the second therapeutic agent potentiates or increases the phagocytic killing of the cancer cell by the myeloid cell.

[0067] In some [embodimentexamples](#), the second or third binding domain binds to an extracellular of IgA, IgD, IgE, IgG, IgM, FcγRI, FcγRIIA, FcγRIIB, FcγRIIC, FcγRIIIA, FcγRIIIB, FcRn, TRIM21, FcRL5.

[0068] In some [embodimentexamples](#), the second or the third binding domain comprises an M2 domain.

[0069] In some [embodimentexamples](#), the second or the third binding domain comprises a LIGHT domain or an HVEM binding domain.

[0070] In some [embodimentexamples](#), the second or the third binding domain comprises a HVEM binding domain.

[0071] In some [embodimentexamples](#), the second or the third binding domain comprises a GITR binding domain.

[0072] In some [embodimentexamples](#), the first binding domain comprises a sequence having an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 27, 28, 111, 112, 113, 115, 143 and 144.

[0073] In some [embodimentexamples](#), the second binding domain comprises a sequence having an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to a sequence selected from the group 141 and 142.

[0074] In some [embodimentexamples](#), the first component comprises an amino acid sequence GGQEINSSYGG (SEQ ID NO: 105) or QEINSSY (SEQ ID NO: 129).

[0075] In some [embodimentexamples](#), the first component comprises an amino acid sequence GGAPPHALSGG (SEQ ID NO: 109) or APPHALS (SEQ ID NO: 137).

[0076] In some [embodimentexamples](#), the linker comprises an amino acid sequence GGQEINSSYGG (SEQ ID NO: 105), or QEINSSY (SEQ ID NO: 129) or GGAPPHALSGG (SEQ ID NO: 109) or APPHALS (SEQ ID NO: 137).

[0077] In some [embodimentexamples](#), provided herein is a bispecific or trispecific engager, comprising a sequence having an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 151.

[0078] In some [embodimentexamples](#), provided herein is a bispecific or trispecific engager, comprising a sequence having an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 152.

[0079] Provided herein is a pharmaceutical composition comprising: a first therapeutic agent, wherein the therapeutic agent comprises one or more polypeptides or recombinant nucleic acids encoding the one or more polypeptides, wherein the one or more polypeptides comprise: a first binding domain, wherein the first binding domain is a first antibody or functional fragment thereof that specifically interacts with an antigen of a target cell, and a second binding domain, wherein the second binding domain is a second antibody or functional fragment thereof that specifically interacts with a myeloid cell; wherein, (i) the first therapeutic agent is coupled to a first component, wherein the first component is an additional therapeutic agent or a third binding domain, or (ii) the composition comprises an additional therapeutic agent; and an acceptable pharmaceutical salt or excipient.

[0080] In some [embodimentexamples](#), the first therapeutic agent of the pharmaceutical composition comprises a single polypeptide. In some [embodimentexamples](#), the first therapeutic agent of the pharmaceutical composition comprises multiple polypeptides. In some [embodimentexamples](#), the first therapeutic agent of the pharmaceutical composition is a recombinant nucleic acid encoding the one or more polypeptides. In some [embodimentexamples](#), the pharmaceutical composition further comprises a second therapeutic agent.

[0081] Provided herein is a method of treatment, comprising: administering to the subject in need thereof, a pharmaceutical composition, comprising: a first therapeutic agent, wherein the therapeutic agent comprises one or more polypeptides or recombinant nucleic acids encoding the one or more polypeptides, wherein the one or more polypeptides comprise: a first binding domain, wherein the first binding domain is a first antibody or functional fragment thereof that specifically interacts with an antigen

of a target cell, and a second binding domain, wherein the second binding domain is a second antibody or functional fragment thereof that specifically interacts with a myeloid cell; wherein, (i) the first therapeutic agent is coupled to a first component, wherein the first component is an additional therapeutic agent or a third binding domain, or (ii) the composition comprises an additional therapeutic agent; and an acceptable pharmaceutical salt or excipient.

[0082] In some [embodimentexamples](#), the method of treatment further comprises administering a second therapeutic agent. In some [embodimentexamples](#), the method of treatment further comprises administering the pharmaceutical composition comprises administering the pharmaceutical composition intravenously.

[0083] In some [embodimentexamples](#), the method of treatment further comprises the administering the pharmaceutical composition comprises administering the pharmaceutical composition subcutaneously. In some [embodimentexamples](#), the method of treatment further comprises administering the pharmaceutical composition comprises injecting the pharmaceutical composition.

In some [embodimentexamples](#), the target cell is a cancer cell.

[0084] In some [embodimentexamples](#), the target cell is a cancer cell that is a lymphocyte.

[0085] In some [embodimentexamples](#), the target cell is a cancer cell that is an ovarian cancer cell.

[0086] In some [embodimentexamples](#), the target cell is a cancer cell that is an ovarian pancreatic cell.

[0087] In some [embodimentexamples](#), the target cell is a cancer cell that is a glioblastoma cell.

[0088] In some [embodimentexamples](#), the recombinant nucleic acid is DNA.

[0089] In some [embodimentexamples](#), the recombinant nucleic acid is RNA.

[0090] In some [embodimentexamples](#), the recombinant nucleic acid is mRNA.

[0091] In some [embodimentexamples](#), the recombinant nucleic acid is a circRNA.

[0092] In some [embodimentexamples](#), the recombinant nucleic acid is a tRNA.

[0093] In some [embodimentexamples](#), the recombinant nucleic acid is a microRNA.

[0094] Provided herein is a vector, comprising the composition described above.

[0095] In some [embodimentexamples](#), vector is viral vector. In some [embodimentexamples](#), the viral vector is retroviral vector or a lentiviral vector. In some [embodimentexamples](#), the vector further comprises a promoter operably linked to at least one nucleic acid sequence encoding one or more polypeptides. In some [embodimentexamples](#), the vector is polycistronic. In some [embodimentexamples](#), each of the at least one nucleic acid sequence is operably linked to a separate promoter. In some [embodimentexamples](#), the vector further comprises one or more internal ribosome entry sites (IRESs). In some [embodimentexamples](#), the vector further comprises a 5'UTR and/or a 3'UTR flanking the at least one nucleic acid sequence encoding one or more polypeptides. In some [embodimentexamples](#), the vector further comprises one or more regulatory regions.

[0096] Provided herein is a polypeptide encoded by the recombinant nucleic acid of the composition described above.

[0097] Additional aspectexamples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodimentexamples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodimentexamples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0098] 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. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0099] 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 (also “FIG.” herein), of which:

[00100] **FIG. 1A** is a graphical representation of the exemplary extracellular stimulants, receptors and pathways generating a dual signal for a myeloid cell: a signal 1 and a signal 2.

[00101] **FIG. 1B** is a graphical representation of a simplified engager construct with a binder A, a linker L and a second binder B. In this simplified diagram, binder A binds to a cell surface biomolecule on a target cell; binder B binds to a cell surface biomolecule on a myeloid cell.

[00102] **FIG. 2A** is a graphical representation of bispecific scFv engager with protease cleavable masked site, that are the antigen binding domains.

[00103] **FIG. 2B** is a graphical representation of bispecific V_{HH} engager with protease cleavable masked antigen binding domains.

[00104] **FIG. 3A** is a graphical representation of an exemplary bispecific scFv engager with protease cleavable masked site, and a peptide linker connecting the two scFv engagers that are the antigen binding domains; in this case, the peptide linker is an additional therapeutic agent or a third binding domain, specifically a TLR4 ligand peptide.

[00105] **FIG. 3B** is a graphical representation of an exemplary bispecific V_{HH} engager with protease cleavable masked antigen binding domains, and a peptide linker connecting the two protease cleavable

[00262] In some embodiments, the bi- or trispecific engager binds to an extracellular domain of a monocyte or macrophage receptor selected from a group consisting of lectin, dectin 1, mannose receptor (CD206), scavenger receptor A1 (SRA1), MARCO, CD36, CD163, MSR1, SCARA3, COLEC12, SCARA5, SCARB1, SCARB2, CD68, OLR1, SCARF1, SCARF2, CXCL16, STAB1, STAB2, SRCRB4D, SSC5D, CD205, CD207, CD209, RAGE, CD14, CD64, CCR2, CX3CR1, CSF1R, Tie2, HuCRIg(L), and CD169 receptor.

[00263] It may be understood that in some embodiments, a binder or any part of an engager can be a molecule other than an antibody or a fragment thereof. For example, a binder that binds to a surface molecule of a cell, such as a target cell or an effector myeloid cell, and may be a ligand for a receptor, where the cell surface molecule is a receptor specific for the ligand. In some embodiments a ligand may be a chimeric protein or a fusion protein, or a naturally occurring ligand of the receptor. In some embodiments one binder in an engager recombinant protein may be a ligand, and another may be an antibody or a fragment thereof.

[00264] In some embodiments, an engager molecule may comprise one or more linkers or spacers. Linkers or spacers may be made up of 2-50 amino acids. In some embodiments, the linker is a 3-30 amino acid spacer. In some embodiments, the linker is a 4-20 or 5-10 amino acid long peptide. In some embodiments, the spacer may be made of nonreactive amino acid moieties, for example, a series of glycine or serine or alanine residues. An exemplary linker may comprise an amino acid sequence GSGS ([SEQ ID NO: 153](#)), or SGGG ([SEQ ID NO: 154](#)), or SGGGGSG ([SEQ ID NO: 155](#)). An exemplary linker may comprise an amino acid sequence SSGGGGSGGGGSGGGGS ([SEQ ID NO: 114](#)). A linker may link the V_H and V_L domains of an scFv. A linker may link the binder domains of a bi- or trispecific engager. The linkers generally serve as structural elements that connect the effective binder sites. In some embodiments, the linker may be flexible. In some embodiments, the linker may be rigid. The length of the linker is adjusted as per the need of the design and the length that is optimal or necessary to space the binders at the opposite ends. In some embodiments, the linker may comprise a peptide that has a unique function, other than linking two domains. Exemplary peptides discussed below may be part of the design of a bi- or trispecific or multispecific engager, and may or may not be a part of a linker.

[00265] In some embodiments, the bi- or trispecific engager comprises a peptide that specifically targets the tumor associated macrophages, such as, for instance, the M2 macrophages. In some embodiments, the M2-specific peptide is M2-pep, having an amino acid sequence, YEQDPWGVKWWY. (**SEQ ID NO: 116**).

[00266] In some embodiments, an M2-specific peptide may comprise a sequence HLSWLDPVYAW, (hereafter, HLS pep) (**SEQ ID NO: 117**).

[00267] A peptide such as the M2- pep or the HLS pep described above can form a part of the bi-specific engager or a trispecific engager, such as a linker between two binding domains or a part of a linker. In some embodiments, the first and the second binding domains of an engager are coupled to an M2- pep or

of Glycine and one or more Serine residues. Other amino acids preferred for a peptide linker include but are not limited to threonine (Thr), serine (Ser), proline (Pro), glycine (Gly), aspartic acid (Asp), lysine (Lys), glutamine (Gln), asparagine (Asn), and alanine (Ala) arginine (Arg), phenylalanine (Phe), glutamic acid (Glu). Of these Pro, Thr, and Gln are frequently used amino acids for natural linkers. Pro is a unique amino acid with a cyclic side chain which causes a very restricted conformation. Pro-rich sequences are used as interdomain linkers, including the linker between the lipoyl and E3 binding domain in pyruvate dehydrogenase (GA₂PA₃PAKQEA₃PAPA₂KAEAPA₃PA₂KA [\(SEQ ID NO: 156\)](#)). For the purpose of the disclosure, the empirical linkers may be flexible linkers, rigid linkers, and cleavable linkers. Sequences such as (G4S)_x [\(SEQ ID NO: 157\)](#) (where x is multiple copies of the moiety, designated as 1, 2, 3, 4, and so on) comprise a flexible linker sequence. Other flexible sequences used herein include several repeats of glycine, e.g., (Gly)₆ [\(SEQ ID NO: 158\)](#) or (Gly)₈ [\(SEQ ID NO: 159\)](#). On the other hand, a rigid linker may be used, for example, a linker (EAAAK)_x [\(SEQ ID NO: 160\)](#), where x is an integer, 1, 2, 3, 4 etc. gives rise to a rigid linker.

[00359] The length of a linker peptide can be crucial in the design of a multi-specific engager. For example, limiting the linker peptide length to less than 10 amino acids restricts the association between two adjacent domains. In some embodiments, the linker may comprise an anchoring or clasping function and may comprise a crosslinking moiety. The cross linking moiety may be a peptide or a chemical cross linking moiety, several of which are described in the previous section.

[00360] Specific peptides with specific functions have been discussed elsewhere in the document. In some embodiments, a peptide linker may further function as an activator or a signal in a myeloid cell. For example, a TLR4 activation peptide may be incorporated within the linker between two binding domains of an engager, and the TLR4 activation peptide binds to and activates a TLR4 signal in a monocyte or macrophage.

[00361] In some embodiments, a peptide linker may further function as a conditionally cleavable linker. By conditionally cleavable it is understood that the peptide is cleaved when the agent that cleaves it is available. For example, an MMP2 cleavable peptide is described herein, which is readily cleaved only when the peptide is in a region rich in MMP2. An exemplary MMP2 cleavable peptide is GPLGVR [\(SEQ ID NO: 118\)](#).

[00362] In some embodiments, a peptide linker may further function as a targeting peptide. For example, an M2 peptide is described, which can bind to a M2 monocyte or macrophage, which is the predominant tumor associated monocyte or macrophage phenotype. An exemplary peptide is YEQDPWGVKWWY [\(SEQ ID NO: 116\)](#).

[00363] Any one or more peptide linkers may comprise specialized functions, such as they can dimerize, trimerize or multimerize. In some embodiments, one or more linkers may comprise leucine zipper sequences.

[00364] In some embodiments, the peptide linker is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length. In some embodiments, a peptide linker or the two linker peptides with an

macrophage thereby enhancing phagocytosis and activating an inflammatory cascade in this exemplary figure, which lead to phagocytic killing of the target cell.

Example 5. Antigen binding domain masking design

[00431] In this example, a generalized exemplary design for an engager having masked antigen binding domains is described in further detail. **FIG. 5Ai** is a diagrammatic representation of a bispecific engager with two scFV binders, scFv1, and scFv2. SdAb or diabody engagers can also be likewise constructed with necessary structural modifications, an exemplary diabody construct with two binders is represented in **FIG. 5Aii**. The antigen binding domains are masked by a peptide mask (1) that remains bound to the antigen binding portions of the diabody ABD1 and ABD2, linked at the N terminal portion of the light chain variable domain of ABD1 (3) of the first chain, or the light chain variable region of ABD2 of the second chain by a peptide linker (2). The peptide linker joining the mask with the light chain variable domains is a substrate for matrix metalloproteinase 2 (MMP2) substrate, having an amino acid sequence GPLGVR ([SEQ ID NO: 118](#)). The design allows passing of the masked diabody engager to pass through the circulation without binding to any substrate until MMP2 is available to cleave the linking peptide. It is understood that the cancer microenvironment is rich in MMP2. Therefore, the diabody engager is activated in a cancer microenvironment to bind its target cancer cell and the monocyte or macrophage with ABD1 and ABD2 respectively in a tumor environment. **FIG. 5B** exemplifies the nucleic acid construct of a single chain of a diabody. The nucleic acid construct comprises from 5'-3' end a nucleic acid sequence encoding the mask peptide, a MMP2 linker, a sequence encoding ABD1 light chain (ABD1-LC), which is linked to a nucleic acid sequence encoding a peptide linker that joins with the ABD1-LC and ABD2-HC; followed by the nucleic acid sequence encoding the ABD2 HC.

Example 6. Modular antigen binding engager designs

[00432] In this example, several modular designs of binding domains represented by light chain heavy chain domains arranged on an antibody-like polypeptide structure as shown in **FIG. 6**. In one design, a common light chain is used to pair with two non-identical heavy chains in an IgG like structure, thereby rendering a bispecific binding domain that could be used in a bispecific or a trispecific engager design. In another model depicted herein, a chimeric bi- or trispecific engager uses a combination of an scFv joined to one arm of an usual antibody light and heavy chain combination. In one design, two scFvs replace the heavy chain-light chain paired regions, while the scFvs are connected by the constant regions of the heavy chains. In other designs as depicted herein, one or more scFvs may be conjugated to the Fc region. In yet other designs, one or more scFvs may be conjugated to the constant regions as side chains of an IgG like polypeptide.

Example 7. Use of monocyte or macrophage specific activators in an engager for activating inflammatory signal in monocyte or macrophage an potentiating phagocytosis

CLAIMS

What is claimed is:

1. A composition comprising a first therapeutic agent, wherein the therapeutic agent comprises:
 - (a) a first binding domain, wherein the first binding domain is a binding domain of a first antibody ~~or functional fragment thereof~~ that specifically interacts with an antigen on a target cell, and
 - (b) a second binding domain, wherein the second binding domain is a binding domain of a second antibody ~~or functional fragment thereof~~ that specifically interacts with a myeloid cell; wherein, the first and second binding domains are operably linked by one or more linkers.
 - (i) ~~the first therapeutic agent is coupled to a first component, wherein the first component is an additional therapeutic agent or a third binding domain, or~~
 - (ii) ~~the composition comprises an additional therapeutic agent.~~
- ~~2. A composition comprising a therapeutic agent, wherein the therapeutic agent comprises: (a) a first binding domain that specifically interacts with an antigen of a target cell, (b) a second binding domain that specifically interacts with a myeloid cell, and (c) a third binding domain that specifically interacts with the myeloid cell.~~
- ~~3.2. The composition of claim 1-or 2, wherein the myeloid cell is a monocyte cell or a macrophage cell.~~
- ~~4. The composition of any one of claims 1-3, wherein the second binding domain that specifically interacts with a myeloid cell interacts with a phagocytic or tethering receptor of the myeloid cell.~~
- ~~5. The composition of claim 2, wherein the third binding domain that specifically interacts with a myeloid cell interacts with an extracellular region of a first phagocytic or tethering receptor of the myeloid cell.~~
- ~~6.3. The composition of any one of claims 1-5, wherein any one of the first binding domains of the therapeutic agent comprises the binding domain of a an antibody, a functional fragment of an antibody, a variable domain thereof, is a V_H domain, a V_L domain, a VNAR domain, a V_{HH} domain, a single chain variable fragment (scFv), an Fab, a single-domain antibody (sdAb), a nanobody, a bispecific antibody or, a diabody,; or a functional fragment or a combination thereof. and wherein the second binding domain is a V_H domain, a V_L domain, a VNAR domain, a V_{HH} domain, a single chain variable fragment (scFv), a Fab, a single-domain antibody (sdAb), a nanobody, a bispecific antibody or a diabody.~~
- ~~7.4. The composition of any one of claims 1-6, wherein the antigen on the target cell to which the first binding domain binds, is a cancer antigen, a viral or a pathogenic antigen-on the target cell or an~~

autoimmune antigen.

~~8. The composition of any one of claims 1-7, wherein the antigen on the target cell to which the first binding domain binds, is a viral antigen.~~

~~9.5. The composition of any one of claims 41-8, wherein the antigen on the target cell to which the first binding domain binds is a cancer antigen selected from the group consisting of an ovarian cancer antigen or a T-lymphocyte antigen.~~

~~10. The composition of any one of claims 1-9, wherein the antigen on the target cell to which the first binding domain binds is an extracellular antigen.~~

~~11. The composition of any one of claims 1-9, wherein the antigen on the target cell to which the first binding domain binds is an intracellular antigen.~~

~~12.6. The composition of any one of claims 1-11, wherein the antigen on the target cell to which the first binding domain binds to a protein is selected from the group consisting of Thymidine Kinase (TK1), Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT), Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1), Mucin-1, Mucin-16 (MUC16), MUC1, Epidermal Growth Factor Receptor vIII (EGFRvIII), Mesothelin, Human Epidermal Growth Factor Receptor 2 (HER2), Mesothelin, EBNA-1, LEMD1, Phosphatidyl Serine, Carcinoembryonic Antigen (CEA), B-Cell Maturation Antigen (BCMA), Glypican 3 (GPC3), Follicular Stimulating Hormone receptor, Fibroblast Activation Protein (FAP), Erythropoietin-Producing Hepatocellular Carcinoma A2 (EphA2), EphB2, a Natural Killer Group 2D (NKG2D) ligand, Disialoganglioside 2 (GD2), CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD24, CD30, CD33, CD38, CD44v6, CD45, CD56CD79b, CD97, CD117, CD123, CD133, CD138, CD171, CD179a, CD213A2, CD248, CD276, PSCA, CS-1, CLECL1, GD3, PSMA, FLT3, TAG72, EPCAM, IL-1, an integrin receptor, PRSS21, VEGFR2, PDGFR-beta, SSEA-4, EGFR, NCAM, prostate, PAP, ELF2M, GM3, TEM7R, CLDN6, TSHR, GPRC5D, ALK, IGLL1 and combinations thereof.~~

~~7. The composition of claim 6, wherein the first binding domain has a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 27, 28, 111, 112, 113, 115, 143 and 144.~~

~~8. The composition of claim 1, wherein the second binding domain promotes phagocytic activity of the myeloid cell, promotes inflammatory signaling of the myeloid cell, promotes adhesion of the myeloid cell to the target cell, inhibits anti-phagocytic activity of the myeloid cell mediated by the target cell or inhibits anti-inflammatory activity of the myeloid cell mediated by the target cell.~~

~~9. The composition of any claim 1, wherein upon binding of the therapeutic agent to the myeloid cell, the killing or phagocytosis activity of the myeloid cell is increased by at least 10%, compared to a myeloid cell not bound by the therapeutic agent, as measured by a particle uptake assay~~

~~13. The composition of any one of claims 1-12, wherein the antigen on the target cell to which the first binding domain binds is selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CCR4, CD8, CD30, CD45, and CD56.~~

- ~~14. The composition of any one of claims 12 or 13, wherein the antigen on the target cell to which the first binding domain binds is an ovarian cancer antigen or a T-lymphoma antigen.~~
- ~~15. The composition of any one of preceding claims, wherein the antigen on the target cell to which the first binding domain binds is an integrin receptor.~~
- ~~16. The composition of claim 1 or 2, wherein the second binding domain or the third binding domain binds to an integrin receptor.~~
- ~~17. The composition of claim 16, wherein the second binding domain or the third binding domain binds to an integrin receptor selected from the group consisting of $\alpha 1$, $\alpha 2$, αHb , $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 7$, $\alpha 8$, $\alpha 9$, $\alpha 10$, $\alpha 11$, αD , αE , αL , αM , αV , αX , $\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$, $\beta 5$, $\beta 6$, $\beta 7$, and $\beta 8$.~~
- ~~18. The composition of any one of the preceding claims, wherein the therapeutic agent binds to a phagocytic or tethering receptor that comprises a phagocytosis activation domain.~~
- ~~19. The composition of claim 18, wherein the therapeutic agent binds to a receptor or a protein selected from the group consisting of the receptors listed in Table 2A and Table 2B, or a fragment thereof.~~
- ~~20.10. The composition of claim 18, wherein the second binding domain binds to therapeutic agent binds to a phagocytic receptor selected from the group consisting of lectin, dectin 1, CD206, scavenger receptor A1 (SRA1), MARCO, CD36, CD163, MSR1, SCARA3, COLEC12, SCARA5, SCARB1, SCARB2, CD68, OLR1, SCARF1, SCARF2, CXCL16, STAB1, STAB2, SRCRB4D, SSC5D, CD205, CD207, CD209, RAGE, CD14, CD64, F4/80, CCR2, CX3CR1, CSF1R, Tie2, HuCRIg(L), CD64, CD32a, CD16a, CD89, Fc-alpha receptor I, CR1, CD35, CR3, CR4, Tim-1, Tim-4 and CD169.~~
11. The composition of claim 1, wherein the second binding domain binds to an extracellular domain of Fc γ RI, Fc γ RIIA, Fc γ RIIB, Fc γ RIIC, Fc γ RIIIA, Fc γ RIIIB, FcRn, TRIM21 or FcRL5.
12. The composition of claim 11, wherein the second binding domain has a sequence with at least 80% sequence identity to SEQ ID NO: 141 or 142
- ~~21. The composition of any one of claims 1-20, wherein the therapeutic agent binds to a receptor comprising an intracellular signaling domain that comprises a pro-inflammatory signaling domain.~~
- ~~22. The composition of any one of claims 1-21, wherein the first therapeutic agent comprises a polypeptide that is less than 1000 amino acids or 1000 nm in length.~~
- ~~23. The composition of any one of claims 1-22, wherein the first therapeutic agent comprises a polypeptide that is less than 500 amino acids or 500 nm in length.~~
- ~~24. The composition of any one of claims 1-23, wherein the first therapeutic agent comprises a polypeptide that is 200-1000 amino acids or 200-1000 nm in length.~~
- ~~25. The composition of any one of claims 1-24, wherein engagement of the binding domains of the first therapeutic agent contacts the cancer cell to the myeloid cell.~~

26. — The composition of claim 1, wherein the second binding domain specifically interacts with a myeloid cell and promotes phagocytosis activity of the myeloid cell.
27. — The composition of claim 1, wherein the second binding domain specifically interacts with a myeloid cell and promotes inflammatory signaling of the myeloid cell.
28. — The composition of claim 1, wherein the second binding domain specifically interacts with a myeloid cell or an adhesion molecule and promotes adhesion of the myeloid cell to the target cell.
29. — The composition of claim 1, wherein the second binding domain specifically interacts with a myeloid cell and inhibits anti-phagocytic activity of the myeloid cell mediated by the target cell.
30. — The composition of claim 1, wherein the second binding domain specifically interacts with a myeloid cell and inhibits anti-inflammatory activity of the myeloid cell mediated by the target cell.
31. — The composition of claim 2, wherein the second and/or the third binding domain promotes phagocytic activity of the myeloid cell.
32. — The composition of claim 2, wherein the second and/or the third binding domain promotes inflammatory signaling of the myeloid cell.
33. — The composition of claim 2, wherein the second and/or the third binding domain specifically interacts with a myeloid cell or an adhesion molecule and promotes adhesion of the myeloid cell to the target cell.
34. — The composition of claim 2, wherein the second and/or the third binding domain inhibits anti-phagocytic activity of the myeloid cell mediated by the target cell.
35. — The composition of claim 2, wherein the second and/or the third binding domain inhibits anti-inflammatory activity of the myeloid cell mediated by the target cell.
36. — The composition of any one of the preceding claims, wherein the therapeutic agent comprises a therapeutic polypeptide.
37. — The composition of any one of the preceding claims, wherein the therapeutic agent comprises a recombinant nucleic acid encoding the therapeutic polypeptide.
38. — The composition of claim 1, wherein the third binding domain or the additional therapeutic agent comprises a CD47 antagonist, a CD47 blocker, an antibody, a chimeric CD47 receptor, a sialidase, a cytokine, a proinflammatory gene, a procaspase, or an anti-cancer agent.
39. — The composition of any one of the preceding claims, wherein the first binding domain, the second binding domain and the third binding domain bind to distinct non-identical target antigens.
40. — The composition of claim 1 or 2, wherein the first binding domain, the second binding domain or the third binding domain is a ligand binding domain.
41. — The composition of any one of the preceding claims, wherein the first, the second or the third binding domains are operably linked by one or more linkers.

- ~~42. The composition of claim 41, wherein the linker is a polypeptide.~~
- ~~43. The composition of claim 42, wherein the linker is a functional peptide.~~
- ~~44. The composition of claim 43, wherein the linker is a ligand for a receptor.~~
- ~~45. The composition of claim 44, wherein the linker is a ligand for a monocyte or macrophage receptor.~~
- ~~46. The composition of claim 43 or 44, wherein the linker activates the receptor.~~
- ~~47. The composition of claim 43 or 44, wherein the linker inhibits the receptor.~~
- ~~48. The composition of claim 44, wherein the linker is a ligand for a M2 macrophage receptor.~~
- ~~49.13. The composition of claim 143 or 44, wherein the one or more linkers comprises a -linker that is a ligand for a TLR receptor,~~
14. The composition of claim 13, wherein the one or more linkers comprises a linker that is a ligand for asuch as TLR4 receptor.
15. The composition of claim 1, wherein the one or more linkers comprises a linker with an amino acid sequence selected from the group consisting of SEQ ID NOs: 105-110 and 123-140.
16. The composition of claim 1, wherein the first binding domain binds to HER2 or CD5, the second binding domain binds to FcγRIIIA or FcγRIIIB and the linker is a ligand for a TLR4 receptor.
17. The composition of claim 1, wherein the therapeutic agent comprises a sequence having an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 151 or 152.
18. The composition of claim 1, wherein the therapeutic agent is a single polypeptide that is 200-1000 amino acids in length.
- ~~50. The composition of claim any of the claims 43, 44, 45, 46, 48 or 49, wherein the linker activates a TLR receptor.~~
- ~~51. The composition of any one of the preceding claims, wherein the first, the second and /or the third binding domains are associated with a mask that binds to the binding domain.~~
- ~~52. The composition of claim 51, wherein the mask is an inhibitor that inhibits the interaction of binding domain to its target when the mask remains associated with the respective binding domain.~~
- ~~53. The composition of claim 52, wherein the mask is associated with the binding domain via a peptide linker.~~
- ~~54. The composition of claim 53, wherein the peptide linker comprises a cleavable moiety.~~
- ~~55. The composition of claim 53, wherein the cleavable moiety is cleaved by a protein or an enzyme selectively abundant in the site of the cancer or tumor.~~
- ~~56. The composition of any one of claims 1-55, wherein the third binding domain that specifically interacts with an extracellular region of a second receptor of the macrophage activates the macrophage.~~

57. ~~The composition of any one of claims 1-56, wherein upon binding of the therapeutic agent to the myeloid cell, the killing or phagocytosis activity of the myeloid cell is increased by at least 10%, or 20%, or 30%, or 40 %, or 50%, or 60%, or 70% or 90% or 100% compared to a myeloid cell not bound by the therapeutic agent, as measured by a particle uptake assay.~~
58. ~~The composition of any one of claims 1-57, wherein engagement of the binding domains of first therapeutic agent triggers phagocytosis of the cancer cell by the myeloid cell.~~
59. ~~The composition of any one of claims 1-58, wherein engagement of the additional therapeutic agent potentiates or increases the phagocytic killing of the cancer cell by the myeloid cell.~~
60. ~~The composition of any one of claims 1-59, wherein the second or third binding domain binds to an extracellular of IgA, IgD, IgE, IgG, IgM, FcγRI, FcγRIIA, FcγRIIB, FcγRIIC, FcγRIIA, FcγRIIB, FcRn, TRIM21, FcRL5.~~
61. ~~The composition of any one of claims 1-60, wherein the second or the third binding domain comprises an M2 domain.~~
62. ~~The composition of any one of claims 1-61, wherein the second or the third binding domain comprises a LIGHT domain.~~
63. ~~The composition of any one of claims 1-62, wherein the second or the third binding domain comprises a HVEM domain.~~
64. ~~The composition of any one of claims 1-63, wherein the second or the third binding domain comprises a GITR domain.~~

65.19. A pharmaceutical composition comprising the composition of claim 1.:
~~a first therapeutic agent, wherein the therapeutic agent comprises one or more polypeptides or recombinant nucleic acids encoding the one or more polypeptides, wherein the one or more polypeptides comprise:~~

- ~~(a) a first binding domain, wherein the first binding domain is a first antibody or functional fragment thereof that specifically interacts with an antigen of a target cell, and~~
- ~~(b) a second binding domain, wherein the second binding domain is a second antibody or functional fragment thereof that specifically interacts with a myeloid cell;~~

~~wherein,~~

- ~~(i) the first therapeutic agent is coupled to a first component, wherein the first component is an additional therapeutic agent or a third binding domain, or~~
- ~~(ii) the composition comprises an additional therapeutic agent; and~~

~~————— an acceptable pharmaceutical salt or excipient.~~

20. Use of the pharmaceutical composition of claim 19 in the manufacture of a medicament for the treatment of cancer.

21. The pharmaceutical composition of claim 19 for use in therapy.

66. ~~The pharmaceutical composition of claim 65, wherein the first therapeutic agent comprises a single polypeptide.~~
67. ~~The pharmaceutical composition of claim 65, wherein the first therapeutic agent comprises multiple polypeptides.~~
68. ~~The pharmaceutical composition of claim 65, wherein the first therapeutic agent is a recombinant nucleic acid encoding the one or more polypeptides.~~
69. ~~The pharmaceutical composition of claim 65, further comprising a second therapeutic agent.~~
70. ~~A method of treating a disease or condition in a subject in need thereof, comprising:~~
~~administering to the subject a pharmaceutical composition, comprising:~~
~~a first therapeutic agent, wherein the therapeutic agent comprises one or more polypeptides or recombinant nucleic acids encoding the one or more polypeptides, wherein the one or more polypeptides comprise:~~
~~(a) a first binding domain, wherein the first binding domain is a first antibody or functional fragment thereof that specifically interacts with an antigen of a target cell, and~~
~~(b) a second binding domain, wherein the second binding domain is a second antibody or functional fragment thereof that specifically interacts with a myeloid cell;~~
~~wherein,~~
~~(i) the first therapeutic agent is coupled to a first component, wherein the first component is an additional therapeutic agent or a third binding domain, or~~
~~(ii) the composition comprises an additional therapeutic agent; and~~
~~an acceptable pharmaceutical salt or excipient.~~
71. ~~The method of claim 70, further comprising, administering a second therapeutic agent.~~
72. ~~The method of claim 70, wherein the administering the pharmaceutical composition comprises administering the pharmaceutical composition intravenously.~~
73. ~~The method of claim 70, wherein the administering the pharmaceutical composition comprises administering the pharmaceutical composition subcutaneously.~~
74. ~~The method of claim 70, wherein the administering the pharmaceutical composition comprises injecting the pharmaceutical composition.~~
75. ~~The composition of claim 1 or 2, wherein the first binding domain comprises a sequence having an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 27, 28, 111, 112, 113, 115, 143 and 144.~~
76. ~~The composition of claim 1 or 2, wherein the second binding domain comprises a sequence having an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 141 and 142.~~

- ~~77. The composition of claim 1, wherein the first component comprises an amino acid sequence GGQEINSSYGG (SEQ ID NO: 105) or QEINSSY (SEQ ID NO: 129).~~
- ~~78. The composition of claim 1, wherein the first component comprises an amino acid sequence GGAPPHALSGG (SEQ ID NO: 109) or APPHALS (SEQ ID NO: 137).~~
- ~~79. The composition of claim 49 or 50, wherein the linker comprises an amino acid sequence GGQEINSSYGG (SEQ ID NO: 105), or QEINSSY (SEQ ID NO: 129) or GGAPPHALSGG (SEQ ID NO: 109) or APPHALS (SEQ ID NO: 137).~~
- ~~80. A bispecific or trispecific engager, comprising a sequence having an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 151.~~
- ~~81. A bispecific or trispecific engager, comprising a sequence having an amino acid sequence with at least 80%, 85%, 90%, 95% or 100% sequence identity to SEQ ID NO: 152.~~