COMPOSITIONS AND METHODS FOR TARGETED DELIVERY OF THERAPEUTIC AGENTS

SEQUENCE LISTING

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 June 6, 2022, is named 51666-002WO2_Sequence_Listing_6_6_22_ST25 and is 276,968 bytes in size.

BACKGROUND OF THE INVENTION

Undesirable off-target effects are a problem for otherwise desirable therapeutic targets that are present in healthy as well as diseased tissues.

SUMMARY OF THE INVENTION

In a first aspect, the present disclosure provides a macromolecule for use in localizing a macromolecule at the intestine of a subject, wherein the macromolecule comprises a first binding site and a second binding site and is to be administered to the subject, wherein:

(a) the first binding site is specific for an effector target in the subject, and

(b) the second binding site is specific for cadherin 17 (CDH17);

wherein:

(i) the second binding site does not substantially influence signaling upon binding CDH17; and

(ii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site;

allowing the macromolecule to localize at the intestine of the subject.

In a second aspect, the present disclosure provides a macromolecule for use in localizing a macromolecule at the intestine of a subject, wherein the macromolecule comprises a first binding site and a second binding site, and is to be administered to the subject, wherein:

(a) the first binding site is a ligand of an effector target in the subject, wherein the ligand has been modified to decrease its affinity for the effector target, and

(b) the second binding site is specific for CDH17;

wherein:

(i) the second binding site does not substantially influence signaling upon binding CDH17; and

(ii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site;

allowing the macromolecule to localize at the intestine of the subject.

The present disclosure describes, in part, macromolecule compositions and related methods that effect targeted delivery of therapeutic agents to effector targets in a desired cell, tissue and/or organ of interest while minimizing or avoiding undesirable delivery to other cells, tissues or organs. Generally, compositions described herein comprise macromolecules, such as an ANDbody™, that

include an effector target binding domain specific for an effector target, and an address binding domain specific for an address target. The address target is generally sufficiently restricted in the subject to target the macromolecule to the desired cell, tissue or organ. In some embodiments, the effector target binding domain does not influence an effector target in the absence of an address target binding domain. Moreover, the address target binding domain does not influence signaling upon binding the address target. However, localization of the effector target binding domain by the address target binding domain enables the effector target binding domain to bind the effector target sufficiently to elicit an influence on signaling by the effector target in the target cell or tissue. The compositions described herein can be used, e.g., to specifically deliver a therapeutic agent to a desired location, e.g., a cell, tissue or organ, in a subject, while avoiding undesirable off-target effects.

In one example, the present disclosure provides a method of localizing a macromolecule at a target tissue or cell of a subject, the method comprising administering to the subject a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in the subject, and (b) the second binding site is specific for an address target expressed in the target tissue or cell in the subject; wherein: (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site; and allowing the macromolecule to localize at the target tissue or cell of the subject.

In some examples, at least 25% of the macromolecule detectable in the subject is detected at the target tissue or cell at a time point between 1 and 7 days following administration of

the macromolecule to the subject.

In some examples, the potency of the first binding site at the target tissue or cell is substantially increased relative to a reference macromolecule lacking the second binding site.

In some examples, the first binding site has a low affinity for the effector target.

In some examples, the first binding site has a low avidity for the effector target.

In some examples, the affinity of the first binding site for the effector target is lower than the affinity of the second binding site for the address target.

In some examples, the avidity of the first binding site for the effector target is lower than the avidity of the second binding site for the address target.

In some examples, effector target signaling by the macromolecule in a non-target tissue or cell of the subject is substantially decreased relative to a reference macromolecule lacking the second binding site.

In some examples, the address target is regionally expressed in the subject. In some examples, the address target is locally expressed in the subject. In some examples, the expression of the address target is restricted to a cell type in the subject.

In some examples, the address target is expressed only by a cell in the subject when in a specific cell state.

In some examples, the address target is expressed only by a cell in the subject in a disease state.

In some examples, the first binding site or the second binding site comprises a polypeptide.

In some examples, the polypeptide is an antibody or antigen-binding fragment thereof.

In some examples, the macromolecule is an antibody comprising a first binding site that is specific for the effector target in the subject and a second binding site that is specific for the address target.

In some examples, the polypeptide is a ligand of the effector target or a ligand of the address target.

In some examples, (a) the first binding site comprises an antibody or antigen-binding fragment thereof and the second binding site comprises a ligand of the address target; or (b) the first binding site comprises a ligand of the effector target and the second binding site comprises an antibody or antigen-binding fragment thereof.

In some examples, the target tissue is skin and the second binding site is specific for desmoglein-1 (DSG-1).

In some examples, the target tissue is lung tissue and the second binding site is specific for RAGE.

In some examples, the target tissue is kidney tissue and the second binding site is specific for cadherin 16 (CDH16).

In some examples, the target tissue is intestine tissue and the second binding site is specific for cadherin 17 (CDH17).

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein: (a) the first binding site is specific for an effector

target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site.

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein: (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site; and wherein localization of the macromolecule to a non-target tissue or cell is substantially reduced relative to localization of a reference macromolecule lacking the second binding site.

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site; and wherein localization of the macromolecule to a target tissue or cell is substantially increased relative to localization of a reference macromolecule lacking the second binding site.

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site; and wherein at least **25%** of the macromolecule administered to a subject is detected at the target tissue or cell at a time point between 1 and 7 days following administration.

In another example, the present disclosure provides a macromolecule comprising a first binding

site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site; and wherein the affinity of the first binding site for the effector target is lower than the affinity of the second binding site for the address target.

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site; and wherein the avidity of the first binding site for the effector target is lower than the avidity of the second binding site for the address target.

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site; and wherein the potency of the first binding site at the target tissue or cell is substantially increased relative to a reference macromolecule lacking the second binding site.

In some examples, the first binding site has a low affinity for the effector target.

In some examples, the first binding site has a low avidity for the effector target.

In some examples, the affinity of the first binding site for the effector target is lower than the affinity of the second binding site for the address target.

In some examples, the avidity of the first binding site for the effector target is lower than the avidity of the second binding site for the address target.

In some examples, (a) the Kd of the first binding site for the effector target is higher than the Kd of the second binding site for the address target; (b) the EC_{50} of the first binding site for the effector target is higher than the EC_{50} of the second binding site for the address target; or (c) the IC_{50} of the first binding site for the effector target is higher than the IC_{50} of the second binding site for the address target.

In some examples, the first binding site has an affinity to the effector target of at least

about 2 times, at least about 5 times, or at least about 10 times less than the affinity of the second binding site to the address target.

In some examples, the affinity of the second binding site to the address target has a Kd of greater than about 1 nM, greater than about 2 nM, or greater than about 50 nm.

In some examples, the effector target is a protein, lipid, or sugar.

In some examples, the effector target is a cell membrane-associated target.

In some examples, the effector target is a protein. In some examples, the effector target is a secreted protein.

In some examples, the effector target is encoded by a gene selected from the group consisting of the genes recited in Table 1.

In some examples, the macromolecule agonizes the effector target.

In some examples, the macromolecule antagonizes the effector target.

In some examples, the address target is a protein, lipid, or sugar.

In some examples, the address target is a protein.

In some examples, expression of the effector target or the address target is expression of an RNA sequence encoding the effector target or the address target.

In some examples, the expression level of the effector target or the address target is assessed by using a RNA sequence dataset.

In some examples, the RNA sequence dataset is a Genotype-Tissue Expression (GTEx) dataset or a Human Protein Atlas (HPA) dataset.

In some examples, expression of the effector target or the address target is protein expression.

In some examples, the effector target is systemically expressed in the subject.

In some examples, the effector target is regionally expressed in the subject.

In some examples, the effector target is locally expressed in the subject.

In some examples, the address target is regionally expressed in the subject.

In some examples, the address target is locally expressed in the subject.

In some examples, the expression of the address target is restricted to a cell type in the subject.

In some examples, the address target is a soluble protein or an extracellular matrix (ECM)-associated protein and is not present in detectable amounts on the cell surface.

In some examples, the address target is expressed in the ECM and is not present in detectable amounts elsewhere in the subject.

In some examples, the address target is expressed only by a cell in the subject when in a specific cell state.

In some examples, the address target is expressed only by a cell in the subject when in a disease state.

In some examples, the address target is not expressed in a tissue in which binding of the second binding site to the effector target is deleterious to the subject.

In some examples, the binding site for the address target does not bind in detectable amounts to the binding site of a natural ligand of the address target.

In some examples, expression of the effector target or address target includes expression in one or more of minor salivary gland, thyroid, lung, breast, mammary tissue, pancreas, adrenal gland, liver, kidney, kidney cortex, kidney medulla, adipose-visceral tissue, omentum, small intestine, terminal ileum, fallopian tube, ovary, uterus, skin, skin not sun exposed, suprapubic skin, cervix, endocervix, ectocervix, vagina, skin sun exposed, lower leg skin, eneanterior cingulate cortex, Brodmann area 24 (BA24), basal ganglia, caudate nucleus, putamen, nucleus acumbens, hypothalamus, amygdala, hippocampus, cerebellum, cerebellar hemisphere, substantia nigra, pituitary gland, spinal cord, cervical spinal cord, artery, aorta, heart, atrial appendage, coronary artery, left ventricle, esophagus, esophagus mucosa, esophagus muscularis, gastroesophageal junction, spleen, stomach, colon, transverse colon, sigmoid colon, testis, whole blood cells, EBV-transformed lymphocytes, artery-tibial, or nerve-tibial tissues.

In some examples, expression of the effector target or address target includes expression in skin tissue, lung tissue, kidney tissue, or intestine tissue. In some examples, expression of the address target is substantially higher in skin tissue, lung tissue, kidney tissue, or intestine tissue than in any other tissue.

In some examples, the effector target and/or the address target is expressed on a structural tissue in the subject.

In some examples, the effector target and address target are on the same cell.

In some examples, the effector target and address target are on different cells.

In some examples, the effector target and address target are on different cells of the same cell type.

In some examples, the effector target and address target are on different cells of different cell types.

In some examples, the effector target and address target are on different cells in the same tissue.

In some examples, (a) the effector target is on a circulating cell and the address target is on a tissue-restricted cell; or (b) the effector target is on a tissue-restricted cell and the address target is on a circulating cell.

In some examples, the effector target and address target are on different cells located within 100 nm of each other in the subject.

In some examples, either the effector target or the address target is present on a cell surface.

In some examples, the macromolecule is a DNA polynucleotide.

In some examples, the macromolecule comprises an RNA or RNA-polypeptide conjugate.

In some examples, the macromolecule comprises a polypeptide. In some examples, the macromolecule is a polypeptide. In some examples, the polypeptide is an antibody or antigen-binding fragment thereof.

In some examples, the first binding site and the second binding site each comprise a VH and/or a VL.

In some examples, the macromolecule is an antibody comprising a first binding site that is specific for the effector target in the subject and a second binding site that is specific for the address target.

In some examples, the macromolecule is an asymmetric antibody or a symmetric antibody.

In some examples, the antibody or antigen-binding fragment thereof comprises an scFv, BsIgG, a BsAb fragment, a BiTE, a dual-affinity re-targeting protein (DART), a tandem diabody (TandAb), a diabody, an Fab2, a di-scFv, chemically linked F(ab')2, an Ig molecule with 2, 3 or 4 different antigen binding sites, a DVI-IgG four-in-one, an ImmTac, an HSAbody, an IgG-IgG, a Cov-X-Body, an scFv1-PEG-scFv$_2$, an appended IgG, an DVD-IgG, an affibody, an affilin, an affimer, an affitin, an alphabody, an anticalin, an avimer, a DARPin, a Fynomer, a monobody, a nanoCLAMP, a bis-Fab, an Fv, a Fab, a Fab'-SH, a linear antibody, an scFv, an antibody with only a heavy chain (Humabody), an ScFab, an IgG antibody fragment, a single-chain variable region antibody, a single-domain heavy chain antibody. a bispecific triplebody, a BiKE, a CrossMAb, a dsDb, an scDb, tandem a dAb / VHH, a triple dAb VHH, a tetravalent dAb / VHH, a Fab-scFv, a Fab-Fv, or a DART-Fc, an adnectin, a Kunitz-type inhibitor, or a receptor decoy.

In some examples, the polypeptide is a ligand of the effector target or a ligand of the address target.

In some examples, the ligand is a natural ligand, a modified ligand, or a synthetic ligand.

In some examples, the effector target or address target is a receptor and the polypeptide is a ligand thereof.

In some examples, the first binding site comprises an antibody or antigen-binding fragment thereof and the second binding site comprises a ligand of the address target.

In some examples, the first binding site comprises a ligand of the effector target and the second binding site comprises an antibody or antigen-binding fragment thereof.

In some examples, the amino acid sequences of the first and second binding sites are at least about 10% identical, at least about 20% identical, at least about 30% identical, at least about 40% identical, at least about 50% identical, at least about 60% identical, or at least about 70% identical.

In some examples, the address target has a Gini coefficient higher than about 0.4, about 0.5, about 0.57, about 0.65, about 0.7, about 0.85, about 0.90, or about 0.95.

In some examples, the address target has a Tau coefficient higher than about 0.67, about 0.75, about 0.8, about 0.85, about 0.90, or about 0.95.

In some examples, the effector target has a Gini coefficient lower than about 0.25, about 0.20, or about 0.15. In some examples, the effector target has a Tau coefficient lower than about 0.25, about 0.20, or about 0.15.

In some examples, the macromolecule further comprises a third binding site. In some examples, the third binding site is the same as the first binding site. In some examples, the third binding site is the same as the second binding site.

In some examples, the first binding site and second binding site are directly joined to each other in the macromolecule.

In some examples, the first binding site and the second binding site in the macromolecule are joined by a stable domain.

In some examples, the effector target is Notch2 and the address target is RAGE.

In some examples, RAGE signaling is not influenced by the second site binding the RAGE address target.

In some examples, the effector target is Notch2 and the address target is uromodulin (UMOD).

In some examples, UMOD signaling is not influenced by the second site binding the UMOD address target.

In some examples, the effector target is Notch2 and the address target is meprin A subunit beta (MEP1B).

In some examples, MEP1B signaling is not influenced by the second site binding the MEP1B address target.

In some examples, the effector target is IL11Ra and the address target is RAGE. In some examples, RAGE signaling is not influenced by the second site binding the RAGE address target.

In some examples, the effector target is IL 11Ra and the address target is UMOD. In some examples, UMOD signaling is not influenced by the second site binding the UMOD address target.

In some examples, the subject is a human.

In another example, the present disclosure provides a method of delivering a moiety to a target tissue or cell in a subject, comprising administering to the subject a macromolecule of any one of claims 1-86, wherein the target tissue comprises the address target.

In some examples, the moiety is a molecule. In some examples, the moiety is not a toxin. In some examples, the moiety is a cell. In some examples, the moiety is not a T cell or an NK cell. In some examples, the target tissue is not a tumor. In another example, the present disclosure provides a method of modulating an effector target in a target tissue, comprising administering to the tissue a macromolecule of any one of claims 1-86, wherein the target tissue comprises the address target and the effector target. In another example, the present disclosure provides a method of biasing a binding agent away from binding an effector target when the effector target is found in the heart or lungs, comprising administering the macromolecule of any one of claims 1-86, wherein the address target is not substantially expressed in the heart or lungs.

In another example, the present disclosure provides a method of modulating a target tissue in a subject, comprising administering to the subject a macromolecule of any one of claims 1-86, wherein the target tissue comprises the address target and the effector target.

In another example, the present disclosure provides a method of treating a subject having a disease or condition associated with an effector target, comprising administering to the subject a macromolecule of any one of claims 1-86, wherein the first binding site of the macromolecule binds the effector target.

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell, wherein the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site, and wherein the second binding site does not bind to the binding site of the natural ligand of the address target.

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell, wherein the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site, and wherein the first binding site and second binding site are directly joined to each other in the macromolecule.

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell, wherein the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site, and wherein the first binding site and second binding are joined to each other by a stable domain.

In another example, the present disclosure provides a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell, wherein the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site, and wherein the effector target and/or the address target is expressed on a structural tissue in a host.

In another example, the present disclosure provides a pharmaceutical composition comprising the macromolecule of any one of the above examples.

In another example, the present disclosure provides a pharmaceutical composition comprising a macromolecule and one or more pharmaceutically acceptable excipients, wherein the macromolecule comprises a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell, and wherein the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site.

In some examples, the pharmaceutical composition is an RNA pharmaceutical composition.

In some examples, the pharmaceutical composition further comprises a carrier.

In some examples, the carrier is a lipid nanoparticle.

In some examples, the carrier is a viral vector.

In some examples, the carrier is a membrane-based carrier.

In some examples, the membrane-based carrier is a cell.

In some examples, the membrane-based carrier is a vesicle.

In another example, the present disclosure provides a method for modulating activity of an effector target in the skin of a subject, the method comprising administering to the subject a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in the subject, and (b) the second binding site is specific for desmoglein-1 (DSG-1).

In another example, the present disclosure provides a method for modulating activity of an effector target in the lung of a subject, the method comprising administering to the subject a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in the subject, and (b) the second binding site is specific for RAGE.

In another example, the present disclosure provides a method for modulating activity of an effector target in the kidney of a subject, the method comprising administering to the subject a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in the subject, and (b) the second binding site is specific for cadherin 16 (CDH16).

In another example, the present disclosure provides a method for modulating activity of an effector target in the intestine of a subject, the method comprising administering to the subject a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in the subject, and (b) the second binding site is specific for cadherin 17 (CDH17).

In another example, the present disclosure provides a method of localizing a macromolecule at

a target tissue or cell of a subject, the method comprising administering to the subject a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in the subject, and (b) the second binding site is specific for an address target expressed in the target tissue or cell in the subject; wherein: (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site; and allowing the macromolecule to localize at the target tissue or cell of the subject.

In another example, the present disclosure provides a method of concentrating a macromolecule in a target tissue or cell in a subject, the method comprising administering to the subject a macromolecule comprising a first binding site and a second binding site, wherein (a) the first binding site is specific for an effector target in a subject, and (b) the second binding site is specific for an address target expressed in a target tissue or cell in the subject; wherein (i) the second binding site localizes the first binding site to the address target such that the first binding site influences effector target signaling in the target tissue or cell; (ii) the second binding site does not substantially influence signaling upon binding the address target; and (iii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site; and allowing the macromolecule to concentrate at the target tissue or cell of the subject, wherein at least 25% of the macromolecule detectable in the subject is detected at the target tissue or cell at a time point between 1 and 7 days following administration of the macromolecule to the subject.

In some examples, the potency of the first binding site at the target tissue or cell is substantially increased relative to a reference macromolecule lacking the second binding site.

In some examples, effector target signaling by the macromolecule in a non-target tissue or cell of the subject is substantially decreased relative to a reference macromolecule lacking the second binding site.

In some examples, the macromolecule is a macromolecule of any one of the above examples.

The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic illustrating exemplary ANDbody™ molecules and their use as logic-gated medicines. FIG. 1 shows broad distribution of a therapeutic target (right side), such as an effector target, in a human subject with no address targeting, and a localized and restricted distribution with address targeting (left side), which is provided by an address target binding domain. FIG 1. also provides a representative bipartite structure of an ANDbody with an address target binding domain linked to an effector target binding domain, which includes a functional moiety, e.g., a moiety

WHAT IS CLAIMED IS:

1. A macromolecule for use in localizing a macromolecule at the intestine of a subject, wherein the macromolecule comprises a first binding site and a second binding site and is to be administered to the subject, wherein:

(a) the first binding site is specific for an effector target in the subject, and

(b) the second binding site is specific for cadherin 17 (CDH17);

wherein:

(i) the second binding site does not substantially influence signaling upon binding CDH17; and

(ii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site;

allowing the macromolecule to localize at the intestine of the subject.

2. The macromolecule for use of claim 1, wherein at least 25% of the macromolecule detectable in the subject is detectable at the intestine at a time point between 1 and 7 days following administration of the macromolecule to the subject.

3. The macromolecule for use of claim 1, wherein the affinity of the first binding site for the effector target is more than two-fold weaker than the affinity of the second binding site for CDH17.

4. The macromolecule for use of claim 1, wherein the affinity of the first binding site for the effector target is more than five-fold weaker than the affinity of the second binding site for CDH17.

5. The macromolecule for use of claim 1, wherein the first binding site or the second binding site comprises a polypeptide, optionally wherein the polypeptide is an antibody or antigen-binding fragment thereof.

6. The macromolecule for use of claim 5, wherein the macromolecule is an antibody comprising a first binding site that is specific for the effector target in the subject and a second binding site that is specific for CDH17.

7. The macromolecule for use of claim 5, wherein the polypeptide is a ligand of the effector target.

8. The macromolecule for use of claim 7, wherein the first binding site comprises a ligand of the effector target and the second binding site comprises an antibody or antigen-binding fragment thereof.

9. The macromolecule for use of claim 1, wherein the macromolecule modulates activity of the effector target in the intestine.

10. The macromolecule for use of claim 1, wherein the second binding site localizes the first binding site to CDH17 such that the first binding site influences effector target signaling in the intestine.

11. A macromolecule for use in localizing a macromolecule at the intestine of a subject, wherein the macromolecule comprises a first binding site and a second binding site, and is to be administered to the subject, wherein:
(a) the first binding site is a ligand of an effector target in the subject, wherein the ligand has been modified to decrease its affinity for the effector target, and
(b) the second binding site is specific for CDH17;
wherein:
(i) the second binding site does not substantially influence signaling upon binding CDH17; and
(ii) the first binding site does not substantially influence effector target signaling in the absence of localization by the second binding site;
allowing the macromolecule to localize at the intestine of the subject.

12. The macromolecule for use of claim 11, wherein the ligand is a cytokine.

13. The macromolecule for use of claim 11, wherein the affinity of the first binding site for the effector target is lower than the affinity of the second binding site for CDH17.

14. The macromolecule for use of claim 11, wherein the second binding site localizes the first binding site to CDH17 such that the first binding site influences effector target signaling in the intestine.