(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 November 2018 (29.11.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/217989 A1

(51) International Patent Classification:
A61K 38/20 (2006.01) *C07K 14/55* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/US2018/034334

(22) International Filing Date:
24 May 2018 (24.05.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/510,586	24 May 2017 (24.05.2017)	US
62/510,816	25 May 2017 (25.05.2017)	US
62/558,175	13 September 2017 (13.09.2017)	US
62/595,352	06 December 2017 (06.12.2017)	US

(71) Applicant: **PANDION THERAPEUTICS, INC.** [US/US]; 700 Main Street, North, Cambridge, Massachusetts 02139 (US).

(72) Inventors: **VINEY, Joanne L.**; 30 Common Street, Belmont, Massachusetts 02478 (US). **HIGGINSON-SCOTT, Nathan**; 623 Tremont Street, Apt. 3, Boston, Massachusetts 02118 (US).

(74) Agent: **SCOLNICK, Daniel M.**; Pepper Hamilton LLP, 19th Floor, High Street Tower, 125 High Street, Boston, Massachusetts 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: TARGETED IMMUNOTOLERANCE

(57) Abstract: Methods and compounds for conferring site-specific or local immune privilege.

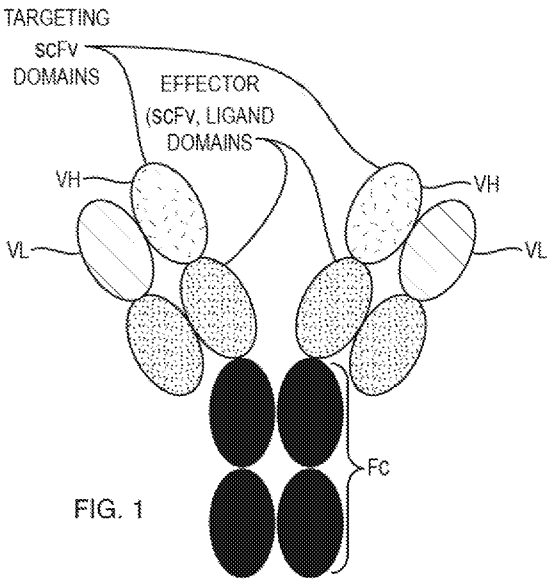

FIG. 1

WO 2018/217989 A1

TARGETED IMMUNOTOLERANCE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/510,586, filed May 24, 2017, U.S. Provisional Application No. 62/510,816, filed May 25, 2017, U.S. Provisional Application No. 62/558,175, filed September 13, 2017, and U.S. Provisional Application No. 62/595,352, filed December 6, 2017, each of which is hereby incorporated by reference in its entirety.

FIELD

The embodiments provided herein relate to, for example, methods and compositions for local or targeted immune-privilege.

BACKGROUND

Instances of unwanted immune responses, e.g., as in the rejection of transplanted tissue or in autoimmune disorders, constitute a major health problem for millions of people across the world. Long-term outcomes for organ transplantation are frequently characterized by chronic rejection, and eventual failure of the transplanted organ. More than twenty autoimmune disorders are known, affecting essentially every organ of the body, and affecting over fifty million people in North America alone. The broadly active immunosuppressive medications used to combat the pathogenic immune response in both scenarios have serious side effects.

SUMMARY

Disclosed herein are methods and therapeutic compounds that provide site-specific immune privilege. Embodiments disclosed herein are incorporated by reference into this section.

In some embodiments, the therapeutic compound comprises an engineered multi-specific compound, e.g., an engineered bi-specific molecule, e.g., an engineered bi-specific antibody molecule, comprising:

1) a specific targeting moiety selected from:

a) a donor specific targeting moiety which, e.g., preferentially binds a donor target (preferentially as compared with binding to a recipient antigen), and is useful for providing site-specific immune privilege for a transplant tissue, e.g., an organ, from a donor; or

b) a tissue specific targeting moiety which, e.g., preferentially binds a subject target tissue (preferentially as compared with subject non-target tissue), and is useful for providing site-specific immune privilege for a subject tissue undergoing unwanted immune attack, e.g., in an autoimmune disorder); and

2) an effector binding/modulating moiety selected from:

(a) an immune cell inhibitory molecule binding/modulating moiety (referred to herein as an ICIM binding/modulating moiety);

(b) an immunosuppressive immune cell binding/modulating moiety (referred to herein as an IIC binding/modulating moiety);

(c) an effector binding/modulating moiety that, as part of a therapeutic compound, promotes an immuno-suppressive local microenvironment, e.g., by providing in the proximity of the target, a substance that inhibits or minimizes attack by the immune system of the target (referred to herein as an SM binding/modulating moiety); or

(d) an immune cell stimulatory molecule binding/modulating moiety (referred to herein as an ICSM binding/modulating moiety), wherein the ICSM inhibits immune activation by, for example, blocking the interaction between a costimulatory molecule and its counterstructure.

An effector binding/modulating moiety can fall into more than one of classes a, b and c. E.g., as is shown below, a CTLA4 binding molecule falls into both of categories a and b.

In some embodiments, the therapeutic compound comprises an ICIM binding/modulating moiety. In some embodiments, an ICIM binding/modulating molecule and binds, and agonizes, an inhibitory molecule, e.g., an inhibitory immune checkpoint molecule, or otherwise inhibits or reduces the activity of an immune cell, e.g., a cytotoxic T cell, a B cell, NK cell, or a myeloid cell, e.g., a neutrophil or macrophage.

In some embodiments, the therapeutic compound comprises an engineered multi-specific compound, e.g., an engineered bi-specific molecule, e.g., an engineered bi-specific antibody molecule, comprising:

1) a specific targeting moiety, e.g., a donor specific targeting moiety (which binds a donor target and is useful for providing site-specific immune privilege for a transplant tissue,

e.g., an organ, from a donor) or a tissue specific targeting moiety (which binds a subject tissue target and is useful for providing site-specific immune privilege for a subject tissue undergoing unwanted immune attack, e.g., in an autoimmune disorder); and

2) an effector binding/modulating moiety comprising an ICIM binding/modulating moiety that binds to an effector molecule on an immune cell, e.g., an inhibitory receptor, e.g., PD-1, wherein, upon binding of the specific targeting moiety to its target, and binding of the ICIM binding/modulating moiety to an effector molecule on the immune cell, an immune cell activity, e.g., the ability of the immune cell to mount an immune attack, is down regulated, e.g., through an inhibitory signal dependent on the clustering of effector molecules on the immune cell. In some embodiments, the engineered multi-specific compound comprises additional binding moieties so that it binds more than two specific molecules, such as, but not limited to, 3 or 4.

In some embodiments, the therapeutic compound comprises an ICIM binding/modulating moiety and has one or both of the following properties: (a) the level of down regulation of an immune cell is greater when the therapeutic compound is bound to its target than when the therapeutic compound is not bound to its target; and (b) the therapeutic compound, when engaged with a cell surface inhibitory receptor, e.g., PD-1, on an immune cell, does not inhibit, or does not substantially inhibit the ability of the cell surface inhibitory receptor to bind an endogenous ligand.

In some embodiments, the level of down regulation of an immune cell is greater when the therapeutic compound is bound to its target than when the therapeutic compound is not bound to its target. In embodiments, the level of down regulation by target bound therapeutic compound is equal to or greater than 1.5-fold, 2-fold, 4-fold, 8-fold or 10-fold greater than what is seen when it is not bound to its target. In embodiments, therapeutic compound does not, or does not significantly down regulate immune cells when it is not bound to target. Thus, indiscriminant or unwanted agonism of an inhibitory receptor, e.g., PD-1, is minimized or eliminated. E.g., when the therapeutic compound is bound to an immune cell, but not bound to the targeted moiety, engagement of a inhibitory immune checkpoint molecule by the therapeutic compound does not result in down regulation or does not result in substantia down regulation, e.g., the inhibitory receptor on the immune cell to which the therapeutic compound is bound, is not clustered or not

clustered sufficiently to result in an inhibitory signal sufficient to give down regulation or substantial inhibition of the immune cell.

In embodiments, the therapeutic compound, when engaged with a cell surface inhibitory receptor, e.g., PD-1, on an immune cell, does not inhibit, or does not substantially inhibit the ability of the cell surface inhibitory receptor to bind an endogenous ligand. In some embodiments, the therapeutic compound can bind to the PD-L1/2 binding site on PD-1. Thus, indiscriminant or unwanted antagonism of an inhibitory receptor, e.g., PD-1, is minimized or eliminated. In embodiments, binding of the therapeutic compound to an inhibitory receptor, e.g. PD-1, on an immune cell does not impede, or substantially impede, the ability of the inhibitory receptor to bind a natural ligand, e.g., PD-L1. In embodiments, binding of the therapeutic compound to an inhibitory receptor, e.g. PD-1, on an immune reduces binding of a natural ligand, e.g., PD-L1, by less than 50, 40, 30, 20, 10, or 5% of what is seen in the absence of therapeutic compound.

In some embodiments, the therapeutic compound comprises an ICIM binding/modulating moiety and, when administered to a subject at a therapeutically effective dose, does not result in unacceptable levels of systemic immune suppression, as would be possible if indiscriminant agonism of the inhibitory receptor in all immune cells of a type, e.g., all T cells, occurred, or unacceptable levels of systemic immune activation, as would be possible if the therapeutic compound antagonized the interaction of the inhibitory receptor with its natural ligand.

While not wishing to be bound by theory, it is believed that, upon administration to a subject, a therapeutic compound comprising an ICIM binding/modulating moiety can exist in any one of four states: i) unbound and in free solution; ii) bound to only an inhibitory receptor expressed on the surface of an immune cell, e.g., a T cell, through the ICIM binding/modulating moiety; iii) bound to only the surface of the target transplant or subject tissue through the targeting moiety; and iv) bound to both the surface of target transplant or subject tissue through the targeting moiety and to an inhibitory receptor expressed by an immune cell, e.g., a T cell, through the ICIM binding/modulating moiety. When the therapeutic compound is bound only to the target transplant or subject tissue (iii) through the targeting moiety, it has no, or no substantial, effect on the target transplant or tissue. When the therapeutic compound is bound to the target transplant or tissue through the targeting moiety and bound to an inhibitory receptor expressed by an immune cell, e.g., a T cell, through the ICIM binding/modulating moiety (iv), it

creates immune privilege at the target organ or tissue. While not wishing to be bound by theory, is believed that this is achieved by the target transplant or donor tissue multimerizing the therapeutic compound molecules on its surface, e.g., by immobilizing a plurality of therapeutic compound molecules at a high density and valency. The multimerization of the therapeutic compound molecules allows the ICIM binding/modulating moieties of the therapeutic compounds to promote clustering of inhibitory receptors expressed on the surface of the immune cell, e.g., a pathogenic T cell, and transmission of an inhibitory signal functioning to silence or down-regulate the immune cell. E.g., in the case of T cells, a therapeutic compound comprising an ICIM binding/modulating moiety comprising a PD-L1 molecule, or an anti-PD-1 Ab, can be used. Binding of a plurality of the therapeutic compound molecules to the target results in multimerization of the therapeutic compound molecules, which in turn, by virtue of the PD-L1 molecule, or a functional anti-PD-1 antibody molecule, leads to clustering of PD-1 on the T cell. If that clustering occurs in the context of antigen presentation by the target MHC, to T cell receptor on the T cell, a negative signal is generated and the T cell will be inactivated. In embodiments the ICIM binding/modulating moiety, e.g., a functional antibody molecule, binds the effector molecule but does not inhibit, or substantially inhibit, interaction of the effector molecule with its native ligand(s).

In some embodiments, the therapeutic compound comprises an IIC binding/modulating moiety, which binds and recruits an immune suppressive immune cell, e.g., a Treg, e.g., a Foxp3+CD25+ Treg, to the proximity of the target tissue.

In some embodiments, the therapeutic compound comprises a SM binding/modulating moiety, which modulates, e.g., binds and inhibits, sequesters, degrades or otherwise neutralizes a substance, e.g., a soluble molecule that modulates an immune response, e.g., ATP or AMP.

In some embodiments, the therapeutic compound comprises a targeting moiety that is specific for a target on an immune cell. In some embodiments, the target is as described herein. In some embodiments, the target is MAdCAM. In some embodiments, the targeting moiety is an antibody that binds to MAdCAM.

In some embodiments the therapeutic compound comprises an ICSM binding/modulating moiety, which binds a stimulatory molecule, e.g., a costimulatory molecule. In some embodiments, the ICSM inhibits the costimulatory molecule counterstructure by. Binding/modulating either the costimulatory molecule or the costimulatory molecule

counterstructure can serve to down regulate the ability of an immune cell to mount an immune response. In some embodiments, the ICSM binding/modulating moiety can bind a stimulatory, e.g., costimulatory molecule on an immune cell, e.g., OX40 on T cells , or the counter member of the stimulatory molecule e.g. OX40L on another cell, such as, but not limited to, immune cells such as NK cells, mast cells, dendritic cells, or, for example, non-immune cells such as endothelial cells, or smooth muscle cells.

In some embodiments, the therapeutic compound comprises a donor specific targeting moiety and provides site-specific immune privilege for donor transplant tissue implanted in a subject. In some embodiments, the therapeutic compound comprises a tissue specific targeting moiety and provides site-specific immune privilege for a tissue of a subject, e.g., a tissue afflicted with an unwanted immune response in an autoimmune disorder.

The targeting moiety is specific for the donor transplant or subject tissue to be protected from the immune system. In some embodiments, the effector molecule binding moiety comprises a de novo generated binding domain, e.g. a functional antibody molecule. In some embodiments, the effector binding/modulating moiety comprises amino acid sequence deriving from the natural ligand that recognizes an inhibitory receptor expressed on the surface of an immune cell, e.g., a T cell.

In some embodiments, the therapeutic compound silences immune cells, e.g., T cells, proximal to the transplant or donor tissue to be protected but does not silence immune cells, e.g., T cells, not proximal to the target, as the therapeutic compound requires the presence of the target transplant or donor tissue for function. This in contrast to when the therapeutic compound binds only to the inhibitory receptor expressed by the immune cell, e.g., T cell, in which case there is no functional consequence.

Methods and therapeutic compounds described here are based at least in part on providing site-specific immune-privilege. Therapeutic compounds and method of using them described herein allow the minimization, e.g., the reduction or elimination of, non-site specific systemic administration of immune-suppressive therapeutic agents in clinical settings, e.g., where reversal and suppression of an immune response is desired, such as in autoimmune diseases or tissue, e.g., organ, transplant. While capable of clinically meaningful response when the underlying pathophysiology driven by an aberrant immune system is impacted, broadly acting immunosuppressants have the undesirable effect of reducing the patient's systemic

immune system function. As the role of a normally functioning immune system is to combat the constant barrage of pathogenic and opportunistic organisms existing in the surrounding environment and to constantly purge healthy individuals of cancerous cells, patients undergoing chronic immunosuppression are at an increased risk to develop infections and cancer. Methods and therapeutic compounds described herein provide therapies that selectively target and attenuate, reduce, or extinguish only the pathogenic immune response at the site of pathology while having minimal inhibition of normal systemic immune system function elsewhere.

In some embodiments, a therapeutic compound is provided as provided herein. In some embodiments, the compound comprises a i) a specific targeting moiety selected from: a) a donor specific targeting moiety which, e.g., preferentially binds a donor target; or b) a tissue specific targeting moiety which, e.g., preferentially binds target tissue of a subject; and ii) an effector binding/modulating moiety selected from: (a) an immune cell inhibitory molecule binding/modulating moiety (ICIM binding/modulating moiety); (b) an immunosuppressive immune cell binding/modulating moiety (IIC binding/modulating moiety); or (c) an effector binding/modulating moiety that, as part of a therapeutic compound, promotes an immuno-suppressive local microenvironment, e.g., by providing in the proximity of the target, a substance that inhibits or minimizes attack by the immune system of the target (SM binding/modulating moiety).

In some embodiments, the effector binding/modulating moiety comprises an ICIM binding/modulating moiety. In some embodiments, the effector binding/modulating moiety comprises an ICIM binding/modulating moiety comprising an inhibitory immune checkpoint molecule ligand molecule. In some embodiments, the inhibitory immune molecule counter-ligand molecule comprises a PD-L1 molecule. In some embodiments, the ICIM is wherein the inhibitory immune molecule counter ligand molecule engages a cognate inhibitory immune checkpoint molecule selected from PD-1, KIR2DL4, LILRB1, LILRB, or CTLA-4. In some embodiments, the ICIM is an antibody. In some embodiments, the ICIM comprises an antibody that binds to PD-1, KIR2DL4, LILRB1, LILRB, or CTLA-4. In some embodiments, the ICIM binding/modulating moiety which comprises a functional antibody molecule to a cell surface inhibitory molecule.

In some embodiments, the cell surface inhibitory molecule is an inhibitory immune checkpoint molecule. In some embodiments, the inhibitory immune checkpoint molecule is selected from PD-1, KIR2DL4, LILRB1, LILRB2, CTLA-4, or selected from Table 1.

In some embodiments, the effector binding/modulating moiety comprises an IIC binding/modulating moiety.

In some embodiments, the compound has the formula from N-terminus to C-terminus:
R1---Linker Region A—R2 or R3—Linker Region B—R4,
wherein, R1, R2, R3, and R4, each independently comprises an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, ICSM binding/modulating moiety, or an SM binding/modulating moiety; a specific targeting moiety; or is absent; provided that an effector binding/modulating moiety and a specific targeting moiety are present.

In some embodiments, polypeptides comprising a targeting moiety that binds to a target cell and an effector binding/modulating moiety, wherein the effector binding/modulating moiety is a IL-2 mutein polypeptide (IL-2 mutein), which is a mutant IL-2 protein, are provided. In some embodiments, the targeting moiety comprises an antibody that binds to a target protein on the surface of a target cell. In some embodiments, the polypeptide comprises two polypeptide chains as provided for herein. In some emboidments, the first chain comprises a VH domain and the second chain comprises a VL domain of an antibody that binds to the target cell or a protein that is expressed on the target cell, such as, but not limited to, MAdCAM. In some embodiments, the targeting moiety is an antibody that binds to MAdCAM. In some embodiments, the targeting moiety binds to OAT1 (SLC22A6) and OCT2 (SLC22A2). In some embodients, the targeting moiety is an antibody that binds to OAT1 (SLC22A6) and OCT2 (SLC22A2). In some embodients, the targeting moiety does not bind to OAT1 (SLC22A6) and OCT2 (SLC22A2). For the avoidance of doubt, the OCT2 referenced herein is not the transcription factor, but rather is the surface protein expressed in kidney tissue. In some embodiments, the targeting moiety is a moiety that specifically binds to a protein found in the pancreas. In some embodiments, the targeting moiety binds to FXYD2, TSPAN7, DPP6, HEPACAM2, TMEM27, or GPR119. In some embodiments, the targetin moiety does not bind to FXYD2, TSPAN7, DPP6, HEPACAM2, TMEM27, or GPR119. In some embodiments, the

targeting moiety is antibody that binds to FXYD2, TSPAN7, DPP6, HEPACAM2, TMEM27, or GPR119.

In some embodiments, the polypeptide comprises a first chain and a second chain that form the polypeptide or therapeutic compound, wherein

the first chain comprises:

V_H-H_c-Linker-C_1, wherein V_H is a variable heavy domain that binds to the target cell with a V_L domain of the second chain; H_c is a heavy chain of antibody comprising CH1-CH2-CH3 domain, the Linker is a glycine/serine amino acid sequence as provided herein or is absent, and C_1 is a IL-2 mutein that can be fused to a Fc protein in either the N-terminal or C-terminal orientation as provided for herein, wherein there can be a glycine/serine linker linking the IL-2 mutein to the Fc protein; and

the second chain comprises:

V_L-L_c, wherein V_L is a variable light chain domain that binds to the target cell with the V_H domain of the first chain, and the Lc domain is a light chain CK domain. In some embodiments, the first chain comprises C_1-Linker-V_H-H_c, with the variables as defined above.

In some embodiments, the the polypeptide comprises the formula of C_1-linker-CH2-CH3-Linker-scFv, wheren C_1 and the Linker are as defined above and herein, the CH2 and CH3 are heavy chain domains and the scFv is a single chain antibody like fragment that acts as the targeting moiety to bind to tissue targets as provided for herein. In some embodiments, the mutein is fused to the Fc region as provided herein and one or more of the linkers are absent. In some embodiments, the Linker is a glycine/serine linker as provided for herein. In some embodiments, the linker is a peptide sequence.

In some embodiments, methods of treating auto-immune diseases or conditions are provided herein, the methods comprising administering one or more of the therapeutic compounds or polypeptides provided herein.

In some embodiments, methods of treating diseases or conditions described herein are provided herein, the methods comprising administering one or more of the therapeutic compounds or polypeptides provided herein.

In some embodiments, methods of treating a subject with inflammatory bowel disease are provided, the methods comprising administering a therapeutic compound or polypeptides

provided herein to the subject to treat the inflammatory bowel disease. In some embodiments, the subject has Crohn's disease and or ulcerative colitis.

In some embodiments, methods of treating a subject with auto-immune hepatitis are provided, the methods comprising administering a therapeutic compound or polypeptides as provided herein to the subject to treat the auto-immune hepatitis.

In some embodiments, methods of treating primary sclerosing cholangitis are provided, the methods comprising administering a therapeutic compound or polypeptides as provided herein to the subject to treat the primary sclerosing cholangitis.

In some embodiments, methods of treating (*e.g.*, reducing) inflammation in the intestine are provided, the methods comprising administering a therapeutic compound or polypeptides as provided herein to the subject to treat the inflammation in the intestine. In some embodiments, the inflammation is in the small intestine. In some embodiments, the inflammation is in the large intesting. In some embodiments, the inflammation is in the bowel or colon.

In some embodiments, methods of treating (*e.g.*, reducing) inflammation in the pancreas are provided, the methods comprising administering a therapeutic compound or polypeptides as provided herein to the subject to treat the inflammation in the pancreas. In some embodiments, the methods treat pancreatitis.

In some embodiments, methods of treating Type 1 diabetes are provided, the methods comprising administering a therapeutic compound or polypeptides as provided herein to the subject to treat the Type 1 diabetes.

In some embodiments, methods of treating a transplant subject are provided, the methods comprising administering a therapeutically effective amount of a therapeutic compound or polypeptides as provided herein to the subject, thereby treating a transplant (recipient) subject.

In some embodiments, methods of treating GVHD in a subject having a transplanted a donor tissue are provided, the methods comprising administering a therapeutically effective amount of a therapeutic compound or polypeptides as provided herein to the subject.

In some embodiments, methods of treating a subject having, or at risk, or elevated risk, for having, an autoimmune disorder are provided, the methods comprising administering a therapeutically effective amount of a therapeutic compound or polypeptides as provided herein, thereby treating the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts non-limiting embodiments of the therapeutic compounds provided herein.

FIG. 2 depicts a non-limiting illustration of how a therapeutic compound provided herein could function.

FIG. 3 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 3A depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 4 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 5 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 6 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 7 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 8 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 9 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 10 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 11 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 12 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 13 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 14 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 15 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 16 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 17 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 18 depicts a non-limiting illustration of the therapeutic compounds provided herein.

FIG. 19 depicts a non-limiting illustration of the therapeutic compounds provided herein.

DETAILED DESCRIPTION

This application incorporates by reference U.S. Application No. 15/922,592 filed March 15, 2018 and PCT Applciation No. PCT/US2018/022675, filed March 15, 2018, each of which is incorporated by ereference in its entirety.

As used herein and unless otherwise indicated, the term "about" is intended to mean ± 5% of the value it modifies. Thus, about 100 means 95 to 105.

As used herein and in the appended claims, the singular forms "a", "an" and "the" include plural reference unless the context clearly dictates otherwise.

As used herein, the term "about" means that the numerical value is approximate and small variations would not significantly affect the practice of the disclosed embodiments. Where

a numerical limitation is used, unless indicated otherwise by the context, "about" means the numerical value can vary by ±10% and remain within the scope of the disclosed embodiments.

As used herein, the term "animal" includes, but is not limited to, humans and non-human vertebrates such as wild, domestic, and farm animals.

As used herein, the term "contacting" means bringing together of two elements in an in vitro system or an in vivo system. For example, "contacting" a therapeutic compound with an individual or patient or cell includes the administration of the compound to an individual or patient, such as a human, as well as, for example, introducing a compound into a sample containing a cellular or purified preparation containing target.

As used herein, the terms "comprising" (and any form of comprising, such as "comprise", "comprises", and "comprised"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include"), or "containing" (and any form of containing, such as "contains" and "contain"), are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. Any composition or method that recites the term "comprising" should also be understood to also describe such compositions as consisting, consisting of, or consisting essentially of the recited components or elements.

As used herein, the term "fused" or "linked" when used in reference to a protein having different domains or heterologous sequences means that the protein domains are part of the same peptide chain that are connected to one another with either peptide bonds or other covalent bonding. The domains or section can be linked or fused directly to one another or another domain or peptide sequence can be between the two domains or sequences and such sequences would still be considered to be fused or linked to one another. In some embodiments, the various domains or proteins provided for herein are linked or fused diretctly to one another or a linker sequences, such as the glycine/serine sequences described herein link the two domains together.

As used herein, the term "individual," "subject," or "patient," used interchangeably, means any animal, including mammals, such as mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, such as humans.

As used herein, the term "inhibit" refers to a result, symptom, or activity being reduced as compared to the activity or result in the absence of the compound that is inhibiting the result, symptom, or activity. In some embodiments, the result, symptom, or activity, is inhibited by

about, or, at least, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99%. An result, symptom, or activity can also be inhibited if it is completely elimination or extinguished.

As used herein, the phrase "in need thereof" means that the subject has been identified as having a need for the particular method or treatment. In some embodiments, the identification can be by any means of diagnosis. In any of the methods and treatments described herein, the subject can be in need thereof. In some embodiments, the subject is in an environment or will be traveling to an environment in which a particular disease, disorder, or condition is prevalent.

As used herein, the phrase "integer from X to Y" means any integer that includes the endpoints. For example, the phrase "integer from X to Y" means 1, 2, 3, 4, or 5.

As used herein, the term "mammal" means a rodent (i.e., a mouse, a rat, or a guinea pig), a monkey, a cat, a dog, a cow, a horse, a pig, or a human. In some embodiments, the mammal is a human.

In some embodiments, therapeutic compounds are provided herein. In some embodiments, the therapeutic compound is a protein or a polypeptide, that has multiple chains that interact with one another. The polypeptides can interact with one another through non-covalent interactions or covalent interactions, such as through disulfide bonds or other covalent bonds. Therefore, if an embodiment refers to a therapeutic compound it can also be said to refer to a protein or polypeptide as provided for herein and vice versa as the context dictates.

As used herein, the phrase "ophthalmically acceptable" means having no persistent detrimental effect on the treated eye or the functioning thereof, or on the general health of the subject being treated. However, it will be recognized that transient effects such as minor irritation or a "stinging" sensation are common with topical ophthalmic administration of drugs and the existence of such transient effects is not inconsistent with the composition, formulation, or ingredient (e.g., excipient) in question being "ophthalmically acceptable" as herein defined. In some embodiments, the pharmaceutical compositions can be ophthalmically acceptable or suitable for ophthalmic administration.

"Specific binding" or "specifically binds to" or is "specific for" a particular antigen, target, or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not

have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

Specific binding for a particular antigen, target, or an epitope can be exhibited, for example, by an antibody having a K_D for an antigen or epitope of at least about 10^{-4M}, at least about 10^{-5M}, at least about $10^{-6\,M}$, at least about 10^{-7M}, at least about 10^{-8M}, at least about 10^{-9M}, alternatively at least about $10^{-10\,M}$, at least about 10^{-11M}, at least about 10^{-12M}, or greater, where K_D refers to a dissociation rate of a particular antibody-target interaction. Typically, an antibody that specifically binds an antigen or target will have a K_D that is, or at least, 2-, 4-, 5-, 10-, 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000-, or more times greater for a control molecule relative to the antigen or epitope.

In some embodiments, specific binding for a particular antigen, target, or an epitope can be exhibited, for example, by an antibody having a K_A or K_a for a target, antigen, or epitope of at least 2-, 4-, 5-, 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the target, antigen, or epitope relative to a control, where K_A or K_a refers to an association rate of a particular antibody-antigen interaction.

As provided herein, the therapeutic compounds and compositions can be used in methods of treatment as provided herein. As used herein, the terms “treat,” “treated,” or “treating” mean both therapeutic treatment and prophylactic measures wherein the object is to slow down (lessen) an undesired physiological condition, disorder or disease, or obtain beneficial or desired clinical results. For purposes of these embodiments, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of extent of condition, disorder or disease; stabilized (i.e., not worsening) state of condition, disorder or disease; delay in onset or slowing of condition, disorder or disease progression; amelioration of the condition, disorder or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

Provided herein are therapeutic compounds, e.g., therapeutic protein molecules, e.g., fusion proteins, including a targeting moiety and an effector binding/modulating moiety, typically as separate domains. Also provided are methods of using and making the therapeutic

compounds. The targeting moiety serves to localize the therapeutic compound, and thus the effector binding/modulating moiety, to a site at which immune-privilege is desired. The effector binding/modulating moiety comprises one or more of: (a) an immune cell inhibitory molecule binding/modulating moiety (an ICIM binding/modulating moiety): (b) an immunosuppressive immune cell binding/modulating moiety (an IIC binding/modulating moiety); (c) a soluble molecule binding/modulating moiety (a SM binding/modulating moiety) or (d) a molecule that blocks or inhibits immune cell stimulatory molecule binding/modulating moiety (referred to herein as an ICSM binding/modulating moiety). In some embodiments, the ICSM inhibits immune activation by, for example, blocking the interaction between a costimulatory molecule and its counterstructure. In some embodiments, a therapeutic compound comprises: (a) and (b); (a) and (c); (a) and (d); (b) and (c); (b) and (d); (c) and (d); or (a), (b), (c), and (d).

The present disclosure provides, for example, molecules that can act as PD-1 agonists. Without being bound to any particular theory, agonism of PD-1 inhibits T cell activation/signaling and can be accomplished by different mechanisms. For example cross-linking can lead to agonism, bead-bound, functional PD-1 agonists have been described (Akkaya. Ph.D. Thesis: Modulation of the PD-1 pathway by inhibitory antibody superagonists. Christ Church College, Oxford, UK, 2012), which is hereby incorporated by reference. Crosslinking of PD-1 with two mAbs that bind non-overlapping epitopes induces PD-1 signaling (Davis, US 2011/0171220), which is hereby incorporated by reference. Another example is illustrated through the use of a goat anti-PD-1 antiserum (e.g. AF1086, R&D Systems) which is hereby incorporated by reference, which acts as an agonist when soluble (Said et al., 2010, Nat Med) which is hereby incorporated by reference. Non-limiting examples of PD-1 agonists that can be used in the present embodiments include, but are not limited to, UCB clone 19 or clone 10, PD1AB-1, PD1AB-2, PD1AB-3, PD1AB-4 and PD1AB-5, PD1AB-6 (Anaptys/Celgene), PD1-17, PD1-28, PD1-33 and PD1-35 (Collins et al, US 2008/0311117 A1 Antibodies against PD-1 and uses therefor, which is incorporated by reference), or can be a bi-specific, monovalent anti-PD-1/anti-CD3 (Ono), and the like. In some embodiments, the PD-1 agonist antibodies can be antibodies that block binding of PD-L1 to PD-1. In some embodiments, the PD-1 agonist antibodies can be antibodies that do not block binding of PD-L1 to PD-1.

PD-1 agonism can be measured by any method, such as the methods described in the examples. For example, cells can be constructed that express, including stably express, constructs that include a human PD-1 polypeptide fused to a b-galactosidase "Enzyme donor" and 2) a SHP-2 polypeptide fused to a b-galactosidase "Enzyme acceptor." Without being bound by any theory, when PD-1 is engaged, SHP-2 is recruited to PD-1. The enzyme acceptor and enzyme donor form a fully active b-galactosidase enzyme that can be assayed. Although, the assay does not directly show PD-1 agonism, but shows activation of PD-1 signaling. PD-1 agonism can also be measured by measuring inhibition of T cell activation because, without being bound to any theory, PD-1 agonism inhibits anti-CD3-induced T cell activation. For example, PD-1 agonism can be measured by preactivating T cells with PHA (for human T cells) or ConA (for mouse T cells) so that they express PD-1. The cells can then be reactivated with anti-CD3 in the presence of anti-PD-1 (or PD-L1) for the PD-1 agonism assay. T cells that receive a PD-1 agonist signal in the presence of anti-CD3 will show decreased activation, relative to anti-CD3 stimulation alone. Activation can be readout by proliferation or cytokine production (IL-2, IFNg, IL-17) or other markers, such as CD69 activation marker. Thus, PD-1 agonism can be measured by either cytokine production or cell proliferation. Other methods can also be used to measure PD-1 agonism.

PD-1 is Ig superfamily member expressed on activated T cells and other immune cells. The natural ligands for PD-1 appear to be PD-L1 and PD-L2. Without being bound to any particular theory, when PD-L1 or PD-L2 bind to PD-1 on an activated T cell, an inhibitory signaling cascade is initiated, resulting in attenuation of the activated T effector cell function. Thus, blocking the interaction between PD-1 on a T cell, and PD-L1/2 on another cell (eg tumor cell) with a PD-1 antagonist is known as checkpoint inhibition, and releases the T cells from inhibition. In contrast, PD-1 agonist antibodies can bind to PD-1 and send an inhibitory signal and attenuate the function of a T cell. Thus, PD-1 agonist antibodies can be incorporated into various embodiments described herein as an effector molecule binding/modulating moiety, which can accomplish localized tissue-specific immunomodulation when paired with a targeting moiety.

The effector molecule binding/modulating moiety can provide an immunosuppressive signal or environment in a variety of ways. In some embodiments, the effector binding/modulating moiety comprises an ICIM binding/modulating moiety that directly binds

and (under the appropriate conditions as described herein) activates an inhibitory receptor expressed by immune cells responsible for driving disease pathology. In another embodiment the effector binding/modulating moiety comprises and IIC binding/modulating moiety and binds and accumulates immunosuppressive immune cells. In some embodiments, the accumulated immune suppressive cells promote immune privilege. In another embodiment the effector binding/modulating moiety comprises an SM binding/modulating moiety which manipulates the surrounding microenvironment to make it less permissible for the function of immune cells, e.g., immune cells driving disease pathology. In some embodiments, the SM binding/modulating moiety depletes an entity that promotes immune attack or activation. In some embodiments the effector binding/modulating moiety comprises an ICSM binding/modulating moiety that binds a member of a pair of stimulatory molecules, e.g., costimulatory molecules, and inhibits the interaction between the costimulatory molecule and the costimulatory molecule counterstructure, such as, but not limited to,OX40 or CD30 or CD40 and OX40L, or CD30L or CD40L and inhibits the immune stimulation of a cell, such as, but not limited to, a T cell, B cell, NK cell, or other immune cell comprising a member of the pair.

The targeting moiety and effector binding/modulating moiety are physically tethered, covalently or non-covalently, directly or through a linker entity, to one another, e.g., as a member of the same protein molecule in a therapeutic protein molecule. In some embodiments, the targeting and effector moieties are provided in a therapeutic protein molecule, e.g., a fusion protein, typically as separate domains. In some embodiments, the targeting moiety, the effector binding/modulating moiety, or both each comprises a single domain antibody molecule, e.g., a camelid antibody VHH molecule or human soluble VH domain . It may also contain a single-chain fragment variable (scFv) or a Fab domain. In some embodiments, the therapeutic protein molecule, or a nucleic acid, e.g., an mRNA or DNA, encoding the therapeutic protein molecule, can be administered to a subject. In some embodiments, the targeting and effector molecule binding/modulating moieties are linked to a third entity, e.g., a carrier, e.g., a polymeric carrier, a dendrimer, or a particle, e.g., a nanoparticle. The therapeutic compounds can be used to down regulate an immune response at or in a tissue at a selected target or site while having no or substantially less immunosuppressive function systemically. The target or site can comprise donor tissue or autologous tissue.

Provided herein are methods of providing site-specific immune privilege for a transplanted donor tissue, e.g., an allograft tissue, e.g., a tissue described herein, e.g., an allograft liver, an allograft kidney, an allograft heart, an allograft pancreas, an allograft thymus or thymic tissue, allograft skin, or an allograft lung, with therapeutic compounds disclosed herein. In embodiments the treatment minimizes rejection of, minimizes immune effector cell mediated damage to, prolongs acceptance of, or prolongs the functional life of, donor transplant tissue.

Also provided herein are methods of inhibiting graft versus host disease (GVHD) by minimizing the ability of donor immune cells, e.g., donor T cells, to mediate immune attack of recipient tissue, with therapeutic compounds disclosed herein.

Also provided herein are methods of treating, e.g., therapeutically treating or prophylactically treating (or preventing), an auto-immune disorder or response in a subject by administration of a therapeutic compound disclosed herein, e.g., to provide site or tissue specific modulation of the immune system. In some embodiments, the method provides tolerance to, minimization of the rejection of, minimization of immune effector cell mediated damage to, or prolonging a function of, subject tissue. In some embodiments, the therapeutic compound includes a targeting moiety that targets, e.g., specifically targets, the tissue under, or at risk for, autoimmune attack. Non-limiting exemplary tissues include, but are not limited to, the pancreas, myelin, salivary glands, synoviocytes, and myocytes.

As used herein, the terms “treat,” “treated,” or “treating” in regards to therapeutic treatment wherein the object is to slow down (lessen) an undesired physiological condition, disorder or disease, or obtain beneficial or desired clinical results. For example, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of extent of condition, disorder or disease; stabilized (i.e., not worsening) state of condition, disorder or disease; delay in onset or slowing of condition, disorder or disease progression; amelioration of the condition, disorder or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. Thus, “treatment of an auto-immune disease/disorder” means an activity that alleviates or ameliorates any of the primary phenomena

or secondary symptoms associated with the auto-immune disease/disorder or other condition described herein. The various disease or conditions are provided herein. The therapeutic treatment can also be administered prophylactically to preventing or reduce the disease or condition before the onset.

In some embodiments, administration of the therapeutic compound begins after the disorder is apparent. In some embodiments, administration of the therapeutic compound, begins prior to onset, or full onset, of the disorder. In some embodiments, administration of the therapeutic compound, begins prior to onset, or full onset, of the disorder, e.g., in a subject having the disorder, a high-risk subject, a subject having a biomarker for risk or presence of the disorder, a subject having a family history of the disorder, or other indicator of risk of, or asymptomatic presence of, the disorder. For example, In some embodiments, a subject having islet cell damage but which is not yet diabetic, is treated.

While not wishing to be bound by theory, it is believed that the targeting moiety functions to bind and accumulate the therapeutic to a target selectively expressed at the anatomical site where immune privilege is desired. In some embodiments, e.g., in the context of donor tissue transplantation, the target moiety binds to a target, e.g., an allelic product, present in the donor tissue but not the recipient. For treatment of autoimmune disorders, the targeting moiety binds a target preferentially expressed at the anatomical site where immune privilege is desired, e.g., in the pancreas. For treatment of GVHD, the targeting moiety targets the host tissue, and protects the host against attack from transplanted immune effector cells derived from transplanted tissue.

Again, while not wishing to be bound by theory it is believed that the effector binding/modulating moiety serves to deliver an immunosuppressive signal or otherwise create an immune privileged environment.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which these embodiments belong. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present embodiments, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Headings, sub-headings or

numbered or lettered elements, e.g., (a), (b), (i) etc, are presented merely for ease of reading. The use of headings or numbered or lettered elements in this document does not require the steps or elements be performed in alphabetical order or that the steps or elements are necessarily discrete from one another. Other features, objects, and advantages of the embodiments will be apparent from the description and drawings, and from the claims.

ADDITIONAL DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the embodiments pertains. In describing and claiming the present embodiments, the following terminology and terminology otherwise referenced throughout the present application will be used according to how it is defined, where a definition is provided.

It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Antibody molecule, as that term is used herein, refers to a polypeptide, e.g., an immunoglobulin chain or fragment thereof, comprising at least one functional immunoglobulin variable domain sequence. An antibody molecule encompasses antibodies (e.g., full-length antibodies) and antibody fragments. In some embodiments, an antibody molecule comprises an antigen binding or functional fragment of a full length antibody, or a full length immunoglobulin chain. For example, a full-length antibody is an immunoglobulin (Ig) molecule (e.g., an IgG antibody) that is naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes). In embodiments, an antibody molecule refers to an immunologically active, antigen-binding portion of an immunoglobulin molecule, such as an antibody fragment. An antibody fragment, e.g., functional fragment, comprises a portion of an antibody, e.g., Fab, Fab′, F(ab′)2, F(ab)2, variable fragment (Fv), domain antibody (dAb), or single chain variable fragment (scFv). A functional antibody fragment binds to the same antigen as that recognized by the intact (e.g., full-length) antibody. The terms “antibody fragment” or “functional fragment” also include isolated fragments consisting of the variable regions, such as the “Fv” fragments consisting of the variable regions of the heavy and light chains or recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (“scFv proteins”). In some embodiments, an antibody fragment does not include portions of antibodies without antigen binding activity, such as Fc fragments or single amino acid

residues. Exemplary antibody molecules include full length antibodies and antibody fragments, e.g., dAb (domain antibody), single chain, Fab, Fab', and F(ab')2 fragments, and single chain variable fragments (scFvs).

The term "antibody molecule" also encompasses whole or antigen binding fragments of domain, or single domain, antibodies, which can also be referred to as "sdAb" or "VHH." Domain antibodies comprise either V_H or V_L that can act as stand-alone, antibody fragments. Additionally, domain antibodies include heavy-chain-only antibodies (HCAbs). Domain antibodies also include a CH2 domain of an IgG as the base scaffold into which CDR loops are grafted. It can also be generally defined as a polypeptide or protein comprising an amino acid sequence that is comprised of four framework regions interrupted by three complementarity determining regions. This is represented as FR1- CDR1 -FR2-CDR2-FR3-CDR3-FR4. sdAbs can be produced in camelids such as llamas, but can also be synthetically generated using techniques that are well known in the art. The numbering of the amino acid residues of a sdAb or polypeptide is according to the general numbering for VH domains given by Kabat et al. ("Sequence of proteins of immunological interest," US Public Health Services, NIH Bethesda, MD, Publication No. 91, which is hereby incorporated by reference). According to this numbering, FR1 of a sdAb comprises the amino acid residues at positions 1-30, CDR1 of a sdAb comprises the amino acid residues at positions 31-36, FR2 of a sdAb comprises the amino acids at positions 36-49, CDR2 of a sdAb comprises the amino acid residues at positions 50-65, FR3 of a sdAb comprises the amino acid residues at positions 66- 94, CDR3 of a sdAb comprises the amino acid residues at positions 95-102, and FR4 of a sdAb comprises the amino acid residues at positions 103-113. Domain antibodies are also described in WO2004041862 and WO2016065323, each of which is hereby incorporated by reference. The domain antibodies can be a targeting moiety as described herein.

Antibody molecules can be monospecific (e.g., monovalent or bivalent), bispecific (e.g., bivalent, trivalent, tetravalent, pentavalent, or hexavalent), trispecific (e.g., trivalent, tetravalent, pentavalent, hexavalent), or with higher orders of specificity (e.g, tetraspecific) and/or higher orders of valency beyond hexavalency. An antibody molecule can comprise a functional fragment of a light chain variable region and a functional fragment of a heavy chain variable region, or heavy and light chains may be fused together into a single polypeptide.

Examples of formats for multispecific therapeutic compounds, e.g., bispecific antibody molecules are shown in the following non-limiting examples. Although illustrated with antibody molecules, they can be used as platforms for therapeutic molecules that include other non-antibody moieties as specific binding or effector moieties. In some embodiments, these non-limiting examples are based upon either a symmetrical or asymmetrical Fc formats.

For example, the figures illustrate non-limiting and varied symmetric homodimer approach. In some embodiments, the dimerization interface centers around human IgG1 CH2-CH3 domains, which dimerize via a contact interface spanning both CH2/CH2 and CH3/CH3. The resulting bispecific antibodies shown have a total valence comprised of four binding units with two identical binding units at the N-terminus on each side of the dimer and two identical units at the C-terminus on each side of the dimer. In each case the binding units at the N-terminus of the homo-dimer are different from those at the C-terminus of the homo-dimer. Using this type of bivalency for both an inhibitory T cell receptor at either terminus of the molecule and bivalency for a tissue tethering antigen can be achieved at either end of the molecule.

For example, in FIG. 3, a non-limiting embodiment is illustrated. The N-terminus of the homodimer contains two identical Fab domains comprised of two identical light chains, which are separate polypeptides, interfaced with the n-terminal VH-CH1 domains of each heavy chain via the VH/VL interaction and Ckappa or Clambda interaction with CH1. The native disulphide bond between the Ckappa or Clambda with CH1 is present providing a covalent anchor between the light and heavy chains. At the c-terminus of this design are two identical scFv units where by (in this example) the c-terminus of the CH3 domain of the Fc, is followed by a flexible, hydrophilic linker typically comprised of (but not limited to) serine, glycine, alanine, and/or threonine residues, which is followed by the VH domain of each scFv unit, which is followed by a glycine/serine rich linker, followed by a VL domain. These tandem VH and VL domains associate to form a single chain fragment variable (scFv) appended at the c-terminus of the Fc. Two such units exist at the c-terminus of this molecule owing to the homodimeric nature centered at the Fc. The domain order of scFvs may be configured to be from N to C terminus either VH-Linker-VL or VL-Linker-VH.

A non-limiting example of a molecule that has different binding regions on the different ends is where, one end is a PD-1 agonist and the antibody that provides target specificity is an

anti-MAdCAM-1 antibody. This can be illustrated as shown, for example, in FIG. 3A, which illustrates the molecules in different orientations.

In some embodiments, the MAdCAM antibody is a blocking or non-blocking antibody as described elsewhere herein. Without being bound to any theory, MAdCAM has been shown to interact with the headpiece of the integrin α4β7 expressed on lymphocytes via multiple residues within its two Ig superfamily I-set domains and the atomic level structural basis for that interaction has been described (Viney JL et al. (1996). J Immunol. 157, 2488-2497; Yu Y et al (2013). J Biol Chem. 288, 6284-6294; Yu Y et al (2012). J Cell Biol. 196, 131-146, each of which is incorporated by reference in its entirety). It has been shown in great structural, mechanistic and functional detail in both the human (Chen J et al (2003). Nat Struct Biol. 10, 995-1001; de Chateau M et al (2001). Biochemistry. 40, 13972-13979) and mouse (Day ES et al (2002). Cell Commun Adhes. 9, 205-219; Hoshino H et al (2011). J Histochem Cytochem. 59, 572-583) molecular systems that any interaction of MAdCAM with α4β7 is dependent on three dication binding sites present in the integrin beta 7 sub unit I-like domain and that these metal binding sites can coordinate with Ca2+, Mn2+, and Mg2+. Using cell adhesion assays, flow cytometry, and/or flow chamber assays in the presence of high levels of Ca2+ with or without Mg2+ or Mn2+, the MAdCAM/α4β7 interaction is shown to be of a lower functional affinity and permits rolling adhesion of lymphocytes, whereas in low Ca2+ but higher Mg2+ or Mn2+ which activates the integrin, the MAdCAM/α4β7 interaction is of a higher functional affinity and mediates firm lymphocyte adhesion (Chen J et al (2003). *Nat Struct Biol.* **10**, 995-1001). A number of groups have shown that various cell:cell, cell:membrane prep, and/or cell:protein based adhesion/interaction assays can be utilized, with FACS, cell flow chamber based counts, or IHC based read-outs to monitor the impact of anti-MAdCAM or anti-α4β7 antibodies upon the interaction of MAdCAM with α4β7, allowing one to identify blocking or non-blocking antibodies (Nakache, M et al (1989). *Nature*. **337**, 179-181; Streeter, PR et al (1988). Nature. **331**. 41-46; Yang Y et al (1995). *Scand J Immunol.* **42**. 235-247; Leung E et al (2004). *Immunol Cell Biol.* **82**. 400-409; Pullen N et al (2009). *B J Pharmacol.* **157**. 281-293; Soler D et al (2009). *J Pharmacol Exp Ther.* **330**. 864-875; Qi J et al (2012). *J Biol Chem.* **287**. 15749-15759).

This has been exemplified in the mouse system setting with the identification of anti-mouse MAdCAM antibodies such as MECA-89 (non-blocking) and MECA-367 (blocking))Nakache, M et al (1989). *Nature*. **337**, 179-181; Streeter, PR et al (1988). Nature. **331**. 41-46;

Yang Y et al (1995). *Scand J Immunol.* **42**. 235-247). In a human system, antibodies have been identified that block the interaction of human MAdCAM with human α4β7 such as anti-human MAdCAM PF-00547659 (Pullen N et al (2009). *B J Pharmacol.* **157**. 281-293) and anti-human α4β7 vedolizumab (Soler D et al (2009). *J Pharmacol Exp Ther.* **330**. 864-875), as well as antibodies that do not block the interaction such as anti-human MAdCAM clone 17F5 (Soler D et al (2009). *J Pharmacol Exp Ther.* **330**. 864-875), and anti-human α4β7 clone J19 (Qi J et al (2012). *J Biol Chem.* **287**. 15749-15759). Thus, the antibody can either be blocking or non-blocking based upon the desired effect. In some embodiments, the antibody is a non-blocking MAdCAM antibody. In some embodiments, the antibody is a blocking MAdCAM antibody. One non-limiting example of demonstrating whether an antibody is blocking or non-blocking can be found in Example 6, but any method can be used. Each of the references described herein are incorporated by reference in its entirety. In some embodiments, the PD-1 Agonist is replaced with an IL-2 mutein, such as, but not limited to, the ones described herein.

In another example, and as depicted in FIG. 4, the N-terminus of the homodimer contains two identical Fab domains comprised of two identical light chains, which are separate polypeptides, interfaced with the n-terminal VH-CH1 domains of each heavy chain via the VH/VL interaction and Ckappa or Clambda interaction with CH1. The native disulphide bond between the Ckappa or Clambda with CH1 is present providing a covalent anchor between the light and heavy chains. At the c-terminus of this design are two identical VH units (though non-antibody moieties could also be substituted here or at any of the four terminal attachment/fusion points) where by (in this example) the c-terminus of the CH3 domain of the Fc, is followed by a flexible, hydrophilic linker typically comprised of (but not limited to) serine, glycine, alanine, and/or threonine residues, which is followed by a soluble independent VH3 germline family based VH domain. Two such units exist at the c-terminus of this molecule owing to the homodimeric nature centered at the Fc.

In another non-limiting example, as depicted in FIG. 5, the N-terminus of the homodimer contains two identical Fab domains comprised of two identical light chains, which, unlike FIG. 3 and FIG. 4, are physically conjoined with the heavy chain at the N-terminus via a linker between the c-terminus of Ckappa or Clambda and the N-terminus of the VH. The linker may be 36-80 amino acids in length and comprised of serine, glycine, alanine and threonine residues. The physically conjoined n-terminal light chains interface with the n-terminal VH-CH1 domains of

each heavy chain via the VH/VL interaction and Ckappa or Clambda interaction with CH1. The native disulphide bond between the Ckappa or Clambda with CH1 is present providing additional stability between the light and heavy chains. At the c-terminus of this design are two identical Fab units where by (in this example) the c-terminus of the CH3 domain of the Fc, is followed by a flexible, hydrophilic linker typically comprised of (but not limited to) serine, glycine, alanine, and/or threonine residues, which is followed by a CH1 domain, followed by a VH domain at the c-terminus. The light chain that is designed to pair with the c-terminal CH1/VH domains is expressed as a separate polypeptide, unlike the N-terminal light chain which is conjoined to the n-terminal VH/CH1 domains as described. The C–terminal light chains form an interface at between VH/VL and Ckappa or Clambda with CH1. The native disulphide anchors this light chain to the heavy chain. Again, any of the antibody moieties at any of the four attachment/fusion points can be substituted with a non-antibody moiety, e.g., a effector binding/modulating moiety that does not comprise an antibody molecule.

The bispecific antibodies can also be asymmetric as shown in the following non-limiting examples. Non-limiting example are also depicted in FIG. 6, FIG. 7, and FIG. 8, which illustrate an asymmetric/heterodimer approach. Again, in any of these formats, any of the antibody moieties at any of the four attachment/fusion points can be substituted with a non-antibody moiety, e.g., a effector binding/modulating moiety that does not comprise an antibody molecule. In some embodiments, the dimerization interface centers around the human IgG1 CH2-CH3 domains, which dimerize via a contact interface spanning both CH2/CH2 and CH3/CH3. However, in order to achieve heterodimerization instead of homodimerization of each heavy chain, mutations are introduced in each CH3 domain. The heterodimerizing mutations include T366W mutation (kabat) in one CH3 domain and T366S, L368A, and Y407V (kabat) mutations in the other CH3 domain. The heterodimerizing interface may be further stabilized with de novo disulphide bonds via mutation of native residues to cysteine residues such as S354 and Y349 on opposite sides of the CH3/CH3 interface.. The resulting bispecific antibodies shown have a total valence comprised of four binding units. With this approach, the overall molecule can be designed to have bispecificity at just one terminus and monospecificity at the other terminus (trispecificity overall) or bispecificity at either terminus with an overall molecular specificity of 2 or 4. In the illustrative examples below, the C-terminus comprises two identical binding domains which could, for example, provide bivalent monospecificity for a tissue tethering target.

At the N-terminus of all three of the illustrative examples, both binding domains comprise different recognition elements/paratopes and which could achieve recognition of two different epitopes on the same effector moiety target, or could recognize for examples a T cell inhibitory receptor and CD3. In some embodiments, the N–terminal binding moieties may be interchanged with other single polypeptide formats such as scFv, single chain Fab, tandem scFv, VH or VHH domain antibody configurations for example. Other types of recognition element may be used also, such as linear or cyclic peptides.

An example of an asymmetric molecule is depicted in FIG. 6. Referring to FIG. 6, the N-terminus of the molecule is comprised of a first light chain paired with a first heavy chain via VH/VL and Ckappa or Clambda / CH1 interactions and a covalent tether comprised of the native heavy/light chain disulphide bond. On the opposite side of this heterodimeric molecule at the N-terminus is a second light chain and a second heavy chain which are physically conjoined via a linker between the c-terminus of Ckappa or Clambda and the N-terminus of the VH. The linker may be 36-80 amino acids in length and comprised of serine, glycine, alanine and threonine residues. The physically conjoined n-terminal light chains interface with the n-terminal VH-CH1 domains of each heavy chain via the VH/VL interaction and Ckappa or Clambda interaction with CH1. The native disulphide bond between the Ckappa or Clambda with CH1 is present providing additional stability between the light and heavy chains. At the c-terminus of the molecule are two identical soluble VH3 germline family VH domains joined via an N-terminal glycine/serine/alanine/threonine based linker to the c-terminus of the CH3 domain of both heavy chain 1 and heavy chain 2.

In some embodiments, an asymmetric molecule can be as illustrated as depicted in FIG. 7. For example, the N-terminus of the molecule is comprised of two different VH3 germlined based soluble VH domains linked to the human IgG1 hinge region via a glycine/serine/alanine/threonine based linker. The VH domain connected to the first heavy chain is different to the VH domain connected to the second heavy chain. At the c-terminus of each heavy chain is an additional soluble VH3 germline based VH domain, which is identical on each of the two heavy chains. The heavy chain heterodimerizes via the previously described knobs into holes mutations present at the CH3 interface of the Fc module.

In some embodiments, an asymmetric molecule can be as illustrated in FIG. 8. This example is similar to the molecule shown in FIG. 7, except both N-terminal Fab units are

configured in a way that light chain 1 and light chain 2 are physically conjoined with heavy chain 1 and heavy chain 2 via a linker between the c-terminus of Ckappa or Clambda and the N-terminus of each respective VH. The linker in each case may be 36-80 amino acids in length and comprised of serine, glycine, alanine and threonine residues. The physically conjoined n-terminal light chains interface with the n-terminal VH-CH1 domains of each heavy chain via the VH/VL interaction and Ckappa or Clambda interaction with CH1. The native disulphide bond between the Ckappa or Clambda with CH1 is present providing additional stability between the light and heavy chains.

Bi-specific molecules can also have a mixed format. This is illustrated, for example, in FIG. 9, FIG. 10, and FIG. 11.

For example, as illustrated in FIG. 9, illustrates a homodimer Fc based approach (see FIGS. 3, 4, and 5), combined with the moiety format selection of FIG. 7, whereby the total molecular valency is four, but specificity is restricted to two specificities. The N-terminus is comprised of two identical soluble VH3 germline based VH domains and the c-terminus is comprised of two identical soluble VH3 germlined based VH domains of different specificity to the N-terminal domains. Therefore, each specificity has a valence of two. Again, in this format, any of the antibody moieties at any of the four attachment/fusion points can be substituted with a non-antibody moiety, e.g., an effector binding/modulating moiety that does not comprise an antibody molecule.

FIG. 10 illustrates another example. In this example, the molecule is comprised of four VH3 germline based soluble VH domains. The first two domains have the same specificity (for example an inhibitory receptor), the 3rd domain from the N-terminus may have specificity for a tissue antigen and the fourth domain from the N-terminus may have specificity for human serum albumin (HSA), thereby granting the molecule extended half-life in the absence of an Ig Fc domain. Three glycine, serine, alanine and/or threonine rich linkers exists between domains 1 and 2, domains 2 and 3, and domains 3 and 4. This format may be configured with up to tetraspecificity, but monovalent in each case, or to have bispecificity with bivalency in each case. The order of domains can be changed. Again, in this format, any of the antibody moieties can be substituted with a non-antibody moiety, e.g., a effector binding/modulating moiety that does not comprise an antibody molecule.

FIG. 11 illustrates yet another approach. This example is similar to FIGS. 3 and 4, in that it is Fc homodimer based with two identical Fab units (bivalent monospecificity) at the N-terminus of the molecule. This example differs in that the C-terminus of each heavy chain is appended with a tandem-scFv. Thus, in each case the c-terminus of the CH3 domain of the Fc is linked via a glycine/serine/alanine/threonine based linker to the N-terminus of a first VH domain, which is linked via the C-terminus by a 12-15 amino acid glycine/serine rich linker to the N-terminus of a first VL domain, which linked via a 25-35 amino acid glycine/serine/alanine/threonine based linker at the c-terminus to the N-terminus of a second VH domain, which is linked via the c-terminus with a 12-15 amino acid glycine/serine based linker to the N-terminus of a 2nd VL domain. In this Fc homodimer based molecule there are therefore two identical tandem scFvs at the c-terminus of the molecule offering either tetravalency for a single tissue antigen for example or bivalency to two different molecules. This format could also be adapted with a heterodimer Fc core allowing two different tandem-scFvs at the c-terminus of the Fc allowing for monovalent tetraspecificity at the c-terminus while retaining either bivalent monospecificity at the N-terminus or monovalent bispecificity at the N-terminal via usage of single chain Fab configurations as in FIGS. 5, 6, and 7. This molecule can therefore be configured to have 2, 3, 4, 5, or 6 specificities. The domain order of scFvs within the tandem—scFv units may be configured to be from N to C terminus either VH-Linker-VL or VL-Linker-VH. Again, in this format, any of the antibody moieties at any of the four attachment/fusion points can be substituted with a non-antibody moiety, e.g., a effector binding/modulating moiety that does not comprise an antibody molecule.

Bi-specific antibodies can also be constructed to have, for example, shorter systemic PK while having increased tissue penetration. These types of antibodies can be based upon, for example, a human VH3 based domain antibody format. These are illustrated, for example, in FIGS. 12, 13, and 14. FIGS. 12, 13, and 14 each comprised a soluble VH3 germline family based VH domain modules. Each domain is approximately 12.5 kDa allowing for a small overall MW, which, without being bound to any particular theory, should be beneficial for enhanced tissue penetration. In these examples, none of the VH domains recognize any half-life extending targets such as FcRn or HSA. As illustrated in FIG. 12, the molecule is comprised of two VH domains joined with a flexible hydrophilic glycine/serine based linker between the C-terminus of the first domain and N-terminus of the second domain. In this example one domain may

recognize a T cell co-stimulatory receptor and the second may recognize a tissue tethering antigen. As illustrated in FIG. 13, the molecule is comprised of three VH domains with N-C terminal linkages of hydrophilic glycine/serine based linkers. The molecule may be configured to be trispecific but monovalent for each target. It may be bispecific with bivalency for one target and monovalency for another. As illustrated in FIG. 14, the molecule is comprised of four VH domains with N-C terminal Glycine/Serine rich linkers between each domain. This molecule may be configured to be tetraspecific, trispecific, or bispecific with varying antigenic valencies in each case. Again, in this format, any of the antibody moieties at can be substituted with a non-antibody moiety, e.g., a effector binding/modulating moiety that does not comprise an antibody molecule.

Other embodiments of bi-specific antibodies are illustrated in FIGs. 15 and 16. FIGs. 15 and 16 are comprised of the naturally heterodimerizing core of the human IgG CH1/Ckappa interface, including the c-terminal heavy/light disulphide bond which covalently anchors the interaction. This format does not contain an Fc or any moieties for half life extension. As illustrated in FIG. 15, the molecule, at the N-terminus of the constant kappa domain is appended with an scFv fragment consisting of an N-terminal VH domain, linked at its C-terminus to the N-terminus of a VL domain via a 12-15 amino acid gly/ser based linker, which is linked by its C-terminus to the N-terminus of the constant kappa domain via the native VL-Ckappa elbow sequence. The CH1 domain is appended at the N-terminus with an scFv fragment consisting of an N-terminal VL domain linked at its c-terminus via a 12-15 amino acid gly/ser linker to the N-terminus of a VH domain, which is linked at its c-terminus to the N-terminus of the CH1 domains via the natural VH-CH1 elbow sequence. As illustrated in FIG. 16, the molecule has the same N-terminal configuration to Example 13. However the C-terminus of the constant kappa and CH1 domains are appended with scFv modules which may be in either the VH-VL or VL-VH configuration and may be either specific for the same antigen or specific for two different antigens. The VH/VL inter-domain linkers may be 12-15 amino acids in length and consisting of gly/ser residues. The scFv binding sub-units may be swapped for soluble VH domains, or peptide recognition elements, or even tandem-scFv elements. This approach can also be configured to use variable lambda and/or constant lambda domains. Again, in this format, any of the antibody moieties at any of the attachment/fusion points can be substituted with a non-

antibody moiety, e.g., a effector binding/modulating moiety that does not comprise an antibody molecule.

FIG. 17 illustrates another embodiment. FIG. 17 represents a tandem scFv format consisting of a first N-terminal VL domain linked at its C-terminus to the N-terminus of a first VH domain with a 12-15 amino acid gly/ser rich linker, followed at the first VH c-terminus by a 25-30 amino acid gly/ser/ala/thr based linker to the N-terminus of a second VL domain. The second VL domain is linked at the C-terminus to the N-terminus of a 2nd VH domain by a 12-15 amino acid gly/ser linker. Each scFv recognizes a different target antigen such as a co-stimulatory T cell molecule and a tissue tethering target. Again, in this format, any of the antibody moieties can be substituted with a non-antibody moiety, e.g., a effector binding/modulating moiety that does not comprise an antibody molecule.

FIG. 18 illustrates another embodiment. FIG. 18 is a F(ab')2 scFv fusion. This consists of two identical Fab components joined via two disulphide bonds in the native human IgG1 hinge region c-terminal of the human IgG CH1 domain. The human IgG1 CH2 and CH3 domains are absent. At the c-terminus of heavy chains 1 and 2 are two identical scFv fragments linked via a gly/ser/ala/thr rich linker to the c-terminus of the huIgG1 hinge region. In the configuration shown, the VH is N-terminal in each scFv unit and linked bia a 12-15 amino acid gly/ser rich linker to the N-terminus of a VL domain. An alternative configuration would be N-term- VL-Linker-VH-C-term. In this design, the construct is bispecific with bivalency for reach target. Again, in this format, any of the antibody moieties at any of the four attachment/fusion points can be substituted with a non-antibody moiety, e.g., a effector binding/modulating moiety that does not comprise an antibody molecule.

CD39 molecule, as that term as used herein, refers to a polypeptide having sufficient CD39 sequence that, as part of a therapeutic compound, it phosphohydrolyzes ATP to AMP. In some embodiments, a CD39 molecule phosphohydrolizes ATP to AMP equivalent to, or at least, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95% of the rate of a naturally occurring CD39, e.g., the CD39 from which the CD39 molecule was derived. In some embodiments, a CD39 molecule has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity ,or substantial sequence identity, with a naturally occurring CD39.

Any functional isoform can be used (with CD39 or other proteins discussed herein). Exemplary CD39 sequence include Genbank accession # NP_001767.3 or a mature form from the following sequence:

MEDTKESNVKTFCSKNILAILGFSSIIAVIALLAVGLTQNKALPENVKYGIVLDAGSSHT
SLYIYKWPAEKENDTGVVHQVEECRVKGPGISKFVQKVNEIGIYLTDCMERAREVIPRS
QHQETPVYLGATAGMRLLRMESEELADRVLDVVERSLSNYPFDFQGARIITGQEEGAYG
WITINYLLGKFSQKTRWFSIVPYETNNQETFGALDLGGASTQVTFVPQNQTIESPDNALQ
FRLYGKDYNVYTHSFLCYGKDQALWQKLAKDIQVASNEILRDPCFHPGYKKVVNVSDL
YKTPCTKRFEMTLPFQQFEIQGIGNYQQCHQSILELFNTSYCPYSQCAFNGIFLPPLQGDF
GAFSAFYFVMKFLNLTSEKVSQEKVTEMMKKFCAQPWEEIKTSYAGVKEKYLSEYCFS
GTYILSLLLQGYHFTADSWEHIHFIGKIQGSDAGWTLGYMLNLTNMIPAEQPLSTPLSHS
TYVFLMVLFSLVLFTVAIIGLLIFHKPSYFWKDMV (SEQ ID NO: 1).

In some embodiments, a CD39 molecule comprises a soluble catalytically active form of CD39 found to circulate in human or murine serum, see, e.g., Metabolism of circulating ADP in the bloodstream is mediated via integrated actions of soluble adenylate kinase-1 and NTPDase1/CD39 activities, Yegutkin et al. FASEB J. 2012 Sep; 26(9):3875-83. A soluble recombinant CD39 fragment is also described in Inhibition of platelet function by recombinant soluble ecto-ADPase/CD39, Gayle, et al., J Clin Invest. 1998 May 1; 101(9): 1851–1859.

CD73 molecule, as that term as used herein, refers to a polypeptide having sufficient CD73 sequence that, as part of a therapeutic compound, it dephosphorylates extracellular AMP to adenosine. In some embodiments, a CD73 molecule dephosphorylates extracellular AMP to adenosine equivalent to, or at least, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95% of the rate of a naturally occurring CD73, e.g., the CD73 from which the CD73 molecule was derived. In some embodiments, a CD73 molecule has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity, or substantial sequence identity, with a naturally occurring CD73. Exemplary CD73 sequences include GenBank AAH65937.1 5'-nucleotidase, ecto (CD73) [Homo sapiens] or a mature form from the following sequence,

MCPRAARAPATLLLALGAVLWPAAGAWELTILHTNDVHSRLEQTSEDSSKCVNASRCM
GGVARLFTKVQQIRRAEPNVLLLDAGDQYQGTIWFTVYKGAEVAHFMNALRYDAMAL
GNHEFDNGVEGLIEPLLKEAKFPILSANIKAKGPLASQISGLYLPYKVLPVGDEVVGIVGY
TSKETPFLSNPGTNLVFEDEITALQPEVDKLKTLNVNKIIALGHSGFEMDKLIAQKVRGV

DVVVGGHSNTFLYTGNPPSKEVPAGKYPFIVTSDDGRKVPVVQAYAFGKYLGYLKIEFD
ERGNVISSHGNPILLNSSIPEDPSIKADINKWRIKLDNYSTQELGKTIVYLDGSSQSCRFRE
CNMGNLICDAMINNNLRHADETFWNHVSMCILNGGGIRSPIDERNNGTITWENLAAVLP
FGGTFDLVQLKGSTLKKAFEHSVHRYGQSTGEFLQVGGIHVVYDLSRKPGDRVVKLDV
LCTKCRVPSYDPLKMDEVYKVILPNFLANGGDGFQMIKDELLRHDSGDQDINVVSTYIS
KMKVIYPAVEGRIKFSTGSHCHGSFSLIFLSLWAVIFVLYQ (SEQ ID NO: 2).

In some embodiments, a CD73 molecule comprises a soluble form of CD73 which can be shed from the membrane of endothelial cells by proteolytic cleavage or hydrolysis of the GPI anchor by shear stress see, e.g., Reference: Yegutkin G, Bodin P, Burnstock G. Effect of shear stress on the release of soluble ecto-enzymes ATPase and 5'-nucleotidase along with endogenous ATP from vascular endothelial cells. Br J Pharmacol 2000; 129: 921–6. For CD73 fucntion see Colgan et al., Physiological roles for ecto-5'-nucleotidase (CD73), Purinergic Signalling, June 2006, 2:351.

Cell surface molecule binder, as that term is used herein, refers to a molecule, typically a polypeptide, that binds, e.g., specifically, to a cell surface molecule on a cell, e.g., an immunosuppressive immune cell, e.g., a Treg. In some embodiments, the cell surface binder has sufficient sequence from a naturally occurring ligand of the cell surface molecule, that it can specifically bind the cell surface molecule (a cell surface molecule ligand). In some embodiments, the cell surface binding is an antibody molecule that binds, e.g., specifically binds, the cell surface molecule.

Donor specific targeting moiety, as that term is used herein, refers to a moiety, e.g., an antibody molecule, that as a component of a therapeutic compound, localizes the therapeutic compound preferentially to an implanted donor tissue, as opposed to tissue of a recipient. As a component of a therapeutic compound, the donor specific targeting moiety provides site-specific immune privilege for a transplant tissue, e.g., an organ, from a donor.

In some embodiments, a donor specific targeting moiety it binds to the product, e.g., a polypeptide product, of an allele present at a locus, which allele is not present at the locus in the (recipient) subject. In some embodiments, a donor specific targeting moiety binds to an epitope on product, which epitope is not present in the (recipient) subject.

In some embodiments, a donor specific targeting moiety, as a component of a therapeutic compound, preferentially binds to a donor target or antigen, e.g., has a binding affinity for the

donor target that is greater for donor antigen or tissue, e.g., at least 2, 4, 5, 10, 50, 100, 500, 1,000, 5,000, or 10,000 fold greater, than its affinity for than for subject antigen or tissue. In some embodiments, a donor specific targeting moiety, has a binding affinity for a product of an allele of a locus present in donor tissue (but not present in the subject) at least 2, 4, 5, 10, 50, 100, 500, 1,000, 5,000, or 10,000 fold greater, than its affinity for the product of the allele of the locus present in the subject (which allele is not present in donor tissue). Affinity of a therapeutic compound of which the donor specific moiety is a component, can be measured in a cell suspension, e.g., the affinity for suspended cells having the allele is compared with its affinity for suspended cells not having the allele. In some embodiments, the binding affinity for the donor allele cells is below 10nM. In some embodiments, the binding affinity for the donor allele cells is below 100 pM, 50 pM, or 10 pM.

In some embodiments, the specificity for a product of a donor allele is sufficient that when the donor specific targeting moiety is coupled to an immune-down regulating effector: i) immune attack of the implanted tissue, e.g., as measured by histological inflammatory response, infiltrating T effector cells, or organ function, in the clinical setting – e.g. creatinine for the kidney, is substantially reduced, e.g., as compared to what would be seen in an otherwise similar implant but lacking the donor specific targeting moiety is coupled to an immune-down regulating effector; and/or ii) immune function in the recipient, outside or away from the implanted tissue, is substantially maintained. In some embodiments, one or more of the following is seen: at therapeutic levels of therapeutic compound, peripheral blood lymphocyte counts are not substantially impacted, e.g., the level of T cells is within 25, 50, 75, 85, 90, or 95 % of normal, the level of B cells is within 25, 50, 75, 85, 90, or 95 % of normal, and/or the level of granuloctyes (PMNs) cells is within 25, 50, 75, 85, 90, or 95 % of normal, or the level of monocytes is within 25, 50, 75, 85, 90, or 95 % of normal; at therapeutic levels of therapeutic compound, the ex vivo proliferative function of PBMCs (peripheral blood mononuclear cells) against non-disease relevant antigens is substantially normal or is within 70, 80, or 90% of normal; at therapeutic levels of therapeutic compound, the incidence or risk of risk of opportunistic infections and cancers associated with immunosuppression is not substantially increased over normal; or at therapeutic levels of therapeutic compound, the incidence or risk of risk of opportunistic infections and cancers associated with immunosuppression is substantially less than would be seen with standard of care, or non-targeted, immunosuppression. In some

embodiments, the donor specific targeting moiety comprises an antibody molecule, a target specific binding polypeptide, or a target ligand binding molecule.

Effector, as that term is used herein, refers to an entity, e.g., a cell or molecule, e.g., a soluble or cell surface molecule, which mediates an immune response.

Effector ligand binding molecule, as used herein, refers to a polypeptide that has sufficient sequence from a naturally occurring counter-ligand of an effector, that it can bind the effector with sufficient specificity that it can serve as an effector binding/modulating molecule. In some embodiments, it binds to effector with at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95% of the affinity of the naturally occurring counter-ligand. In some embodiments, it has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity ,or substantial sequence identity, with a naturally occurring counter-ligand for the effector.

Effector specific binding polypeptide, as used herein, refers to a polypeptide that can bind with sufficient specificity that it can serve as an effector binding/modulating moiety. In some embodiments, a specific binding polypeptide comprises a effector ligand binding molecule.

Elevated risk, as used herein, refers to the risk of a disorder in a subject, wherein the subject has one or more of a medical history of the disorder or a symptom of the disorder, a biomarker associated with the disorder or a symptom of the disorder, or a family history of the disorder or a symptom of the disorder.

Functional antibody molecule to an effector or inhibitory immune checkpoint molecule, as that term is used herein, refers to an antibody molecule that when present as the ICIM binding/modulating moiety of a multimerized therapeutic compound, can bind and agonize the effector or inhibitory immune checkpoint molecule. In some embodiments, the anti-effector or inhibitory immune checkpoint molecule antibody molecule, when binding as a monomer (or binding when the therapeutic compound is not multimerized), to the effector or inhibitory immune checkpoint molecule, does not antagonize, substantially antagonize, prevent binding, or prevent substantial binding, of an endogenous counter ligand of the inhibitory immune checkpoint molecule molecule to inhibitory immune checkpoint molecule. In some embodiments, the anti- effector or inhibitory immune checkpoint molecule antibody molecule when binding as a monomer (or binding when the therapeutic compound is not multimerized), to the inhibitory immune checkpoint molecule, does not agonize or substantially agonize, the effector or inhibitory molecule.

ICIM binding/modulating moiety, as that term is used herein, refers to an effector binding/modulating moiety that, as part of a therapeutic compound, binds and agonizes a cell surface inhibitory molecule, e.g., an inhibitory immune checkpoint molecule, e.g., PD-1, , or binds or modulates cell signaling, e.g., binds a FCRL, e.g., FCRL1-6, or binds and antagonizes a molecule that promotes immune function.

IIC binding/modulating moiety, as that term is used herein, refers to an effector binding/modulating moiety that, as part of a therapeutic compound, binds an immunosuppressive immune cell. In some embodiments, the IIC binding/modulating moiety increases the number or concentration of an immunosuppressive immune cell at the binding site.

ICSM binding/modulating moiety, as that term is used herein, refers to an effector binding/modulating moiety that antagonizes an immune stimulatory effect of a stimulatory, e.g., co-stimulatory, binding pair. A stimulatory or co-stimulatory binding pair, as that term is used herein, comprises two members, 1) a molecule on the surface of an immune cell; and 2) the binding partner for that cell molecule, which may be an additional immune cell, or a non-immune cell. Ordinarily, upon binding of one member to the other, assuming other requirements are met, the member on the immune cell surfaces stimulates the immune cell, e.g., a costimulatory molecule, and an immune response is promoted. In situations where the costimulatory molecule and the costimulatory molecule counterstructure are both expressed on immune cells, bi-directional activation of both cells may occur. In an embodiment an ICSM binding/modulating moiety binds and antagonizes the immune cell expressed member of a binding pair. For example, it binds and antagonizes OX40. In another embodiment, an ICSM binding/modulating moiety binds and antagonizes the member of the binding pair that itself binds the immune cell expressed member, e.g., it binds and antagonizes OX40L. In either case, inhibition of stimulation or co-stimulation of an immune cell is achieved. In an embodiment the ICSM binding/modulating moiety decreases the number or the activity of an immunostimulating immune cell at the binding site.

IL-2 mutein molecule, as that term is used herein, refers to an IL2 variant that binds with high affinity to the CD25 (IL-2R alpha chain) and with low affinity to the other IL-2R sigalling components CD122 (IL-2R beta) and CD132 (IL-2R gamma). Such an IL-2 mutein molecule preferentially activates Treg cells. In embodiments, either alone, or as a component of a therapeutic compound, an IL-2 mutein activates Tregs at least 2, 5, 10, or 100 fold more than

cytotoxic or effector T cells. Exemplary IL-2 mutein molecules are described in WO2010085495, WO2016/164937, US2014/0286898A1, WO2014153111A2, WO2010/085495, cytotoxic WO2016014428A2, WO2016025385A1, and US20060269515. Muteins disclosed in these references that include additional domains, e.g., an Fc domain, or other domain for extension of half life can be used in the therapeutic compounds and methods described herein without such additional domains. In another embodiment an IIC binding/modulating moiety comprises an IL-2 mutein, or active fragment thereof, coupled, e.g., fused, to another polypeptide, e.g., a polypeptide that extends in vivo half life, e.g., an immunoglobulin constant region, or a multimer or dimer thereof, e.g., AMG 592. In an embodiment the therapeutic compound comprises the IL-2 portion of AMG 592. In an embodiment the therapeutic compound comprises the IL-2 portion but not the immunoglobulin portion of AMG 592. In some embodiments, the mutein does not comprise a Fc region. For some IL-2 muteins, the muteins are engineered to contain a Fc region because such region has been shown to increase the half-life of the mutein. In some embodiments, the extended half-life is not necessary for the methods described and embodied herein. In some embodiments, the Fc region that is fused with the IL-2 mutein comprises a N297 mutations, such as, but not limited to, N297A. In some embodiments, the Fc region that is fused with the IL-2 mutein does not comprise a N297 mutation, such as, but not limited to, N297A.

An "inhibitory immune checkpoint molecule ligand molecule," as that term is used herein, refers to a polypeptide having sufficient inhibitory immune checkpoint molecule ligand sequence, e.g., in the case of a PD-L1 molecule, sufficient PD-L1 sequence, that when present as an ICIM binding/modulating moiety of a multimerized therapeutic compound, can bind and agonize its cognate inhibitory immune checkpoint molecule, e.g., again in the case of a PD-L1 molecule, PD-1.

In some embodiments, the inhibitory immune checkpoint molecule ligand molecule, e.g., a PD-L1 molecule, when binding as a monomer (or binding when the therapeutic compound is not multimerized), to its cognate ligand, e.g., PD-1, does not antagonize or substantially antagonize, or prevent binding, or prevent substantial binding, of an endogenous inhibitory immune checkpoint molecule ligand to the inhibitory immune checkpoint molecule. E.g., in the case of a PD-L1 molecule, the PD-L1 molecule does not antagonize binding of endogenous PD-L1 to PD-1.

In some embodiments, the inhibitory immune checkpoint molecule ligand when binding as a monomer, to its cognate inhibitory immune checkpoint molecule does not agonize or substantially agonize the inhibitory immune checkpoint molecule. By way of example, e.g., a PD-L1 molecule when binding to PD-1, does not agonize or substantially agonize PD-1.

In some embodiments, an inhibitory immune checkpoint molecule ligand molecule has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity ,or substantial sequence identity, with a naturally occurring inhibitory immune checkpoint molecule ligand.

Exemplary inhibitory immune checkpoint molecule ligand molecules include: a PD-L1 molecule, which binds to inhibitory immune checkpoint molecule PD-1, and in embodiments has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity ,or substantial sequence identity, with a naturally occurring PD-L1, e.g., the PD-L1 molecule comprising the sequence of MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWE MEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMI SYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVL SGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNE RTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET (SEQ ID NO: 3), or an active fragment thereof; in some embodiments, the active fragment comprises residues 19 to 290 of the PD-L1 sequence; ; a HLA-G molecule, which binds to any of inhibitory immune checkpoint molecules KIR2DL4, LILRB1, and LILRB2, and in embodiments has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity, or substantial sequence identity, with a naturally occurring HLA-G. Exemplary HLA-G sequences include, e.g., a mature form found in the sequence at GenBank P17693.1 RecName: Full=HLA class I histocompatibility antigen, alpha chain G; AltName: Full=HLA G antigen; AltName: Full=MHC class I antigen G; Flags: Precursor, or in the sequence MVVMAPRTLFLLLSGALTLTETWAGSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFV RFDSDSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEAS SHTLQWMIGCDLGSDGRLLRGYEQYAYDGKDYLALNEDLRSWTAADTAAQISKRKCE AANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRADPPKTHVTHHPVFDYEATLRCW ALGFYPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQ HEGLPEPLMLRWKQSSLPTIPIMGIVA (SEQ ID NO: 4).

Inhibitory molecule counter ligand molecule, as that term is used herein, refers to a polypeptide having sufficient inhibitory molecule counter ligand sequence such that when present as the ICIM binding/modulating moiety of a multimerized therapeutic compound, can bind and agonize a cognate inhibitory molecule. In some embodiments, the inhibitory molecule counter ligand molecule, when binding as a monomer (or binding when the therapeutic compound is not multimerized), to the inhibitory molecule, does not antagonize, substantially antagonize, prevent binding, or prevent substantial binding, of an endogenous counter ligand of the inhibitory molecule to the inhibitory molecule. In some embodiments, the inhibitory molecule counter ligand molecule when binding as a monomer (or binding when the therapeutic compound is not multimerized), to the inhibitory molecule, does not agonize or substantially agonize, the inhibitory molecule.

Sequence identity, percentage identity, and related terms, as those terms are used herein, refer to the relatedness of two sequences, e.g., two nucleic acid sequences or two amino acid or polypeptide sequences. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain having at least about 85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, e.g., a sequence provided herein.

In the context of nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, e.g., a sequence provided herein.

The term “functional variant” refers to polypeptides that have a substantially identical amino acid sequence to the naturally-occurring sequence, or are encoded by a substantially

identical nucleotide sequence, and are capable of having one or more activities of the naturally-occurring sequence.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology").

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) J. Mol. Biol. 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller ((1989) CABIOS, 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to for example any a nucleic acid sequence provided herein. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules provided herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

As used herein, the term “hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions” describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, which is incorporated by reference. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified.

It is understood that the molecules and compounds of the present embodiments may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on their functions.

The term "amino acid" is intended to embrace all molecules, whether natural or synthetic, which include both an amino functionality and an acid functionality and capable of being included in a polymer of naturally-occurring amino acids. Exemplary amino acids include naturally-occurring amino acids; analogs, derivatives and congeners thereof; amino acid analogs having variant side chains; and all stereoisomers of any of any of the foregoing. As used herein the term "amino acid" includes both the D- or L- optical isomers and peptidomimetics.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). CD39 molecule, a CD73 molecule, a Cell surface molecule binder, Donor specific targeting moiety Effector ligand binding molecule, ICIM binding/modulating moiety IIC binding/modulating moiety, an inhibitory immune checkpoint molecule ligand molecule, Inhibitory molecule counter ligand molecule, SM binding/modulating moiety, or ICSM binding/modulating moiety.

SM binding/modulating moiety, as that term is used herein, refers to an effector binding/modulating moiety that, as part of a therapeutic compound, promotes an immuno-suppressive local microenvironment, e.g., by providing in the proximity of the target, a substance that inhibits or minimizes attack by the immune system of the target. In some embodiments, the SM binding/modulating moiety comprises, or binds, a molecule that inhibits or minimizes attack by the immune system of the target. In some embodiments, a therapeutic compound comprises an SM binding/modulating moiety that binds and accumulates a soluble substance, e.g., an endogenous or exogenous substance, having immunosuppressive function. In some embodiments, a therapeutic compound comprises an SM binding/modulating moiety that binds and inhibits, sequesters, degrades or otherwise neutralizes a substance, e.g., a soluble substance,

typically and endogenous soluble substance, that promotes immune attack. In some embodiments, a therapeutic compound comprises an SM binding/modulating moiety that comprises an immune-suppressive substance, e.g. a fragment of protein known to be immunosuppressive. By way of example, an effector molecule binding moiety that binds, or comprises, a substance e.g., a CD39 molecule or a CD73 molecule, that depletes a component, that promotes immune effector cell function, e.g., ATP or AMP.

Specific targeting moiety, as that term is used herein, refers to donor specific targeting moiety or a tissue specific targeting moiety.

Subject, as that term is used herein, refers to a mammalian subject, e.g., a human subject. In some embodiments, the subject is a non-human mammal, e.g., a horse, dog, cat, cow, goat, or pig.

Target ligand binding molecule, as used herein, refers to a polypeptide that has sufficient sequence from a naturally occurring counter-ligand of a target ligand that it can bind the target ligand on a target tissue (e.g., donor tissue or subject target tissue) with sufficient specificity that it can serve as a specific targeting moiety. In some embodiments, it binds to target tissue or cells with at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95% of the affinity of the naturally occurring counter-ligand. In some embodiments, it has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity ,or substantial sequence identity, with a naturally occurring counter-ligand for the target ligand.

Target site, as that term is used herein, refers to a site which contains the entity, e.g., epitope, bound by a targeting moiety. In some embodiments, the target site is the site at which immune privilege is established.

Tissue specific targeting moiety, as that term is used herein, refers to a moiety, e.g., an antibody molecule, that as a component of a therapeutic molecule, localizes the therapeutic molecule preferentially to a target tissue, as opposed to other tissue of a subject. As a component of a therapeutic compound, the tissue specific targeting moiety provides site-specific immune privilege for a target tissue, e.g., an organ or tissue undergoing or at risk for autoimmune attack. In some embodiments, a tissue specific targeting moiety binds to a product, e.g., a polypeptide product, which is not present outside the target tissue, or is present at sufficiently low levels that, at therapeutic concentrations of therapeutic molecule, unacceptable levels of immune suppression are absent or substantially absent. In some embodiments, a tissue specific targeting

moiety binds to an epitope, which epitope is not present outside, or not substantially present outside, the target site.

In some embodiments, a tissue specific targeting moiety, as a component of a therapeutic compound, preferentially binds to a target tissue or target tissue antigen, e.g., has a binding affinity for the target tissue or antigen that is greater for target antigen or tissue, e.g., at least 2, 4, 5, 10, 50, 100, 500, 1,000, 5,000, or 10,000 fold greater, than its affinity for than for non-target tissue or antigen present outside the target tissue. Affinity of a therapeutic compound of which the tissue specific moiety is a component, can be measured in a cell suspension, e.g., the affinity for suspended cells having the target antigen is compared with its affinity for suspended cells not having the target antigen. In some embodiments, the binding affinity for the target antigen bearing cells is below 10nM.

In some embodiments, the binding affinity for the target antigen bearing cells is below 100 pM, 50 pM, or 10 pM. In some embodiments, the specificity for a target antigen is sufficient, that when the tissue specific targeting moiety is coupled to an immune-down regulating effector: i) immune attack of the target tissue, e.g., as measured by histological inflammatory response, infiltrating T effector cells, or organ function, in the clinical setting – e.g. creatinine for kidney, is substantially reduced, e.g., as compared to what would be seen in an otherwise similar implant but lacking the tissue specific targeting moiety is coupled to an immune-down regulating effector; and/or ii) immune function in the recipient, outside or away from the target tissue, is substantially maintained.

In some embodiments, one or more of the following is seen: at therapeutic levels of therapeutic compound, peripheral blood lymphocyte counts are not substantially impacted, , e.g., the level of T cells is within 25, 50, 75, 85, 90, or 95 % of normal, the level of B cells is within 25, 50, 75, 85, 90, or 95 % of normal, and/or the level of granulocytes (PMNs) cells is within 25, 50, 75, 85, 90, or 95 % of normal, or the level of monocytes is within 25, 50, 75, 85, 90, or 95 % of normal l; at therapeutic levels of therapeutic compound, the ex vivo proliferative function of PBMCs (peripheral blood mononuclear cells) against non-disease relevant antigens is substantially normal or is within 70, 80, or 90% of normal; at therapeutic levels of therapeutic compound, the incidence or risk of risk of opportunistic infections and cancers associated with immunosuppression is not substantially increased over normal; or at therapeutic levels of therapeutic compound, the incidence or risk of risk of opportunistic infections and cancers

associated with immunosuppression is substantially less than would be seen with standard of care, or non-targeted, immunosuppression. In some embodiments, the tissue specific targeting moiety comprises an antibody molecule. In some embodiments, the donor specific targeting moiety comprises an antibody molecule, a target specific binding polypeptide, or a target ligand binding molecule. In some embodiments, the tissue specific targeting moiety binds a product, or a site on a product, that is present or expressed exclusively, or substantially exclusively, on target tissue.

ICIM BINDING/MODULATING MOIETIES: EFFECTOR BINDING/MODULATING MOIETIES THAT BIND INHIBITORY RECEPTORS

Methods and compounds described herein provide for a therapeutic compound having an effector binding/modulating moiety comprising an ICIM binding/modulating moiety, that directly binds and activates an inhibitory receptor on the surface of an immune cell, e.g., to reduce or eliminate, or substantially eliminate, the ability of the immune cell to mediate immune attack. Coupling of the ICIM binding/modulating moiety to a targeting entity, promotes site-specific or local down regulation of the immune cell response, e.g., confined substantially to the locations having binding sites for the targeting moiety. Thus, normal systemic immune function is substantially retained. In some embodiments, an ICIM binding/modulating moiety comprises an inhibitory immune checkpoint molecule counter ligand molecule, e.g., a natural ligand, or fragment of a natural ligand (e.g., PD-L1 or HLA-G) of the inhibitory immune checkpoint molecule. In some embodiments, an ICIM binding/modulating moiety comprises a functional antibody molecule, e.g., a functional antibody molecule comprising an scFv binding domain, that engages inhibitory immune checkpoint molecule.

In some embodiments, the ICIM binding/modulating moiety, comprising, e.g., a functional antibody molecule, or inhibitory immune checkpoint molecule ligand molecule, binds the inhibitory receptor but does not prevent binding of a natural ligand of the inhibitory receptor to the inhibitory receptor. In embodiments a format is used wherein a targeting moiety is coupled, e.g., fused, to an ICIM binding/modulating moiety, comprising, e.g., an scFv domain, and configured so that upon binding of an inhibitory receptor while in solution (e.g., in blood or lymph) (and presumably in a monomeric format), the therapeutic molecule: i) fails to agonize, or fails to substantially agonize (e.g., agonizes at less than 30, 20, 15, 10, or 5% of the level seen with a full agonizing molecule) the inhibitory receptor on the immune cell; and/or ii) fails to

antagonize, or fails to substantially antagonize (e.g., antagonizes at less than 30, 20, 15, 10, or 5% of the level seen with a full antagonizing molecule) the inhibitory receptor on the immune cell. A candidate molecule can be evaluated for its ability to agonize or not agonize by its ability to either increase or decrease the immune response in an in vitro cell based assay wherein the target is not expressed, e.g., using an MLR-based assay (mixed lymphocyte reaction).

In some embodiments, candidate ICIM binding/modulating moieties can reduce, completely or substantially eliminate systemic immunosuppression and systemic immune activation. In some embodiments, the targeting domain of the therapeutic compound, when bound to target, will serve to cluster or multimerize the therapeutic compound on the surface of the tissue desiring immune protection. In some embodiments, the ICIM binding/modulating moiety, e.g., an ICIM binding/modulating moiety comprising a scFv domain, requires a clustered or multimeric state to be able to deliver an agonistic and immunosuppressive signal, or substantial levels of such signal, to local immune cells. This type of therapeutic can, for example, provide to a local immune suppression whilst leaving the systemic immune system unperturbed or substantially unperterbed. That is, the immune suppression is localized to where the suppression is needed as opposed to being systemic and not localized to a particular area or tissue type.

In some embodiments, upon binding to the target e.g., a target organ, tissue or cell type, the therapeutic compound coats the target, e.g., target organ, tissue or cell type. When circulating lymphocytes attempt to engage and destroy the target, this therapeutic will provide an 'off' signal only at, or to a greater extent at, the site of therapeutic compound accumulation.

A candidate therapeutic compound can be evaluated for the ability to bind, e.g., specifically bind, its target, e.g., by ELISA, a cell based assay, or surface plasmon resonance. by. This property should generally be maximized, as it mediates the site-specificity and local nature of the immune privilege. A candidate therapeutic compound can be evaluated for the ability to down regulate an immune cell when bound to target, e.g., by a cell based activity assay. This property should generally be maximized, as it mediates the site-specificity and local nature of the immune privilege. The level of down regulation effected by a candidate therapeutic compound in monomeric (or non-bound) form can be evaluated, e.g., by a cell based activity assay. This property should generally be minimized, as could mediate systemic down regulation of the immune system. The level of antagonism of a cell surface inhibitory molecule, e.g., an

inhibitory immune checkpoint molecule, effected by a candidate therapeutic compound in monomeric (or non-bound) form can be evaluated , e.g.,by , e.g., by a cell based activity assay. This property should generally be minimized, as could mediate systemic unwanted activation of the immune system. Generally, the properties should be selected and balanced to produce a sufficiently robust site specific immune privilege without unacceptable levels of non-site specific agonism or antagonism of the inhibitory immune checkpoint molecule.

EXEMPLARY INHIBITORY IMMUNE CHECKPOINT MOLECULES

Exemplary inhibitory molecules (e.g., an inhibitory immune checkpoint molecule) (together with their counter ligands) can be found in Table 1. This table lists molecules to which exemplary ICIM binding moieties can bind.

Table 1: Cell surface inhibitory molecules, e.g., inhibitory immune checkpoint molecules (column A), counter ligands (column B) and cell types affected (column C).

A	B	C
PD-1	PD-L1, PD-L2	T cells, B cells
Alkaline phosphatase		
B7-H3	Unknown	T cells
B7-H4	Neuropilin 1, neuropilin 2, Plexin4A	T cells
BTLA	HVEM	T cells, B cells
CTLA-4	CD80, CD86	T cells
IDO1	Tryptophan	Lymphocytes
TDO2	Tryptophan	Lymphocytes
KIR2DL1, KIR2DL2/3, KIR3DL1, KIR3DL2	HLA MHC class I	NK cells
LAG3	HLA MHC class II	T cells
TIM-3	Galectin-9	T cells

VISTA	Unknown	T cells, myeloid cells
TIGIT	CD155	T cells
KIR2DL4	HLA-G	NK cells
LILRB1	HLA-G	T cells, NK cells, B cells, monocytes, dendritic cells
LILRB2	HLA-G	Monocytes, dendritic cells, neutrophils, some tumor cells
NKG2A	nonclassical MHC glycoproteins class I	T cells, NK cells
FCRL1-6	FCRL1 – 2 not known FCRL4 = IgA FCRL5 = IgG FCRL6 = MHC Class II	B cells
	BUTYROPHILINS, for example BTN1A1, BTN2A2, BTNL2, BTNL1, BTNL8	Modulation of immune cells

THE PD-L1/PD-1 PATHWAY

Programmed cell death protein 1, (often referred to as PD-1) is a cell surface receptor that belongs to the immunoglobulin superfamily. PD-1 is expressed on T cells and other cell types including, but not limited to, B cells, myeloid cells, dendritic cells, monocytes, T regulatory cells, iNK T cells. PD-1 binds two ligands, PD-L1 and PD-L2, and is an inhibitory immune checkpoint molecule. Engagement with a cognate ligand, PD-L1 or PD-L2, in the context of engagement of antigen loaded MCH with the T Cell Receptor on a T cell minimizes or prevents the activation and function of T cells. The inhibitory effect of PD-1 can include both promoting apoptosis (programmed cell death) in antigen specific T-cells in lymph nodes and reducing apoptosis in regulatory T cells (suppressor T cells).

In some embodiments, a therapeutic compound comprises an ICIM binding/modulating moiety which agonizes PD-1 inhibition. An ICIM binding/modulating moiety can include an inhibitory molecule counter ligand molecule, e.g., comprising a fragment of a ligand of PD-1 (e.g., a fragment of PD-L1 or PD-L2) or another moiety, e.g., a functional antibody molecule, comprising, e.g., an scFv domain that binds PD-1.

In some embodiments, a therapeutic compound comprises a targeting moiety that is preferentially binds a donor antigen not present in, present in substantially lower levels in the subject, e.g., a donor antigen from Table 2, and is localized to donor graft tissue in a subject. In some embodiments, it does not bind, or does not substantially bind, other tissues. In some embodiments, a therapeutic compound can include a targeting moiety that is specific for HLA-A2 and specifically binds donor allograft tissue but does not bind, or does not substantially bind, host tissues. In some embodiments, the therapeutic compound comprises an ICIM binding/modulating moiety, e.g., an inhibitory molecule counter ligand molecule, e.g., comprising a fragment of a ligand of PD-1 (e.g., a fragment of PD-L1 or PD-L2) or another moiety, e.g., a functional antibody molecule, comprising, e.g., an scFv domain that binds PD-1, such that the therapeutic compound, e.g., when bound to target, activates PD-1. The therapeutic compound targets an allograft and provides local immune privilege to the allograft.

In some embodiments, a therapeutic compound comprises a targeting moiety that is preferentially binds to an antigen of Table 3, and is localized to the target in a subject, e.g., a subject having an autoimmune disorder, e.g., an autoimmune disorder of Table 3. In some embodiments, it does not bind, or does not substantially bind, other tissues. In some embodiments, the therapeutic compound comprises an ICIM binding/modulating moiety, e.g., an inhibitory molecule counter ligand molecule, e.g., comprising a fragment of a ligand of PD-1 (e.g., a fragment of PD-L1 or PD-L2) or another moiety, e.g., a functional antibody molecule, comprising, e.g., an scFv domain that binds PD-1, such that the therapeutic compound, e.g., when bound to target, activates PD-1. The therapeutic compound targets a tissue subject to autoimmune attack and provides local immune privilege to the tissue.

PD-L1 and PDL2, or polypeptides derived therefrom, can provide candidate ICIM binding moieties. However, in monomer form, e.g., when the therapeutic compound is circulating in blood or lymph, this molecule could have an undesired effect of antagonizing the PD-L1/PD-1 pathway, and may only agonize the PD-1 pathway when clustered or multimerized

on the surface of a target, e.g., a target organ. In some embodiments, a therapeutic compound comprises an ICIM binding/modulating moiety comprising a functional antibody molecule, e.g., a scFv domain, that is inert, or substantially inert, to the PD-1 pathway in a soluble form but whch agonizes and drives an inhibitory signal when multimerized (by the targeting moiety) on the surface of a tissue.

THE HLA-G: KIR2DL4 / LILRB1 / LILRB2 PATHWAY

KIR2DL4, LILRB1, and LILRB2 are inhibitory molecules found on T cells, NK cells, and myeloid cells. HLA-G is a counter ligand for each.

KIR2DL4 is also known as CD158D, G9P, KIR-103AS, KIR103, KIR103AS, KIR, KIR-2DL4, killer cell immunoglobulin like receptor, and two Ig domains and long cytoplasmic tail 4. LILRB1 is also known as LILRB1, CD85J, ILT-2, ILT2, LIR-1, LIR1, MIR-7, MIR7, PIR-B, PIRB, leukocyte immunoglobulin like receptor B1. LILRB2 is also known as CD85D, ILT-4, LIR-2, LIR2, MIR-10, MIR10, and ILT4.

A therapeutic compound comprising an HLA-G molecule can be used to provide inhibitory signals to an immune cell comprising any of KIR2DL4, LILRB1, and LILRB2, e.g., with multimerized therapeutic compound molecules comprising an HLA-G molecule and thus provide site-specific immune privilege.

A therapeutic compound comprising an agonistic anti-KIR2DL4, anti-LILRB1, or anti-LILRB2 antibody molecule can be used to provide inhibitory signals to an immune cell comprising any of KIR2DL4, LILRB1, and LILRB2.

HLA-G only delivers an inhibitory signal when multimerized, for example, when expressed on the surface of a cell or when conjugated to the surface of a bead. In embodiments, a therapeutic compound comprising an HLA-G molecule which therapeutic compound does not multimerize in solution (or does not multimerize sufficiently to result in significant levels of inhibitory molecule agonization), is provided. The use of HLA-G molecules that minimize mulitmerization in solution will minimize systemic agonization of immune cells and unwanted immune suppression.

While not wishing to be bound by theory it is believed that HLA-G is not effective in down regulation unless multimerized, that binding of the therapeutic compound to target, through the targeting moiety, multimerizes the ICIM binding entity, and that the multimerized ICIM binding entity, binds and clusters inhibitory molecules on the surface of an immune cell,

thus mediating a negative signal that down regulates the immune cell. Thus, infiltrating immune cells attempting to damage the target tissue, including antigen presenting cells and other myeloid cells, NK cells and T cells, are down regulated.

While HLA-G molecules minimize antagonism when in monomeric form are desirable, the redundancy of LILRB1 and LILRB2 will minimize, the impact on systemic even with some monomeric antagonism.

In some embodiments, the therapeutic compound comprises an ICIM binding/modulating moiety that comprises a HLA-G molecule, e.g., an B2M-free isoform (e.g., HLA-G5), see Carosella et al., Advances in Immunology, 2015, 127:33. In a B2M-free format, HLA-G preferentially binds LILRB2.

Suitable sequences for the construction of HLA-G molecules include GenBank P17693.1 RecName: Full=HLA class I histocompatibility antigen, alpha chain G; AltName: Full=HLA G antigen; AltName: Full=MHC class I antigen G; Flags: Precursor, or MVVMAPRTLFLLLSGALTLTETWAGSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFV RFDSDSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEAS SHTLQWMIGCDLGSDGRLLRGYEQYAYDGKDYLALNEDLRSWTAADTAAQISKRKCE AANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRADPPKTHVTHHPVFDYEATLRCW ALGFYPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQ HEGLPEPLMLRWKQSSLPTIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD (SEQ ID NO: 5). A candidate HLA-G molecule can be tested for suitability for use in methods and compounds, e.g., by methods analogous to those described in "Synthetic HLA-G proteins for therapeutic use in transplantation," LeMaoult et al., 2013 The FASEB Journal 27:3643.

In some embodiments, a therapeutic compound comprises a targeting moiety that is preferentially binds a donor antigen not present in, present in substantially lower levels in the subject, e.g., a donor antigen from Table 2, and is localized to donor graft tissue in a subject. In some embodiments, it does not bind, or does not substantially bind, other tissues. In some embodiments, a therapeutic compound can include a targeting moiety that is specific for HLA-A2 and specifically binds a donor allograft but does not bind host tissues and is combined with an ICIM binding/modulating moiety that comprises a HLA-G molecule that binds KIR2DL4, LILRB1, or LILRB2, such that the therapeutic compound, e.g., when bound to target, activates

KIR2DL4, LILRB1, or LILRB2. The therapeutic compound targets an allograft and provides local immune privilege to the allograft.

In some embodiments, a therapeutic compound comprises a targeting moiety that is preferentially binds a tissue specific antigen, e.g., an antigen from Table 3, and is localized to the target site in a subject, e.g., a subject having an autoimmune disorder, e.g., an anutoimmune disorder from Table 3. In some embodiments, it does not bind, or does not substantially bind, other tissues. In embodiments the therapeutic compound comprises an ICIM binding/modulating moiety that comprises a HLA-G molecule binds KIR2DL4, LILRB1, or LILRB2, such that the therapeutic compound, e.g., when bound to target, activates KIR2DL4, LILRB1, or LILRB2. The therapeutic compound targets an tissue subject to autoimmune attack and provides local immune privilege to the tissue.

It is likely possible to engineer a stable and soluble HLA-G-B2M fusion protein that can also bind LILRB1. For example, the crystal structure of HLA-G was determined using HLA-G/B2M monomers (Clements et al. 2005 PNAS 102:3360)

FCRL FAMILY

FCRL1-6 generally inhibit B cell activation or function. These type 1 transmembrane glycoproteins are composed of different combinations of 5 types of immunoglobulin-like domains, with each protein consisting of 3 to 9 domains, and no individual domain type conserved throughout all of the FCRL proteins. In general, FCRL expression is restricted to lymphocytes, with the primary expression in B-lymphocytes. Generally, FCRLs function to repress B-cell activation.

An ICIM binding/modulating moiety can comprise an agonistic anti-BCMA antibody molecule. In some embodiments, the therapeuticcompound comprises an anti-FCRL antibody molecule and an anti-B cell receptor (BCR) antibody molecule. While not wishing to be bound be theory is is believed that a therapeutic compound comprising anti-body molecules of both specificities will bring the FCRL into close proximity with the BCR and inhibit BCR signaling.

BUTYROPHILINS AND BUTYROPHILIN-LIKE MOLECULES

Effector binding/modulating moiety can comprise an agonist or antagonist of a butyrophilin. In some embodiments, an effector binding/modulating moiety an agonistic or functional BTN1A1 molecule, BTN2A2 molecule, BTNL2 molecule, or BTNL1 molecule.

A functional BTNXi molecule (where Xi=1A1, 2A2, L2 or L1), as that term as used herein, refers to a polypeptide having sufficient BTNXi sequence that, as part of a therapeutic compound, it inhibits T cells. In some embodiments, a BTNXi molecule has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity, or substantial sequence identity, with a naturally occurring butyrophilin or butyrophilin-like molecule.

In some embodiments, an effector binding/modulating moiety an antagonistic BTNL8 molecule.

An antagonistic BTNL8 molecule, as that term as used herein, refers to a polypeptide having sufficient BTNL8 sequence that, as part of a therapeutic compound, it inhibits the activation, proliferation, or secretion of cytokine by a resting T cell. In some embodiments, a BTNL8 molecule has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity, or substantial sequence identity, with a naturally occurring butyrophilin.

IIC BINDING/MODULATING MOIETIES: EFFECTOR BINDING/MODULATING MOIETIES THAT RECRUIT IMMUNOSUPPRESSIVE T CELLS

In some embodiments, a therapeutic compound comprises an effector binding/modulating moiety, e.g., an IIC binding/modulating moiety, that binds, activates, or retains immunosuppressive cells, e.g., immunosuppressive T cells, at the site mediated by the targeting moiety, providing site-specific immune privilege. The IIC binding/modulating moiety, e.g., an IIC binding/modulating moiety comprising an antibody molecule, comprising, e.g., an scFv binding domain, binds immunosuppressive cell types, e.g., Tregs, e.g., Foxp3+CD25+ Tregs. Organ, tissue or specific cell type tolerance is associated with an overwhelming increase of Tregs proximal and infiltrating the target organ; in embodiments, the methods and compounds described herein synthetically re-create and mimic this physiological state. Upon accumulation of Tregs, an immunosuppressive microenvironment is created that serves to protect the organ of interest from the immune system.

GARP-BINDERS AS A TREG AND TGFB TARGETING MOLECULE

GARP is a membrane protein receptor for latent TGF-beta expressed on the surface of activated Tregs (Tran et al. 2009 PNAS 106:13445 and Wang et al. 2009 PNAS 106:13439). In some embodiments, a therapeutic compound comprises an IIC binding entity that binds one or both of soluble GARP and GARP-expressing cells, such as activated human Tregs, and a targeting moiety that targets the therapeutic compound to the target tissue of interest. IIC

binding/modulating moieties that comprises a GARP-Binder include, e.g., an IIC binding/modulating moiety that comprises an anti-GARP antibody molecule, e.g., an anti-GARP scFv domain. While not wishing to be bound by theory, it is believed that the therapeutic compound that comprises a GARP binder effects accumulation of GARP-expressing Tregs at the site targeted by the targeting moiety of the therapeutic compound, e.g., a transplant or site of organ injury. Again, while not wishing to be bound by theory, it is believed that a therapeutic compound that comprises a GARP binder effects can also effect accumulation of soluble GARP at site of organ injury, which will serve to bind and activate TGFB1, an immuno-suppressive cytokine, in a local manner (Fridrich et al. 2016 PLoS One 11:e0153290; doi: 10.1371/journal.pone.0153290 and Hahn et al. 2013 Blood 15:1182). Thus, an effector binding/modulating moiety that comprises a GARP binder can act as either a IIC binding/modulating moiety or an SM binding/modulating moiety.

CTLA4 AS A TREG TARGETING AND T EFFECTOR CELL SILENCING MOLECULE

In some embodiments, an effector binding/modulating moiety, e.g., comprises an antibody molecule, e.g., an scFv domain, that binds CTLA4 expressed on the surface of Tregs. The therapeutic molecule accumulates or retains CTLA4+ Tregs at the target site, with local immunosuppression the consequence.

Though expressed more highly on Tregs, CTLA4 is also expressed on activated T cells. A therapeutic compound comprising an effector binding/modulating moiety, e.g., an anti-CTLA4 antibody, or a functional anti-CTLA4 antibody, can down regulate the CTLA4 expressing T cell. Thus, in a therapeutic compound comprising an effector binding/modulating moiety that binds CTLA4, the effector moiety can also act as an ICIM binding/modulating moiety.

In some embodiments, the anti-CTLA4 binder is neither antagonizing or agonizing when in monomeric format, and is only agonizing when clustered or multimerized upon binding to the target.

While not wishing to be bound by theory it is believed that the binding of the therapeutic compound, via the targeting moiety, to the target, effects multimerization of therapeutic compound. In the case of memory and activated T cells, CTLA4 bound by the effector binding/modulating moiety of the therapeutic compound, is clustered, and an inhibitory signal by engagement of CTLA4 expressed by memory and activated T cells

In some embodiments, the anti-CTLA4 binder is neither antagonizing or agonizing when in monomeric format, and is only agonizing when clustered or multimerized upon binding to the target.

IL-2 MUTEIN MOLECULES: IL2 RECEPTOR BINDERS THAT ACTIVATE TREGS

IL-2 mutein molecules that preferentially expand or stimulate Treg cells (over cytotoxic T cells) can be used as an IIC binding/modulating moiety.

In some embodiments, IIC binding/modulating moiety comprises a IL-2 mutein molecule. As used herein, the term "IL-2 mutein molecule" or "IL-2 mutein" refers to an IL-2 variant that preferentially activates Treg cells. In some embodiments, either alone, or as a component of a therapeutic compound, an IL-2 mutein molecule activates Tregs at least 2, 5, 10, or 100 fold more than cytotoxic T cells. A suitable assay for evaluating preferential activation of Treg cells can be found in U.S. Patent No. 9,580,486 at, for example, Examples 2 and 3, or in WO2016014428 at, for example, Examples 3, 4, and 5, each of which is incorporated by reference in its entirety. The sequence of mature IL-2 is

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLT
FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRP
RDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFC
QSIISTLT (mature IL-2 sequence) (SEQ ID NO: 6)

The immature sequence of IL-2 can be represented by

MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDL
QMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE
ELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF
MCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 15).

In some embodiments, an IIC binding/modulating moiety comprises an IL-2 mutein, or active fragment thereof, coupled, e.g., fused, to another polypeptide, e.g., a polypeptide that extends in vivo half life, e.g., an immunoglobulin constant region, or a multimer or dimer thereof.

An IL-2 mutein molecule can be prepared by mutating one or more of the residues of IL-2. Non-limiting examples of IL-2-muteins can be found in WO2016/164937, US9580486, US7105653, US9616105, US 9428567, US2017/0051029, US2014/0286898A1, WO2014153111A2, WO2010/085495, WO2016014428A2, WO2016025385A1, and US20060269515, each of which are incorporated by reference in its entirety.

In some embodiments, the alanine at position 1 of the sequence above is deleted. In some embodiments, the IL-2 mutein molecule comprises a serine substituted for cysteine at position 125 of the mature IL-2 sequence. Other combinations of mutations and substitutions that are IL-2 mutein molecules are described in US20060269515, which is incorporated by reference in its entirety. In some embodiments, the cysteine at position 125 is also substituted with a valine or alanine. In some embodiments, the IL-2 mutein molecule comprises a V91K substitution. In some embodiments, the IL-2 mutein molecule comprises a N88D substitution. In some embodiments, the IL-2 mutein molecule comprises a N88R substitution. In some embodiments, the IL-2 mutein molecule comprises a substitution of H16E, D84K, V91N, N88D, V91K, or V91R, any combinations thereof. In some embodiments, these IL-2 mutein molecules also comprise a substitution at position 125 as described herein. In some embodiments, the IL-2 mutein molecule comprises one or more substitutions selected from the group consisting of: T3N, T3A, L12G, L12K, L12Q, L12S, Q13G, E15A, E15G, E15S, H16A, H16D, H16G, H16K, H16M, H16N, H16R, H16S, H16T, H16V, H16Y, L19A, L19D, L19E, L19G, L19N, L19R, L19S, L19T, L19V, D20A, D20E, D20H, D20I, D20Y, D20F, D20G, D20T, D20W, M23R, R81A, R81G, R81S, R81T, D84A, D84E, D84G, D84I, D84M, D84Q D84R, D84S, D84T, S87R, N88A, N88D, N88E, N88I, N88F, N88G, N88M, N88R, N88S, N88V, N88W, V91D, V91E, V91G, V91S, I92K, I92R, E95G, and Q126. In some embodiments, the amino acid sequence of the IL-2 mutein molecule differs from the amino acid sequence set forth in mature IL-2 sequence with a C125A or C125S substitution and with one substitution selected from T3N, T3A, L12G, L12K, L12Q L12S, Q13G, E15A, E15G, E15S, H16A, H16D, H16G, H16K, H16M, H16N, H16R, H16S, H16T, H16V, H16Y, L19A, L19D, L19E, L19G, L19N, L19R, L19S, L19T, L19V, D20A, D20E, D20F, D20G, D20T, D20W, M23R, R81A, R81G, R81S, R81T, D84A, D84E, D84G, D84I, D84M, D84Q, D84R, D84S, D84T, S87R, N88A, N88D, N88E, N88F, N88I, N88G, N88M, N88R, N88S, N88V, N88W, V91D, V91E, V91G, V91S, I92K, I92R, E95G, Q126I, Q126L, and Q126F. In some embodiments, the IL-2 mutein molecule differs from the amino acid sequence set forth in mature IL-2 sequence with a C125A or C125S substitution and with one substitution selected from D20H, D20I, D20Y, D20E, D20G, D20W, D84A, D84S, H16D, H16G, H16K, H16R, H16T, H16V, I92K, I92R, L12K, L19D, L19N, L19T, N88D, N88R, N88S, V91D, V91G, V91K, and V91S. In some embodiments, the IL-2 mutein comprises N88R and/or D20H mutations.

In some embodiments, the IL-2 mutein molecule comprises a mutation in the polypeptide sequence at a position selected from the group consisting of amino acid 30, amino acid 31, amino acid 35, amino acid 69, and amino acid 74. In some embodiments, the mutation at position 30 is N30S. In some embodiments, the mutation at position 31 is Y31H. In some embodiments, the mutation at position 35 is K35R. In some embodiments, the mutation at position 69 is V69A. In some embodiments, the mutation at position 74 is Q74P. In some embodiments, the mutein comprises a V69A mutation, a Q74P mutation, a N88D or N88R mutation, and one or more of L53I, L56I, L80I, or L118I mutations. In some embodiments, the mutein comprises a V69A mutation, a Q74P mutation, a N88D or N88R mutation, and a L to I mutation selected from the group consisting of: L53I, L56I, L80I, and L118I mutation. In some embodiments, the IL-2 mutein comprises a V69A, a Q74P, a N88D or N88R mutation, and a L53I mutation. In some embodiments, the IL-2 mutein comprises a V69A, a Q74P, a N88D or N88R mutation, and a L56I mutation. In some embodiments, the IL-2 mutein comprises a V69A, a Q74P, a N88D or N88R mutation, and a L80I mutation. In some embodiments, the IL-2 mutein comprises a V69A, a Q74P, a N88D or N88R mutation, and a L118I mutation. As provided for herein, the muteins can also comprise a C125A or C125S mutation.

In some embodiments, the IL-2 mutein molecule comprises a substitution selected from the group consisting of: N88R, N88I, N88G, D20H, D109C, Q126L, Q126F, D84G, or D84I relative to mature human IL-2 sequence provided above. In some embodiments, the IL-2 mutein molecule comprises a substitution of D109C and one or both of a N88R substitution and a C125S substitution. In some embodiments, the cysteine that is in the IL-2 mutein molecule at position 109 is linked to a polyethylene glycol moiety, wherein the polyethylene glycol moiety has a molecular weight of between 5 and 40 kDa.

In some embodiments, any of the substitutions described herein are combined with a substitution at position 125. The substitution can be a C125S, C125A, or C125V substitution.

In addition to the substitutions or mutations described herein, in some embodiments, the IL-2 mutein has a subsitutiton/mutation at one or more of positions 73, 76, 100, or 138 that correspond to SEQ ID NO: 15 or positions at one or more of positions 53, 56, 80, or 118 that correspond to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a mutation at positions 73 and 76; 73 and 100; 73 and 138; 76 and 100; 76 and 138; 100 and 138; 73, 76, and 100; 73, 76, and 138; 73, 100, and 138; 76, 100 and 138; or at each of 73, 76, 100, and 138 that

correspond to SEQ ID NO: 15. In some embodiments, the IL-2 mutein comprises a mutation at positions 53 and 56; 53 and 80; 53 and 118; 56 and 80; 56 and 118; 80 and 118; 53, 56, and 80; 53, 56, and 118; 53, 80, and 118; 56, 80 and 118; or at each of 53, 56, 80, and 118 that correspond to SEQ ID NO: 6. As the IL-2 can be fused or tethered to other proteins, as used herein, the term corresponds to as reference to a SEQ ID NOs: 6 or 15 refer to how the sequences would align with default settings for alignment software, such as can be used with the NCBI website. In some embodiments, the mutation is leucine to isoleucine. Thus, the IL-2 mutein can comprise one more isoleucines at positions 73, 76, 100, or 138 that correspond to SEQ ID NO: 15 or positions at one or more of positions 53, 56, 80, or 118 that correspond to SEQ ID NO: 6. In some embodiments, the mutein comprises a mutation at L53 that correspond to SEQ ID NO: 6. In some embodiments, the mutein comprises a mutation at L56 that correspond to SEQ ID NO: 6. In some embodiments, the mutein comprises a mutation at L80 that correspond to SEQ ID NO: 6. In some embodiments, the mutein comprises a mutation at L118 that correspond to SEQ ID NO: 6. In some embodiments, the mutation is leucine to isoleucine. In some embodiments, the mutein also comprises a mutation as position 69, 74, 88, 125, or any combination thereof in these muteins that correspond to SEQ ID NO: 6. In some embodiments, the mutation is a V69A mutation. In some embodiments, the mutation is a Q74P mutation. In some embodiments, the mutation is a N88D or N88R mutation. In some embodiments, the mutation is a C125A or C125S mutation.

In some embodiments, the IL-2 mutein comprises a mutation at one or more of positions 49, 51, 55, 57, 68, 89, 91, 94, 108, and 145 that correspond to SEQ ID NO: 15 or one or more positions 29, 31, 35, 37, 48, 69, 71, 74, 88, and 125 that correspond to SEQ ID NO: 6. The substitutions can be used alone or in combination with one another. In some embodiments, the IL-2 mutein comprises substitutions at 2, 3, 4, 5, 6, 7, 8, 9, or each of positions 49, 51, 55, 57, 68, 89, 91, 94, 108, and 145. Non-limiting examples such combinations include, but are not limited to, a mutation at positions 49, 51, 55, 57, 68, 89, 91, 94, 108, and 145; 49, 51, 55, 57, 68, 89, 91, 94, and 108; 49, 51, 55, 57, 68, 89, 91, and 94; 49, 51, 55, 57, 68, 89, and 91; 49, 51, 55, 57, 68, and 89; 49, 51, 55, 57, and 68; 49, 51, 55, and 57; 49, 51, and 55; 49 and 51; 51, 55, 57, 68, 89, 91, 94, 108, and 145; 51, 55, 57, 68, 89, 91, 94, and 108; 51, 55, 57, 68, 89, 91, and 94; 51, 55, 57, 68, 89, and 91; 51, 55, 57, 68, and 89; 55, 57, and 68; 55 and 57; 55, 57, 68, 89, 91, 94, 108, and 145; 55, 57, 68, 89, 91, 94, and 108; 55, 57, 68, 89, 91, and 94; 55, 57, 68, 89, 91, and 94;

55, 57, 68, 89, and 91; 55, 57, 68, and 89; 55, 57, and 68; 55 and 57; 57, 68, 89, 91, 94, 108, and 145; 57, 68, 89, 91, 94, and 108; 57, 68, 89, 91, and 94; 57, 68, 89, and 91; 57, 68, and 89; 57 and 68; 68, 89, 91, 94, 108, and 145; 68, 89, 91, 94, and 108; 68, 89, 91, and 94; 68, 89, and 91; 68 and 89; 89, 91, 94, 108, and 145; 89, 91, 94, and 108; 89, 91, and 94; 89 and 91; 91, 94, 108, and 145; 91, 94, and 108; 91, and 94; or 94 and 108. Each mutation can be combined with one another. The same substitutions can be made in SEQ ID NO: 6, but the numbering would adjusted appropriately as is clear from the present disclosure (20 less than the numbering for SEQ ID NO: 15 corresponds to the positions in SEQ ID NO: 6).

In some embodiments, the IL-2 mutein comprises a mutation at one or more positions of 35, 36, 42, 104, 115, or 146 that correspond to SEQ ID NO: 15 or the equivalent positions at SEQ ID NO: 6 (*e.g.* positions 15, 16, 22, 84, 95, or 126). These mutations can be combined with the other leucine to isoleucine mutations described herein or the mutation at positions 73, 76, 100, or 138 that correspond to SEQ ID NO: 15 or at one or more of positions 53, 56, 80, or 118 that correspond to SEQ ID NO: 6. In some emboidments, the mutation is a E35Q, H36N, Q42E, D104N, E115Q, or Q146E, or any combination thereof. In some embodiments, one or more of these substitutions is wildtype. In some embodiments, the mutein comprises a wild-type residue at one or more of positions 35, 36, 42, 104, 115, or 146 that correspond to SEQ ID NO: 15 or the equivalent positions at SEQ ID NO: 6 (*e.g.* positions 15, 16, 22, 84, 95, and 126).

The mutations at these positions can be combined with any of the other mutations described herein, including, but not limited to substitutions at positions 73, 76, 100, or 138 that correspond to SEQ ID NO: 15 or positions at one or more of positions 53, 56, 80, or 118 that correspond to SEQ ID NO: 6 described herein and above. In some embodiments, the IL-2 mutein comprises a N49S mutation that corresponds to SEQ ID NO: 15. In some embodiments, the IL-2 mutein comprises a Y51S or a Y51H mutation that corresponds to SEQ ID NO: 15. In some embodiments, the IL-2 mutein comprises a K55R mutation that corresponds to SEQ ID NO: 15. In some embodiments, the IL-2 mutein comprises a T57A mutation that corresponds to SEQ ID NO: 15. In some embodiments, the IL-2 mutein comprises a K68E mutation that corresponds to SEQ ID NO: 15. In some embodiments, the IL-2 mutein comprises a V89A mutation that corresponds to SEQ ID NO: 15. In some embodiments, the IL-2 mutein comprises a N91R mutation that corresponds to SEQ ID NO: 15. In some embodiments, the IL-2 mutein comprises a Q94P mutation that corresponds to SEQ ID NO: 15. In some embodiments, the IL-2

mutein comprises a N108D or a N108R mutation that corresponds to SEQ ID NO: 15. In some embodiments, the IL-2 mutein comprises a C145A or C145S mutation that corresponds to SEQ ID NO: 15. These substitutions can be used alone or in combination with one another. In some embodiments, the mutein comprises each of these substitutions. In some embodiments, the mutein comprises 1, 2, 3, 4, 5, 6, 7, or 8 of these mutations. In some embodiments, the mutein comprises a wild-type residue at one or more of positions 35, 36, 42, 104, 115, or 146 that correspond to SEQ ID NO: 15 or the equivalent positions at SEQ ID NO: 6 (*e.g.* positions 15, 16, 22, 84, 95, and 126).

In some embodiments, the IL-2 mutein comprises a N29S mutation that corresponds to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a Y31S or a Y31H mutation that corresponds to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a K35R mutation that corresponds to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a T37A mutation that corresponds to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a K48E mutation that corresponds to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a V69A mutation that corresponds to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a N71R mutation that corresponds to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a Q74P mutation that corresponds to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a N88D or a N88R mutation that corresponds to SEQ ID NO: 6. In some embodiments, the IL-2 mutein comprises a C125A or C125S mutation that corresponds to SEQ ID NO: 6. These substitutions can be used alone or in combination with one another. In some embodiments, the mutein comprises 1, 2, 3, 4, 5, 6, 7, or 8 of these mutations. In some embodiments, the mutein comprises each of these substitutions. In some embodiments, the mutein comprises a wild-type residue at one or more of positions 35, 36, 42, 104, 115, or 146 that correspond to SEQ ID NO: 15 or the equivalent positions at SEQ ID NO: 6 (*e.g.* positions 15, 16, 22, 84, 95, and 126).

For any of the IL-2 muteins described herein, in some embodiments, one or more of positions 35, 36, 42, 104, 115, or 146 that correspond to SEQ ID NO: 15 or the equivalent positions at SEQ ID NO: 6 (*e.g.* positions 15, 16, 22, 84, 95, or 126) are wild-type (*e.g.*are as shown in SEQ ID NOs: 6 or 15). In some embodiments, 2, 3, 4, 5, 6, or each of positions 35, 36, 42, 104, 115, or 146 that correspond to SEQ ID NO: 15 or the equivalent positions at SEQ ID NO: 6 (*e.g.* positions 15, 16, 22, 84, 95, and 126) are wild-type.

In some embodiments, the IL-2 mutein comprises a sequence of:

MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDL
QMILNGISNHKNPRLARMLTFKFYMPEKATEIKHLQCLEEE
LKPLEEALRLAPSKNFHLRPRDLISDINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFSQSIISTLT (SEQ ID NO: 16)

In some embodiments, the IL-2 mutein comprises a sequence of:

MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDL
QMILNGISNHKNPRLARMLTFKFYMPEKATELKHIQCLEEE
LKPLEEALRLAPSKNFHLRPRDLISDINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFSQSIISTLT (SEQ ID NO: 17)

In some embodiments, the IL-2 mutein comprises a sequence of:

MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDL
QMILNGISNHKNPRLARMLTFKFYMPEKATELKHLQCLEEE
LKPLEEALRLAPSKNFHIRPRDLISDINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFSQSIISTLT (SEQ ID NO: 18)

In some embodiments, the IL-2 mutein comprises a sequence of:

MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDL
QMILNGISNHKNPRLARMLTFKFYMPEKATELKHLQCLEEE
LKPLEEALRLAPSKNFHLRPRDLISDINVIVLELKGSETTFMC
EYADETATIVEFINRWITFSQSIISTLT (SEQ ID NO: 19)

In some embodiments, the IL-2 mutein sequences described herein do not comprise the IL-2 leader sequence. The IL-2 leader sequence can be represented by the sequence of MYRMQLLSCIALSLALVTNS (SEQ ID NO: 20). Therefore, in some embodiments, the sequences illustrated above can also encompass peptides without the leader sequence. Although SEQ ID NOs; 16-20 are illustrated with only mutation at one of postions 73, 76, 100, or 138 that correspond to SEQ ID NO: 15 or positions at one or more of positions 53, 56, 80, or 118 that correspond to SEQ ID NO: 6, the peptides can comprises one, two, three or 4 of the mutations at these positions. In some embodiments, the substitution at each position is isoleucine or other type of conservative amino acid substitution. In some embodiments, the leucine at the recited positions are substituted with, independently, isoleucine, valine, methionine, or phenylalanine.

In some embodiments, the IL-2 mutein molecule is fused to a Fc Region or other linker region as described herein. Examples of such fusion proteins can be found in US9580486, US7105653, US9616105, US 9428567, US2017/0051029, WO2016/164937, US2014/0286898A1, WO2014153111A2, WO2010/085495, WO2016014428A2, WO2016025385A1, US2017/0037102, and US2006/0269515, each of which are incorporated by reference in its entirety.

In some embodiments, the Fc Region comprises what is known as the LALA mutation. Using the Kabat numbering of the Fc region, this would correspond to L247A, L248A, and G250A. In some embodiments, using the EU numbering of the Fc region, the Fc region comprises a L234A mutation, a L235A mutation, and/or a G237A mutatoin mutation. Regardless of the numbering system used, in some embodiments, the Fc portion can comprise mutations that correspond to these residues. In some embodiments, the Fc Region comprises N297G or N297A (kabat numbering) mutations. The Kabat numbering is based upon a full-length sequence, but would be used in a fragment based upon a traditional alignment used by one of skill in the art for the Fc region.

In some embodiments, the Fc Region comprises a sequence of:

DKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPG. (SEQ ID NO: 21)

or

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPG. (SEQ ID NO: 28)

In some embodiments, the IL-2 mutein is linked to the Fc Region. Non-limiting examples of linkers are glycine/serine linkers. For example, a glycine/serine linkers can be a

sequence of GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 22) or GGGGSGGGGSGGGGS (SEQ ID NO: 30). This is simply a non-limiting example and the linker can have varying number of GGGGS (SEQ ID NO: 23) or GGGGA repeats (SEQ ID NO: 29). In some embodiments, the linker comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the GGGGS (SEQ ID NO: 23) or GGGGA repeats (SEQ ID NO: 29) repeats.

Thus, the IL-2/Fc Fusion can be represented by the formula of $Z_{IL\text{-}2M}$-L_{gs}-Z_{Fc}, wherein $Z_{IL\text{-}2M}$ is a IL-2 mutein as described herein, L_{gs} is a linker sequence as described herein (*e.g.* glycine/serine linker) and Z_{Fc} is a Fc region described herein or known to one of skill in the art. In some embodiments, the formula can be in the reverse orientation Z_{Fc}-L_{gs}-$Z_{IL\text{-}2M}$.

In some embodiments, the IL-2/Fc fusion comprises a sequence of

MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDL
QMILNGISNHKNPRLARMLTFKFYMPEKATEIKHLQCLEEE
LKPLEEALRLAPSKNFHLRPRDLISDINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGG
SGGGGSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 24)

MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDL
QMILNGISNHKNPRLARMLTFKFYMPEKATELKHIQCLEEE
LKPLEEALRLAPSKNFHLRPRDLISDINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGG
SGGGGSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 25)

MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDL
QMILNGISNHKNPRLARMLTFKFYMPEKATELKHLQCLEEE
LKPLEEALRLAPSKNFHIRPRDLISDINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGG
SGGGGSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 26)

MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDL
QMILNGISNHKNPRLARMLTFKFYMPEKATELKHLQCLEEE
LKPLEEALRLAPSKNFHLRPRDLISDINVIVLELKGSETTFMC
EYADETATIVEFINRWITFSQSIISTLTGGGGSGGGGSGGGGS
GGGGSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 27).

In some embodiments, the IL-2/Fc Fusion comprises a sequence selected from the following table, Table 2:

Table 2: IL-2/Fc Fusion Protein Amino Acid Sequences

Sequence Identification	Sequence
SEQ ID NO: 7	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE ELKPLEEVLNLAQSKNFHLRPRDLISRINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFSQSIISTLTGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK

Sequence Identification	Sequence
SEQ ID NO: 8	APTSSSTKKTQLQLEHLLLHLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE ELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFSQSIISTLTVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ FNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP MLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 9	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE ELKPLEEVLNLAQSKNFHLRPRDLISRINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFSQSIISTLTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
SEQ ID NO: 10	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE ELKPLEEVLNLAQSKNFHLRPRDLISRINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFSQSIISTLTGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
SEQ ID NO: 11	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE ELKPLEEVLNLAQSKNFHLRPRDLISRINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFSQSIISTLTGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG
SEQ ID NO: 12	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE ELKPLEEVLNLAQSKNFHLRPRDLISRINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFSQSIISTLTGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNYHTQ KSLSLSPG
SEQ ID NO: 13	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE ELKPLEEVLNLAQSKNFHLRPRDLISRINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFSQSIISTLTGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
SEQ ID NO: 14	APTSSSTKKTQLQLEHLLLHLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE ELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFSQSIISTLTGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYPVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPG

In some embodiments, the IL-2 muteins comprises one or more of the seuqences provided in the following table, which, in some embodiments, shows the IL-2 mutein fused with other proteins or linkers. The table also provides sequences for a variety of Fc domains or variants that the IL-2 can be fused with:

SEQ ID	Brief	Amino Acid Sequence

NO:	Description	
31	Human IL-2 with C125S mutation	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
32	Human IL-2 with C125S and T3A mutations	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
33	Human IL-2 with N88R and C125S	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISRINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
34	Human IL-2 with V69A, Q74P and C125S mutations	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
35	Human IL-2 with V69A, Q74P, N88D and C125S mutations	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
36	Human IL-2 with V69A, Q74P, N88R and C125S mutations	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISRINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
37	Human IL-2 with N88D and C125S	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
38	Human IL-2 with L53I, V69A, Q74P, N88D and C125S mutations	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TEIKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
39	Human IL-2 with L56I, V69A, Q74P, N88D and C125S mutations	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHIQCLEEELKPLEEALNLAPSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
40	Human IL-2 with V69A, Q74P, L80I, N88D and C125S mutations	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHIRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
41	Human IL-2 with V69A, Q74P, N88D, L118I, and C125S mutations	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADx`ETATIVEFINRWITFSQSIISTLT
42	Human IgG1 Fc (N-terminal fusions) with	DKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK

	L234A, L235A, and G237A mutations	GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG
30	GGGGSGGGGSGGG GS linker (15 amino acids)	GGGGSGGGGSGGGGS
22	GGGGSGGGGSGGG GSGGGGS linker (20 amino acids)	GGGGSGGGGSGGGGSGGGGS
23	GGGGS linker (5 amino acids)	GGGGS
43	Human IgG1 Fc (truncated) with N297G mutation	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG
44	Antibody Heavy Chain CH1-CH2-CH3 domains (human IgG1 with L234A, L235A, and G237A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
45	Antibody Kappa Constant Domain (human)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
46	IL-2-G4Sx3-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSDK THTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG
47	IL-2 T3A-G4Sx3-Fc	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSDK THTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG
48	IL-2 N88R-G4Sx3-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISRINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSDK THTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF

		YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG
49	IL-2 V69A, Q74P,-G4Sx3-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSDK THTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG
50	IL-2 N88D V69A, Q74P-G4Sx3-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSDK THTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG
51	IL-2 N88R V69A, Q74P-G4Sx3-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISRINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSDK THTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG
52	IL-2 N88D-G4Sx3-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSDK THTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG
53	IL-2 L53I N88D V69A, Q74P-G4Sx4-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TEIKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSGG GGSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
54	IL-2 L56I N88D V69A, Q74P-G4Sx4-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHIQCLEEELKPLEEALNLAPSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSGG GGSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG

55	IL-2 L80I N88D V69A, Q74P-G4Sx4-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHIRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSGG GGSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
56	IL-2 L118I N88D V69A, Q74P-G4Sx4-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFINRWITFSQSIISTLTGGGGSGGGGSGGGGSGG GGSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
57	IL-2 N88D V69A, Q74P-G4Sx4-Fc	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA TELKHLQCLEEELKPLEEALNLAPSKNFHLRPRDLISDINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLTGGGGSGGGGSGGGGSGG GGSDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
58	Fc-G4S-IL-2 N88D V69A, Q74P	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGGGGGSAPTSSSTKKTQLQLEHLLL DLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEA LNLAPSKNFHLRPRDLISDINVIVLELKGSETTFMCEYADETATIVEFLN RWITFAQSIISTLT

In some embodiments, the sequences shown in the table or throughout comprise or don't comprise one or more mutations that correspond to positions L53, L56, L80, and L118. . In some embodiments, the sequences shown in the table or throughout the present application comprise or don't comprise one or more mutations that correspond to positions L59I, L63I, I24L, L94I, L96I or L132I or other substitutions at the same positions. In some embodiments, the mutation is leucine to isoleucine. In some embodiments, the mutein does not comprise another mutation other than as shown or described herein. In some embodiments, the peptide comprises a sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO:

46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, or SEQ ID NO: 56, SEQ ID NO: 57, or SEQ ID NO: 58.

In some embodiments, the Fc portion of the fusion is not included. In some embodiments, the peptide consists essentially of a IL-2 mutein provided for herein. In some embodiments, the protein is free of a Fc portion.

For illustrative purposes only, embodiments of IL-2 mutein fused with a Fc and with a targeting moiety are illustrated in FIG. 19.

The sequences are for illustrative purposes only and are not intended to be limiting. In some embodiments, the compound comprises an amino acid sequence of SEQ ID NO: 53, 54, 55, or 56. In some embodiments, the compound comprises an amino acid sequence of SEQ ID NO: 53, 54, 55, or 56.with or without a C125A or C125S mutation.

In an embodiment, an IL-2 mutein molecule comprises at least 60, 70, 80, 85, 90, 95, or 97% sequence identity or homology with a naturally occurring human IL-2 molecule, e.g., a naturally occurring IL-2 sequence disclosed herein or those that incorporated by reference.

As described herein the IL-2 muteins can be part of a bi-specific molecule with a tethering moiety, such as a MAdCAM antibody that will target the IL-2 mutein to a MAdCAM expressing cell. As described herein, the bispecific molecule can be produced from two polypeptide chains. In some embodiments, the following can be used:

Table of MAdCAM-IL-2 Mutein Bispecific Compounds

	Chain 1 N-terminal to C-terminal Molecule Component Sequence IDs				Chain 2 N-terminal to C-terminal Molecule Component Sequence IDs	
Detail	Antibody VH Domain	Antibody Heavy Chain CH1-CH2-CH3 Domains	Linker 1	C-terminal Moiety	Light Chain VK Domain	Light Chain CK Domain
1. Anti-MAdCam-Fc-IL-2 N88D V69A, Q74P	Rat Anti-MAdCam -VH1	SEQ ID NO: 44	SEQ ID NO: 23	SEQ ID NO: 35	Rat Anti-MAdCam -VK1	SEQ ID NO: 45
2. Anti-MAdCam -Fc-IL-2 N88D V69A, Q74P	Rat Anti-MAdCam-VH2	SEQ ID NO: 44	SEQ ID NO: 23	SEQ ID NO: 35	Rat Anti-MAdCam -VK2	SEQ ID NO: 45
3. Anti-	Rat Anti-	SEQ ID	SEQ ID	SEQ ID	Rat Anti-	SEQ

MAdCam - Fc-IL-2 L118I N88D V69A, Q74P	MAdCam -VH1	NO: 44	NO: 23	NO: 41	MAdCam -VK3	ID NO: 45
4. TTJ2-Fc-IL-2 L118I N88D V69A, Q74P	Human TTJ2-VH	SEQ ID NO: 44	SEQ ID NO: 23	SEQ ID NO: 41	Human TTJ2-VK	SEQ ID NO: 45
5. anti hu.MAdCAM-Fc-IL-2 L118I N88D V69A, Q74P	Anti-MAdCam Human VH3	SEQ ID NO: 44	SEQ ID NO: 23	SEQ ID NO: 41	Anti-MAdCam Human VK3	SEQ ID NO: 45
6. anti hu.MAdCAM-Fc-IL-2 L118I N88D V69A, Q74P	Anti-MAdCam Human VH4	SEQ ID NO: 44	SEQ ID NO: 23	SEQ ID NO: 41	Anti-MAdCam Human VK4	SEQ ID NO: 45
7. anti hu.MAdCAM-Fc-IL-2 L118I N88D V69A, Q74P	Anti-MAdCam Human VH5	SEQ ID NO: 44	SEQ ID NO: 23	SEQ ID NO: 41	Anti-MAdCam Human VK5	SEQ ID NO: 45

The proteins can be produced with or without a C125A or C125S mutation in the IL-2 mutein.

In some embodiments, the constant kappa domain in any of the light chains can be replaced with a constant lambda domain.

GITR-Binders

GITR(CD357) is a cell surface marker present on Tregs. Blockade of the GITR-GITRL interaction maintains Treg function. In some embodiments, a therapeutic compound comprises an IIC binding entity that binds GITR-expressing Treg cells and a targeting moiety that targets the therapeutic compound to the target tissue of interest.

In some embodiments, a therapeutic compound comprises an anti-GITR antibody molecule, e.g., anti-GITR antibody molecule that inhibit binding of GITR to GITRL.

In some embodiments, a therapeutic compound comprises an anti-GITR antibody molecule, anti-GITR antibody molecule that inhibit binding of GITR to GITRL, and PD-1 agonist, IL-2 mutein molecule, or other effector described herein.

While not wishing to be bound by theory, it is believed that the therapeutic compound that comprises a GITR binder effects accumulation of GITR-expressing Tregs at the site targeted by the targeting moiety of the therapeutic compound, e.g., a transplant or site of organ injury.

Butyrophilins/BUTYROPHILIN-LIKE MOLECULES

Effector binding/modulating moiety can comprise an agonistic BTNL2 molecule. While not wishing to be bound by theory it is believed that agonistic BTNL2 molecules induce Treg cells.

An agonistic BTNL2 molecule as that term as used herein, refers to a polypeptide having sufficient BTNL2 sequence that, as part of a therapeutic compound, it induces Treg cells. In some embodiments, a BTNL2 molecule has at least 60, 70, 80, 90, 95, 99, or 100% sequence identity, or substantial sequence identity, with a naturally occurring butyrophilin.

In some embodiments, an effector binding/modulating moiety an antagonistic BTNL8 molecule.

THERAPEUTIC COMPOUNDS COMPRISING AN SM BINDING/MODULATING MOIETY: MANIPULATION OF LOCAL MICROENVIRONMENT

A therapeutic compound can comprise an effector binding/modulating moiety that promotes an immuno-suppressive local microenvironment, e.g., by providing in the proximity of the target, a substance that inhibits or minimizes attack by the immune system of the target, referred to herein a SM binding/modulating moiety.

In some embodiments, the SM binding/modulating moiety comprises a molecule that inhibits or minimizes attack by the immune system of the target (referred to herein as an SM binding/modulating moiety). In some embodiments, a therapeutic compound comprises an SM binding/modulating moiety that binds and accumulates a soluble substance, e.g., an endogenous or exogenous substance having immunosuppressive function. In some embodiments, a therapeutic compound comprises an SM binding/modulating moiety, e.g., a CD39 molecule or a CD73 molecule or alkaline phosphatase molecule, that binds, inhibits, sequesters, degrades or otherwise neutralizes a soluble substance, typically and endogenous soluble substance, e.g., ATP in the case of a CD39 molecule or alkaline phosphatase molecule, or AMP in the case of a CD73 molecule, that promotes immune attack. In some embodiments, a therapeutic compound comprises an SM binding/modulating moiety that comprises an immune-suppressive substance, e.g. a fragment of protein that is immunosuppressive.

THERAPEUTIC COMPOUNDS COMPRISING AN ICSM BINDING/MODULATING MOIETY: INHIBITION OF STIMULATION, E.G., INHIBITION OF CO-STIMULATION OF IMMUNE CELLS

A therapeutic compound can comprise an ICSM binding/modulating moiety that inhibits or antagonizes a stimulatory, e.g., co-stimulatory binding pair, e.g., OX40 and OX40L. The ICSM binding/modulating moiety can bind and antagonize either member of the pair.

In an embodiment, the ICSM binding/modulating moiety comprises an antibody molecule that binds and antagonizes either member of a stimulatory, e.g., co-stimulatory binding pair. In an embodiment the ICSM binding/modulating moiety comprises antagonistic analog of one of the members of the binding pair. In such embodiments the ICSM binding/modulating moiety can comprise a soluble fragment of one of the members that binds the other. Typically the analog will have at least 50, 60, 70, 80, 90, 95, or 98% homology or sequence identity with a naturally occurring member that binds the target member of the pair. In the case of an ICSM binding/modulating moiety that binds the member present on the surface of an immune cell, the ICSM binding/modulating moiety typically binds but does not activate, or allow endogenous counter member to bind and activate.

Thus, in the case of the binding pair that includes, for example, the OX40 immune cell member and the OX40L counter member, an ICSM binding/modulating member can comprise any of the following:

a) an antibody molecule that binds the OX40 immune cell member and antagonizes stimulation, e.g., by blocking binding of endogenous OX40L counter member;

b) an antibody molecule that binds OX40L counter member and antagonizes stimulation, e.g., by blocking effective binding of the endogenous OX40L counter member to the OX40 immune cell member;

c) a soluble fragment or analog of OX40L counter member which binds OX40 immune cell member and antagonizes stimulation; and

c) a soluble fragment or analog of OX40 immune cell member which binds OX40L counter member and antagonizes stimulation.

For example, the ICSM binding/modulating moiety, e.g., an antibody molecule or an antagonistic analog or of the counter member, can bind to CD2, ICOS, CD40L, CD28, LFA1, SLAM, TIM1, CD30, OX40 (CD134), 41BB (CD137), CD27, HVEM, DR3, GITR, BAFFR, TACI, BCMA, or CD30, CD40. In another embodiment, the ICSM binding/modulating moiety , e.g., an antibody molecule or an antagonistic analog or of the counter member, can bind to B7.1,

B7.2, ICOSL (B7-H2, B7RP1), LFA3, CD48, CD58, ICAM1, SLAM, TIM4, CD40, CD30L, OX40L (CD252), 41BBL (CD137L), CD70, LIGHT, TL1A, GITRL, BAFF, APRIL, or CD30, CD40L.

In some embodiments, the ICSM binding/modulating molecule binds, and antagonizes, an activating or costimulatory molecule, e.g., a costimulatory molecule, present on an immune cell, or binds the counter member preventing the counter member from activating the costimulatory molecule present on the immune cell. In some embodiments, the ICSM comprises an antagonistic antibody molecule e.g., an antibody molecule that binds the costimulatory molecule on an immune cell or binds the counter member of the ICSM, preventing the counter member from activating the costimulatory molecule on the immune cell, and results in inhibiting the activity of the costimulatory molecule. In some embodiments, the ICSM comprises an antagonistic counterpart molecule, e.g., a fragment of a molecule that binds the costimulatory molecule, and results in the inhibition of the costimulatory molecule activity.

In some embodiments, one member of the binding pair will be on the surface of an immune cell, e.g., a T, B, or NK cell or dendritic cell, while the counter member will be on another immune cell, or an APC such as a dendritic cell_ or on non-immune cells such as smooth cells, or endothelial cells.

The following table provides non-limiting examples of costimulatory molecule and counterstructure pairs

TABLE 2: Costimulatory molecule and counterstructure pairs

Costimulatory Molecule (eg on T cells)	Counterstructure
CD28	B7.1 or B7.2
ICOS	ICOSL (B7H-2, B7RP1)
CD2	LFA3, CD48, CD58
LFA1	ICAM1
SLAM	SLAM
TIM1	TIM4

CD40L	CD40
CD30	CD30L
OX40/CD134	OX40L (CD252)
41BB/CD137	41BBL (CD137L)
CD27	CD70
HVEM	LIGHT
DR3	TL1A
GITR	GITRL
Costimulatory Molecule (eg on B cells)	Counterstructure
BAFFR	BAFF
TACI	BAFF and APRIL
BCMA	BAFF and APRIL
CD40	CD40L
CD30L	CD30

DONOR TISSUE

Therapeutic compounds and methods described herein can be used in conjunction with a transplantation of donor tissue into a subject and can minimizes rejection of, minimizes immune effector cell mediated damage to, prolongs acceptance of, or prolongs the functional life of, donor transplant tissue. The tissue can be xenograft or allograft tissue. Transplanted tissue can comprise all or part of an organ, e.g., a liver, kidney, heart, pancreas, thymus, skin or lung. In embodiments, therapeutic compounds described herein reduce, or eliminate the need for systemic immune suppression. Therapeutic compounds and methods described herein can also be used to treat GVHD. In some embodiments, host cells are coated with a therapeutic compound that comprises, as an effector binding/modulating moiety, a PD-L1 molecule.

Table 2 provides target molecules for transplant indications. A target molecule is the target to which a targeting moiety binds. As discussed elsewhere herein, In some embodiments, a targeting moiety is selected that binds a product of an allele present on donor tissue and which

is not expressed by the subject (recipient) or at expressed at a different level (e.g. reduced or substantially reduced).

Table 2: Target Molecules for Transplant Indications		
Indication	Organ / cell type	Target
Allograft transplant tissue, e.g., allograft solid organ transplant, GvHD	All	HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ or HLA-DR
Transplant	Kidney	Antigens expressed in the kidney where immune cells infiltrate, for example including but not limited to the tubular interstitial region eg Uromodulin, SLC22A2, SLC22A6, FXYD4, SLC5A10, SLC6A13, AQP6, SLC13A3, TMEM72, BSND, NPR3, and the proximal and distal tubular epithelium, such as OAT1, OCT2

AUTO-IMMUNE DISORDERS

Therapeutic compounds and methods described herein can be used to treat a subject having or at risk for having an unwanted autoimmune response, e.g., an auto immune response in Type 1 Diabetes, Multiple Sclerosis, Cardiomyositis, vitiligo, alopecia, inflammatory bowel disease (IBD, e.g. Crohn's disease or ulcerative colitis), Sjogren's syndrome, focal segmented glomerular sclerosis (FSGS), scleroderma/systemic sclerosis (SSc) or rheumatoid arthritis. In some embodiments, the treatment minimizes rejection of, minimizes immune effector cell mediated damage to, prolongs the survival of subject tissue undergoing, or a risk for, autoimmune attack. Table 3 provides target molecules for several autoimmune indications and organ/cell types. A target molecule is the target to which a targeting moiety binds.

Table 3: Target Molecules for autoimmune indications		
Indication	Organ / cell type	Target Molecule
Type 1 Diabetes and Transplant	Pancreas/Pancreatic islets, beta cells	SEZ6L2, LRP11, DISP2, SLC30A8, FXYD2 TSPAN7

		TMEM27 (reference Hald et al. 2012 Diabetelogia 55:154); FXYD2; GPR119; HEPACAM2, DPP6, or MAdCAM
Multiple Sclerosis	CNS / myelin sheath of oligodendrocytes	MOG, PLP, MBP
Cardiomyositis, rheumatoid arthritis	Cardiomyocytes, monocytes, macrophages, myeloid cells	SIRPA (CD172a)
Inflammatory bowel disease (ulcerative colitis, Crohn's disease) or GVHD; Celiac disease	Intestine	MAdCAM
Autoimmune hepatitis (AIH); Primary Sclerosing Cholangitis (PSC); Primary Biliary Sclerosis ; (PBC); transplant	liver	MAdCAM
Focal Segmented Glomerular Sclerosis (FSGS) and other diseases that can affect kidney for example lupus nephritis, systemic scleroderma, membranous glomerular nephropathy (MGN); Membranous nephropathy (MN); Minimal Change Disease (MCD); IgA nephropathy; ANCA-associated vasculitis (AAV)	Kidney, podocytes, tubules, epithelial cells	COL1A1, Cadherin 2, VCAM-1, Thy1, Podocin, KIM1 (Hodgin et al, Am J Pathol 177:1675 2010); PLA2R; OAT1; OCT2; K-cadherin 6

Sjogren's syndrome	Salivary glands, epithelial cells, kidney	FCGR3B, HLAB, KIM1 (Hu et al Arth and Rheum 56:3588 2007
Scleroderma, systemic sclerosis (SSc)	skin, kidney, lung, Fibroblasts, connective tissue	Collagen I, III, VI, VII, fibronectin (Wang et al Arth and Rheum 54:2271 2006)
vitiligo	Skin, epidermis, Langerhans cells, keratinocytes, melanocytes	COL17A1, CD1A, CD207, desmoglein 1–4, keratin 1
Alopecia areata	Skin, Hair follicle/hair bulb, dermis	CD133 (Yang and Cotsarelis, J Dermatol Sci 57:2 2010)

Other examples of autoimmune disorders and diseases that can be treated with the compounds described herein include, but are not limited to, Myocarditis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Subacute bacterial endocarditis, Anti-Glomerular Basement Membrane nephritis, Interstitial cystitis, Lupus nephritis, membranous glomerulonephropathy, Chronic Kidney Disease ("CKD"), Autoimmune hepatitis, Primary biliary cirrhosis, Primary sclerosing cholangitis, Antisynthetase syndrome, alopecia areata, autoimmune angioedema, autoimmune progesterone dermatitis, autoimmune urticaria, bullous pemphigoid, cicatricial pemphigoid, dermatitis herpetiformis, discoid lupus erythematosus, epidermolysis bullosa acquisita, erythema nodosum, gestational pemphigoid, hidradenitis suppurativa, lichen planus, lichen sclerosus, linear iga disease (lad), morphea, pemphigus vulgaris, pityriasis lichenoides et varioliformis acuta, mucha-habermann disease, psoriasis, systemic scleroderma, vitiligo, Addison's disease, Autoimmune polyendocrine syndrome (APS) type 1, Autoimmune polyendocrine syndrome (APS) type 2, Autoimmune polyendocrine syndrome (APS) type 3, Autoimmune pancreatitis (AIP), Diabetes mellitus type 1, Autoimmune thyroiditis, Ord's thyroiditis, Graves' disease, Autoimmune Oophoritis, Endometriosis, Autoimmune orchitis, Sjogren's syndrome, Autoimmune enteropathy, Coeliac disease, Crohn's disease, Microscopic colitis, Ulcerative colitis, thrombocytopenia, Adiposis, dolorosa, Adult-onset Still's, disease, Ankylosing, Spondylitis, CREST syndrome, Drug-induced lupus, Enthesitis-related arthritis, Eosinophilic fasciitis, Felty syndrome, IgG4-related disease, Juvenile,

Arthritis, Lyme disease (Chronic), Mixed connective tissue disease (MCTD), Palindromic rheumatism, Parry Romberg syndrome, Parsonage-Turner syndrome, Psoriatic arthritis, Reactive arthritis, Relapsing polychondritis, Retroperitoneal fibrosis, Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Schnitzler syndrome, Systemic Lupus Erythematosus (SLE), Undifferentiated connective tissue disease (UCTD), Dermatomyositis, Fibromyalgia, Inclusion body myositis, Myositis, Myasthenia gravis, Neuromyotonia, Paraneoplastic cerebellar degeneration, Polymyositis, Acute disseminated encephalomyelitis (ADEM), Acute motor axonal neuropathy, Anti-N-Methyl-D-Aspartate (anti-NMDA) Receptor Encephalitis, Balo concentric sclerosis, Bickerstaff's encephalitis, Chronic inflammatory demyelinating polyneuropathy, Guillain-Barre syndrome, Hashimoto's encephalopathy, Idiopathic inflammatory demyelinating diseases, Lambert-Eaton myasthenic syndrome, Multiple sclerosis, Oshtoran syndrome, Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus (PANDAS), Progressive inflammatory neuropathy, Restless leg syndrome, Stiff person syndrome, Sydenham chorea, Transverse myelitis, Autoimmune retinopathy, Autoimmune uveitis, Cogan syndrome, Graves ophthalmopathy, Intermediate uveitis, Ligneous conjunctivitis, Mooren's ulcer, Neuromyelitis optica, Opsoclonus myoclonus syndrome, Optic neuritis, Scleritis, Susac's syndrome, Sympathetic ophthalmia, Tolosa-Hunt syndrome, Autoimmune inner ear disease (AIED), Ménière's disease, Behcet's disease, Eosinophilic granulomatosis with polyangiitis (EGPA), Giant cell arteritis, Granulmatosis with polyangiitis (GPA), IgA vasculitis (IgAV), Kawasaki's disease, Leukocytoclastic vasculitis, Lupus vasculitis, Rheumatoid vasculitis, Microscopic polyangiitis (MPA), Polyarteritis nodosa (PAN), Polymyalgia rheumatica, Vasculitis, Primary Immune Deficiency, and the like.

Other examples of potential autoimmune disorders and diseases, as well as autoimmune comorbidities that can be treated with the compounds described herein include, but are not limited to, Chronic fatigue syndrome, Complex regional pain syndrome, Eosinophilic esophagitis, Gastirtis, Interstitial lung disease, POEMS syndrome, Raynaud's phenomenon, Primary immunodeficiency, Pyoderma gangrenosum, Agammaglobulinemia, Anyloidosis, Anyotrophic lateral sclerosis, Anti-tubular basement membrane nephritis, Atopic allergy, Atopic dermatitis, Autoimmune peripheral neuropathy, Blau syndrome, Castleman's disease, Chagas disease, Chronic obstructive pulmonary disease, Chronic recurrent multifocal osteomyelitis, Complement component 2 deficiency, Contact dermatitis, Cushing's syndrome, Cutaneous

leukocytoclastic angiitis, Dego' deiase, Eczema, Eosinophilic gastroenteritis, Eosinophilic pneumonia, Erythroblastosis fetalsis, Fibrodysplasia ossificans progressive, Gastrointestinal pemphigoid, Hypogammaglobulinemia, Idiopathic giant-cell myocarditis, Idiopathic pulmonary fibrosis, IgA nephropathy, Immunregulatory lipoproteins, IPEX syndrome, Ligenous conjunctivitis, Majeed syndrome, Narcolepsy, Rasmussen's encephalitis, Schizophrenia, Serum sickness, Spondyloathropathy, Sweet's syndrome, Takayasu's arteritis, and the like.

In some embodients, the the autoimmune disorder does not comprise pemphigus Vulgaris, pemphigus. In some embodmeints, the autoimmune disorder does not comprise pemphigus foliaceus. In some embodiments, the autoimmune disorder does not comprise bullous pemphigoid. In some embodiments, the autoimmune disorder does not comprise Goodpasture's Disease. In some embodiments, the autoimmune disorder does not comprise psoriasis. In some embodiments, the autoimmune disorder does not comprise a skin disorder. In some embodiments, the disorder does not comprise a neoplastic disorder, e.g., cancer.

THERAPEUTIC COMPOUNDS

A therapeutic compound comprises a specific targeting moiety functionally associated with an effector binding/modulating moiety. In some embodiments, the specific targeting moiety and effector binding/modulating moiety are linked to one another by a covalent or noncovalent bond, e.g., a covalent or non-covalent bond directly linking the one to the other. In other embodiments, a specific targeting moiety and effector binding/modulating moiety are linked, e.g., covalently or noncovalently, through a linker moiety. E.g., in the case of a fusion polypeptide, a polypeptide sequence comprising the specific targeting moiety and a polypeptide sequence can be directly linked to one another or linked through one or more linker sequences. In some embodiments, the linker moiety comprises a polypeptide. Linkers are not, however, limited to polypeptides. In some embodiments, a linker moiety comprises other backbones, e.g., a non-peptide polymer, e.g., a PEG polymer. In some embodiments, a linker moiety can comprise a particle, e.g., a nanoparticle, e.g., a polymeric nanoparticle. In some embodiments, a linker moiety can comprise a branched molecule, or a dendrimer. However, in embodiments where the effector binding/modulating moiety comprises an ICIM binding/modulating moiety (which binds an effector like PD-1) structures that result in clustering in the absence of target

binding should be avoided as they may cause clustering in the absence of target binding. Thus in embodiments, the therapeutic compound has a structure, e.g., the copies of an ICIM are sufficiently limited, such that clustering in the absence of target binding is minimized or substantially eliminated, or eliminated, or is sufficiently minimized that substantial systemic immune suppression does not occur.

In some embodiments, a therapeutic compound comprises a polypeptide comprising a specific targeting moiety covalently or non-covalently conjugated to an effector binding/modulating moiety. In some embodiments, a therapeutic molecule comprises a fusion protein having comprising a specific targeting moiety fused, e.g., directly or through a linking moiety comprising one or more amino acid residues, to an effector binding/modulating moiety. In some embodiments, a therapeutic molecule comprises a polypeptide comprising a specific targeting moiety linked by a non-covalent bond or a covalent bond, e.g., a covalent bond other than a peptide bond, e.g., a sulfhydryl bond, to an effector binding/modulating moiety.

In some embodiments, a therapeutic compound comprises polypeptide, e.g., a fusion polypeptide, comprising:

1.a) a specific targeting moiety comprising a target specific binding polypeptide;

1.b) a specific targeting moiety comprising a target ligand binding molecule;

1.c) a specific targeting moiety comprising an antibody molecule;

1.d) a specific targeting moiety comprising a single chain antibody molecule, e.g., a scFv domain; or

1.e) a specific targeting moiety comprising a first of the light or heavy chain variable region of an antibody molecule, and wherein the other variable region is covalently or non covalently associated with the first;

and

2.a) an effector binding/modulating moiety comprising an effector specific binding polypeptide;

2.b) an effector binding/modulating moiety comprising an effector ligand binding molecule;

2.c) an effector binding/modulating moiety comprising an antibody molecule;

2.d) an effector binding/modulating moiety comprising a single chain antibody molecule, e.g., a scFv domain; or

2.e) an effector binding/modulating moiety comprising a first of the light or heavy chain variable region of an antibody molecule, and wherein the other variable region is covalently or non covalently associated with the first.

In some embodiments, a therapeutic compound comprises 1.a and 2.a.

In some embodiments, a therapeutic compound comprises 1.a and 2.b.

In some embodiments, a therapeutic compound comprises 1.a and 2.c.

In some embodiments, a therapeutic compound comprises 1.a and 2.d.

In some embodiments, a therapeutic compound comprises 1.a and 2.e.

In some embodiments, a therapeutic compound comprises 1.b and 2.a.

In some embodiments, a therapeutic compound comprises 1.b and 2.b.

In some embodiments, a therapeutic compound comprises 1.b and 2.c.

In some embodiments, a therapeutic compound comprises 1.b and 2.d.

In some embodiments, a therapeutic compound comprises 1.b and 2.e.

In some embodiments, a therapeutic compound comprises 1.c and 2.a.

In some embodiments, a therapeutic compound comprises 1.c and 2.b.

In some embodiments, a therapeutic compound comprises 1.c and 2.c.

In some embodiments, a therapeutic compound comprises 1.c and 2.d.

In some embodiments, a therapeutic compound comprises 1.c and 2.e.

In some embodiments, a therapeutic compound comprises 1.d and 2.a.

In some embodiments, a therapeutic compound comprises 1.d and 2.b.

In some embodiments, a therapeutic compound comprises 1.d and 2.c.

In some embodiments, a therapeutic compound comprises 1.d and 2.d.

In some embodiments, a therapeutic compound comprises 1.d and 2.e.

In some embodiments, a therapeutic compound comprises 1.e and 2.a.

In some embodiments, a therapeutic compound comprises 1.e and 2.b.

In some embodiments, a therapeutic compound comprises 1.e and 2.c.

In some embodiments, a therapeutic compound comprises 1.e and 2.d.

In some embodiments, a therapeutic compound comprises 1.e and 2.e.

Therapeutic compounds disclosed herein can, for example, comprise a plurality of effector binding/modulating and specific targeting moieties. Any suitable linker or platform can be used to present the plurality of moieties. The linker is typically coupled or fused to one or more effector binding/modulating and targeting moieties.

In some embodiments, two (or more) linkers associate, either covalently or noncovelaently, e.g., to form a hetero or homo-dimeric therapeutic compound. E.g., the linker can comprise an Fc region and two Fc regions associate with one another. In some embodiments of a therapeutic compound comprising two linker regions, the linker regions can self associate, e.g., as two identical Fc regions. In some embodiments of a therapeuticcompound comprising two linker regions, the linker regions are not capable of, or not capable of substantial, self association, e.g., the two Fc regions can be members of a knob and hole pair.

Non-limiting exemplary configurations of therapeutic compounds comprise the following (e.g., in N to C terminal order):

R1---Linker Region A—R2

R3---Linker Region B---R4,

wherein,

R1, R2, R3, and R4, each independently comprises an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, ICSM binding/modulating moiety, or an SM binding/modulating moiety; a specific targeting moiety; or is absent;

Linker Region A and Linker B comprise moieties that can associate with one another, e.g., Linker A and Linker B each comprises an Fc moiety provided that an effector binding/modulating moiety and a specific targeting moiety are present.

In some embodiments:

R1 comprises an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, ICSM binding/modulating moiety, or an SM binding/modulating moiety, or is absent;

R2 comprises a specific targeting moiety, or is absent;

R3 comprises an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, ICSM binding/modulating moiety, or an SM binding/modulating moiety, or is absent;

R4 comprises a specific targeting moiety, or is absent;

Linker Region A and Linker B comprise moieties that can associate with one another, e.g., Linker A and Linker B each comprises an Fc moiety, provided that one of R1 or R3 is present and one of R2 or R4 is present.

In some embodiments:

R1 comprises a specific targeting moiety, or is absent;

R2 comprises an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, ICSM binding/modulating moiety, or an SM binding/modulating moiety, or is absent;

R3 comprises a specific targeting moiety, or is absent;

R4 comprises an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, ICSM binding/modulating moiety, or an SM binding/modulating moiety, or is absent;

Linker Region A and Linker B comprise moieties that can associate with one another, e.g., Linker A and Linker B each comprises an Fc moiety, provided that one of R1 or R3 is present and one of R2 or R4 is present.

Non-limiting examples include, but are not limited to:

R1	Linker Region A	R2	R3	Linker Region B	R4	Other
HCVR and LCVR	Fc Region	fcFv	HCVR and LCVR	Fc Region	scFv	Self Pairing Linker Regions
HCVR and LCVR	Fc Region	fcFv	HCVR and LCVR	Fc Region	scFv	Non-Self Pairing linker regions
HCVR and	Fc Region	fcFv	HCVR	Fc Region	scFv	Self Pairing

LCVR (or absent)			and LCVR (or absent)			Linker Regions One of R1 or R3 is absent.
HCVR and LCVR (or absent)	Fc Region	fcFv	HCVR and LCVR (or absent)	Fc Region	scFv	Non-Self Pairing Linker Regions One of R1 or R3 is absent.
HCVR and LCVR	Fc Region	fcFv (or absent)	HCVR and LCVR	Fc Region	scFv (or absent)	Self Pairing linker regions One of R2 or R4 is absent.
HCVR and LCVR	Fc Region	fcFv (or absent)	HCVR and LCVR	Fc Region	scFv (or absent)	Non-Self Pairing linker regions One of R2 or R4 is absent.
HCVR and LCVR	Fc Region	fcFv	HCVR and LCVR	Fc Region	scFv	Self Pairing Linker Regions R1 and R3 are the same
HCVR and LCVR	Fc Region	fcFv	HCVR and LCVR	Fc Region	scFv	Non-Self Pairing linker regions R1and R3 are different

HCVR and LCVR	Fc Region	fcFv	HCVR and LCVR	Fc Region	scFv	Self Pairing Linker Regions R2 and R4 are the same
HCVR and LCVR	Fc Region	fcFv	HCVR and LCVR	Fc Region	scFv	Non-Self Pairing linker regions R2and R4 are different
HCVR and LCVR: refers to an moiety comprising an antigen binding portion of a heavy and light chian variable region, typically with the heavy chain fused to the Linker region. Self pairing: wherein a liker region can pair with itself, e.g., an Fc region that can pair a copy of itself. Non-Self Pairing: wherein a Linker Region does not pair with itself, or does not substantially pair with itself, e.g., an Fc region does not or does not significantly pair with itself, e.g., wherein Linker Region A and Linker Region B are members of a knob and hole pair.						

In some embodiments,:

R1, R2, R3 and R4 each independently comprise: an effector binding modulating moiety that activates an inhibitory receptor on an immune cell, e.g., a T cell or a B cell, e.g., a PD-L1 molecule or a functional anti-PD-1 antibody molecule (an agonist of PD-1); a specific targeting moiety; or is absent;

provided that an effector binding moiety and a specific targeting moiety are present.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties).

In some embodiments,:

R1 and R3 independently comprise an effector binding modulating moiety that activates an inhibitory receptor on an immune cell, e.g., a T cell or a B cell, e.g., a PD-L1 molecule or an functional anti-PD-1 antibody molecule (an agonist of PD-1); and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties).

In some embodiments,:

R1 and R3 independently comprise a functional anti-PD-1 antibody molecule (an agonist of PD-1); and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties).

In some embodiments,:

R1 and R3 independently comprise specific targeting moieties, e.g., an anti-tissue antigen antibody; and

R2 and R4 independently comprise a functional anti-PD-1 antibody molecule (an agonist of PD-1), e.g., an scFv molecule.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties).

In some embodiments,:

R1 and R3 independently comprise a PD-L1 molecule (an agonist of PD-1); and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen; and

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties).

In some embodiments,:

R1 and R3 independentlycomprise specific targeting moieties, e.g., an anti- tissue antigen antibody; and

R2 and R4 independently comprise a PD-L1 molecule (an agonist of PD-1).

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties).

In some embodiments,:

R1, R2, R3 and R4 each independently comprise: an SM binding/modulating moiety which modulates, e.g., binds and inhibits, sequesters, degrades or otherwise neutralizes a substance, e.g., a soluble molecule that modulates an immune response, e.g., ATP or AMP, e.g., a CD39 molecule or a CD73 molecule; a specific targeting moiety; or is absent;

provided that an SM binding/modulating moiety and a specific targeting moiety are present.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1 and R3 independently comprise an SM binding/modulating moiety which modulates, e.g., binds and inhibits, sequesters, degrades or otherwise neutralizes a substance, e.g., a soluble molecule that modulates an immune response, e.g., ATP or AMP, e.g., a CD39 molecule or a CD73 molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1 and R3 independently comprise a CD39 molecule or a CD73 molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1 and R3 each comprises a CD39 molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen; and

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1 and R3 each comprises a CD73 molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

One of R1 and R3 comprises a CD39 molecule and the other comprises a CD73 molecule; and R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1, R2, R3 and R4 each independently comprise: an HLA-G molecule; a specific targeting moiety; or is absent;

provided that an HLA-G molecule and a specific targeting moiety are present.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1 and R3 each comprise an HLG-A molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1 and R3 each comprise an agonistic anti-LILRB1 antibody molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1 and R3 each comprise an agonistic anti-KIR2DL4 antibody molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1 and R3 each comprise an agonistic anti-LILRB2 antibody molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1 and R3 each comprise an agonistic anti-NKG2A antibody molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

one of R1 and R3 comprises a first moiety chosen from, and the other comprises a different moiety chosen from: an antagonistic anti-LILRB1 antibody molecule, an agonistic anti-KR2DL4 antibody molecule, and an agonistic anti-NKG2A antibody molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

one of R1 and R3 comprises an antagonistic anti-LILRB1 antibody molecule and the other comprises an agonistic anti-KR2DL4 antibody molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

one of R1 and R3 comprises an antagonistic anti-LILRB1 antibody molecule and the other comprises an agonistic anti-NKG2A antibody molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In an embodiment:

R1, R2, R3 and R4 each independently comprise: an IL-2 mutein molecule; a specific targeting moiety; or is absent;

provided that an IL-2 mutein molecule and a specific targeting moiety are present.

In an embodiment Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

One of R1, R2, R3 and R4 comprises an IL-2 mutein molecule, one comprises an anti-GITR antibody molecule, e.g., an anti-GITR antibody molecule that inhibits binding of GITRL to GITR, and one comprises a specific targeting moiety;

In an embodiment Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In an embodiment:

R1 and R3 each comprise an IL-2 mutein molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In an embodiment Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In an embodiment:

one of R1 and R3 comprises a GARP binding molecule, e.g., an anti-GARP antibody molecule or a GITR binding molecule, e.g., an anti-GITR antibody molecule and the other comprises an IL-2 mutein molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In an embodiment Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In an embodiment:

one of R1 and R3 comprises a GARP binding molecule, e.g., an anti-GARP antibody molecule and the other comprises an IL-2 mutein molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In an embodiment Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In an embodiment:

one of R1 and R3 comprises a GITR binding molecule, e.g., an anti-GITR antibody molecule, and the other comprises an IL-2 mutein molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In an embodiment Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments,:

R1, R2, R3 and R4 each independently comprise: an effector binding modulating moiety that activates an inhibitory receptor on a B cell, e.g., an anti-FCRL antibody molecule, e.g., an agonistic anti-FCRL antibody molecule; a specific targeting moiety; or is absent;

provided that an effector binding moiety and a specific targeting moiety are present.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In embodiment the anti-FCRL molecule comprises: an anti-FCRL antibody molecule, e.g., an agonistic anti-FCRL antibody molecule, directed to FCRL1, FCRL2, FCRL3, FCRL4, FCRL5, or FCRL6.

In some embodiments,:

R1 and R3 each comprises an agonistic anti-FCRL antibody molecule; and

R2 and R4 independentlycomprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In embodiment the anti-FCRL molecule comprises: an anti-FCRL antibody molecule, e.g., an agonistic anti-FCRL antibody molecule directed to FCRL1, FCRL2, FCRL3, FCRL4, FCRL5, or FCRL6.

In some embodiments,:

R1 and R3 independentlycomprise specific targeting moieties, e.g., antibody molecules against a tissue antigen; and

R2 and R4 each comprises an anti-FCRL antibody molecule, e.g., an agonistic anti-FCRL antibody molecule, e.g., an scFv molecule.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In embodiment the anti-FCRL molecule comprises: an anti-FCRL antibody molecule, e.g., an agonistic anti-FCRL antibody molecule directed to FCRL1, FCRL2, FCRL3, FCRL4, FCRL5, or FCRL6.

In some embodiments,:

One of R1, R2, R3 and R4comprises an anti-BCR antibody molecule, e.g., an antagonistic anti-BCR antibody molecule, one comprises an anti FCRL antibody molecule, and one comprises a specific targeting moiety.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In some embodiments, the anti-FCRL molecule comprises: an anti-FCRL antibody molecule, e.g., an agonistic anti-FCRL antibody molecule directed to FCRL1, FCRL2, FCRL3, FCRL4, FCRL5, or FCRL6.

In some embodiments,:

One of R1, R2, R3 and R4comprises a bispecfic antibody molecule comprising an anti-BCR antibody molecule, e.g., an antagonistic anti- BCR antibody molecule, and an anti FCRL antibody molecule, and one comprises a specific targeting moiety;

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

In embodiment the anti-FCRL molecule comprises: an anti-FCRL antibody molecule, e.g., an agonistic anti-FCRL antibody molecule directed to FCRL1, FCRL2, FCRL3, FCRL4, FCRL5, or FCRL6.

In some embodiments,:

R1, R2, R3 and R4 each independently comprise:

i) an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, ICSM binding/modulating moiety, or an SM binding/modulating moiety, that minimizes or inhibits T cell activity, expansion, or function (a T cell effector binding/modulating moiety);

ii) an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, ICSM binding/modulating moiety, or an SM binding/modulating moiety, that minimizes or inhibits B cell activity, expansion, or function (a B cell effector binding/modulating moiety);

iii) a specific targeting moiety; or

iv) is absent;

provided that, a T cell effector binding/modulating moiety, a B cell effector binding/modulating moiety, and a specific targeting moiety are present.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties).

In some embodiments, one of R1, R2, R3, and R4 comprises an agonistic anti-PD-1 antibody and one comprises an HLA-G molecule.

In some embodiments, one of R1, R2, R3, and R4 comprises an SM binding/modulating moiety, e.g., a CD39 molecule or a CD73 molecule. In some embodiments, one of R1, R2, R3, and R4 comprises an entity that binds, activates, or maintains, a regulatory immune cell, e.g., a Treg cell or a Breg cell, for example, an IL-2 mutein molecule.

In some embodiments, one of R1, R2, R3, and R4 comprises an agonistic anti-PD-1 antibody, or one comprises an HLA-G molecule, and one comprises an IL-2 mutein molecule. In some embodiments, the PD-1 antibody is replaced with a IL-2 mutein molecule. In some embodiments, one of R1, R2, R3, and R4 comprises an agonistic anti-PD-1 antibody, one comprises an HLA-G molecule, and one comprises CD39 molecule or a CD73 molecule. In some embodiments, the PD-1 antibody is replaced with a IL-2 mutein molecule.

Linker Regions

As discussed elsewhere herein specific targeting and effector binding/modulating moieties can be linked by linker regions. Any linker region described herein can be used as a linker. For example, linker Regions A and B can comprise Fc regions. In some embodiments, a therapeutic compound comprises a Linker Region that can self-associate. In some embodiments, a therapeutic compound comprises a Linker Region that has a moiety that minimizes self association, and typically Linker Region A and Linker Region B are heterodimers. Linkers also include glycine/serine linkers. In some embodiments, the linker can comprise one or more repeats of GGGGS (SEQ ID NO: 23). In some embodiments, the linker comprises 1, 2, 3, 4, or 5 repeats of SEQ ID NO: 23. In some embodiments, the linker comprises or GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 22) GGGGSGGGGSGGGGS (SEQ ID NO: 30). These linkers can be used in any of the therapeutic compounds or compositions provided herein.

The linker region can comprise a Fc region that has been modified (*e.g.* mutated) to produce a heterodimer. In some embodiments, the CH3 domain of the Fc region can be mutated. Examples of such Fc regions can be found in, for example, U.S. Patent No. 9,574,010, which is hereby incorporated by reference in its entirety. The Fc region as defined herein comprises a CH3 domain or fragment thereof, and may additionally comprise one or more addition constant region domains, or fragments thereof, including hinge, CH1, or CH2. It will be understood that the numbering of the Fc amino acid residues is that of the EU index as in Kabat et al., 1991, NIH Publication 91-3242, National Technical Information Service, Springfield, Va. The "EU index as set forth in Kabat" refers to the EU index numbering of the human IgG1 Kabat antibody. For convenience, Table B of U.S. Patent No. 9,574,010 provides the amino acids numbered according to the EU index as set forth in Kabat of the CH2 and CH3 domain from human IgG1, which is hereby incorporated by reference. Table 1.1 of U.S. Patent No. 9,574,010 provides mutations of variant Fc heterodimers that can be used as linker regions. Table 1.1 of U.S. Patent No. 9,574,010 is hereby incorporated by reference.

In some embodiments, the Linker Region A comprises a first CH3 domain polypeptide and a the Linker Region B comprises a second CH3 domain polypeptide, the first and second CH3 domain polypeptides independently comprising amino acid modifications as compared to a wild-type CH3 domain polypeptide, wherein the first CH3 domain polypeptide comprises amino

acid modifications at positions T350, L351, F405, and Y407, and the second CH3 domain polypeptide comprises amino acid modifications at positions T350, T366, K392 and T394, wherein the amino acid modification at position T350 is T350V, T3501, T350L or T350M; the amino acid modification at position L351 is L351Y; the amino acid modification at position F405 is F405A, F405V, F405T or F405S; the amino acid modification at position Y407 is Y407V, Y407A or Y407I; the amino acid modification at position T366 is T366L, T366I, T366V, or T366M, the amino acid modification at position K392 is K392F, K392L or K392M, and the amino acid modification at position T394 is T394W, and wherein the numbering of amino acid residues is according to the EU index as set forth in Kabat.

In some embodiments, the amino acid modification at position K392 is K392M or K392L. In some embodiments, the amino acid modification at position T350 is T350V. In some embodiments, the first CH3 domain polypeptide further comprises one or more amino acid modifications selected from Q347R and one of S400R or S400E. In some embodiments, the second CH3 domain polypeptide further comprises one or more amino acid modifications selected from L351Y, K360E, and one of N390R, N390D or N390E. In some embodiments, the first CH3 domain polypeptide further comprises one or more amino acid modifications selected from Q347R and one of S400R or S400E, and the second CH3 domain polypeptide further comprises one or more amino acid modifications selected from L351Y, K360E, and one of N390R, N390D or N390E. In some embodiments, the amino acid modification at position T350 is T350V. In some embodiments, the amino acid modification at position F405 is F405A. In some embodiments, the amino acid modification at position Y407 is Y407V. In some embodiments, the amino acid modification at position T366 is T366L or T366I. In some embodiments, the amino acid modification at position F405 is F405A, the amino acid modification at position Y407 is and Y407V, the amino acid modification at position T366 is T366L or T366I, and the amino acid modification at position K392 is K392M or K392L. In some embodiments, the first CH3 domain polypeptide comprises the amino acid modifications T350V, L351Y, S400E, F405V and Y407V, and the second CH3 domain polypeptide comprises the amino acid modifications T350V, T366L, N390R, K392M and T394W. In some embodiments, the first CH3 domain polypeptide comprises the amino acid modifications T350V, L351Y, S400E, F405T and Y407V, and the second CH3 domain polypeptide comprises the amino acid modifications T350V, T366L, N390R, K392M and T394W. In some embodiments,

the first CH3 domain polypeptide comprises the amino acid modifications T350V, L351Y, S400E, F405S and Y407V, and the second CH3 domain polypeptide comprises the amino acid modifications T350V, T366L, N390R, K392M and T394W. In some embodiments, the first CH3 domain polypeptide comprises the amino acid modifications T350V, L351Y, S400E, F405A and Y407V, and the second CH3 domain polypeptide comprises the amino acid modifications T350V, L351Y, T366L, N390R, K392M and T394W. In some embodiments, the first CH3 domain polypeptide comprises the amino acid modifications Q347R, T350V, L351Y, S400E, F405A and Y407V, and the second CH3 domain polypeptide comprises the amino acid modifications T350V, K360E, T366L, N390R, K392M and T394W. In some embodiments, the first CH3 domain polypeptide comprises the amino acid modifications T350V, L351Y, S400R, F405A and Y407V, and the second CH3 domain polypeptide comprises the amino acid modifications T350V, T366L, N390D, K392M and T394W. In some embodiments, the first CH3 domain polypeptide comprises the amino acid modifications T350V, L351Y, S400R, F405A and Y407V, and the second CH3 domain polypeptide comprises the amino acid modifications T350V, T366L, N390E, K392M and T394W. In some embodiments, the first CH3 domain polypeptide comprises the amino acid modifications T350V, L351Y, S400E, F405A and Y407V, and the second CH3 domain polypeptide comprises the amino acid modifications T350V, T366L, N390R, K392L and T394W. In some embodiments, the first CH3 domain polypeptide comprises the amino acid modifications T350V, L351Y, S400E, F405A and Y407V, and the second CH3 domain polypeptide comprises the amino acid modifications T350V, T366L, N390R, K392F and T394W.

In some embodiments, an isolated heteromultimer comprising a heterodimeric CH3 domain comprising a first CH3 domain polypeptide and a second CH3 domain polypeptide, the first CH3 domain polypeptide comprising amino acid modifications at positions F405 and Y407, and the second CH3 domain polypeptide comprising amino acid modifications at positions T366 and T394, wherein: (i) the first CH3 domain polypeptide further comprises an amino acid modification at position L351, and (ii) the second CH3 domain polypeptide further comprises an amino acid modification at position K392, wherein the amino acid modification at position F405 is F405A, F405T, F405S or F405V; and the amino acid modification at position Y407 is Y407V, Y407A, Y407L or Y407I; the amino acid modification at position T394 is T394W; the amino acid modification at position L351 is L351Y; the amino acid modification at position K392 is

K392L, K392M, K392V or K392F, and the amino acid modification at position T366 is T366I, T366L, T366M or T366V, wherein the heterodimeric CH3 domain has a melting temperature (Tm) of about 70.degree. C. or greater and a purity greater than about 90%, and wherein the numbering of amino acid residues is according to the EU index as set forth in Kabat.

In some embodiments, the Linker Region A comprises a first CH3 domain polypeptide and a t Linker Region B comprises a second CH3 domain polypeptide, wherein the first CH3 domain polypeptide comprising amino acid modifications at positions F405 and Y407, and the second CH3 domain polypeptide comprising amino acid modifications at positions T366 and T394, wherein: (i) the first CH3 domain polypeptide further comprises an amino acid modification at position L351, and (ii) the second CH3 domain polypeptide further comprises an amino acid modification at position K392, wherein the amino acid modification at position F405 is F405A, F405T, F405S or F405V; and the amino acid modification at position Y407 is Y407V, Y407A, Y407L or Y407I; the amino acid modification at position T394 is T394W; the amino acid modification at position L351 is L351Y; the amino acid modification at position K392 is K392L, K392M, K392V or K392F, and the amino acid modification at position T366 is T366I, T366L, T366M or T366V, wherein the heterodimeric CH3 domain has a melting temperature (Tm) of about 70 C. or greater and a purity greater than about 90%, and wherein the numbering of amino acid residues is according to the EU index as set forth in Kabat. In some embodiments, the amino acid modification at position F405 is F405A. In some embodiments, the amino acid modification at position T366 is T366I or T366L. In some embodiments, the amino acid modification at position Y407 is Y407V. In some embodiments, the amino acid modification at position F405 is F405A, the amino acid modification at position Y407 is Y407V, the amino acid modification at position T366 is T366I or T366L, and the amino acid modification at position K392 is K392L or K392M. In some embodiments, the amino acid modification at position F405 is F405A, the amino acid modification at position Y407 is Y407V, the amino acid modification at position T366 is T366L, and the amino acid modification at position K392 is K392M. In some embodiments, the amino acid modification at position F405 is F405A, the amino acid modification at position Y407 is Y407V, the amino acid modification at position T366 is T366L, and the amino acid modification at position K392 is K392L. In some embodiments, the amino acid modification at position F405 is F405A, the amino acid modification at position Y407 is Y407V, the amino acid modification at position T366 is T366I, and the amino acid modification

at position K392 is K392M. In some embodiments, the amino acid modification at position F405 is F405A, the amino acid modification at position Y407 is Y407V, the amino acid modification at position T366 is T366I, and the amino acid modification at position K392 is K392L. In some embodiments, the first CH3 domain polypeptide further comprises an amino acid modification at position S400 selected from S400D and S400E, and the second CH3 domain polypeptide further comprises the amino acid modification N390R. In some embodiments, the amino acid modification at position F405 is F405A, the amino acid modification at position Y407 is Y405V, the amino acid modification at position S400 is S400E, the amino acid modification at position T366 is T366L, and the amino acid modification at position K392 is K392M.

In some embodiments, the modified first and second CH3 domains are comprised by an Fc construct based on a type G immunoglobulin (IgG). The IgG can be an IgG1, IgG2, IgG3 or IgG4.

Other Linker Region A and Linger Region B comprising variant CH3 domains are described in U.S. Patent Nos. 9,499,634 and 9,562,109, each of which is incorporated by reference in its entirety.

A Linker Region A and Linker Region B can be complementary fragments of a protein, e.g., a naturally occurring protein such as human serum albumin. In embodiments, one of Linker Region A and Linker Region B comprises a first, e.g., an N terminal fragment of the protein, e.g., hSA, and the other comprises a second, e.g., a C terminal fragment of the protein, e.g., has. In an embodiment the fragments comprise an N terminal and a C terminal fragment. In an embodiment the fragments comprise two internal fragments. Typically the fragments do not overlap. In an embodiment the First and second fragment, together, provide the entire sequence of the original protein, e.g., hSA. The first fragment provides a N terminus and a C terminus for linking, e.g., fusing, to other sequences, e.g., sequences of R1, R2, R3, or R4 (as defined herein).

The Linker Region A and the Linker Region B can be derived from albumin polypeptide. In some embodiments, the albumin polypeptide is selected from native human serum albumin polypeptide and human alloalbumin polypeptide. The albumin polypeptide can be modified such that the Linker Region A and Linker Region B interact with one another to form heterodimers. Examples of modified albumin polypeptides are described in U.S. Patent Nos. 9,388,231 and 9,499,605, each of which is hereby incorporated by reference in its entirety.

Accordingly, provided herein are multifunctional heteromultimer proteins of the formula R1---Linker Region A—R2 and R3---Linker Region B---R4,. wherein the Linker Region A and Linker Region B form a heteromultimer. In some embodiments, the Linker Region A comprises a first polypeptide and the Linker Region B comprises a second polypeptide; wherein each of said first and second polypeptides comprises an amino acid sequence comprising a segment of an albumin polypeptide selected from native human serum albumin polypeptide and human alloalbumin polypeptide; wherein said first and second polypeptides are obtained by segmentation of said albumin polypeptide at a segmentation site, such that the segmentation results in a deletion of zero to 3 amino acid residues at the segmentation site; wherein said first polypeptide comprises at least one mutation selected from A194C, L198C, W214C, A217C, L331C and A335C, and said second polypeptide comprises at least one mutation selected from L331C, A335C, V343C, L346C, A350C, V455C, and N458C; and wherein said first and second polypeptides self-assemble to form a quasi-native structure of the monomeric form of the albumin polypeptide.

In some embodiments, the segmentation site resides on a loop of the albumin polypeptide that has a high solvent accessible surface area (SASA) and limited contact with the rest of the albumin structure, b) the segmentation results in a complementary interface between the transporter polypeptides. These segmentation sites are described, for example, in U.S. Patent No. 9,388,231, which is hereby incorporated by reference in its entirety.

In some embodiments, the first polypeptide comprises residues 1-337 or residues 1-293 of the albumin polypeptide with one or more of the mutations described herein. In some embodiments, the second polypeptide comprises residues of 342-585 or 304-585 of the albumin polypeptide with one or more of the mutations described herein. In some embodiments, the first polypeptide comprises residues 1-339, 1-300, 1-364, 1-441, 1-83, 1-171, 1-281, 1-293, 1-114, 1-337, or 1-336 of the albumin protein. In some embodiments, the second polypeptide comprises residues 301-585, 365-585, 442-585, 85-585, 172-585, 282-585, or 115-585, 304-585, 340-585, or 342-585 of the albumin protein.

In some embodiments, the first and second polypeptide comprise the residues of the albumin protein as shown in the table below. The sequence of the albumin protein is described below.

First Polypeptide Residues	Second Polypeptide Residues

1-300	301-585
1-364	365-585
1-441	442-585
1-83	85-585
1-171	172-585
1-281	282-585
1-114	115-585
1-339	340-585
1-337	342-585
1-293	304-585
1-336	342-585

In some embodiments, the first and second polypeptides comprise a linker that can form a covalent bond with one another, such as a disulfide bond. A non-limiting example of the linker is a peptide linker. In some embodiments, the peptide linker comprises GGGGS. The linker can be fused to the C-terminus of the first polypeptide and the N-terminus of the second polypeptide. The linker can also be used to attach the moieties described herein without abrogating the ability of the linkers to form a disulfide bond. In some embodiments, the first and second polypeptides do not comprise a linker that can form a covalent bond. In some embodiments, the first and second polypeptides have the following substitutions.

First Polypeptide Substitution	Second Polypeptide Substitution
A217C	V343C
L331C	A350C
A217C	L346C
W214C	V343C
A335C	L346C
L198C	V455C
A217C	A335C
A217C	L331C

L198C	N458C
A194C	V455C

The sequence of the albumin polypeptide can be The sequence of human albumin is as shown, in the post-protein form with the N-terminal signaling residues removed (MKWVTFISLLFLFSSAYSRGVFRR)

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHV
KLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATL
RETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEV
DVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRY
KAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCA
SLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKV
HTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKP
LLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEA
KDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCA
AADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKF
QNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEA
KRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVN
RRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKK
QTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDK
ETCFAEEGKKLVAASQAALGL (human albumin)

In some embodiments, the Linker Region A and the Linker Region B form a heterodimer as described herein.

In some embodiments, the polypetide comprises at the N-terminus an antibody comprised of F(ab')2 on an IgG1 Fc backbone fused with scFvs on the C-terminus of the IgG Fc backbone. In some embodiments, the IgG Fc backbone is a IgG1 Fc backbone. In some embodiments, the IgG1 backbone is replaced with a IgG4 backbone, IgG2 backbone, or other similar IgG backbone. The IgG backbones described in this paragraph can be used throughout this application where a Fc region is referred to as part of the therapeutic compound. Thus, in some embodiments, the antibody comprised of F(ab')2 on an IgG1 Fc backbone can be an anti-MAdCAM antibody or an anti-PD-1 antibody on an IgG1 Fc or any other targeting moiety or effector binding/modulating moiety provided herein. In some embodiments, the The scFV segments fused to the C-terminus could be an anti-PD-1 antibody, if the N-terminus region is an anti-MAdCAM antibody, or anti-MAdCAM antibody, if the N-terminus region is an anti-PD-1 antibody. In this non-limiting example, the N-terminus can be the targeting moiety, such as any one of the ones provided for herein, and the C-terminus can be the effector binding/modulating moiety, such as any of the ones provided for herein. Alternatively, in some embodiments, the N-

terminus can be the effector binding/modulating moiety, such as any one of the ones provided for herein, and the C-terminus can be the targeting moiety, such as any of the ones provided for herein.

In some embodiments, the N-terminus can be the targeting moiety, such as any one of the ones provided for herein, and the C-terminus can be the effector binding/modulating moiety, such as any of the ones provided for herein.

In some embodiments, the therapeutic compound comprises two polypeptides that homodimerize. In some embodiments, the N-terminus of the polypeptide comprises an effector binding/modulating moiety that is fused to a human IgG1 Fc domain (*e.g.* CH2 and/or CH3 domains). In some embodiments, the C-terminus of the Fc domain is another linker that is fused to the targeting moiety. Thus, in some embodiments, the molecule could be represented using the formula of R1-Linker A-Fc Region-Linker B-R2, wherein R1 can be an effector binding/modulating moiety, R2 is a targeting moiety, Linker A and Linker B are independently linkers as provided for herein. In some embodiments, Linker 1 and Linker 2 are different.

In some embodiments, the molecule could be represented using the formula of R1-Linker A-Fc Region-Linker B-R2, wherein R1 can be a targeting moiety, R2 is an effector binding/modulating moiety, Linker A and Linker B are independently linkers as provided for herein. In some embodiments, Linker A and Linker B are different. The linkers can be chosen from the non-limiting exmaples provided for herein. In some embodiments, R1 and R2 are independently selected from F(ab')2 and scFV antibody domains. In some embodiments, R1 and R2 are different antibody domains. In some embodiments, the scFV is in the VL-VH domain orientation.

In some embodiments, the therapeutic compound is a bispecific antibody. In some embodiments, the bispecific antibodies are comprised of four polypeptide chains comprising the following:

Chain 1: nt-VH1-CH1-CH2-CH3-Linker A-scFv[VL2-Linker B-VH2]-ct

Chain 2: nt-VH1-CH1-CH2-CH3-Linker A-scFv[VL2-Linker B-VH2]-ct

Chain 3: nt-VL1-CL-ct

Chain 4: nt-VL1-CL-ct,

wherein chains 1 and 2 are identical to eachother, and chains 3 and 4 are identical to each other,

wherein chain 1 forms a homodimer with chain 2; and chain 3 and 4 associate with chain 1 and chain 2. That is, when each light chain associates with each heavy chain, VL1 associates with VH1 and CL associates with CH1 to form two functional Fab units. Without being bound to any particular theory, each scFv unit is intrinsically functional since VL2 and VH2 are covalently linked in tandem with a linker as provided herein (*e.g.* GGGGSG (SEQ ID NO: 23), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 22), or GGGGSGGGGSGGGGS (SEQ ID NO: 30). The sequences of Linker A and Linker B, which are independent of one another can be the same or different and as otherwise described throughout the present application. Thus, in some embodiments, Linker A comprises GGGGS (SEQ ID NO: 23), or two repeats thereof, GGGGSGGGGSGGGGS (SEQ ID NO: 30), or GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 22). In some embodiments, Linker B comprises GGGGS (SEQ ID NO: 23), or two repets thereof, GGGGSGGGGSGGGGS (SEQ ID NO: 30), or GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 22). The scFv may be arranged in the NT-VH2-VL2-CT or NT-VL2-VH2-CT orientation. NT or nt stands for N-terminus and CT or ct stands for C-terminus of the protein. CH1, CH2, and CH3 are the domains from the IgG Fc region, and CL stands for Constant Light chain, which can be either kappa or lambda family light chains. The other definitions stand for the way they are normally used in the art.

In some embodiments, the VH1 and VL1 domains are derived from the effector molecule and the VH2 and VL2 domains are derived from the targeting moiety. In some embodiments the VH1 and VL1 domains are derived from a targeting moiety and the VH2 and VL2 domains are derived from an effector binding/modulating moiety.

In some embodiments, the VH1 and VL1 domains are derived from an anti-PD-1 antibody, and the VH2 and VL2 domains are derived from an anti-MAdCAM antibody. In some embodiments the VH1 and VL1 domains are derived from an anti-MAdCAM antibody and the VH2 and VL2 domains are derived from an anti-PD-1 antibody.

In some embodiments, Linker A comprises 1, 2, 3, 4, or 5 GGGGS (SEQ ID NO: 23) repeats. In some embodiments, Linker B comprises 1, 2, 3, 4, or 5 GGGGS (SEQ ID NO: 23) repeats. For the avoidance of doubt, the sequences of Linker A and Linker B, which are used throughout this application, are independent of one another. Therefore, in some embodiments, Linker A and Linker B can be the same or different. In some embodiments, Linker A comprises GGGGS (SEQ ID NO: 23), or two repeats thereof, GGGGSGGGGSGGGGS (SEQ ID NO: 30),

or GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 22). In some embodiments, Linker B comprises GGGGS (SEQ ID NO: 23), or two repeats thereof, GGGGSGGGGSGGGGS (SEQ ID NO: 30), or GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 22).

In some embodiments, the therapeutic compound comprises a light chain and a heavy chain. In some embodiments, the light and heavy chain begin at the N-terminus with the VH domain of a targeting moiety followed by the CH1 domain of a human IgG1, which is fused to a Fc region (*e.g.* CH2-CH3) of human IgG1. In some embodiments, at the c-terminus of the Fc region is fused to a linker as provided herein, such as but not limited to, GGGGS (SEQ ID NO: 23), or two or three repeats thereof, or GGGGSGGGGSGGGGS (SEQ ID NO: 22). The linekr can then be fused to an effector binding/modulating moiety, such as any one of the effector moieties provided for herein. The polypeptides can homodimerize because through the heavy chain homodimerization, which results in a therapeutic compound having two effector moieties, such as two anti-PD-1 antibodies. In this orientation, the targeting moiety is an IgG format, there are two Fab arms that each recognize binding partner of the targeting moiety, for example, MAdCAM being bound by the anti-MAdCAM targeting moiety.

In some embodiments, if the therapeutic compound comprises a Fc portion, the Fc domain, (portion) bears mutations to render the Fc region "effectorless," that is unable to bind FcRs. The mutations that render Fc regions effectorless are known. In some embodiments, the mutations in the Fc region, which is according to the known numbering system, are selected from the group consisting of: K322A, L234A, L235A, G237A, L234F, L235E, N297, P331S, or any combination thereof. In some embodiments, the Fc mutations comprises a mutation at L234 and/or L235 and/or G237. In some embodiments, the Fc mutations comprise L234A and/or L235A mutations, which can be referred to as LALA mutations. In some emobdimetns, the Fc mutations comprise L234A, L235A, and G237A mutations.

Disclosed herein are Linker Region polypeptides, therapeutic peptides, and nucleic acids encoding the polypeptides (*e.g.* therapeutic compounds), vectors comprising the nucleic acid sequences, and cells comprising the nucleic acids or vectors

Therapeutic compounds can comprise a plurality of specific targeting moieties. In some embodiments, the therapeutic compound comprises a plurality one specific targeting moiety, a plurality of copies of a donor specific targeting moiety or a plurality of tissue specific targeting moieties. In some embodiments, a therapeutic compound comprises a first and a second donor

specific targeting moiety, e.g., a first donor specific targeting moiety specific for a first donor target and a second donor specific targeting moiety specific for a second donor target, e.g., wherein the first and second target are found on the same donor tissue. In some embodiments, the therapeutic compound comprises e.g., a first specific targeting moiety for a tissue specific target and a second specific targeting moiety for a second target, e.g., wherein the first and second target are found on the same or different target tissue,

In some embodiments, a therapeutic compound comprises a plurality of effector binding/modulating moieties each comprising an ICIM binding/modulating moiety, the number of ICIM binding/modulating moieties is sufficiently low that clustering of the ICIM binding/modulating moiety's ligand on immune cells (in the absence of target binding) is minimized, e.g., to avoid systemic agonizing of immune cells in the absence of binding of the therapeutic compound to target.

POLYPEPTIDES DERIVED FROM REFERENCE, E.G., HUMAN POLYPEPTIDES

In some embodiments, a component of a therapeutic molecule is derived from or based on a reference molecule, e.g., in the case of a therapeutic molecule for use in humans, from a naturally occurring human polypeptide. E.g., In some embodiments, all or a part of a CD39 molecule, a CD73 molecule, a cell surface molecule binder, a donor specific targeting moiety, an effector ligand binding molecule, an ICIM binding/modulating moiety, an IIC binding/modulating moiety, an inhibitory immune checkpoint molecule ligand molecule, an inhibitory molecule counter ligand molecule, a SM binding/modulating moiety, a specific targeting moiety, a target ligand binding molecule, or a tissue specific targeting moiety, can be based on or derived from a naturally occurring human polypeptide. E.g., a PD-L1 molecule can be based on or derived from a human PD-L1 sequence.

In some embodiments, a therapeutic compound component, e.g., a PD-L1 molecule:

a) comprises all or a portion of, e.g., an active portion of, a naturally occurring form of the human polypeptide;

b) comprises all or a portion of, e.g., an active portion of, a human polypeptide having a sequence appearing in a database, e.g., GenBank database, on January 11, 2017, a naturally occurring form of the human polypeptide that is not associated with a disease state ;

c) comprises a human polypeptide having a sequence that differs by no more than 1, 2, 3, 4, 5, 10, 20, or 30 amino acid residues from a sequence of a) or b);

d) comprises a human polypeptide having a sequence that differs at no more than by 1, 2, 3, 4, 5 10, 20, or 30 % its amino acids residues from a sequence of a) or b);

e) comprises a human polypeptide having a sequence that does not differ substantially from a sequence of a) or b); or

f) comprises a human polypeptide having a sequence of c), d),or e) that does not differ substantially in a biological activity, e.g., ability to enhance or inhibit an immune response, from a human polypeptide having the sequence of a) or b).

In some embodiments, therapeutic compounds can comprise a plurality of effector binding/modulating moieties. For example, a therapeutic compound can comprise two or more of the following selected from:

(a) an ICIM binding/modulating moiety; (b) an IIC binding/modulating moiety; (c) an SM binding/modulating moiety, or (d) an ICSM binding/modulating moiety. In some embodiments, for example, a therapeutic compound can comprise a plurality, e.g., two, ICIM binding/modulating moieties (wherein they are the same or different); by way of example, two that activate or agonize PD-1; a plurality, e.g., two, IIC binding/modulating moieties; (wherein they are the same or different); a plurality, e.g., two, SM binding/modulating moieties (wherein they are the same or different), or a plurality, e.g., tow, ICSM binding/modulating moieties (wherein they are the same or different). In some embodiments, the therapeutic compound can comprise an ICIM binding/modulating moiety and an IIC binding/modulating moiety; an ICIM binding/modulating moiety and an SM binding/modulating moiety; an IIC binding/modulating moiety and an SM binding/modulating moiety, an ICIM binding/modulating moiety and an ICSM binding/modulating moiety; an IIC binding/modulating moiety and an ICSM binding/modulating moiety; or an ICSM binding/modulating moiety and an SM binding/modulating moiety. In some embodiments, the therapeutic compound comprises a plurality of targeting moieties. In some embodiments, the targeting moieties can be the same or different.

PHARMACEUTICAL COMPOSITIONS AND KITS

In another aspect, the present embodiments provide compositions, e.g., pharmaceutically acceptable compositions, which include a therapeutic compound described herein, formulated together with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically

acceptable carrier" includes any and all solvents, dispersion media, isotonic and absorption delaying agents, and the like that are physiologically compatible.

The carrier can be suitable for intravenous, intramuscular, subcutaneous, parenteral, rectal, local, ophthalmic, topical, spinal or epidermal administration (e.g. by injection or infusion). As used herein, the term “carrier” means a diluent, adjuvant, or excipient with which a compound is administered. In some embodiments, pharmaceutical carriers can also be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical carriers can also be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. The carriers can be used in pharmaceutical compositions comprising the therapeutic compounds provided for herein.

The compositions and compounds of the embodiments provided for herein may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical compositions are in the form of injectable or infusible solutions. In some embodiments, the mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In some embodiments, the therapeutic molecule is administered by intravenous infusion or injection. In another embodiment, the therapeutic molecule is administered by intramuscular or subcutaneous injection. In another embodiment, the therapeutic molecule is administered locally, e.g., by injection, or topical application, to a target site.
The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high therapeutic molecule

concentration. Sterile injectable solutions can be prepared by incorporating the active compound (i.e., therapeutic molecule) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, a therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. Therapeutic compositions can also be administered with medical devices known in the art.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of a therapeutic compound is 0.1-30 mg/kg, more preferably 1-25 mg/kg. Dosages and therapeutic regimens of the therapeutic compound can be determined by a skilled artisan. In certain embodiments, the therapeutic compound is administered by injection (e.g., subcutaneously or intravenously) at a dose of about 1 to 40 mg/kg, e.g., 1 to 30 mg/kg, e.g., about 5 to 25 mg/kg, about 10 to 20 mg/kg, about 1 to 5 mg/kg, 1 to 10 mg/kg, 5 to 15 mg/kg, 10 to 20 mg/kg, 15 to 25 mg/kg, or about 3 mg/kg. The dosing schedule can vary from e.g., once a week to once every 2, 3, or 4 weeks. In one embodiment, the therapeutic compound is administered at a dose from about 10 to 20 mg/kg every other week. The therapeutic compound can be administered by intravenous infusion at a rate of more than 20 mg/min, e.g., 20-40 mg/min, and typically greater than or equal to 40 mg/min to reach a dose of about 35 to 440 mg/m2, typically about 70 to 310 mg/m2, and more typically, about 110 to 130 mg/m2. In embodiments, the infusion rate of about 110 to 130 mg/m2 achieves a level of about 3 mg/kg. In other embodiments, the therapeutic compound can be administered by intravenous infusion at a rate of less than 10 mg/min, e.g., less than or equal to 5 mg/min to reach a dose of about 1 to 100 mg/m2, e.g., about 5 to 50 mg/m2, about 7 to 25 mg/m2, or, about 10 mg/m2. In some embodiments, the therapeutic compound is infused over a period of about 30 min. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the

individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

The pharmaceutical compositions may include a "therapeutically effective amount" or a "prophylactically effective amount" of a therapeutic molecule. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of a therapeutic molecule may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the therapeutic compound to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of a therapeutic molecule t is outweighed by the therapeutically beneficial effects. A "therapeutically effective dosage" preferably inhibits a measurable parameter, e.g., immune attack at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit a measurable parameter, e.g., immune attack, can be evaluated in an animal model system predictive of efficacy in transplant rejection or autoimmune disorders. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition in vitro by assays known to the skilled practitioner.

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Also within the scope of the embodiments is a kit comprising a therapeutic compound described herein. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, a therapeutic agent, or an agent useful for chelating, or otherwise coupling, a therapeutic molecule to a label or other therapeutic agent, or a radioprotective composition; devices or other materials for preparing the a therapeutic molecule for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

In some embodiments, embodiments provided herein also include, but are not limited to:

1. A therapeutic compound comprising:

i) a specific targeting moiety selected from:

a) a donor specific targeting moiety which, e.g., preferentially binds a donor target; or

b) a tissue specific targeting moiety which, e.g., preferentially binds target tissue of a subject; and

ii) an effector binding/modulating moiety selected from:

(a) an immune cell inhibitory molecule binding/modulating moiety (ICIM binding/modulating moiety);

(b) an immunosuppressive immune cell binding/modulating moiety (IIC binding/modulating moiety); or

(c) an effector binding/modulating moiety that, as part of a therapeutic compound, promotes an immuno-suppressive local microenvironment, e.g., by providing in the proximity of the target, a substance that inhibits or minimizes attack by the immune system of the target (SM binding/modulating moiety).

2. The therapeutic compound of embodiment 1, wherein the effector binding/modulating moiety directly binds and activates an inhibitory receptor.

3. The therapeutic compound of embodiment 2, wherein the effector binding/modulating moiety is an inhibitory immune checkpoint molecule.

4. The therapeutic compound of any of embodiments 1-3, wherein the effector binding/modulating moiety is expressed by an immune cell.

5. The therapeutic compound of embodiment 4, wherein the immune cell contributes to an unwanted immune response.

6. The therapeutic compound of embodiments 4 or 5, wherein the immune cell causes a disease pathology.

7. The therapeutic compound of embodiment 1, wherein the ability of the therapeutic molecule to agonize the molecule to which the effector binding/modulating binds is greater, e.g., 2, 5, 10, 100, 500, or 1,000 times greater, when the therapeutic compound is bound to a target through the targeting moiety than when the therapeutic compound is not bound to target through the targeting moiety.

8. The therapeutic compound of embodients 1-7, wherein when binding as a monomer (or binding when the therapeutic compound is not multimerized), to its cognate ligand, e.g., an

inhibitory immune checkpoint molecule, does not agonize or substantially agonize, the cognate ligand.

9. The therapeutic compound of embodiments 1-8, wherein at a therapeutically effective dose of the therapeutic compound, there is significant, systemic agonization of the molecule to which the effector binding/modulating moiety binds.

10. The therapeutic compound of embodiments 1-9, wherein at a therapeutically effective dose of the therapeutic compound, the agonization of the molecule to which the effector binding/modulating moiety binds occurs substantially only at a target site to which the targeting moiety binds to.

11. The therapeutic compound of embodiments 1-9, wherein binding of the therapeutic compound to its cognate ligand, e.g., an inhibitory immune checkpoint molecule, does not inhibit, or does not substantially inhibit, binding of an endogenous counter ligand to the cognate ligand, e.g., an inhibitory immune checkpoint molecule.

12. The therapeutic compound of embodiments 1-11, wherein binding of the effector binding/modulating moiety to its cognate ligand, inhibits the binding of an endogenous counter ligand to the cognate ligand of the effector binding/modulating moiety by less than 60, 50, 40, 30, 20, 10, or 5%.

14. The therapeutic compound of embodiments 1-11, wherein binding of the effector binding/modulating moiety to the cognate ligand, results in substantially no antagonism of the cognate ligand of the effector binding/modulating molecule.

15. The therapeutic compound of embodiment 1, wherein the effector binding/modulating moiety comprises an ICIM binding/modulating moiety.

16. The therapeutic compound of embodiment 15, wherein the effector binding/modulating moiety comprises an ICIM binding/modulating moiety comprising an inhibitory immune checkpoint molecule ligand molecule.

17. The therapeutic compound of embodiment 16, wherein the inhibitory immune molecule counter-ligand molecule comprises a PD-L1 molecule.

18. The therapeutic compound of embodiment 15, wherein the ICIM is wherein the inhibitory immune molecule counter ligand molecule engages a cognate inhibitory immune checkpoint molecule selected from PD-1, KIR2DL4, LILRB1, LILRB, or CTLA-4.

19. The therapeutic compound of embodiment 18, wherein the ICIM is an antibody.

20. The therapeutic compound of embodiment 18, wherein the ICIM comprises an antibody that binds to PD-1, KIR2DL4, LILRB1, LILRB, or CTLA-4.

21. The therapeutic compound of embodiment 20, wherein the antibody is an antibody that binds to PD-1.

22. The therapeutic compound of embodiment 20,wherein the antibody is an antibody that binds to PD-1 and is a PD-1 agonist.

23. The therapeutic compound of embodiment 20,wherein the antibody is an antibody that binds to PD-1 and is a PD-1 agonist when tethered at a target site.

24. The therapeutic compound of embodiment 16, wherein the inhibitory immune molecule counter-ligand molecule comprises a HLA-G molecule.

25. The therapeutic compound of embodiment 15, wherein the ICIM is wherein the inhibitory immune molecule counter ligand molecule engages a cognate inhibitory immune checkpoint molecule selected from PD-1, KIR2DL4, LILRB1, LILRB, or CTLA-4.

26. The therapeutic compound of embodiment 15, wherein the inhibitory immune molecule counter ligand molecule engages a cognate inhibitory immune checkpoint molecule selected from Table 1.

27. The therapeutic compound of embodiment 15, wherein when binding as a monomer, to its cognate inhibitory immune checkpoint molecule, does not agonize or substantially agonize the inhibitory immune checkpoint molecule.

28. The therapeutic compound of embdoiment 15, wherein the inhibitory immune molecule counter ligand has at least 60, 70, 80, 90, 95, 99, or 100% homology with a naturally occurring inhibitory immune checkpoint molecule ligand.

29 The therapeutic compound of embodiment 1, wherein the effector binding/modulating moiety comprises a ICIM binding/modulating moiety which comprises a functional antibody molecule to a cell surface inhibitory molecule.

30. The therapeutic compound of embodiment 1, wherein the cell surface inhibitory molecule is an inhibitory immune checkpoint molecule.

31. The compound of of embodiment 30, wherein the inhibitory immune checkpoint molecule is selected from PD-1, KIR2DL4, LILRB1, LILRB2, CTLA-4, or selected from Table 1.

32. The therapeutic compound of any of embodiments 1-31, wherein the level of systemic immune suppressionat a therapeutically effective dose of the therapeutic compound, is less than that given by the standard of care with a systemic immune suppressant (if relevant), or is less than that given by an equimolar amount of free (not as a component of a therapeutic compound), effector binding/modulating molecule.

33. The therapeutic compound of embodiment 1-32, wherein the level of systemic immune activation, e.g., at a therapeutically effective dose of the therapeutic compound, is less than that given by a equimolar amount of free (not as a component of a therapeutic compound), effector binding/modulating molecule.

34. The therapeutic compound of any one of embodiments 1-33, further comprising a second effector binding/modulating moiety.

35. The therapeutic compound of embodiment 34, wherein the second effector binding/modulating moiety, binds a different target than the effector binding/modulating moiety.

36. The therapeutic compound embodiments 34 or 35, wherein the second effector binding/modulating moiety comprises a IIC binding/modulating moiety.

. The therapeutic compound embodiments 34 or 35, wherein the second effector binding/modulating moiety comprises an SM binding/modulating moiety.

37. The therapeutic compound of embodiment 1, wherein the effector binding/modulating moiety comprises an IIC binding/modulating moiety.

38. The therapeutic compound of embodiment 1, wherein the effector binding/modulating moiety comprises an IIC binding/modulating moiety, which, increases, recruits or accumulates an immunosuppressive immune cell at the target site.

39. The therapeutic compound of embodiment 1, wherein the effector binding/modulating moiety comprises a cell surface molecule binder which binds or specifically binds, a cell surface molecule on an immunosuppressive immune cell.

40. The therapeutic compound of embodiment 1, wherein the effector binding/modulating moiety comprises a cell surface molecule ligand molecule that binds or specifically binds, a cell surface molecule on an immunosuppressive immune cell.

41. The therapeutic compound of embodiment 1, wherein the effector binding/modulating moiety comprises an antibody molecule that binds a cell surface molecule on an immunosuppressive immune cell.

42. The therapeutic compound of any of embodiments 38-41, wherein the immunosuppressive immune cell comprises a T regulatory cell, such as a a Foxp3+CD25+ T regulatory cell.

43. The therapeutic compound of any of embodiments 1-42, wherein the effector binding/modulating moiety binds GARP, and e.g., comprises an antibody molecule that binds GARP on GARP expressing immunosuppressive cells, e.g., Tregs.

44. The therapeutic compound of embodiment 1, wherein the effector binding/modulating moiety comprises an SM binding/modulating moiety.

45. The therapeutic compound of embodiment 44, wherein SM binding/modulating moiety promotes an immuno-suppressive local microenvironment.

46. The therapeutic compound of any of embodiments 44 and 45, wherein the effector molecule binding moiety increases the availability, e.g., by increasing the local concentration or amount, of a substance which inhibits immune cell function, e.g., a substance that inhibits the activation of an immune cell or the function of an activated immune cell.

47. The therapeutic compound of any of embodiments 44-46, wherein the effector molecule binding moiety binds and accumulate a soluble substance, e.g., an endogenous or exogenous substance, having immunosuppressive function.

48. The therapeutic compound of any of embodiments 44-47, wherein the effector molecule binding moiety decreases the availability, e.g., by decreasing the local concentration or amount, or sequestering, of a substance which promotes immune cell function, e.g., a substance that promotes the activation of an immune cell or the function of an activated immune cell.

49. The therapeutic compound of any one of embodiments 44-48, wherein SM binding/modulating moiety promotes an immuno-suppressive local microenvironment, e.g., by providing in the proximity of the target, a substance that inhibits or minimizes attack by the immune system of the target.

50. The therapeutic compound of any one of embodiments 44-49, wherein the SM binding/modulating moiety comprises a molecule that inhibits or minimizes attack by the immune system of the target.

51. The therapeutic compound any one of embodiments 44-50, wherein the SM binding/modulating moiety binds and/or accumulate a soluble substance, e.g., an endogenous or exogenous substance having immunosuppressive function.

52. The therapeutic compound any one of embodiments 44-51, wherein the SM binding/modulating moiety binds and/or inhibits, sequesters, degrades or otherwise neutralizes a substance, e.g., a soluble substance, typically and endogenous soluble substance, that promotes immune attack.

53. The therapeutic compound any one of embodiments 44-52, wherein the effector molecule binding moiety decreases the availability of ATP or AMP.

54. The therapeutic compound any one of embodiments 44-53, wherein SM binding/modulating moiety binds, or comprises, a substance, e.g., CD39 or CD73, that depletes a component that promotes immune effector cell function, e.g., ATP or AMP.

55. The therapeutic compound any one of embodiments 44-54, wherein the SM binding/modulating moiety comprises a CD39 molecule.

56. The therapeutic compound any one of embodiments 44-54, wherein the SM binding/modulating moiety comprises a CD73 molecule.

57. The therapeutic compound any one of embodiments 44-54, wherein the SM binding/modulating moiety comprises an anti-CD39 molecule.

58. The therapeutic compound any one of embodiments 44-54, wherein the SM binding/modulating moiety comprises an anti-CD73 antibody molecule.

59. The therapeutic compound any one of embodiments 44-54, wherein the effector molecule binding moiety comprises an immune-suprressive substance, e.g. a fragment an immunosuppressive protein.

60. The therapeutic compound any one of embodiments 44-54, wherein SM binding/modulating moiety comprises alkaline phosphatase molecule.

61. The therapeutic compound of embodiment 1, wherein the compound has the formula from N-terminus to C-terminus:

R1---Linker Region A—R2 or R3—Linker Region B—R4,

wherein,

R1, R2, R3, and R4, each independently comprises an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, or an SM binding/modulating moiety; a specific targeting moiety; or

is absent; provided that an effector binding/modulating moiety and a specific targeting moiety are present.

62. The therapeutic compound of embodiment 61, wherein each of Linker Region A and Linker Region B comprises an Fc region.

63. The therapeutic compound of embodiment 61, wherein one of R1 and R2 is anti-PD-1 antibody and one of R1 and R2 is an anti-MAdCAM antibody.

64. The therapeutic compound of embodiment 61, wherein one of R1 is anti-PD-1 antibody and one R2 is an anti-MAdCAM antibody.

65. The therapeutic compound of embodiment 61, wherein one of R1 is anti-MAdCAM antibody and one R2 is an anti-PD-1 antibody.

66. The therapeutic compound of embodiment 61, wherein one of R3 and R4 is anti-PD-1 antibody and one of R3 and R4 is an anti-MAdCAM antibody.

67. The therapeutic compound of embodiment 61, wherein one of R3 is anti-PD-1 antibody and one R4 is an anti-MAdCAM antibody.

68. The therapeutic compound of embodiment 61, wherein one of R3 is anti-MAdCAM antibody and one R4 is an anti-PD-1 antibody.

69. The therapeutic compound of any of embodiments 61-68, wherein the linker is absent.

70. The therapeutic compound of any of embodiments 61-68, wherein the linker is a Fc region.

71. The therapeutic compound of any of embodiments 61-68, wherein the linker is a glycine/serine linker, such as 1, 2, 3, 4, or 5 repeats of GGGGS (SEQ ID NO: 23).

72. The therapeutic compound of any of embodiments 61-68, wherein the linker comprises a Fc region and a glycine/serine linker, such as 1, 2, 3, 4, or 5 repeats of GGGGS (SEQ ID NO: 23).

73. The therapeutic compound of any of embodiments 61-72, wherein the PD-1 antibody is a PD-1 agonist.

74. The therapeutic compound of embodiment 61, wherein:

R1 and R3 independently comprise a functional anti-PD-1 antibody molecule (an agonist of PD-1); and R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

75. The therapeutic compound of any of embodiments 73 and 74, wherein:

R1 and R3 independently comprise specific targeting moieties, e.g., an anti-tissue antigen antibody; and R2 and R4 independently comprise a functional anti-PD-1 antibody molecule (an agonist of PD-1).

76. The therapeutic compound of any of embodiments 73 and 74, wherein:

R1, R2, R3 and R4 each independently comprise: an SM binding/modulating moiety which modulates, e.g., binds and inhibits, sequesters, degrades or otherwise neutralizes a substance, e.g., a soluble molecule that modulates an immune response, e.g., ATP or AMP, e.g., a CD39 molecule or a CD73 molecule; a specific targeting moiety; or is absent;

provided that an SM binding/modulating moiety and a specific targeting moiety are present.

77. The therapeutic compound of embodiment 61, wherein:

R1 and R3 independently comprise a CD39 molecule or a CD73 molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

78. The therapeutic compound of embodiment 77, wherein:

R1 and R3 each comprises a CD39 molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

79. The therapeutic compound of embodiments 61 or 77, wherein:

R1 and R3 each comprises a CD73 molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

80. The therapeutic compound of embodiment 61, wherein:

one of R1 and R3 comprises a CD39 molecule and the other comprises a CD73 molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

81. The therapeutic compound of embodiment 61, wherein:

R1, R2, R3 and R4 each independently comprise: an HLA-G molecule; a specific targeting moiety; or is absent;

provided that an HLA-G molecule and a specific targeting moiety are present.

82. The therapeutic compound of embodiments 61 or 81, wherein:

R1 and R3 each comprise an HLG-A molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

83. The therapeutic compound of any of embodiments 81 and 82, wherein:

R1 and R3 each comprise an agonistic anti-LILRB1 antibody molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

84. The therapeutic compound of any of embodiments 81 and 82, wherein:

R1 and R3 each comprise an agonistic anti-KIR2DL4 antibody molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

In some embodiments, Linker A and Linker B comprise Fc moieties (e.g., self pairing Fc moieties or Fc moieties that do not, or do not substantially self pair).

85. The therapeutic compound of any of embodiments 81-84, wherein:

R1 and R3 each comprise an agonistic anti-LILRB2 antibody molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

86. The therapeutic compound of any of embodiments 81-84, wherein:

R1 and R3 each comprise an agonistic anti-NKG2A antibody molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

87. The therapeutic compound of any of embodiments 81-84, wherein:

one of R1 and R3 comprises a first moiety chosen from, and the other comprises a different moiety chosen from: an antagonistic anti-LILRB1 antibody molecule, an agonistic anti-KR2DL4 antibody molecule, and an agonistic anti-NKG2A antibody molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

88. The therapeutic compound of any of embodiments 81-84, wherein:

one of R1 and R3 comprises an antagonistic anti-LILRB1 antibody molecule and the other comprises an agonistic anti-KR2DL4 antibody molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

89. The therapeutic compound of any of embodiments 81-84, wherein:

one of R1 and R3 comprises an antagonistic anti-LILRB1 antibody molecule and the other comprises an agonistic anti-NKG2A antibody molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

89A. The therapeutic compound of any of embodiments 81-84wherein:

R1, R2, R3 and R4 each independently comprise: an IL-2 mutein molecule; a specific targeting moiety; or is absent; and

provided that an IL-2 mutein molecule and a specific targeting moiety are present.

89B. The therapeutic compound of embodiment 89A, wherein:

R1 and R3 each comprise an IL-2 mutein molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

89C. The therapeutic compound of embodiments 89A or 89B, wherein:

one of R1 and R3 comprises a MAdCAM binding molecule, e.g., an anti- MAdCAM antibody molecule or a GITR binding molecule, e.g., an anti-GITR antibody molecule and the other comprises an IL-2 mutein molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

89D. The therapeutic compound of embodiments 89A or 89B, wherein:

one of R1 and R3 comprises a GARP binding molecule, e.g., an anti-GARP antibody molecule and the other comprises an IL-2 mutein molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

89E. The therapeutic compound of embodiments 89A or 89B, wherein:

one of R1 and R3 comprises a GARP binding molecule, e.g., an anti-GARP antibody

molecule or a GITR binding molecule, e.g., an anti-GITR antibody molecule and the other comprises an IL-2 mutein molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

89F. The therapeutic compound of embodiments 89A or 89B, wherein:

one of R1 and R3 comprises a GARP binding molecule, e.g., an anti-GARP antibody molecule and the other comprises an IL-2 mutein molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

89G. The therapeutic compound of embodiments 89A or 89B, wherein:

one of R1 and R3 comprises a GITR binding molecule, e.g., an anti-GITR antibody molecule, and the other comprises an IL-2 mutein molecule; and

R2 and R4 independently comprise specific targeting moieties, e.g., scFv molecules against a tissue antigen.

89H. The therapeutic compound of embodiment 1, wherein the compound is a polypeptide or protein, wherein the polypeptide or protein comprises a targeting moiety that binds to a target cell and an effector binding/modulating moiety, wherein the effector binding/modulating moiety is a IL-2 mutant polypeptide (IL-2 mutein).

89I. The therapeutic compound of embodiment 89H, wherein the targeting moiety comprises an antibody that binds to a target protein on the surface of a target cell.

89J. The therapeutic compound of embodiment 89I, wherein the antibody is an antibody that binds to MAdCAM, OAT1 (SLC22A6), OCT2 (SLC22A2), FXYD2, TSPAN7, DPP6, HEPACAM2, TMEM27, or GPR119.

89K. The therapeutic compound of embodiment 89I, wherein the IL-2 mutein binds to a receptor expressed by an immune cell.

89L. The therapeutic compound of embodiment 89I, wherein the the immune cell contributes to an unwanted immune response.

89M. The therapeutic compound of any of embodiments 89H-89L, wherein the immune cell causes a disease pathology.

89N. The therapeutic compound of any of embodiments 89H-89M, wherein the targeting moiety comprises an anti-MAdCAM antibody.

89O. The therapeutic compound of embodiment 89H, wherein the compound has the formula from N-terminus to C-terminus:

R1---Linker Region A—R2 or R3—Linker Region B—R4,

wherein,

R1, R2, R3, and R4, each independently comprises the effector binding/modulating moiety, the targeting moiety, or is absent.

89P. The therapeutic compound of embodiment 89O, wherein each of Linker Region A and Linker Region B comprises an Fc region.

89Q. The therapeutic compound of embodiments 89O or 89P or, wherein one of R1 and R2 is the IL-mutein antibody and one of R1 and R2 is an anti-MAdCAM antibody.

89R. The therapeutic compound of embodiments 89O, 89P, or 89Q, wherein R1 is the IL-mutein and R2 is an anti-MAdCAM antibody.

89S. The therapeutic compound of embodiments 89O, 89P, or 89Q, wherein one of R1 is anti-MAdCAM antibody and one R2 is an anti-PD-1 antibody.

89T. The therapeutic compound of embodiments 89O, 89P, or 89Q, wherein one of R3 and R4 is the IL-2 mutein and one of R3 and R4 is an anti-MAdCAM antibody.

89U. The therapeutic compound of embodiments 89O, 89P, or 89Q, wherein R3 is the IL-2 mutein and R4 is an anti-MAdCAM antibody.

89V. The therapeutic compound of embodiments 89O, 89P, or 89Q, wherein R3 is an anti-MAdCAM antibody and one R4 is the IL-2 mutein.

89W. The therapeutic compound of any of embodiments 89O-89W, wherein the linker is absent.

89X. The therapeutic compound of any of embodiments 89O-89W, wherein the linker is or comprises a Fc region.

89Y. The therapeutic compound of any of embodiments 89O-89W, wherein the linker comprises a glycine/serine linker.

89X. The therapeutic compound of any of embodiments 89O-89W, wherein the linker comprises a sequence of GGGGSGGGGSGGGGSGGGGS, GGGGSGGGGSGGGGS, GGGGSGGGGS, or GGGGS.

89Y. The therapeutic compound of embodiment 89H, wherein the IL-2 mutein comprises a IL-2 sequence of SEQ ID NO: 6, wherein peptide comprises a mutation at a position that corresponds to position 53, 56, 80, or 118 of SEQ ID NO: 6.

89Z. The therapeutic compound of any of embodiments 89H-89Z, wherein the IL-2 mutein comprises a IL-2 sequence of SEQ ID NO: 6, wherein peptide comprises a mutation at a position that corresponds to position 53, 56, 80, or 118 of SEQ ID NO: 6.

89AA. The therapeutic compound of embodiment 89Y, wherein the mutation is a L to I mutation at position 53, 56, 80, or 118.

89BB. The therapeutic compound of embodiment 89Z, wherein the mutation is a L to I mutation at position 53, 56, 80, or 118.

89CC. The therapeutic compound of any of embodiments 89H-89BB, wherein the IL-2 mutein further comprises a mutation at one or more positions of 29, 31, 35, 37, 48, 69, 71, 74, 88, and 125 corresponding to those positions in SEQ ID NO: 6.

89DD. The therapeutic compound of any of embodiments 89H-89CC, wherein the IL-2 mutein further comprises a mutation at one or more of positions E15, H16, Q22, D84, E95, or Q126 or 1, 2, 3, 4, 5, or each of positions E15, H16, Q22, D84, E95, or Q126 is wild-type.

89EE. The therapeutic compound of any of embodiments 89H-89DD, wherein the mutation in the mutein is one or more of E15Q, H16N, Q22E, D84N, E95Q, or Q126E.

89FF. The therapeutic compound of any of embodiments 89H-89EE, wherein the mutein comprises a N29S mutation in SEQ ID NO: 6.

89GG. The therapeutic compound of any of embodiments 89H-89FF, wherein the mutein comprises a Y31S or a Y51H mutation.

89HH. The therapeutic compound of any of embodiments 89H-89GG, wherein the mutein comprises a K35R mutation.

89II. The therapeutic compound of any of embodiments 89H-89HH, wherein the mutein comprises a T37A mutation.

89JJ. The therapeutic compound of any of embodiments 89H-89II, wherein the mutein comprises a K48E mutation.

89KK. The therapeutic compound of any of embodiments 89H-89JJ, wherein the mutein comprises a V69A mutation.

89LL. The therapeutic compound of any of embodiments 89H-89KK, wherein the mutein comprises a N71R mutation.

89MM. The therapeutic compound of any of embodiments 89H-89LL, wherein the mutein comprises a Q74P mutation.

89NN. The therapeutic compound of any of embodiments 89H-89MM, wherein the mutein comprises a N88D or a N88R mutation.

89OO. The therapeutic compound of any of embodiments 89H-89NN,wherein the mutein comprises a C125A or C125S mutation.

89PP. The therapeutic compound of any of embodiments 89H-89OO, wherein the IL-2 mutein is fused or linked to a Fc peptide.

89PP1. The therapeutic compound of embodiment 89PP,wherein the Fc peptide comprises a mutation at one or more of positions of L234, L247, L235, L248, G237, and G250.

89PP2. The therapeutic compound of embodiment 89PP1, wherein the mutation is L to A or G to A mutation.

89PP3. The therapeutic compound of embodiment 89PP1, wherein the Fc peptide comprises L247A, L248A, and/or a G250A mutations (Kabat numbering).

89PP4. The therapeutic compound of embodiment 89PP1, wherein the Fc peptide comprises a L234A mutation, a L235A mutation, and/or a G237A mutation (EU numbering).

89QQ. The therapeutic compound of embodiment 89H, wherein the compound comprises a polypeptide comprising a first chain and a second chain that form the polypeptide, wherein

the first chain comprises:

V_H-H_c-Linker-C_1, wherein V_H is a variable heavy domain that binds to the target cell with a V_L domain of the second chain; H_c is a heavy chain of antibody comprising CH1-CH2-CH3 domain, the Linker is a glycine/serine linker, and C_1 is a IL-2 mutein fused or linked to a Fc protein in either the N-terminal or C-terminal orientation; and

the second chain comprises:

V_L-L_c, wherein V_L is a variable light chain domain that binds to the target cell with the V_H domain of the first chain, and the Lc domain is a light chain CK domain.

89QQ1. The therapeutic compound of embodiment 89QQ, wherein the VH and VL domain are anti-MAdCAM variable domains that bind to MAdCAM expressed on a cell.

89QQ2. The therapeutic compound of embodiment 89QQ or 89QQ1, wherein the IL-2 mutein comprises a mutation at a position that corresponds to position 53, 56, 80, or 118 of SEQ ID NO: 6.

89QQ3. The therapeutic compound of embodiment 89QQ2, wherein the mutation is a L to I mutation at position 53, 56, 80, or 118.

89QQ4. The therapeutic compound of embodiments 89QQ2 or 89QQ3, wherein the mutein further comprises a mutation at a position that corresponds to position 69, 75, 88, and/or 125, or any combination thereof.

89QQ5. The therapeutic compound of embodiments 89QQ2 or 89QQ3, wherein the IL-2 mutein comprises a mutation selected from the group consisting of: at one of L53I, L56I, L80I, and L118I and the mutations of V69A, Q74P, N88D or N88R, and optionally C125A or C125S.

89QQ6. The therapeutic compound of embodiment 89QQ5, wherein the IL-2 mutein comprises a L53I mutation.

89QQ7. The therapeutic compound of embodiment 89QQ5, wherein the IL-2 mutein comprises a L56I mutation.

89QQ8. The therapeutic compound of embodiment 89QQ5, wherein the IL-2 mutein comprises a L80I mutation.

89QQ9. The therapeutic compound of embodiment 89QQ5, wherein the IL-2 mutein comprises a L118I mutation.

89QQ10. The therapeutic compound of embodiment 89QQ5, wherein the IL-2 mutein does not comprises any other mutations.

89QQ11. The therapeutic compound of any one of embodiments 89QQ-89QQ10, wherein the Fc protein comprises L247A, L248A, and G250A mutations or a L234A mutation, a L235A mutation, and/or a G237A mutation according to KABAT numbering.

89QQ12. The therapeutic compound of any one of embodiments 89QQ-89QQ11, wherein the Linker comprises a sequence of GGGGSGGGGSGGGGS or GGGGSGGGGSGGGGSGGGGS.

89QQ13. The therapeutic compound of any one of embodiments 89QQ-89QQ11, wherein the polypeptide comprises a Fc peptide comprising a sequence described herein.

90. The therapeutic compound of any of embodiments 81-84, wherein:

one of R1, R2, R3 and R4comprises an anti-BCR antibody molecule, e.g., an antagonistic anti-BCR antibody molecule, one comprises an anti FCRL antibody molecule, and one comprises specific targeting moiety.

91. The therapeutic compound of embodiment 90, wherein:

the anti-FCRL molecule comprises: an anti-FCRL antibody molecule, e.g., an agonistic anti-FCRL antibody molecule, directed to FCRL1, FCRL2, FCRL3, FCRL4, FCRL5, or FCRL6.

92. The therapeutic compound of any of embodiments 81-84, wherein:

R1, R2, R3 and R4 each independently comprise:

i) an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, or an SM binding/modulating moiety, that minimizes or inhibits T cell activity, expansion, or function (a T cell effector binding/modulating moiety);

ii) an effector binding/modulating moiety, e.g., an ICIM binding/modulating moiety, an IIC binding/modulating moiety, or an SM binding/modulating moiety, that minimizes or inhibits B cell activity, expansion, or function (a B cell effector binding/modulating moiety);

iii) a specific targeting moiety; or

iv) is absent; provided that, a T cell effector binding/modulating moiety, a B cell effector binding/modulating moiety, and a specific targeting moiety are present.

93. The therapeutic compound of embodiment 92, wherein:

one of R1, R2, R3, and R4 comprises an agonistic anti-PD-1 antibody and one comprises an HLA-G molecule.

94. The therapeutic compound embodiments 92-93, wherein:

one of R1, R2, R3, and R4 comprises an SM binding/modulating moiety, e.g., a CD39 molecule or a CD73 molecule.

95. The therapeutic compound of any of embodiments 92-94, wherein:

one of R1, R2, R3, and R4 comprises an entity that binds, activates, or maintains, a regulatory immune cell, e.g., a Treg cell or a Breg cell.

96. The therapeutic compound of any of embodiments 92-95, wherein:

one of R1, R2, R3, and R4 comprises an agonistic anti-PD-1 antibody or one comprises an HLA-G molecule.

97. The therapeutic compound of embodiment 96, wherein:

one of R1, R2, R3, and R4 comprises an agonistic anti-PD-1 antibody, one comprises an HLA-G molecule, and one comprises CD39 molecule or a CD73 molecule.

98. The therapeutic compound of any of embodiments 1-97, wherein the effector binding/modulating moiety comprises a polypeptide.

99. The therapeutic compound of any of embodiments 1-98, wherein the effector binding/modulating moiety comprises a polypeptide having at least 5, 10, 20, 30, 40, 50, 150, 200 or 250 amino acid residiues.

100. The therapeutic compound of any of embodiments 1-99, wherein the effector binding/modulating moiety has a molecular weight of 5, 10, 15, 20, or 40 Kd.

101. The therapeutic compound of any of embodiments 1-100, wherein the effector binding/modulating moiety does not comprise an inhibitor of the expression of apolipoprotien CIII, protein kinase A, Src kinase, or Beta1 integrin.

102. The therapeutic compound of any of embodiments 1-100, wherein the effector binding/modulating moiety does not comprise an inhibitor of the activity of apolipoprotien CIII, protein kinase A, Src kinase, or Beta1 integrin.

103. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target a tissue selected from lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut tissue.

104. The therapeutic compound of any of embodiments 1-101, whewherein the therapeutic compound does not specficially target tubular cells, e.g., proximal tubular epithelial cells

105. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target TIE-2, APN, TEM4, TEM6, ICAM-1, nucleolin P2Z receptor, Trk-A, FLJ10849, HSPA12B, APP, or OX-45.

106. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target a luminally expressed protein.

107. The therapeutic compound of any of embodiments 1-101, wherein the donor target does not comprise a heart specific target.

108. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target lung tissue.

109. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target kidney tissue.

110. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially pancreas lung tissue.

111. The therapeutic compound of any of embodiments 1-101,, wherein the therapeutic compound does not specficially target gut tissue.

112. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target prostate tissue.

113. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target brain tissue.

114. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target CD71.

115. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target CD90.

116. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target MAdCAM.

117. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target albumin.

118. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target carbonic anhydrase IV.

119. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target ZG16-p.

120. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target dipeptidyl peptidase IV.

121. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target the luminal surface of a vascular endothelial cell membrane.

121. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target heart tissue.

122. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target a tumor, solid tumor, or the vascular of a solid tumor.

123. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target skin tissue.

124. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target epidermal tissue.

125. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target the basement membrane.

126. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target a Dsg polypeptide.

127. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target Dsg1.

128. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target Dsg3.

129. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target BP180.

130. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not specficially target desmoglein.

131. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not comprise a complement modulator, e.g., a compliment inhibitor, such as, but not limited to, those described in U.S. Patent No. 8,454,963, which is hereby incorporated by reference in its entirety.

133. The therapeutic compound of any of embodiments 1-101,wherein the therapeutic compound does not comprise an imaging agent.

134. The therapeutic compound of any of embodiments 1-101,wherein the therapeutic compound does not comprise an imaging agent selected from the group of: a radioactive agent, a radioisotope, a radiopharmaceutical, a contrast agent, a nanoparticle; an enzyme, a prosthetic group, a fluorescent material, a luminescent material, and a bioluminescent material, such as, but not limited to, those described in U.S. Patent No. 8,815,235, which is hereby incorporated by reference in its entirety.

135. The therapeutic compound of any of embodiments 1-101,wherein the therapeutic compound does not comprise a radionuclide, such as, but not limited to, those described in U.S. Patent No. 6,232,287, which is hereby incorporated by reference in its entirety.

136. The therapeutic compound of any of embodiments 1-101,which is not internalized by a donor cell to which it binds.

137. The therapeutic compound of any of embodiments 1-101,wherein the therapeutic compound does not enter the cell which is targeted by the specific targeting moiety.

138. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not kill the cell which is targeted by the specific targeting moiety.

139. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not enter the cell to which the effector binding/modulating moiety binds.

140. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not kill the cell to which the effector binding/modulating moiety binds.

141. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not comprise an autoantigenic peptide or polypeptide.

142. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not comprise an autoantigenic peptide or polypeptide, e.g., does not comprise a peptide or polypeptide against which the subject has autoantibodies.

143. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not comprise an antibody molecule derived from a mammal, e.g., a human, having an autoimmune disorder.

144. The therapeutic compound of any of embodiments 1-101, wherein the therapeutic compound does not comprise an antibody molecule derived from a mammal, e.g., a human, having acute mucocutaneous PV.

145. The therapeutic compound of any of embodiments 1-101, wherein the the therapeutic compound does not comprise an antibody molecule derived from a mammal, e.g., a human, having Goodpasture's Disease

146. The therapeutic compound of any of embodiments 1-101, wherein the the therapeutic compound does not comprise an antibody molecule derived from a mammal, e.g., a human, having pemphigus vulgaris.

141. The therapeutic compound of any of embodiments 1-146, comprising a donor specific targeting moiety.

142. The therapeutic compound of any of embodiments 141, that localizes preferentially to an implanted donor tissue, as opposed to tissue of a recipient.

143. The therapeutic compound of embodiments 141-142, wherein, the donor specific targeting moiety provides site-specific immune privilege for a transplant tissue, e.g., an organ, from a donor.

144. The therapeutic compound of embodiments 141-143, wherein the donor specific targeting moiety binds to a product, e.g., a polypeptide, of an allele present at a locus in the donor, which allele is not present at the locus in the recipient

145. The therapeutic compound of any of embodiments 141-144, wherein, the donor specific targeting moiety preferentially binds to an allele of a gene expressed on donor tissue, e.g., a transplant tissue, e.g., an organ, as compared with an allele of the gene expressed on subject tissue.

146. The therapeutic compound of embodiments 141-145, wherein, the donor specific targeting moiety has a binding affinity for an allele of a gene expressed on donor tissue, e.g., a transplant tissue, e.g., an organ, which is at least 2, 4, 5, 10, 50, 100, 500, 1,000, 5,000, or 10,000 fold greater than its affinity for an allele of the gene expressed on subject tissue.

147. The therapeutic compound of any of embodiments 141-146, wherein the donor specific targeting moiety binds to the product, e.g., a polypeptide, of an allele present at a locus in the donor, which allele is not present at the locus in the recipient.

148. The therapeutic compound of any one of embodiments 141-147, wherein the binding is sufficiently specific that, e.g., at a clinically effective dose of the therapeutic compound, unwanted, substantial, or clinically unacceptable, systemic immune suppression occurs.

149. The therapeutic compound of any one of embodiments 141-148, wherein the therapeutic compound accumulates at the target site, e.g., binding of the donor specific targeting moiety to results in accumulation of the therapeutic compound at the target site.

150. The therapeutic compound of any one of embodiments 141-149, wherein the donor specific targeting moiety binds a product of an allele of a locus selected from Table 2, e.g., the HLA locus, e.g., the HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ or HLA-DR locus, which allele is present in the donor but not the recipient. HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ or HLA-DR locus.

151. The therapeutic compound of any one of embodiments 141-150, wherein the donor specific targeting moiety binds an allele of HLA A , an allele of HLA-B , an allele of HLA-C ,, an allele of HLA-DP ,, an allele of HLA-, or an allele of HLA-.

152. The therapeutic compound of any one of embodiments 141-151, wherein the therapeutic compound is suitable for treating a subject that has, will have, or is in need of, a transplant.

153. The therapeutic compound of embodiment 152, wherein the transplant comprises all or part of an organ, e.g., a liver, kidney, heart, pancreas, thymus, skin or lung.

154. The therapeutic compound of any one of embodiments 141-153, wherein the donor specific targeting moiety comprises an antibody molecule.

155. The therapeutic compound of any one of embodiments 141-153, wherein the donor specific targeting moiety comprises a target specific binding polypeptide, or a target ligand binding molecule.

156. The therapeutic compound of any one of embodiments 1-155, comprising a tissue specific targeting moiety.

157. The therapeutic compound of embodiment 156, wherein the tissue specific targeting moiety is a molecule that specifically binds to MAdCAM.

158. The therapeutic compound of embodiment 156, wherein the tissue specific targeting moiety is an antibody that specifically binds to MAdCAM.

159. The therapeutic compound of any one of embodiments, 156-158, wherein the therapeutic compound is suitable for treating a subject having, or is at risk, or elevated risk, for having, an autoimmune disorder, e.g., an autoimmune disorder described herein.

160. The therapeutic compound of any of embodiments 156-159, wherein the therapeutic compound accumulates at the target site, e.g., binding of the tissue specific targeting moiety results in accumulation of the therapeutic compound at the target site.

161. The therapeutic compound of any of embodiments 156-160, wherein the therapeutic compound which localizes, preferentially to a target tissue, as opposed to other tissue of a subject.

162. The therapeutic compound of any of embodiments 156-161, wherein the therapeutic compound provides site-specific immune privilege for a subject target tissue, e.g., a target tissue undergoing, or at risk, or elevated risk, for, unwanted immune attack, e.g., in an autoimmune disorder.

163. The therapeutic compound of any of embodiments 156-161, wherein the tissue specific targeting moiety, as a component of the therapeutic compound, preferentially binds a subject target tissue undergoing unwanted immune attack, e.g., in an autoimmune disorder.

164. The therapeutic compound of any of embodiments 156-163, wherein a tissue specific targeting moiety binds to the product, e.g., a polypeptide, which is not present outside the target tissue, or is present at sufficiently low levels that, at therapeutic concentrations of therapeutic molecule, unacceptable levels of immune suppression are absent or substantially absent.

165. The therapeutic compound of any of embodiments 156-164, wherein, the tissue specific targeting moiety binds a product, or site on a product, which is more abundant in target tissue than in non-target tissue.

166. The therapeutic compound of any of embodiments 156-165, wherein, therapeutic compound binds a product, or a site on a product, that is present or expressed substantially exclusively on target tissue.

167 The therapeutic compound of any of embodiments 156-166, wherein the product, or site on a product, to which the specific targeting moiety binds, is sufficiently limited to the target tissue, that at therapeutically effective level of therapeutic compound, the subject does not suffer an unacceptable level, e.g., a clinically significant level, of systemic immune suppression.

168. The therapeutic compound of any of embodiments 156-167, wherein the therapeutic compound, preferentially binds to a target tissue or target tissue antigen, e.g., has a binding affinity for the target tissue or antigen that is greater for target antigen or tissue, e.g., at least 2, 4, 5, 10, 50, 100, 500, 1,000, 5,000, or 10,000 fold greater, than its affinity for than for non-target tissue or antigen present outside the target tissue.

169. The therapeutic compound of any of embodiments 156-168, wherein the tissue specific targeting moiety binds to a product, e.g., a polypeptide product, or site on a product, present at a preselected site, e.g., a site of unwanted immune response in an autoimmune disorder.

170. The therapeutic compound of any of embodiments 156-169, wherein therapeutic compound is suitable for the treatment of a subject having, or at risk, or elevated risk, for having, type1diabetes.

171. The therapeutic compound of any of embodiments 156-170, wherein the target tissue comprises pancreatic tissue, e.g., pancreatic islets or pancreatic beta cells, gut tissue (*e.g.* gut

endothelial cells), kidney tissue (*e.g.* kidney epithelial cells), or liver tissue (*e.g.* liver epithelial cells).

172. The therapeutic compound of any of embodiments 156-171, wherein the effector binding/modulating moiety or targeting moiety binds a polypeptide selected from those described herein, such as those listed in Table 3, e.g., SEZ6L2, LRP11, DISP2, SLC30A8, FXYD2, TSPAN7, or TMEM27.

173. The therapeutic compound of any of embodiments 156-168, wherein therapeutic compound is suitable for the treatment of a subject having, or at risk, or elevated risk, for having, multiple sclerosis.

174. The therapeutic compound of embodiment 173, wherein the target tissue comprises CNS tissue, myelin sheath, or myelin sheath of oligodendrocytes.

175. The therapeutic compound of any of embodiments 173-174, wherein the effector binding/modulating moiety or targeting moiety binds a polypeptide selected from those described herein and including, but not limited to, Table 3, e.g., MOG, PLP, or MBP.

176. The therapeutic compound of any of embodiments 156-168, wherein therapeutic compound is suitable for the treatment of a subject having, or at risk, or elevated risk, for having, cardiomyositis.

177. The therapeutic compound of embodiment 176, wherein the target tissue comprises cardiomyocytes, monocytes, macrophages, or myeloid cells.

178. The therapeutic compound of embodiments 176-177, wherein the effector binding/modulating moiety binds or the targeting moiety a polypeptide as described herein, incldugin, but not limited to those selected from Table 3, e.g., SIRPA (CD172a).

179. The therapeutic compound of any of embodiments 156-168, wherein therapeutic compound is suitable for the treatment of a subject having, or at risk, or elevated risk, for having, inflammatory bowel disease, autoimmune hepatitis (AIH); Primary Sclerosing Cholangitis (PSC); Primary Biliary Sclerosis ; (PBC); or transplant.

180. The therapeutic compound of any of embodiments 156-168, wherein the subject with has, is at risk or elevated risk for having Crohn's disease or ulcerative colitis.

181. The therapeutic compound of embodiments 179 or 180, wherein the target tissue comprises gut cells, such as gut epithelial cells or liver cells, such as liver epithelial cells.

182. The therapeutic compound of embodiments 179-181, wherein the effector binding/modulating moiety binds a polypeptide as described herein, including, but not limited to those selected from Table 3, e.g., PD-1.

182. The therapeutic compound of embodiments 179-181, wherein the targeting moiety binds a polypeptide as described herein, including, but not limited to MAdCAM.

183. The therapeutic compound of any of embodiments 156-168, wherein therapeutic compound is suitable for the treatment of a subject having, or at risk, or elevated risk, for having, rheumatoid arthritis.

184. The therapeutic compound of embodiment 183, wherein the target tissue comprises cardiomyocytes, monocytes, macrophages, or myeloid cells.

185. The therapeutic compound of embodiments 183 or 184, wherein the effector binding/modulating moiety or targeting moiety binds a polypeptide selected from Table 3, e.g., SIRPA (CD172a).

186. The therapeutic compound of any of embodiments 156-185, wherein the tissue specific targeting moiety comprises an antibody molecule.

187. The therapeutic compound of any of embodiments 156-185, wherein the tissue specific targeting moiety comprises a target specific binding polypeptide, or a target ligand binding molecule.

188. The therapeutic compound of any of embodiments 156-185, wherein the tissue specific targeting moiety comprises a target specific binding polypeptide binds to MAdCAM.

189. The therapeutic compound of any of embodiments 1-188, wherein the therapeutic compound binds a cell surface molecule of an immune effector cell, e.g., a T cell, B cell, NK cell, or other immune cell, which cell propagates a pro-immune response.

190. The therapeutic compound of any of embodiments 1-189, wherein the therapeutic compound reduces the ability of an immune effector cell, e.g., a T cell, B cell, NK cell, or other immune cell, to propagate a pro-immune response.

191. The therapeutic compound of any of embodiments 1-190, wherein the specific targeting moiety targets a mammalian target, e.g., a mammalian polypeptide, and the effector binding/modulating moiety binds/modulates a mammalian immune component, e.g., a human immune cell, e.g., a mammalian B cell, T cell, or macrophage.

192. The therapeutic compound of any of embodiments 1-192, wherein the specific targeting moiety targets a human target, e.g., a human polypeptide, and the effector binding/modulating moiety binds/modulates a human immune component, e.g., a human immune cell, e.g., a ahuman B cell, T cell, or macrophage.

193. The therapeutic compound of any of embodiments 1-193, wherein the therapeutic compound is configured for use in a human.

194. The therapeutic compound of any of embodiments 1-191, wherein the therapeutic compound is configured for use in a non-human mammal.

195. The therapeutic compound of any of embodiments 1-194, wherein the therapeutic compound, e.g., the effector binding/modulating moiety, comprises a PD-1 agonist.

195.1. The therapeutic compound of any of the preceding embodiments, wherein the therapeutic compound comprises a IL-2 mutein of SEQ ID NO: 15, wherein the mutein comprises a mutation at position 73, 76, 100, or 138.

195.2. The therapeutic compound of embodiment 195.1, wherein the mutation is a L to I mutation at position 73, 76, 100, or 138.

195.3. The therapeutic compound of embodiments 195.1 or 195.2, wherein the IL-2 mutein further comprises a mutation at one or more of positions 49, 51, 55, 57, 68, 89, 91, 94, 108, and 145.

195.4. The therapeutic compound of any of embodiments 195.1-195.3, wherein the mutein further comprises a mutation at one or more of positions E35, H36, Q42, D104, E115, or Q146 or 1, 2, 3, 4, 5, or each of E35, H36, Q42, D104, E115, or Q146 is wild-type.

195.5. The therapeutic compound of embodiment 195.4, wherein the mutation is one or more of E35Q, H36N, Q42E, D104N, E115Q, or Q146E.

195.6. The therapeutic compound of any one of embodiments 195.1-195.5, wherein the IL-2 mutein comprises a N49S mutation.

195.7. The therapeutic compound of any one of embodiments 195.1-195.6, wherein the IL-2 mutein comprises a Y51S or a Y51H mutation.

195.8. The therapeutic compound of any one of embodiments 195.1-195.7, wherein the IL-2 mutein comprises a K55R mutation.

195.9. The therapeutic compound of any one of embodiments 195.1-195.8, wherein the IL-2 mutein comprises a T57A mutation.

195.10. The therapeutic compound of any one of embodiments 195.1-195.8, wherein the IL-2 mutein comprises a K68E mutation, V89A (V69A) mutation, a N91R (N71R) mutation, a Q94P or Q74P mutation, a (N88D) or a N108R (N88R) mutation, a C145A(C125A) or C145S (C125S) mutation.

195.11. The therapeutic compound of any one of embodiments 195.1-195.10, wherein the therapeutic compound comprises a IL-2 mutein of SEQ ID NO: 6, wherein the mutein comprises a mutation at position 53, 56, 80, or 118 and one or more of the mutations recited in embodiments 195.1-195.10.

195.12 The therapeutic compound of any one of embodiments 195.1-195.11, wherein the IL-2 mutein is fused or linked to a Fc peptide.

195.13 The therapeutic compound of embodiment 195.12 wherein the Fc peptide comprise a mutation at one or more of positions of L234, L247, L235, L248, G237, and G250 (EU numbering).

196. A method of treating a subject with inflammatory bowel disease, the method comprising administering a therapeutic compound of any of embodiments 1-195.13.13 to the subject to treat the inflammatory bowel disease.

197. The method of embodiment 196, wherein the subject with inflammatory bowel disease has Crohn's disease.

198. The method of embodiment 196, wherein the subject with inflammatory bowel disease has ulcerative colitis.

199. A method of treating a subject with auto-immune hepatitis, the method comprising administering a therapeutic compound of any of embodiments 1-195.13 to the subject to treat the auto-immune hepatitis.

200. A method of treating primary sclerosing cholangitis the method comprising administering a therapeutic compound of any of embodiments 1-195.13 to the subject to treat the primary sclerosing cholangitis.

201. A method of treating Type 1 diabetes the method comprising administering a therapeutic compound of any of embodiments 1-195.13, therby treating the subject to treat the Type 1 diabetes.

202. A method of treating a transplant subject comprising administering a therapeutically effective amount of a therapeutic compound of any of embodiments 1-195.13 to the subject, thereby treating a transplant (recipient) subject.

203. A method of treating GVHD in a subject having a transplanted a donor tissue comprising administering a therapeutically effective amount of a therapeutic compound of any of embodiments 1-195.13 to the subject.

204. The method of embodiment 203, wherein the therapeutic compound is administered to the subject: prior to receiving the transplant; prior to developing a symptom of GVHD; after or concurrent with receiving the transplant; or after or concurrent with developing a symptom of GVHD.

205. A method of treating a subject having, or at risk, or elevated risk, for having, an autoimmune disorder, comprising administering a therapeutically effective amount of a therapeutic compound of any embodiments 1-195.13, thereby treating the subject.

206. The method of embodiment 205, wherein the subject has received, will receive, or is in need of, allograft donor tissue.

207. The method of any of embodiments 205-206, wherein the donor tissue comprises a solid organ, e.g., a liver, kidney, heart, pancreas, thymus, or lung.

208. The method of any of embodiments 205-206, wherein the donor tissue comprises all or part of an organ, e.g., a liver, kidney, heart, pancreas, thymus, or lung.

209. The method of any of embodiments 205-206, wherein the donor tissue comprises skin.

210. The method of any of embodiments 205-206, wherein the donor tissue does not comprises skin.

211. The method of any of embodiments 205-210, wherein the donor tissue presents or expresses a product of an allele of a locus locus, which allele is not present or expressed in the subject.

212. The method of any of embodiments 205-210,, wherein the donor tissue presents or expresses a product of an allele of a locus selected from Table 2, e.g., the HLA locus, e.g., the HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ or HLA-DR locus, which allele is not present or expressed in the subject.

213 The method of any of embodiments 205-212, comprising introducing the transplant tissue into the subject.

214. The method of any of embodiments 196-213, comprising monitoring the subject for immune cell inactivation (e.g., to monitor unwanted agonization of an immune inhibitory checkpoint molecule) at a site distant from the target site, e.g., in the peripheral circulation or the lymphatic system.

215. The method of any of embodiments 196-214, comprising monitoring the subject for immune cell activation (e.g., to monitor unwanted antagonization of an immune inhibitory checkpoint molecule) at a site distant from the target site, e.g., in the peripheral circulation or the lymphatic system.

216. The method of any of embodiments 196-215, wherein responsive to the result of monitoring, selecting a course of treatment for the subject, e.g., increasing the dose of the therapeutic compound, decreasing the dose of the therapeutic compound, continuing treatment with the therapeutic compound without a change in dose.

217. The method of any of embodiments 196-216, comprising administering the compound of embodiments 1-195.13, to the recipient.

218. The method of any of embodiments 196-216, wherein administering comprises systemic administration, e.g., to the peripheral circulatory system.

219. The method of any of embodiments 196-216, wherein administering comprises local administration, e.g., to the target tissue, the donor tissue or the site of at which the target tissue or the donor tissue is, or will be located.

220. The method of any of embodiments 219, comprising administering the therapeutic compound to the recipient prior to introduction of the donor tissue into the recipient.

221. The method of any of embodiments 219, comprising administering the therapeutic compound, to the recipient after introduction of the donor tissue into the recipient.

222. The method of any of embodiments 213, comprising administering the therapeutic compound to the recipient concurrent with introduction of the donor tissue into the recipient.

223. The method of embodiment 213, comprising contacting the therapeutic compound with the donor tissue prior to introduction of the donor tissue into the recipient.

224. The method of any of embodiments 213, comprising providing the therapeutic compound to the subject, wherein the transplant tissue has been contacted with therapeutic compound prior to introduction into the subject.

225. The method of any of embodiments 213, comprising contacting the therapeutic compound with the donor tissue after introduction of the donor tissue into the recipient, e.g., by local administration to the donor tissue.

226. The method of any of embodiments 196-226, comprising administering a therapeutic comound as provided for herein such that therapeutic levels are present for at least 1, 5, 10, 14, or 28 days, for example, consecutive or non-consequitive days.

227. The method of any of embodiments 196-226, wherein the subject does not receive a non-targeted immune suppressive agent.

228. The method of any of embodiments 196-226, wherein for the subject has not received a non-targeted immune suppressive agent for at least 1, 15, 30, 60, or 90 days prior to the initial administration of the therapeutic compound.

229. The method of any of embodiments 213, wherein the subject has not received a non-targeted immune suppressive agent for at least 1, 15, 30, 60, or 90 days prior to introduction of the transplant tissue.

230. The method of any of embodiments 196-229, wherein the subject does not receive a non-targeted immune suppressive agent for at least 1, 15, 30, 60, 90, or 180 days after the initial administration of the therapeutic compound.

231. The method of any of embodiments 196-229, wherein the subject does not receive a non-targeted immune suppressive agent for at least 1, 15, 30, 60, 90, or 180 days after introduction of the transplant tissue.

232. The method of any of embodiments 196-231, comprising administering a non-targeted immune suppressive agent to the subject.

233. The method of any of embodiments 196-232, wherein for the subject receives a non-targeted immune suppressive agent for at least 1, 15, 30, 60, or 90 days prior to the initial administration of the therapeutic compound.

234. The method of embodiment 213, wherein the subject receives a non-targeted immune suppressive agent for at least 1, 15, 30, 60, or 90 days prior to introduction of the transplant tissue.

235. The method of embodiment 234, wherein the subject receives a non-targeted immune suppressive agent for at least 1, 15, 30, 60, 90 or 180 days after the initial administration of the therapeutic compound.

236. The method of any of embodiments 196-235, wherein the subject receives a non-targeted immune suppressive agent for at least 1, 15, 30, 60, 90 or 180days after introduction of the transplant tissue.

237. The method of any of embodiments 196-235, wherein for the subject receives a non-targeted immune suppressive agent prior to the initial administration of the therapeutic compound but for no more than 1, 15, 30, 60, 90 or 180 days.

238. The method of embodiment 213, wherein the subject receives a non-targeted immune suppressive agent prior to introduction of the transplant tissue but for no more than 1, 15, 30, 60, 90 or 180 days.

239. The method of any of embodiments 196-238, wherein the subject receives a non-targeted immune suppressive agent after the initial administration of the therapeutic compound but for no more than 1, 15, 30, 60, 90 or 180 days.

240. The method of embodiment 213, wherein the subject receives a non-targeted immune suppressive agent after introduction of the transplant tissue but for no more than 1, 15, 30, 60, 90 or 180 days.

241. The method of embodiment 213, wherein the subject is monitored for rejection of the transplant tissue.

242. The method of any of embodiments 196-242, a dosage of a non-targeted immune suppressive agent is selected, or wherein responsive to the monitoring, a dosage of a non-targeted immune suppressive agent is selected.

243. The method of embodiment 242, wherein the dosage is administered.

244. The method of embodiment 243, wherein the selected dosage is zero, i.e., a non-targeted immune suppressive agent is not administered.

245. The method of embodiment 243, wherein the selected dosage is non-zero, i.e., a non-targeted immune suppressive agent is administered.

246. The method of embodiment 243, wherein the dosage is less than what would be administered in the absence of administration of a therapeutic compound.

247. The method of any of embodiments 196-246, wherein the subject is a mammal, e.g., a non-human mammal.

248. The method of any of embodiments 196-246, wherein the subject is a human.

249. The method of embodiment 213, wherein the donor and subject are mismatched at an HLA locus, e.g., a major or minor locus.

250. The method of embodiment 249, wherein the subject is a mammal, e.g., a non-human mammal.

251. The method of embodiment 249, wherein the subject is a human.

252. A method of treating a subject having, or at risk, or elevated risk, for having, an autoimmune disorder, comprising administering a therapeutically effective amount of a therapeutic compound of any embodiments 1-195.13, thereby treating the subject.

253. The method of embodiment 252, wherein provision of the therapeutic compound is initiated prior to the onset, or prior to identification of onset, of symptoms of the autoimmune disorder.

254. The method of any of embodiments 252-253, wherein provision of the therapeutic compound is initiated after onset, or after identification of onset, of symptoms of the autoimmune disorder.

255. The method of embodiments 252-254, wherein autoimmune disorder comprises type1diabetes.

256. The therapeutic compound of any of embodiments 252-255, wherein the target tissue comprises pancreatic islets or pancreatic beta cells, gut tissue (e.g. gut endothelial cells), kidney tissue (e.g. kidney epithelial cells), or liver tissue (e.g. liver epithelial cells).

257. The therapeutic compound of any of embodiments 252-256, wherein the effector binding/modulating moiety or targeting moiety binds a polypeptide selected from Table 3, e.g., MAdCAM, OAT1, OCT, DPP6, SEZ6L2, LRP11, DISP2, SLC30A8, FXYD2, TSPAN7, or TMEM27 polypeptide.

258. The method of any of embodiments 252-257, wherein provision of the therapeutic compound is initiated prior to the onset, or prior to identification of onset, of symptoms of type1diabetes.

259. The method of any of embodiments 252-258, wherein provision of the therapeutic compound is initiated prior to, or prior to identification of the subject having a preselected characteristic or symptom.

260. The method of any of embodiments 252-259, wherein provision of the therapeutic compound is initiated after onset, or after identification of onset, of symptoms of type1diabetes.

261. The method of any of embodiments 252-260, wherein provision of the therapeutic compound is initiated after, or after identification of the subject having a preselected characteristic or symptom.

262, The method of any of embodiments 252-261, wherein the therapeutic compound is a therapeutic compound of any of embodiments 1-195.13

263 The method of any of embodiments 252-257, wherein therapeutic compound is suitable for the treatment of a subject having, or at risk, or elevated risk, for having, multiple sclerosis.

264. The method of embodiment 263, wherein the target tissue comprises CNS tissue, myelin sheath, or myelin sheath of oligodendrocytes.

265. The method of any of embodiments 263 or 264, wherein the effector binding/modulating moiety or targeting moiety binds a polypeptide selected from Table 3, e.g., a MOG, PLP, or MBP polypeptide.

266. The method of any of embodiments 263-265, wherein provision of the therapeutic compound is initiated prior to the onset, or prior to identification of onset, of symptoms of multiple sclerosis.

267. The method of any of clai embodiments ms 263-265, wherein provision of the therapeutic compound is initiated prior to, or prior to identification of the subject a preselected characteristic or symptom.

268. The method of any of embodiments 263-265, wherein provision of the therapeutic compound is initiated after onset, or after identification of onset, of symptoms of multiple sclerosis.

269. The method of any of embodiments 263-265, wherein provision of the therapeutic compound is initiated after, or after identification of the subject having a preselected characteristic or symptom.

270. The method of any of embodiments 263-269, wherein the therapeutic compound is a therapeutic compound of any of embodiments 1-195.13

271. The method of any of embodiments 252-257, wherein the therapeutic compound is suitable for the treatment of a subject having, or at risk, or elevated risk, for having, cardiomyositis.

272. The method of embodiment 271, wherein the target tissue comprises cardiomyocytes, monocytes, macrophages, or myeloid cells.

273. The method of embodiments 271 or 272,, wherein the effector binding/modulating moiety or targeting moiety binds a polypeptide selected from Table 3, e.g., a SIRPA (CD172a) polypeptide.

274. The method of any of embodiments 271-273, wherein provision of the therapeutic compound is initiated prior to the onset, or prior to identification of onset, of symptoms of cardiomyositis.

275. The method of any of embodiments 271-273, wherein provision of the therapeutic compound is initiated prior to, or prior to identification of the subject having a preselected characteristic or symptom.

276. The method of any of embodiments 271-273, wherein provision of the therapeutic compound is initiated after onset, or after identification of onset, of symptoms of cardiomyositis.

277. The method of any of embodiments 271-273, wherein provision of the therapeutic compound is initiated after, or after identification of the subject having a preselected characteristic or symptom.

278. The method of any of embodiments 271-277, wherein the therapeutic compound is a therapeutic compound of any of embodiments 1-195.13.

279. The method of any of embodiments 252-257, wherein therapeutic compound is suitable for the treatment of a subject having, or at risk, or elevated risk, for having, rheumatoid arthritis.

280. The method of embodiment 279, wherein the target tissue comprises cardiomyocytes, monocytes, macrophages, or myeloid cells.

281. The method of embodiments 279 or 280, wherein the effector binding/modulating moiety or targeting moiety binds a polypeptide selected from Table 3, e.g., a SIRPA (CD172a) polypeptide.

282. The method of embodiments 279-281, wherein provision of the therapeutic compound is initiated prior to the onset, or prior to identification of onset, of symptoms of rheumatoid arthritis.

283. The method of embodiments 279-281, wherein provision of the therapeutic compound is initiated prior to, or prior to identification of the subject having a preselected characteristic or symptom.

284. The method of embodiments 279-281, wherein provision of the therapeutic compound is initiated after onset, or after identification of onset, of symptoms of rheumatoid arthritis.

285. The method of embodiments 279-281, wherein provision of the therapeutic compound is initiated after, or after identification of the subject having a preselected characteristic or symptom.

286. The method of embodiments 279-285, wherein the therapeutic compound is a therapeutic compound of any of embodiments 1-195.13.

287. The method of any of embodiments 196-286, comprising monitoring the subject for immune cell inactivation (e.g., to monitor unwanted agonization of an immune inhibitory checkpoint molecule) at a site distant from the target site, e.g., in the peripheral circulation or the lymphatic system.

288. The method of any of embodiments 196-287, comprising monitoring the subject for immune cell activation (e.g., to monitor unwanted antagonization of an immune inhibitory checkpoint molecule) at a site distant from the target site, e.g., in the peripheral circulation or the lymphatic system.

289. The method of any of embodiments 196-288, wherein responsive to the result of monitoring, selecting a course of treatment for the subject, e.g., increasing the dose of the therapeutic compound, decreasing the dose of the therapeutic compound, continuing treatment with the therapeutic compound without a change in dose.

290. The method of any of embodiments 196-289, wherein the subject monitored for autoimmune attack of the target tissue.

291. The method of embodiment 290, wherein responsive to the monitoring, a dosage of the therapeutic compound is selected.

292. The method of embodiment 291, wherein the dosage is administered.

293. The method of embodiment 290, wherein the selected dosage is zero, i.e., administration of therapeutic compound is ceased.

294. The method of embodiment 290, wherein the selected dosage is non-zero.

295. The method of embodiment 290, wherein the selected dosage is an increased dosage.

296. The method of embodiment 290, wherein the selected dosage is an reduced dosage.

297. The method of any of embodiments 196-296, wherein administering comprises systemic administration, e.g., to the peripheral circulatory system.

298. The method of any of embodiments 196-297, wherein administering comprises local administration, e.g., to the target tissue.

299. The method of any of embodiments 196-298, comprising administering a therapeutic comound provided herein such that therapeutic levels are present for at least 1, 5, 10, 14, or 28 days, e.g, consecutive or non-consequitive days.

300. The method of any of embodiments 196-299, wherein the subject is a mammal, e.g., a non-human mammal.

301. The method of any of embodiments 196-299, wherein the subject is a human.

302. A nucleic acid molecule or a plurality of nucleic acid molecules encoding a therapeutic compound of any of embodiments 1-195.13.

303. A vector or a plurality of vectors comprising the nucleic acid molecules of embodiment 302.

304. A cell comprising the nucleic acid molecules of embodiment 302 or the vector of embodiment 303.

305. A method of making a therapeutic compound comprising culturing a cell of embodiment 304 to make the therapeutic compound.

306. A method of making a nucleic acid sequence encoding a therapeutic compound of any of embodiments 1-195.13, comprising

a) providing a vector comprising sequence encoding a targeting moiety and inserting into the vector sequence encoding an effector binding/modulating moiety to form a sequence encoding a therapeutic compound; or

b) providing a vector comprising sequence encoding an effector binding/modulating moiety and inserting into the vector sequence encoding a targeting moiety to form a sequence encoding a therapeutic compound,

thereby making a sequence encoding a therapeutic compound.

307. The method of embodiment 306, wherein the targeting moiety is selected in response to the need of a subject.

308. The method of embodiment 306 or 307, wherein the effector binding/modulating moiety is selected in response to the need of a subject.

309. The method of any of embodiments 306 or 307, further comprising expressing the sequence encoding the therapeutic compound to produce the therapeutic compound.

310. The method of any of embodiments 306-309, further comprising transferring the sequence, or a polypeptide made from the sequence, to another entity, e.g., a health care provider who will administer the therapeutic compound to a subject.

311. A method of treating a subject comprising:

acquiring, e.g., receiving from another entity, a therapeutic compound, or a nucleic acid encoding a therapeutic compound, made by the method of any of provided herein, but not limited to embodiments 306-310;

administering the therapeutic compound, or a nucleic acid encoding a therapeutic compound to the subject,

thereby treating the subject.

312. The method of embodiment 311, further comprising identifying the therapeutic compound, or nucleic acid encoding a therapeutic compound to another entity, e.g., the entity that will make the therapeutic compound, or nucleic acid encoding a therapeutic compound.

313. The method of embodiments 311 or 312, further comprising requesting the therapeutic compound, or nucleic acid encoding a therapeutic compound from another entity, e.g., the entity that made the therapeutic compound, or nucleic acid encoding a therapeutic compound.

314. The method of any of embodiments 311-333, wherein the subject has an autoimmune disorder and the therapeutic compound does not comprise an autoantigenic peptide or polypeptide characteristic of the autoimmune disorder, e.g., does not comprise a peptide or polypeptide against which the subject has autoantibodies.

The following examples are illustrative, but not limiting, of the compounds, compositions and methods described herein. Other suitable modifications and adaptations known to those skilled in the art are within the scope of the following embodiments.

EXAMPLES

EXAMPLE 1: HLA-TARGETED PD-1 AGONIZING THERAPEUTIC COMPOUNDS.

Engineering of a HLA-targeted PD-1-agonizing therapeutic

Binding domains specific for HLA-A2 are obtained by cloning the variable regions of the Ig heavy and light chains from the BB7.2 hybridoma (ATCC) and converting into a single-chain Ab (scFv). Activity and specificity of the scFv can be confirmed by assessing binding of BB7.2

to HLA-A2 expressing cells in comparison to cells expressing other HLA-A alleles. The minimal PD-L1 residues required for PD-1 binding activity are identified by systematically evaluating the requirement of amino acids 3' and 5'of the PD-L1 IgV domain corresponding to amino acids 68-114. Expression constructs are designed and proteins synthesized and purified, with PD-1 binding activity tested by Biacore. The minimum essential amino acids required for PD-1 binding by the PD-L1 IgV domain are referred to as PD-L1-IgV. To generate a BB7.2 scFv and PD-L1-IgV bi-specific molecule, a DNA fragment is synthesized encoding the bispecific single-chain antibody BB7.2 x PD-L1-IgV with the domain arrangement $VL_{BB7.2}$-$VH_{BB7.2}$-PD-L1-IgV-IgG4 Fc and cloned into an expression vector containing a DHFR selection cassette.

Expression vector plasmid DNA is transiently transfected into 293T cells, and BB7.2 x PD-L1-IgV bispecific antibodies are purified from supernatants using a protein A/G column. BB7.2 x PD-L1-IgV bispecific antibody integrity is assessed by polyacrylamide gel. Binding of the BB7.2 scFv domain to HLA-A2 and PD-L1-IgV domain to PD-1 is assessed by ELISA and cell-based FACS assay.

The in vitro function of BB7.2 x PD-L1-IgV bispecific antibodies is assessed using mixed lymphocyte reaction (MLR) assay. In a 96-well plate format, 100,000 irradiated human PBMCs from an HLA-$A2^+$ donor are aliquoted per well and used as activators. HLA-$A1^-$ responder T cells are then added together with increasing amounts of BB7.2 x PD-L1-IgV bispecific antibody. The ability of responder T cells to proliferate over a period of 72 hours is assessed by BrdU incorporation, and with IFNg and IL2 cytokine production additionally evaluated in the co-culture supernatant as assessed by ELISA. BB7.2 x PD-L1-IgV bispecific antibody is found to suppress MLR reaction as demonstrated by inhibiting HLA-$A2^-$ responder T cell proliferation and cytokine production.

The in vivo function of BB7.2 x PD-L1-IgV bispecific antibody is assessed using a murine mouse model of skin allograft tolerance. The C57BL/6-Tg(HLA-A2.1)1Enge/J (Jackson Laboratories, Bar Harbor Maine) strain of mouse is crossed with Balb/cJ, with F1 progeny expressing the HLA-A2.1 transgene and serving as allograft donors. C57BL/6J mice are shaved and surgically engrafted with skin removed from euthanized C57BL/6-Tg(HLA-A2.1)1Enge/J x Balb/cJ F1 mice. At the same time, host mice start receiving intraperitoneal injections of the BB7.2 x PD-L1-IgV bispecific antibody engineered to contain a murine IgG1 Fc or BB7.2 only

or PD-L1-IgV only controls. Skin allograft rejection or acceptance is monitored over a period of 30 days, wherein hosts were euthanized and lymph node and allograft-resident lymphocyte populations quantified.

EXAMPLE 2: CD39 AND/OR CD73 AS EFFECTOR DOMAINS CREATING A PURINERGIC HALO SURROUNDING A CELL TYPE OR TISSUE OF INTEREST

A catalytically active fragment of CD39 and/or CD73 is fused to a targeting domain. Upon binding and accumulation at the target site, CD39 phosphohydrolyzes ATP to AMP. Upon binding and accumulation at the target site, CD73 dephosphorylates extracellular AMP to adenosine. A soluble catalytically active form of CD39 suitable for use herein has been found to circulate in human and murine blood, see, e.g., Yegutkin et al. FASEB J. 2012 Sep; 26(9):3875-83. A soluble recombinant CD39 fragment is also described in Inhibition of platelet function by recombinant soluble ecto-ADPase/CD39, Gayle, et al., J Clin Invest. 1998 May 1; 101(9): 1851–1859.. A suitable CD73 molecule comprises a soluble form of CD73 which can be shed from the membrane of endothelial cells by proteolytic cleavage or hydrolysis of the GPI anchor by shear stress see, e.g., Reference: Yegutkin G, Bodin P, Burnstock G. Effect of shear stress on the release of soluble ecto-enzymes ATPase and 5'-nucleotidase along with endogenous ATP from vascular endothelial cells. Br J Pharmacol 2000; 129: 921–6.

The local catalysis of ATP to AMP or AMP to adenosine will deplete local energy stores required for fulminant T effector cell function. Treg function should not be impacted by ATP depletion due to their reliance on oxidative phosphorylation for energy needs (which requires less ATP), wherein T memory and other effector cells should be impacted due their reliance on glycolysis (requiring high ATP usage) for fulminant function.

Example 3: Measuring Antibody-induced PD-1 signaling.

Jurkat cells that stably express 2 constructs, 1) a human PD-1 polypeptide fused to a b-galactosidase, which can be referred to as an "Enzyme donor" and 2) a SHP-2 polypeptide fused to a b-galactosidase, which can be referred to as an "Enzyme acceptor." A PD-1 antibody is contacted with the cell and when the PD-1 is engaged, SHP-2 is recruited to PD-1. The enzyme acceptor and enzyme donor form a fully active b-galactosidase enzyme that can be assayed. This assay can be used to show activation of PD-1 signaling.

Example 4: Measuring PD-1 Agonism. PD-1 Agonists inhibit T cell activation. Without being bound to any particular theory, PD-1 agonism inhibits anti-CD3-induced T cell activation. Human or mouse cells are preactivated with PHA (for human T cells) or ConA (for mouse T cells) so that they express PD-1. The T cells are then "reactivated" with anti-CD3 in the presence of anti-PD-1 (or PD-L1) for the PD-1 agonism assay. T cells that receive a PD-1 agonist signal in the presence of anti-CD3 will show decreased activation, relative to anti-CD3 stimulation alone. Activation can be readout by proliferation or cytokine production (IL-2, IFNg, IL-17) and possibly by other markers, such as CD69 activation marker.

Example 5. Expression and Function of Anti-MAdCAM / mouse PD-L1 Fusion Protein is not impacted by molecular configuration.

A bispecific fusion molecule comprising an anti-mouseMAdCAM Ab/mouse PD-L1 molecule was expressed in two orientations. The first orientation consisted of an anti-mouse MAdCAM IgG with mouse PD-L1 fused at the c-terminus of it's heavy chain. The second orientation consisted of mouse PD-L1 fused at the N-terminus of an Ig Fc domain, with a c-terminally fused anti-mouse MAdCAM scFv. Both molecules were found to be well expressed in a mammalioan expression system. It was also found that the molecules can bind to their respective binding partners, MAdCAM or PD-1 in both orientations, simultaneously. These results demonstrate that a molecule consisting of an anti-MAdCAM antibody fused to PD-L1, can be expressed in configurations whereby PD-L1 is N or C-terminally fused to the Fc and retain proper functional binding activity.

Briefly, a pTT5 vector containing the single gene encoding a single polypeptide with mouse PD-L1 fused N-terminally of human IgG1 Fc domain and with c-terminal fused anti-MAdCAM scFv MECA-89 was transfected into HEK293 Expi cells. Alternatively, two plasmids were co-transfected at equimolar ratios. The first plasmid encoded the light chain of MECA-89 and the 2nd encoded the full length IgG1 heavy chain of MECA-89 with c-terminally fused mouse PD-L1. After 5-7 days, cell culture supernatants expressing the molecules were harvested, and clarified by centrifugation and filtration through a 0.22um filtration device. The bi-specific molecules were captured on proA resin. The resin was washed with PBS pH 7.4 and the captured molecule was eluted using 100mM Glycine pH 2.5, with neutralization using a tenth volume of 1M Tris pH 8.5. The protein was buffer exchanged into PBS pH 7.4, and analyzed by

size exclusion chromatography on a Superdex 200 3.2/300. Analysis of 1ug of purified material by reducing and non-reducing SDS-PAGE on a Bis-Tris 4-12% gel was conducted.

Both proteins, regardless of orientation were expressed at over 10mg/L, and were over 95% monodispersed after purification as shown by size exclusion chromatography and reducing/non-reducing SDS-PAGE. Accordingly, this demonstrates the production and activity of dual function bispecific molecules with different immunomodulators and tissue targeting moieties at the N and C terminus of an Fc domain. This also shows specifically that a PD-1 agonist and binding partner can be expressed at the N or C terminus of an Ig Fc domain.

Example 6. A Bispecific molecule comprising a PD-1 agonist protoytpe tethered to MAdCAM can bind MAdCAM and PD-1 simultaneously.

Briefly, an immunosorbent plate was coated with mouse PD-1 at a concentration of 1 µg/mL in PBS pH 7.4, 75 ul/well, and incubated overnight at 4°C. Wells were washed with PBS pH 7.4 containing 0.05% Tween-20 (wash buffer) three times, and then blocked with 200ul/well 1% BSA in PBS pH 7.4 (block buffer) for two hours at room temperature. After three washes with wash buffer, two bispecific molecules that comprises the PD-1 Agonist prototype at either the N-terminus or C-terminus were diluted to 1nM, 10nM, and 100nM in PBS containing 1% BSA and 0.05% Tween-20 (assay buffer). The diluted material was added to the mouse PD-1 coated plate at 75ul/well for 1 hour at room temperature. After three washes with wash buffer, mouse MAdCAM was added to the plate at 75ul/well, at a concentration of 10nM in assay buffer for 1 hr at room temperature. After three washes with wash buffer, a goat biotinylated anti-mouse MAdCAM polyclonal antibody, diluted to 0.5µg/mL in assay buffer, was added to the plate at 75ul/well for 1 hr at room temperature. After three washes with wash buffer high sensitivity streptavidin HRP diluted in assay buffer at 1:5000 was added to the plate at 75ul / well for 15 minutes at room temperature. After three washes with wash buffer and 1 wash with wash buffer (with no tween-20), the assay was developed with TMB, and stopped with 1N HCL. OD 450nm was measured. The experiment included appropriate controls for non-specific binding to the plate/block in the absence of mouse PD-1, as well as no MAdCAM controls, and mono-specific controls, that are unable to form a bridge between mouse PD-1 and mouse MAdCAM.

The results demonstrated that at concentrations of 1nM, 10nM, and 100nM, both bispecific molecules, are able to simultaneously interact with mouse MAdCAM and mouse PD-L1, whilst the monospecific controls did not create a bridging signal. Additionally, there was no binding of any compound to MAdCAM at any concentration tested, when mouse PD-1 was not present on the plate surface, indicating none of the test compounds were interacting non-specifically with the plate surface. Thus, these results demonstrate that a bispecific molecule that is targeting binding to both MAdCAM and PD-1 can successfully bind to both molecules. Although the experiments were performed with PD-L1 as a substitute for a PD-1 antibody, it is expected that the PD-1 antibody will function in a similar manner.

Example 7. A Bispecific PD-L1 Prototype Molecule Inhibits T cells in a PD-1 Agonist Assay.

A bispecific molecule that mimics a PD-1 agonist antibody was tested to demonstrate that PD-1 agonsim can inhibit T cells. Briefly, 7 week old female C57LB/6 mice were sacrificed and their splenocytes were isolated. The splenocytes were exposed to ConA for 3 days and then exposed to anti-CD3 in the presense or absence of the PD-1 type molecule, which in this example was a PD-L1 bispecific molecule that was tethered to a plate using anti-human IgG. T cells were then introduced to the PD-L1 bispecific molecule. The PD-L1, which mimics a PD-1 antibody were found to be a T cell agonist and inhibit T cell activation. The same experiments were repeated using a PD-L1 bispecific molecule that was fused with an anti-MAdCAM antibody, which were tethered to a plate by interacting with a MAdCAM coated plate. The PD-1 agonist mimic, the PD-L1/anti-MAdCAM antibody were found to be effective agonists of T cell activity. These results demonstrate that a bispecific molecule that mimics a PD-1 antibody/MAdCAMAb fusion protein can exert functional inhibitory signaling into primary mouse T cell blasts when the molecule is captured via the MAdCAM antibody component at the end of the molecule.

Example 8: A bispecific PD-1 prototype molecule with a different tissue tether can inhibit T cells in a PD-1 agonist assay. A fusion molecule of a PD-L1 was used as a substitute for a PD-1 antibody and linked to a Class I H-2Kk antibody. The MHC Class I H-2Kk tethered PD-L1 molecule had functional binding, similar to the data described in Examples 6 and 7. Briefly,

splenocytes from C57Bl/6 mice were stimulated with Concanavalin A (ConA) and IL-2 for 3 days. Plates were coated with anti-CD3 (2C11) overnight at 4 C, washed. Plates were coated with anti-human IgG for 3 hrs at 37 C and washed. Mono-specific anti-H-2Kk (16-3-22) or bi-specific anti-H-2Kk:mPD-L1 were added and incubated for 3 hr at 37 C and washed. All test articles contained a human IgG1-Fc portion. PBS (No Tx) was added to determine the assay background. ConA blasts were washed 2 times, added to the plate and incubated at 37 C. Supernatants were removed after 24 hrs. IFNg levels were determined by MSD. After 48 hrs, cell viability/metabolism was analyzed by Cell Titer-glo. When captured via the IgG Fc domain, an MHC Class I tethered PD-L1 bispecific can attenuate T cell activation in a mouse PD-1 agonism assay. Therefore, this example demonstrates that a different bispecific prototype molecule can exert functional inhibitory signaling into primary mouse T cell blasts – when the molecule is captured via a different tissue tether – in this case a mouse antibody to MHC Class I H-2Kk. Accordingly, this data demonstrates that the tethering is not specific to MAdCAM and is possible with other molecules that can act as targeting moieties as provided herein.

Example 9. PD-1 Agonists Can Induce Signaling in Jurkat Cells

Jurkat cells expressing both human PD-1 fused to a beta-galactosidase enzyme donor and SHP-2 fused to a beta-galactosidase enzyme acceptor are added to test conditions in a plate and incubated for 2 hrs. Agonist PD-1 antibodies induce signaling and SHP-2 recruitment, enzyme complementation and formation of an active beta-galactosidase enzyme. Beta-galactosidase substrate was added and chemiluminescence can be measured on a standard luminescence plate reader. Agonism is measured by chemiluminescence, where the more chemiluminescence that is measured indicates the greater agonism.

Agonism of a PD-1/MAdCAM bi-specific molecule was measured in this assay. Cl10 (UCB) and CC-90006 (Celgene/Anaptys) were used as PD-1 agonist antibodies. Both are active and exhibit PD-1 agonism in functional assay in Ig-capture assay format. Briefly, plates were coated with anti-human IgG for overnight at 4 C and washed. Anti-tetanus toxin (TT) or benchmark agonist anti-PD-1 monoclonal antibodies, Cl10 or CC-90006 were added and incubated for 1 hr at 37 C and washed. All test articles contained a human IgG1-Fc. Media (No Tx) was added to determine the assay background. Plates were washed 3 times. Jurkat cells expressing both human PD-1 fused to a b-galactosidase enzyme donor and SHP-2 fused to a b-

galactosidase enzyme acceptor were added and incubated for 2 hrs. Agonist PD-1 antibodies induce signaling and SHP-2 recruitment, enzyme complementation and formation of an active b-galactosidase enzyme. B-galactosidase substrate was added and chemiluminescence was measured on a standard luminescence plate reader. The two human PD-1 agonist antibodies (Cl10 and CC-90006) bind and induce signaling (a surrogate for agonism) in the modified Jurkat reporter assay. Thus, this assay is a functional PD-1 agonism assay. Cl10:MECA89 (MECA89 is a known MAdCAM antibody) is a novel bispecific molecule created by fusing MAdCAM antibody, MECA89[scFv], to C-terminus of the heavy chain of Cl10. This fusion protein was found to be active and exhibit PD-1 agonism in functional assay when captured via IgG Fc domain, as was Cl10 only protein. However, only Cl10:MECA89 is active in functional assay format using MAdCAM protein as capture (the monospecific components do not signal).

Briefly, plates were coated with either anti-human IgG or recombinant mMAdCAM-1 overnight at 4 C and washed. Mono-specific Anti-tetanus toxin (TT), anti-MAdCAM-1 (MECA89) or agonist anti-PD-1 (Cl10) or bi-specific Cl10:MECA89 were added and incubated for 1 hr at 37 C and washed. All test articles contained a human IgG1-Fc portion. PBS (No Tx) was added to determine the assay background. Plates were washed 2 times. Jurkat cells expressing both human PD-1 fused to a b-galactosidase enzyme donor and SHP-2 fused to a b-galactosidase enzyme acceptor were added and incubated for 2 hrs. Agonist PD-1 antibodies induce signaling and SHP-2 recruitment, enzyme complementation and formation of an active b-galactosidase enzyme. B-galactosidase substrate was added and chemiluminescence was measured on a standard luminescence plate reader. Results: Both Cl10, and the MAdCAM-tethered Cl10 bispecific molecules can induce PD-1 signaling in the Jurkat reporter assay when the plate is coated with an anti-IgG Fc capture, but only the MAdCAM-tethered bispecific can induce PD-1 signaling in the reporter assay when the plate is coated with recombinant MAdCAM protein. These results demonstrate that the molecule tethered with MAdCAM and contains a PD-1 agonist antibody are functional, which is similar to the results shown with the PD-L1 as the PD-1 agonist surrogate.

Example 10: Generation of PD-1 Agonist Antibodies

PD-1 deficient mice immunized with mouse PD-1 under conditions to generate an immune response against PD-1. 54 hybridomas were generated and identified that bind mouse

PD-1. The antibodies produced by the different hybridomas were analyzed for T cell agonism according to the methods described in Examples 4 and 6. Out of the 54 hybridomas at least 6 were identified as PD-1 agonists. The antibodies were also tested for binding on PD-1 and were found to bind at the same site as the PD-L1 binding site.

Briefly, binding to the PD-L1 binding site was determined using the following assay. Immunosorbent plates were coated overnight with 75 µL of recombinant mouse PD-L1-Fc (2 µg/mL) in 1x PBS, pH 7.4. Plates were then washed 3x with 1x PBS and blocked for 2 hours at room temperature with 1x PBS supplemented with 1% BSA. Recombinant mouse PD-1-Fc (1 nM) was incubated with 100 nM of the indicated anti-mouse PD-1 antibody in 1x PBS supplemented with 1% BSA and 0.05% Tween20 (Assay Buffer) for 1 hour at room temperature, shaking. After blocking, plates were washed 3x with 1x PBS supplemented with 0.05% Tween20 PBST and the antibody-PD-1 conjugates were incubated with plate-bound mouse PD-L1. After washing away unbound PD-1 with PBST, plates were incubated with 75 µL of biotinylated, polyclonal anti-PD-1 antibody (0.5 µg/mL) in assay buffer, followed by amplification with 1:5000 streptavidin HRP also diluted in assay buffer. Plates were washed three times with PBST followed by three washes with 1x PBS before addition of 100 µL TMB followed by 100 µL 1M HCl to stop the developing. Absorbance read at 450 nm and normalized to binding of PD-1 to PD-L1 in the absence of antibody. The results showed that the active antibodies bind to the PD-L1 binding site. The inactive antibodies did not bind to the PD-L1 binding site. Therefore, this example demonstrates the ability to produce anti-PD-1 antibodies that are agonists, in addition to the prevoiusly identified PD-1 agonist antibodies described herein.

Example 11: Tethered anti-PD-1 antibodies acts as PD-1 agonists.

A human antibody scFv phage library was panned against recombinant human, mouse, and cyno PD-1 proteins across iterative selection rounds to enrich for antibody clones that recognize all three aforementioned species orthologues of PD-1. The scFv clones were configured in nt-VH-Linker-VL-ct format and fused to the M13 phage surface via the pIII coat protein. After selections, clonal scFvs were screened for binding to human, mouse, and cyno PD-1 expressed on the cell surface of CHO cells. Clones that were found to be cross reactive to all three cell surface expressed PD-1 species orthologues were converted using standard molecular biology techniques, into a human IgG1 format whereby each molecule was comprised

of four polypeptide chains in total (2 heavy, and 2 light chains). The two light chains were identical to each other and the two heavy chains were identical to each other as provided.

The two identical heavy chains homodimerize and the two identical light chains pair with each heavy chain to form an intact human IgG1. The Fc domain contains the L234A, L235A, and G237A mutations to ablate FcγR interactions. The converted human IgG1 anti-PD-1 antibodies were transfected and expressed in HEK293 Expi cells, and purified by protein A chromatography. The protein concentration was determined using a nanodrop spectrophotometer in conjunction with antibody specific extinction coefficients. Antibodies were formulated in PBS pH 7.4.

The anti-PD-1 antibodies were next tested in the Jurkat assay described herein for agonist activity. Briefly, tissue culture plates were coated with anti-IgG or left uncoated. For captured format, test articles or controls were added to the anti-IgG coated wells at 100 nM, 25 nM or 12.5 nM and incubated for 3 hrs at 37 C. Plates were washed and Jurkat PD-1 cells were added. For the soluble format, soluble test articles or controls were added to wells at 100 nM, 25 nM or 12.5 nM already containing Jurkat PD1 cells. Luminescence was measured in a plate reader. The results demonstrated that nine of the twelve human/mouse cross-reactive PD-1 antibodies showed dose-dependent activity in the Jurkat assay when the anti-PD-1 antibodies were captured via anti-IgG, but not in the soluble format. This data demonstrates that the anti-PD-1 antibody can act as an agonist when tethered to its target by a targeting moiety.

In conclusion, without being bound to any particular theory, the data presented herein demonstrate that a PD-1 Agonist/MAdCAM bi-specific molecule can bind to both MAdCAM and PD-1 and also agonize T cell activity. Thus, the molecules can be used to treat the various conditions provided herein and provide for localized and/or tissue specific immunomodulation and the down regulation of a T-Cell response.

Example 12: Generation of IL-2 muteins

A pTT5 vector containing the single gene encoding the human IL-2M polypeptide fused N-terminally (SEQ ID NO: 57) or C-terminally (SEQ ID NO: 58) to human IgG1 Fc domain was transfected into HEK293 Expi cells. After 5-7 days, cell culture supernatants expressing IL-2Ms were harvested, and clarified by centrifugation and filtration through a 0.22um filtration device. IL-2Ms were captured on proA resin. The resin was washed with PBS pH 7.4 and the captured protein was eluted using 0.25% acetic acid pH 3.5, with neutralization using a tenth volume of

1M Tris pH 8.0. The protein was buffer exchanged into 30mM HEPES 150mM NaCl pH 7, and analyzed by size exclusion chromatography on a Superdex 200 3.2/300 column. Analysis of 5ug of purified material by reducing and non-reducing SDS-PAGE on a Bis-Tris 4-12% gel was conducted. The IL-2Ms were expressed at over 10mg/L, and were over 95% monodispersed after purification as shown by size exclusion chromatography and reducing/non-reducing SDS-PAGE.

Example 13: IL-2M Molecules can bind CD25

An immunosorbent plate was coated with CD25 at a concentration of 0.5μg/mL in PBS pH 7.4, 75 ul/well, and incubated overnight at 4°C. Wells were washed with PBS pH 7.4 containing 0.05% Tween-20 (wash buffer) three times, and then blocked with 200ul/well 1% BSA in PBS pH 7.4 (block buffer) for two hours at room temperature. After three washes with wash buffer IL-2M molecules of Example 12 were diluted to eleven –two fold serial dilution in PBS containing 1% BSA and 0.05% Tween-20 (assay buffer) with 2nM being the highest concentration. The diluted material was added to the CD25 coated plate at 75ul/well for 1 hour at room temperature. After three washes with wash buffer, a goat biotinylated anti-IL-2 polyclonal antibody, diluted to 0.05μg/mL in assay buffer, was added to the plate at 75ul/well for 1 hr at room temperature. After three washes with wash buffer high sensitivity streptavidin HRP diluted in assay buffer at 1:5000 was added to the plate at 75ul / well for 15 minutes at room temperature. After three washes with wash buffer and 1 wash with wash buffer (with no tween-20), the assay was developed with TMB, and stopped with 1N HCL. OD 450nm was measured. The experiment included appropriate controls for non-specific binding of IL-2M molecules to the plate/block in the absence of CD25 and a negative control molecule that is unable to bind CD25.

The results indicate that at concentrations of 2nM-1.9pM, IL-2M molecules are able to bind CD25 with sub nanomolar EC50s. Additionally, there was no detection of any compound at any concentration tested, when CD25 was not present on the plate surface, indicating none of the test compounds were interacting non-specifically with the plate surface (data not shown).

Example 14: In Vitro P-STAT5 Assay To Determine Potency and Selectivity of IL-2M Molecules. Peripheral blood mononuclear cells (PBMCs) were prepared using FICOLL-PAQUE Premium and Sepmate tubes from freshly isolated heparinized human whole blood. PBMCs were cultured in 10% fetal bovine serum RPMI medium in the presence of wild-type IL-2 or IL-2M of Example 12 for 20 minutes and then fixed for 10 minutes with BD Cytofix.

Fixed cells were sequentially permeabilized with BD Perm III and then BioLegend FOXP3 permeabilization buffer. After blocking with human serum for 10 minutes, cells were stained for 30 minutes with antibodies for phospho-STAT5 FITC, CD25 PE, FOXP3 AF647 and CD4 PerCP Cy5.5 and then acquired on an Attune NXT with plate reader. The IL-2M of Example 12 potently and selectively induces STAT5 phosphorylation in Tregs but not Teffs.

Example 15: Methods for Generation of Bispecific MAdCAM-tethered IL-2M Molecules

A pTT5 vector containing the single gene encoding the single B0001 polypeptide comprising an IL-2 mutein with a N88D, V69A, and Q74P mutations fused to a Fc protein with the LALA mutations as provided for herein with a GGGGS(x3) linker and scFV antibody that binds to MAdCAM or a similar molecule but with a GGGGS(x4) linker B0002 with human IL-2M fused N-terminally of human IgG1 Fc domain and with c-terminal fused anti-mMAdCAM scFv MECA-89 was transfected into HEK293 Expi cells. For B0003, two plasmids were co-transfected at equimolar ratios. The first plasmid encoded the light chain of MECA-89 and the 2nd encoded the full length IgG1 heavy chain of MECA-89 with c-terminally fused human IL-2M. After 5-7 days, cell culture supernatants expressing B0001, B0002, and B0003 were harvested, and clarified by centrifugation and filtration through a 0.22um filtration device. B0001, B0002, and B0003 were captured on proA resin. The resin was washed with PBS pH 7.4 and the captured protein was eluted using 0.25% acetic acid pH 3.5, with neutralization using a tenth volume of 1M Tris pH 8.0. The protein was buffer exchanged into 30mM HEPES 150mM NaCl pH 7, and analyzed by size exclusion chromatography on a Superdex 200 3.2/300. Analysis of 1ug of purified material by reducing and non-reducing SDS-PAGE on a Bis-Tris 4-12% gel was conducted.

B0001, B0002, and B0003 were expressed at over 8mg/L, and were over 95% monodispersed after purification as shown by size exclusion chromatography and reducing/non-reducing SDS-PAGE. This experiment shows that dual function bispecific molecules with immunomodulators at either the N or C terminus can be produced and the position of the IL-2M protein (either at the N or C terminus) did not significantly alter expression and therefore, either format can be used.

Example 16: Bispecific MAdCAM-tethered IL-2M Molecules Can Bind MAdCAM and CD25 Simultaneously

An immunosorbent plate was coated with recombinant mouse MAdCAM-1 at a concentration of 1μg/mL in PBS pH 7.4, 75 ul/well, and incubated overnight at 4°C. Wells were washed with PBS pH 7.4 containing 0.05% Tween-20 (wash buffer) three times, and then blocked with 200ul/well 1% BSA in PBS pH 7.4 (block buffer) for two hours at room temperature. After three washes with wash buffer, B0001, B0002, B0003 were diluted to 1nM, 10nM, and 100nM in PBS containing 1% BSA and 0.05% Tween-20 (assay buffer). The diluted material was added to the mouse MAdCAM-1 coated plate at 75ul/well for 1 hour at room temperature. After three washes with wash buffer, human CD25 was added to the plate at 75ul/well, at a concentration of 10nM in assay buffer for 1 hr at room temperature. After three washes with wash buffer, a goat biotinylated anti-human CD25 polyclonal antibody, diluted to 0.4μg/mL in assay buffer, was added to the plate at 75ul/well for 1 hr at room temperature. After three washes with wash buffer high sensitivity streptavidin HRP diluted in assay buffer at 1:5000 was added to the plate at 75ul / well for 15 minutes at room temperature. After three washes with wash buffer and 1 wash with wash buffer (with no tween-20), the assay was developed with TMB, and stopped with 1N HCL. OD 450nm was measured. The experiment included appropriate controls for non-specific binding of the proteins of Example 15 to the plate/block in the absence of mouse MAdCAM-1, as well as no CD25 controls, and mono-specific controls, that are unable to form a bridge between human CD25 and mouse MAdCAM.

It was found that that at concentrations of 1nM, 10nM, and 100nM, the bi-specific molecules of Example 15 were able to simultaneously interact with mouse MAdCAM and human CD25, whilst the monospecific controls, did not create a bridging signal. Additionally, there was no binding of any compound to CD25 at any concentration tested, when mouse MAdCAM-1 was not present on the plate surface, indicating none of the test compounds were interacting non-specifically with the plate surface. These results demonstrate that the bispecific molecules can bind both MAdCAM and CD25 simultaneously in a functional binding assay, such as an ELISA.

Example 17: In Vitro P-STAT5 Assay Demonstrating Activity and Selectivity of Bispecific MAdCAM-tethered IL-2M When In Solution or When Tethered

Recombinant mouse MAdCAM was coated onto wells of a 96 well high binding plate (Corning) overnight. After washing 2 times with PBS, the plate was blocked for 1 hour with 10% FBS RPMI media. A MAdCAM-tethered IL-2M bispecific of Example 15 or untethered

IL-2M control (such as those prepared in Example 12) were captured for 1 hour. After washing 2 times with PBS, freshly-isolated human PBMCs were stimulated for 60 minutes with captured IL-2M or for comparison IL-2M in solution. Cells were then fixed for 10 minutes with BD Cytofix, permeabilized sequentially with BD Perm III and BioLegend FOXP3 permeabilization buffer, blocked with human serum and stained for 30 minutes with antibodies against phospho-STAT5 FITC (CST), CD25 PE, FOXP3 AF647 and CD4 PerCP Cy5.5 (BD) and acquired on an Attune NXT with plate loader. In solution, both molecules have comparable activity and selectivity on Treg versus Teff. Plates coated with mouse MAdCAM were able to capture the bi-specific molecule of Example 15 and the captured/immobilized bi-specific molecule was still able to selectively activate T_{regs} over T_{effs}. This example demonstrates that MAdCAM-tethered IL-2M molecules can retain biological activity and selectivity when in solution or when captured/immobilized.

Example 18: Immunogenicity of IL-2 muteins

IL-2 Mutein sequences were analyzed using the NetMHCIIPan 3.2 software, which can be found at www "dot" cbs "dot" dtu "dot" dk/services/NetMHCIIpan/. Artificial neural networks were used to determine peptide affinity to MHC class II alleles. In that analysis, 9-residue peptides with potentially direct interaction with the MHC class II molecules were recognized as binding cores. Residues adjacent to binding cores, with potential to influence the binding indirectly, were also examined as masking residues. Peptides comprising both the binding cores and masking residues were marked as strong binders when their predicted K_D to the MHC class II molecule was lower than 50 nM. Strong binders have a greater chance of introducing T cell immunogenicity.

A total of 9 MHCII alleles that are highly represented in North America and Europe were included in the in silico analysis. The panel of IL-2M (IL-2 mutein) molecules tested included the IL-2 Muteins with L53I, L56I, L80I, or L118I mutations. Only MHCII alleles DRB1_1101, DRB1_1501, DRB1_0701, and DRB1_0101 yielded hits with any of the molecules assessed. The peptide hits for DRB_1501 were identical between all constructs tested including wild-type IL-2 with the C125S mutation. The addition of L80I removes 1 T cell epitope for DRB1-0101 [ALNLAPSKNFHLRPR] and modestly reduces the affinity of two other T cell epitopes [EEALNLAPSKNFHLR and EALNLAPSKNFHLRP]. For MHCII allele DRB1-0701, L80I removes 1 T cell epitope [EEALNLAPSKNFHLR]. Therefore, the data demonstrates that a IL-2

mutein comprising the L80I mutation should be less immunogenic, which is a surprising and unexpected result from the *in silico* analysis.

Example 19: Generation of Additional IL-2 Muteins

A pTT5 vector containing the single gene encoding the single IL-2M (IL-2 mutein) of SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 (and IL-2M control; SEQ ID NO: 50) polypeptide with human IL-2M fused N-terminally of human IgG1 Fc domain was transfected into HEK293 Expi cells. After 5-7 days, cell culture supernatants expressing SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 (and IL-2M control; SEQ ID NO: 50) were harvested, and clarified by centrifugation and filtration through a 0.22um filtration device. SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 (and IL-2M control; SEQ ID NO: 50) were captured on proA resin. The resin was washed with PBS pH 7.4 and the captured protein was eluted using 0.25% acetic acid pH 3.5, with neutralization using a tenth volume of 1M Tris pH 8.0. The protein was buffer exchanged into 30mM HEPES 150mM NaCl pH 7, and analyzed by size exclusion chromatography on a Superdex 200 3.2/300 column. Analysis of 5ug of purified material by reducing and non-reducing SDS-PAGE on a Bis-Tris 4-12% gel was conducted.

IL-2Ms SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 (and IL-2M control; SEQ ID NO: 50) expressed at over 45mg/L, and were over 95% monodispersed after purification as shown by size exclusion chromatography and reducing/non-reducing SDS-PAGE.

Example 20: IL-2Ms of Example 19 can bind CD25

An immunosorbent plate was coated with CD25 at a concentration of 0.5 µg/mL in PBS pH 7.4, 75 ul/well, and incubated overnight at 4°C. Wells were washed with PBS pH 7.4 containing 0.05% Tween-20 (wash buffer) three times, and then blocked with 200ul/well 1% BSA in PBS pH 7.4 (block buffer) for two hours at room temperature. After three washes with wash buffer IL-2Ms SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56 were diluted to eleven –two fold serial dilution in PBS containing 1% BSA and 0.05% Tween-20 (assay buffer) with 2nM being the highest concentration. The diluted material was added to the CD25 coated plate at 75ul/well for 1 hour at room temperature. After three washes with wash buffer, a goat biotinylated anti-IL-2 polyclonal antibody, diluted to 0.05µg/mL in assay buffer, was added to the plate at 75ul/well for 1 hr at room temperature. After three washes with wash buffer high sensitivity streptavidin HRP diluted in assay buffer at 1:5000 was added to the plate

at 75ul / well for 15 minutes at room temperature. After three washes with wash buffer and 1 wash with wash buffer (with no tween-20), the assay was developed with TMB, and stopped with 1N HCL. OD 450nm was measured. The experiment included appropriate controls for non-specific binding of the molecules to the plate/block in the absence of CD25. The results indicate that at concentrations of 2nM-1.9pM, the muteins of Example 19 were able to bind CD25 with sub nanomolar EC50s. Additionally, there was no detection of any compound at any concentration tested, when CD25 was not present on the plate surface, indicating none of the test compounds were interacting non-specifically with the plate surface. Thus, the muteins of Example 19 can bind to CD25.

Example 21: IL-2 Muteins of Example 19 are Potent and Selective

Peripheral blood mononuclear cells (PBMCs) were prepared using FICOLL-PAQUE Premium and Sepmate tubes from freshly isolated heparinized human whole blood. PBMCs were cultured in 10% fetal bovine serum RPMI medium in the presence of wild-type IL-2 or the muteins of Example 19 for 20 minutes and then fixed for 10 minutes with BD Cytofix. Fixed cells were sequentially permeabilized with BD Perm III and then BioLegend FOXP3 permeabilization buffer. After blocking with human serum for 10 minutes, cells were stained for 30 minutes with antibodies for phospho-STAT5 FITC (CST), CD25 PE, FOXP3 AF647 and CD4 PerCP Cy5.5 (all BD) and then acquired on an Attune NXT with plate reader. The IL-2 muteins of Example 19 were found to be potent and have selectivity against Treg versus Teff. The mutein comprising the L118I mutation was found to have increased activity and selectivity as compared to the other muteins.

Example 22: IL-2 muteins Expand Tregs in Humanized Mice

NSG mice humanized with human CD34+ hematopoietic stem cells were purchased from Jackson Labs. On days 0 and 7, the mice were dosed subcutaneously with 1ug IL-2 Mutein (SEQ ID NO: 50) or other IL-2 muteins SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, or SEQ ID NO: 56. On Day 7, mice were euthanized and whole blood and spleens were collected. Whole blood was aliquoted into a 96 well deep well plate and fixed for 10 minutes using BD Fix Lyse. Splenocytes were isolated using 70um filters (BD) and red blood cells were lysed using RBC lysis buffer from BioLegend. After washing with 2% fetal bovine serum PBS, splenocytes were labeled with near infrared live dead stain (Invitrogen) for 20 minutes and then fixed for 20 minutes using BioLegend fixation buffer. Both whole blood cells and splenocytes were then

permeabilized using BioLegend FOXP3 permeabilization buffer, blocked with human serum and stained for 30 minutes with antibodies against human CD8a FITC (BL), human CD25 PE (BD), human FOXP3 AF647 (BD) CD4 PerCP Cy5.5 (BD), human Siglec-8 PE Cy7 (BL), human CD3 BV421 (BL), human CD45 BV605 (BL), human CD56 BV785 (BL) and mouse CD45 (BV711) and acquired on an Attune NXT with plate loader.

Compared to vehicle control, IL-2Ms SEQ ID NO: 54 and SEQ ID NO: 56 selectively induced Tregs in mouse spleens and whole blood ($p<0.0005$ by ANOVA with Dunn's Multiple Comparison Test). The other IL-2Ms also increased the frequency of Tregs, though these changes compared to the vehicle group were not statistically significant. There were no significant changes in the frequencies of CD56pos NK cells, CD3pos T cells, CD8pos cytotoxic T lymphocytes, CD4pos helper T cells or CD25lo/FOXP3neg T effectors in mice dosed with SEQ ID NO: 54 and SEQ ID NO: 56. These results demonstrate that the IL-2 muteins increase the frequency of regulatory T cells.

Example 23: Generation of Bispecific mMAdCAM-tethered IL-2M Molecule

A bispecific MAdCAM-IL-2 mutein was produced, with the antibody being the heavy and light chains of MECA89. This was produced using two plasmids encoding both heavy and light chains were co-transfected at equimolar ratios. The first plasmid encoded the light chain of MECA-89 and the second encoded the full length IgG1 heavy chain of MECA-89 with C-terminally fused to a human IL-2M comprising the L118I mutation. After 3-5 days, cell culture supernatants expressing the bispecific were harvested, and clarified by centrifugation and filtration through a 0.22um filtration device. The bispecific was captured on proA resin. The resin was washed with PBS pH 7.4 and the captured protein was eluted using 0.25% acetic acid pH 3.5, with neutralization using a tenth volume of 1M Tris pH 8.0. The protein was buffer exchanged into 30mM HEPES 150mM NaCl pH 7, and analyzed by size exclusion chromatography on an AdvanceBio SEC column. Analysis of 1ug of purified material by reducing and non-reducing SDS-PAGE on a Bis-Tris 4-12% gel was conducted.

The bispecific molecule expressed at 17 mg/L, and was over 95% monodispersed after purification as shown by size exclusion chromatography and reducing/non-reducing SDS-PAGE. These results demonstrate that it was able to produce dual function bispecific molecules with immunomodulators at the C terminus.

Example 24: Generation of MAdCAM Antibodies.

A human antibody scFv phage library was panned against recombinant human, mouse, and cyno MAdCAM proteins across iterative selection rounds to enrich for antibody clones that recognize all three aforementioned species orthologues of MAdCAM. The scFv clones were configured in nt-VH-Linker-VL-ct format and fused to the M13 phage surface via the pIII coat protein. After selections, clonal scFvs were screened by ELISA for binding to human, mouse, and cyno MAdCAM expressed on the cell surface of CHO cells. Clones that were found to be cross reactive to all three cell surface expressed MAdCAM species orthologues were converted using standard molecular biology techniques or gene synthesis, into a human IgG1 format whereby each molecule was comprised of four polypeptide chains in total (2 heavy, and 2 light chains). The two light chains were identical to eachother and the two heavy chains were identical to eachother. The two identical heavy chains (1 and 2) homodimerize and the two identical light chains (3 and 4) pair with each heavy chain to form an intact human IgG1. The Fc domain contains the L234A, L235A, and G237A mutations to ablate FcγR interactions. The format can be illustrated as follows:

Chain 1: nt-VH1-CH1-CH2-CH3-ct
Chain 2: nt-VH1-CH1-CH2-CH3-ct
Chain 3: nt-VK1-CK-ct
Chain 4: nt-VK1-CK-ct

In addition, MAdCAM scFvs were also converted using standard molecular biology techniques (such as Gibson Cloning procedure) or gene synthesis into a bispecific format whereby an IL-2M was situated at the c-terminus of the IgG heavy chain of the MAdCAM antibody, as outlined below:

Chain 1: nt-VH1-CH1-CH2-CH3-ct-Linker-IL-2M
Chain 2: nt-VH1-CH1-CH2-CH3-ct-Linker-IL-2M
Chain 3: nt-VK1-CK-ct
Chain 4: nt-VK1-CK-ct

An ELISA was used to analyze binding of anti-MAdCAM scFvs to captured or plate bound human, cyno, and mouse MAdCAM. Biotinylated human and cyno MAdCAM were captured on a streptavidin coated plate, and mouse MAdCAM-Fc coated directly onto an immunosorbent plate. After a blocking step, the plates were washed and scFv in crude periplasmic lysate was applied to the plate surface. scFv binding was detected using an anti-V5 HRP conjugate. The

assay was developed with TMB substrate and stopped with acid. The absorbance at 450nm was measured. Appropriate wash steps were applied between each step of the ELISA. Human versus cyno and human versus mouse were evaluated. The scFv's were also analyzed using surface plasmon resonance technology. After being captured on a biosensor surface via the V5 tag, soluble monomeric human MAdCAM was titrated and both binding and dissociation measured and fit to a 1:1 binding model allowing the derivation of on and off-rates.

The results measured indicate that the majority of clones tested have human and cyno MAdCAM binding cross reactivity and a small panel have additional cross reactivity to mouse MAdCAM. Biosensor experiments demonstrated that the clones exhibited a range of binding on and off-rates against human MAdCAM with k_a values ranging from 10^3 1/Ms through 10^7 1/Ms and k_d values ranging 10^{-1}through 10^{-4} 1/s. Certain clones have an off-rate slower than 2x10e2 1/s. Thus, MadCAM antibodies were generated and can be used in a bi-specific format.

Example 25: Generation of Bispecific Human MAdCAM-tethered IL-2Ms of Example 19

Two plasmids each were co-transfected at equimolar ratios. The first plasmid in each case encoded the light chain of Hu.MAdCAM and the second encoded the full length IgG1 heavy chain of Hu.MAdCAM with a C-terminally fused human IL-2M comprising the L118I mutation as illustrated in the Table of MAdCAM-IL-2 Mutein Bispecific Compounds provided herein. After 3-5 days, cell culture supernatants expressing the Hu.MAdCAM-IL-2M bispecifics was harvested, and clarified by centrifugation and filtration through a 0.22um filtration device. The Hu.MAdCAM-IL-2M bispecifics were captured on proA resin. The resin was washed with PBS pH 7.4 and the captured proteins were eluted using 0.25% acetic acid pH 3.5, with neutralization using a tenth volume of 1M Tris pH 8.0. The proteins were buffer exchanged into 30mM HEPES 150mM NaCl pH 7, and analyzed by size exclusion chromatography on an AdvanceBio SEC column. Analysis of 1ug of purified material by reducing and non-reducing SDS-PAGE on a Bis-Tris 4-12% gel was conducted. The Hu.MAdCAM-IL-2M bispecifics expressed at over 10 mg/L, and was over 95% monodispersed after purification as shown by size exclusion chromatography and reducing/non-reducing SDS-PAGE. Thus, these results demonstrate that fully human dual function bispecific molecules with immunomodulators at the C terminus can be produced.

Example 26: Durability of Signaling induced by IL-2 muteins

Peripheral blood mononuclear cells (PBMCs) were prepared using FICOLL-PAQUE Premium and Sepmate tubes from freshly isolated heparinized human whole blood. PBMCs were cultured in 10% fetal bovine serum RPMI medium in the presence of IL-2Ms for 60 minutes. Cells were then wash 3 times and incubated for an additional 3 hours. Cells were then fixed for 10 minutes with BD Cytofix. Fixed cells were sequentially permeabilized with BD Perm III and then BioLegend FOXP3 permeabilization buffer. After blocking with human serum for 10 minutes, cells were stained for 30 minutes with antibodies for phospho-STAT5 FITC, CD25 PE, FOXP3 AF647 and CD4 PerCP Cy5.5 and then acquired on an Attune NXT with plate reader. All four IL-2 muteins of Exmaple 19 induced durable signaling in Treg but not in Teff as compared to the control. An IL-2 mutein of SEQ ID NO: 56 is superior to an IL-2 mutein of SEQ ID NO: 55, SEQ ID NO: 54 or SEQ ID NO: 53. These results demonstrate that the IL-2 can induce durable and selective signaling in Treg which should lead to greater Treg expansion in vivo and permit less frequent dosing to achieve Treg expansion.

Example 27: In Vitro P-STAT5 Assay Demonstrates Activity and Selectivity of Bispecific Hu.MAdCAM-tethered IL-2Muteins When In Solution or When Tethered

Recombinant human MAdCAM was coated onto wells of a 96 well high binding plate (Corning) overnight. After washing 2 times with PBS, the plate was blocked for 1 hour with 10% FBS RPMI media. MAdCAM-tethered IL-2M mutein bi-specifics or untethered IL-2M control were captured for 1 hour. After washing 2 times with PBS, freshly-isolated human PBMCs were stimulated for 60 minutes with captured IL-2MM or for comparison IL-2MM in solution. Cells were then fixed for 10 minutes with BD Cytofix, permeabilized sequentially with BD Perm III and BioLegend FOXP3 permeabilization buffer, blocked with human serum and stained for 30 minutes with antibodies against phospho-STAT5 FITC (CST), CD25 PE, FOXP3 AF647 and CD4 PerCP Cy5.5 (BD) and acquired on an Attune NXT with plate loader.

Results: In solution, IL-2M bi-specifics tethered to human MAdCAM and the control have comparable activity and selectivity on Treg versus Teff. Plates coated with MAdCAM were able to capture bi-specifics, and the captured/immobilized bi-specifics were still able to selectively activate Tregs over Teffs. This example demonstrates that IL-2MM bi-specifics targeting human MAdCAM can retain biological activity and selectivity when in solution or when captured/immobilized.

The examples provided for herein demonstrate the surprising and unexpected result that a bispecific molecule comprising a MAdCAM antibody and a IL-2 mutein can function to selectively and potently activate Tregs over Teffs, which demonstrates that the molecules can be used to treat or ameliorate the conditions described herein. The examples also demonstrate that the IL-2 mutein can function to selectively and potently activate Tregs over Teffs when used alone (or linked to a Fc protein) as provided for herein.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While various embodiments have been disclosed with reference to specific aspects, it is apparent that other aspects and variations of these embodiments may be devised by others skilled in the art without departing from the true spirit and scope of the embodiments. The appended claims are intended to be construed to include all such aspects and equivalent variations.

What is claimed is:

1. A polypeptide comprising a targeting moiety that binds to a target cell and an effector binding/modulating moiety, wherein the effector binding/modulating moiety is a IL-2 mutein polypeptide (IL-2 mutein).

2. The polypeptide of claim 1, wherein the targeting moiety comprises an antibody that binds to a target protein on the surface of a target cell.

3. The polypeptide of claim 1, wherein the antibody is an antibody that binds to MAdCAM, OAT1, OCT2, FXYD2, TSPAN7, DPP6, HEPACAM2, TMEM27, or GPR119.

4. The polypeptide of claim 1, wherein the IL-2 mutein binds to a receptor expressed by an immune cell.

5. The polypeptide of claim 1, wherein the the immune cell contributes to an unwanted immune response.

6. The polypeptide of claim 1, wherein the immune cell causes a disease pathology.

7. The polypeptide of claim 1, wherein the targeting moiety comprises an anti-MAdCAM antibody.

8. The polypeptide of claim 1, wherein the compound has the formula from N-terminus to C-terminus:

R1---Linker Region A—R2 or R3—Linker Region B—R4,

wherein,

R1, R2, R3, and R4, each independently comprises the effector binding/modulating moiety, the targeting moiety, or is absent.

9. The polypeptide of claim 8, wherein each of Linker Region A and Linker Region B comprises an Fc region.

10. The polypeptide of claim 9, wherein one of R1 and R2 is the IL-mutein antibody and one of R1 and R2 is an anti-MAdCAM antibody.

11. The polypeptide of claim 8, wherein R1 is the IL-mutein and R2 is an anti-MAdCAM antibody.

12. The polypeptide of claim 8, wherein one of R1 is anti-MAdCAM antibody and one R2 is an anti-PD-1 antibody.

13. The polypeptide of claim 8, wherein one of R3 and R4 is the IL-2 mutein and one of R3 and R4 is an anti-MAdCAM antibody.

14. The polypeptide of claim 8, wherein R3 is the IL-2 mutein and R4 is an anti-MAdCAM antibody.

15. The polypeptide of claim 8, wherein R3 is an anti-MAdCAM antibody and one R4 is the IL-2 mutein.

16. The polypeptide of any of claims 8-15, wherein the linker is absent.

17. The polypeptide of any of claims 8-15, wherein the linker is a Fc region.

18. The polypeptide of any of claims 8-15, wherein the linker is a glycine/serine linker.

19. The polypeptide of claim 8, wherein the linker is GGGGSGGGGSGGGGSGGGGS, GGGGSGGGGSGGGGS, GGGGSGGGGS, or GGGGS.

20. The polypeptide of claim 1, wherein the IL-2 mutein comprises a IL-2 sequence of SEQ ID NO: 6, wherein peptide comprises a mutation at a position that corresponds to position 53, 56, 80, or 118 of SEQ ID NO: 6.

21. The polypeptide of any one of claims 1-20, wherein the IL-2 mutein comprises a IL-2 sequence of SEQ ID NO: 6, wherein peptide comprises a mutation at a position that corresponds to position 53, 56, 80, or 118 of SEQ ID NO: 6.

22. The polypeptide of claim 20, wherein the mutation is a L to I mutation at position 53, 56, 80, or 118.

23. The polypeptide of claim 21, wherein the mutation is a L to I mutation at position 53, 56, 80, or 118.

24. The polypeptide of claim 1, 20, or 23, further comprising a mutation at one or more positions of 29, 31, 35, 37, 48, 69, 71, 74, 88, and 125 in SEQ ID NO: 6.

25. The polypeptide of any of the preceding claims, wherein the mutein further comprises a mutation at one or more of positions E15, H16, Q22, D84, E95, or Q126 or 1, 2, 3, 4, 5, or each of positions E15, H16, Q22, D84, E95, or Q126 is wild-type.

26. The polypeptide of any of the preceding claims, wherein the mutation in the mutein is one or more of E15Q, H16N, Q22E, D84N, E95Q, or Q126E.

27. The polypeptide of any of the preceding claims, wherein the mutein comprises a N29S mutation in SEQ ID NO: 6.

28. The polypeptide of any of the preceding claims, wherein the mutein comprises a Y31S or a Y51H mutation.

29. The polypeptide of any of the preceding claims, wherein the mutein comprises a K35R mutation.

30. The polypeptide of any of the preceding claims, wherein the mutein comprises a T37A mutation.

31. The polypeptide of any of the preceding claims, wherein the mutein comprises a K48E mutation.

32. The polypeptide of any of the preceding claims, wherein the mutein comprises a V69A mutation.

33. The polypeptide of any of the preceding claims, wherein the mutein comprises a N71R mutation.

34. The polypeptide of any of the preceding claims, wherein the mutein comprises a Q74P mutation.

35. The polypeptide of any of the preceding claims, wherein the mutein comprises a N88D or a N88R mutation.

36. The polypeptide of any of the preceding claims, wherein the mutein comprises a C125A or C125S mutation.

37. The polypeptide of any of the preceding claims, wherein the IL-2 mutein is fused or linked to a Fc peptide.

38. The polypeptide of claim 37, wherein the Fc peptide comprises a mutation at one or more of positions of L234, L247, L235, L248, G237, and G250.

39. The polypeptide of claim 37, wherein the mutation is L to A or G to A mutation.

40. The polypeptide of claim 37, wherein the Fc peptide comprises L247A, L248A, and G250A mutations.

41. The polypeptide of claim 37, wherein the Fc peptide comprises a L234A mutation, a L235A mutation, and/or a G237A mutation.

42. The polypeptide of claim 1, wherein the polypeptide comprises a first chain and a second chain that form the polypeptide, wherein

the first chain comprises:

V_H-H_c-Linker-C_1, wherein V_H is a variable heavy domain that binds to the target cell with a V_L domain of the second chain; H_c is a heavy chain of antibody comprising CH1-CH2-CH3 domain, the Linker is a glycine/serine linker, and C_1 is a IL-2 mutein fused to a Fc protein in either the N-terminal or C-terminal orientation; and

the second chain comprises:

V_L-L_c, wherein V_L is a variable light chain domain that binds to the target cell with the V_H domain of the first chain, and the Lc domain is a light chain CK domain.

43. The polypeptide of claim 42, wherein the VH and VL domain are anti-MAdCAM variable domains that bind to MAdCAM expressed on a cell.

44. The polypeptide of claim 42, wherein the IL-2 mutein comprises a mutation at a position that corresponds to position 53, 56, 80, or 118 of SEQ ID NO: 6.

45. The polypeptide of claim 44, wherein the mutation is a L to I mutation at position 53, 56, 80, or 118.

46. The polypeptide of claim 44, wherein the mutein further comprises a mutation at a position that corresponds to position 69, 75, 88, or 125, or any combination thereof.

47. The polypeptide of claim 44, comprises a mutation selected from the group consisting of: at one of L53I, L56I, L80I, and L118I and the mutations of V69A, Q74P, N88D or N88R, and optionally C125A or C125S.

48. The polypeptide of claim 47, wherein the IL-2 mutein comprises a L53I mutation.

49. The polypeptide of claim 47, wherein the IL-2 mutein comprises a L56I mutation.

50. The polypeptide of claim 47, wherein the IL-2 mutein comprises a L80I mutation.

51. The polypeptide of claim 47, wherein the IL-2 mutein comprises a L118I mutation.

52. The polypeptide of claim 47, wherein the IL-2 mutein does not comprises any other mutations.

53. The polypeptide of claim 47, wherein the Fc protein comprises L247A, L248A, and G250A mutations or a L234A mutation, a L235A mutation, and/or a G237A mutation according to KABAT numbering.

54. The polypeptide of claim 47, wherein the Linker comprises a sequence of GGGGSGGGGSGGGGS or GGGGSGGGGSGGGGSGGGGS.

55. The polypeptide of any of the preceding claims, wherein the polypeptide comprises a Fc peptide comprising a sequence described herein.

56. A method of treating a subject with inflammatory bowel disease, the method comprising administering a polypeptide of any of claims 1-55 to the subject to treat the inflammatory bowel disease.

57. The method of claim 56, wherein the subject with inflammatory bowel disease has Crohn's disease.

58. The method of claim 56, wherein the subject with inflammatory bowel disease has ulcerative colitis.

59. A method of treating a subject with auto-immune hepatitis, the method comprising administering a therapeutic compound of any of claims 1-55 to the subject to treat the auto-immune hepatitis.

60. A method of treating primary sclerosing cholangitis the method comprising administering a therapeutic compound of any of claims 1-55 to the subject to treat the primary sclerosing cholangitis.

61. A method of treating Type 1 diabetes the method comprising administering a therapeutic compound of any of claims 1-55 to the subject to treat the Type 1 diabetes.

62. A method of treating a transplant subject comprising administering a therapeutically effective amount of a therapeutic compound of any of claims 1-55 to the subject, thereby treating a transplant (recipient) subject.

63. A method of treating GVHD in a subject having a transplanted a donor tissue comprising administering a therapeutically effective amount of a therapeutic compound of any of claims 1-55 to the subject.

64. A method of treating a subject having, or at risk, or elevated risk, for having, an autoimmune disorder, comprising administering a therapeutically effective amount of a therapeutic compound of any claims 1-55, thereby treating the subject.

65. The method of any of claims 56-64, wherein the subject has an autoimmune disorder and the therapeutic compound does not comprise an autoantigenic peptide or polypeptide characteristic of the autoimmune disorder, e.g., does not comprise a peptide or polypeptide against which the subject has autoantibodies.

66. A nucleic acid encoding a therapeutic compound of any of claims 1-55.

67. A vector comprising the nucleic acid of claim 65.

68. A cell comprising the nucleic acid of claim 66 or the vector of claim 67.

68. A method of making a therapeutic compound comprising culturing a cell of claim 67 to make the therapeutic compound.

69. A method of making a nucleic acid sequence encoding a therapeutic compound of claim 1-55, comprising

a) providing a vector comprising sequence encoding a targeting moiety and inserting into the vector sequence encoding an effector binding/modulating moiety to form a sequence encoding a therapeutic compound; or

b) providing a vector comprising sequence encoding an effector binding/modulating moiety and inserting into the vector sequence encoding a targeting moiety to form a sequence encoding a therapeutic compound,

thereby making a sequence encoding a therapeutic compound.

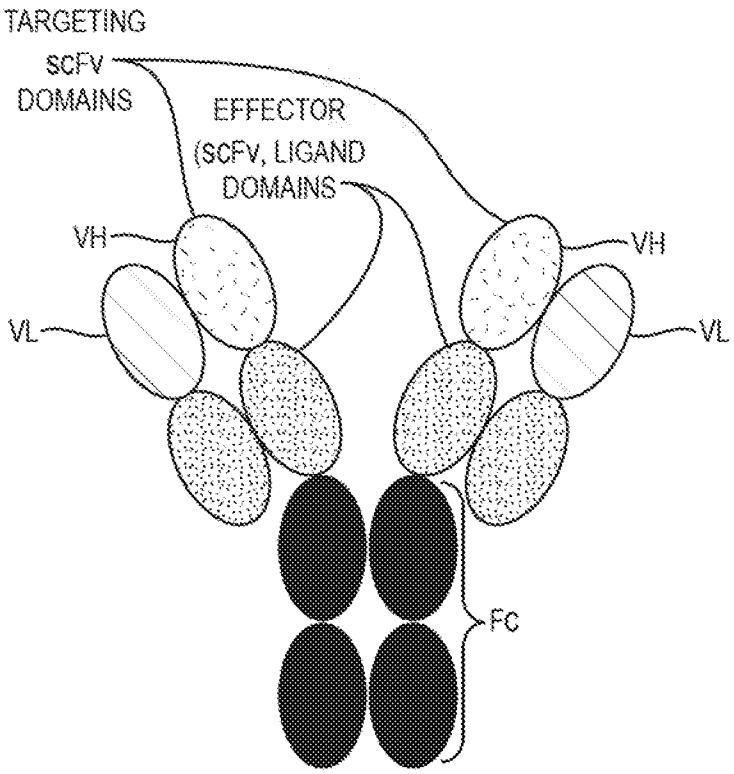
TARGETING
scFv
DOMAINS
EFFECTOR
(scFv, LIGAND
DOMAINS
VH
VL
VH
VL
Fc

FIG. 1

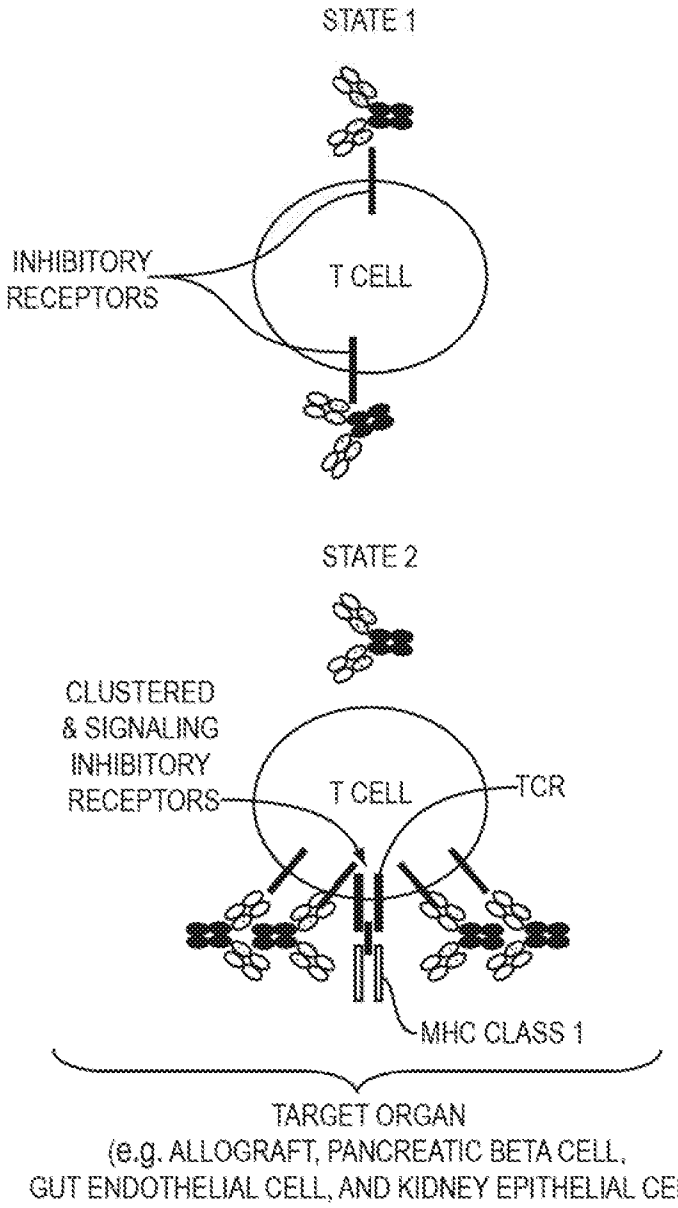
STATE 1
INHIBITORY RECEPTORS
T CELL
STATE 2
CLUSTERED & SIGNALING INHIBITORY RECEPTORS
T CELL
TCR
MHC CLASS 1
TARGET ORGAN
(e.g. ALLOGRAFT, PANCREATIC BETA CELL, GUT ENDOTHELIAL CELL, AND KIDNEY EPITHELIAL CELL)

FIG. 2

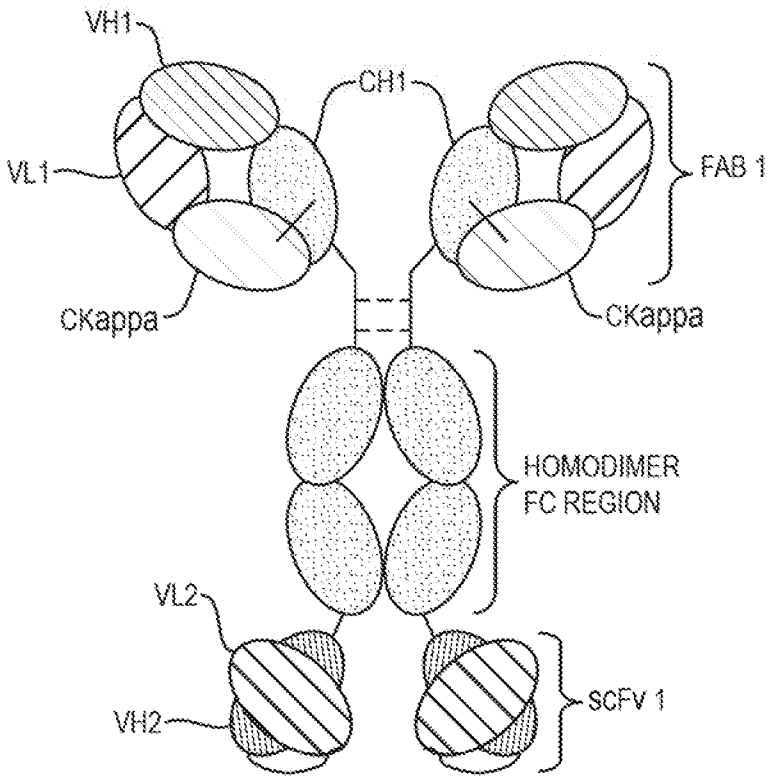
VH1
CH1
VL1
FAB 1
CKappa
CKappa
HOMODIMER
FC REGION
VL2
VH2
SCFv 1

FIG. 3

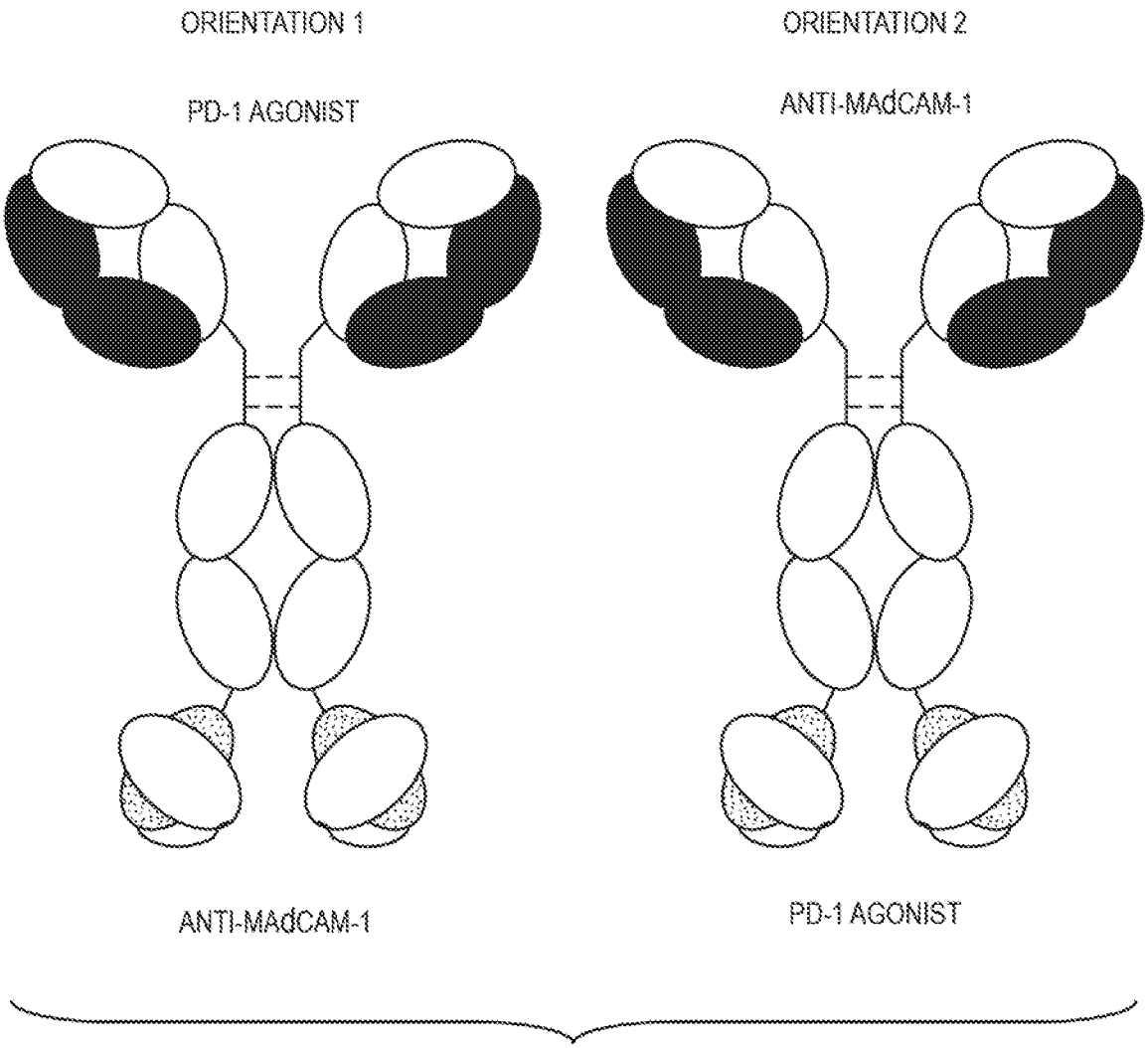
ORIENTATION 1
PD-1 AGONIST
ANTI-MAdCAM-1
ORIENTATION 2
ANTI-MAdCAM-1
PD-1 AGONIST

FIG. 3A

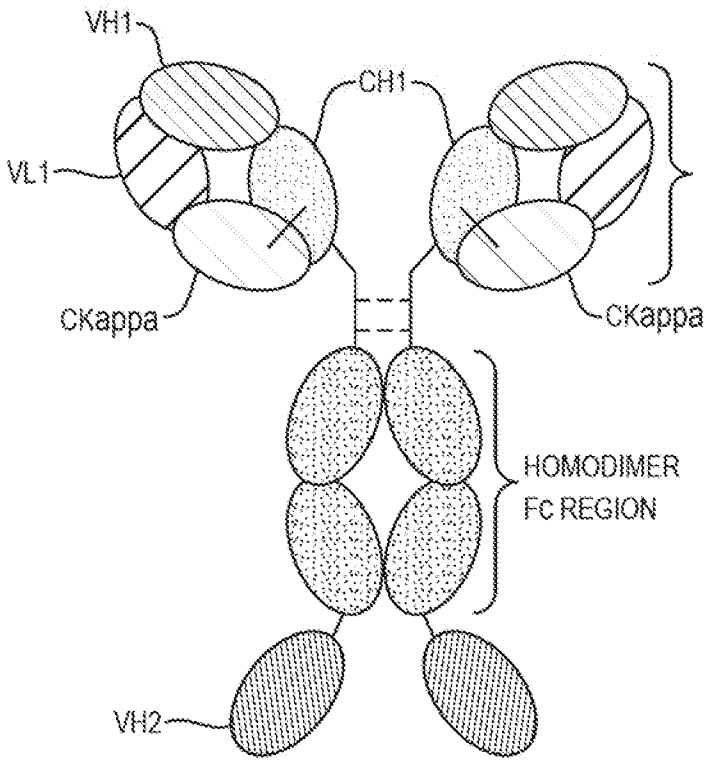

FIG. 4

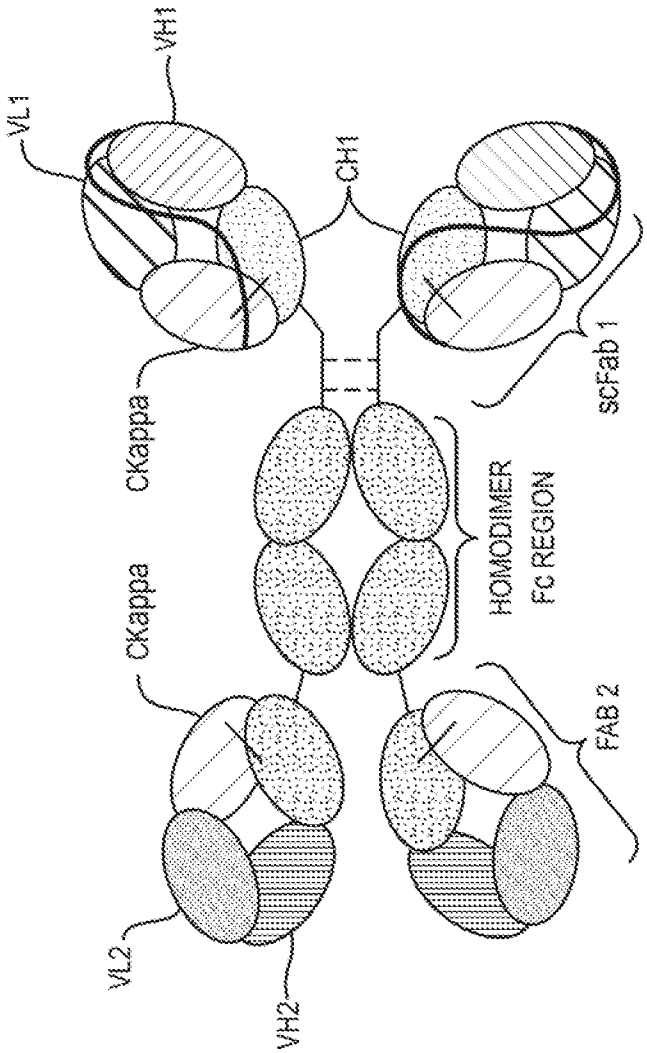
VL1
VH1
CH1
CKappa
scFab 1
HOMODIMER
Fc REGION
CKappa
FAB 2
VL2
VH2

FIG. 5

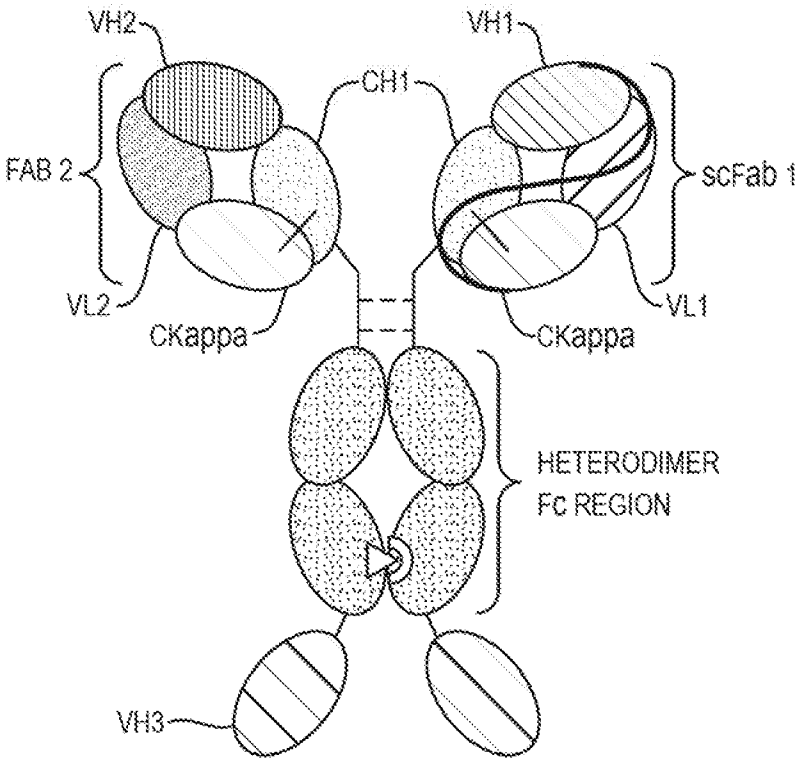
VH2
VH1
CH1
FAB 2
scFab 1
VL2
CKappa
CKappa
VL1
HETERODIMER
FC REGION
VH3

FIG. 6

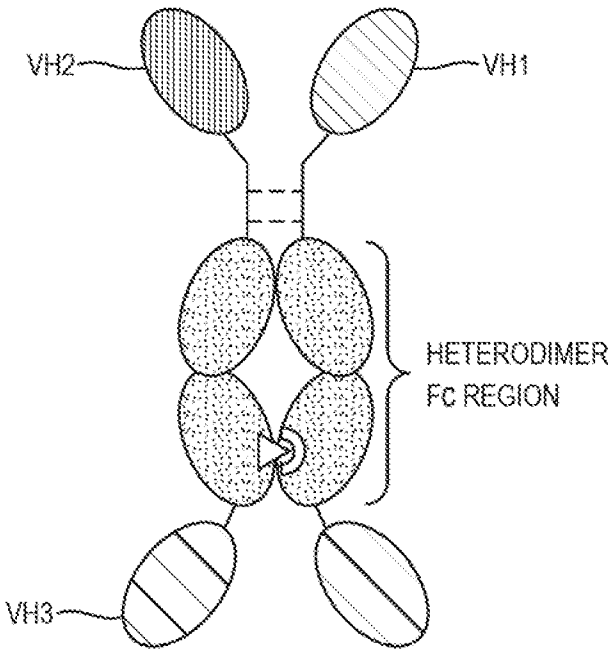

FIG. 7

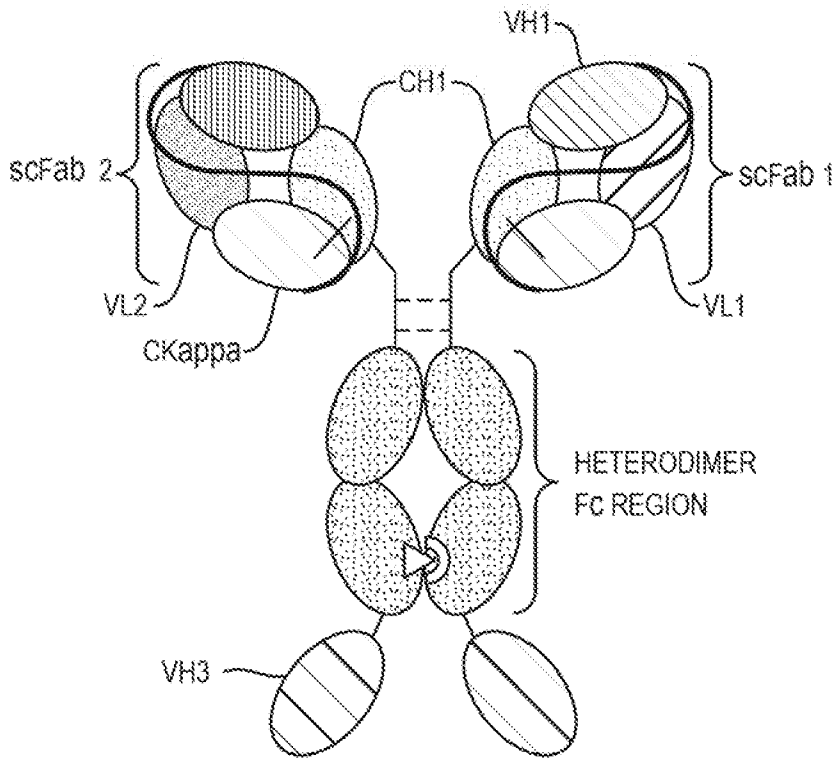
VH1
CH1
scFab 2
scFab 1
VL2
VL1
CKappa
HETERODIMER
FC REGION
VH3

FIG. 8

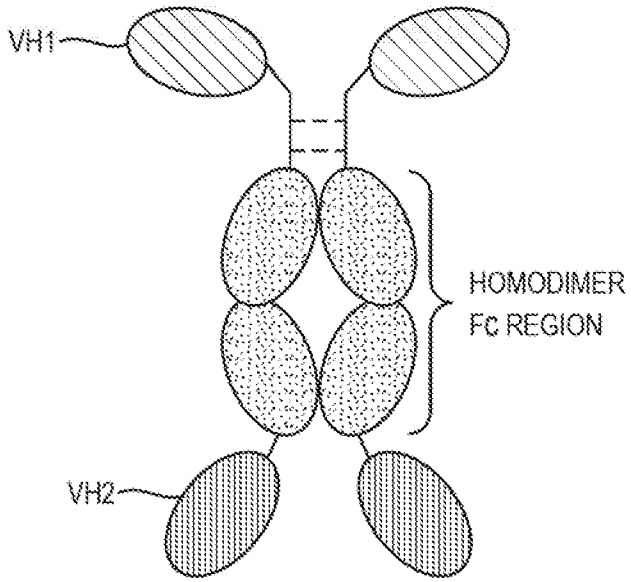

FIG. 9

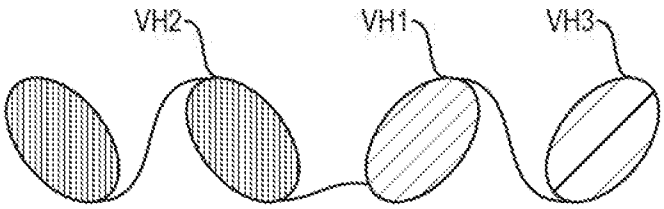

FIG. 10

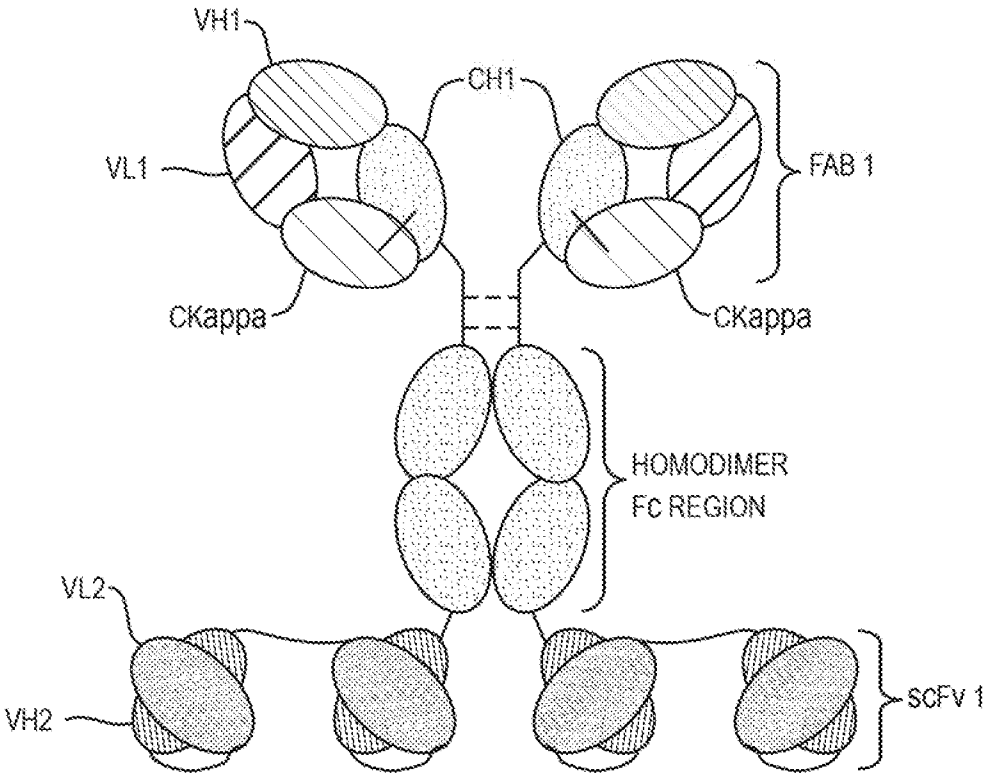

FIG. 11

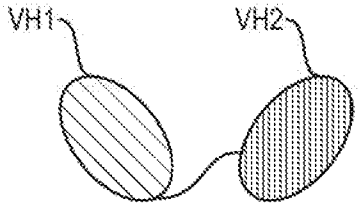

FIG. 12

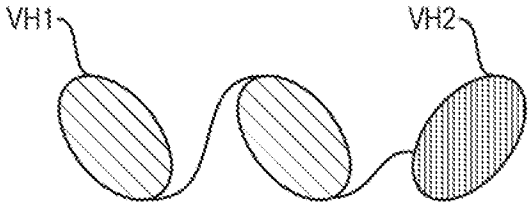

FIG. 13

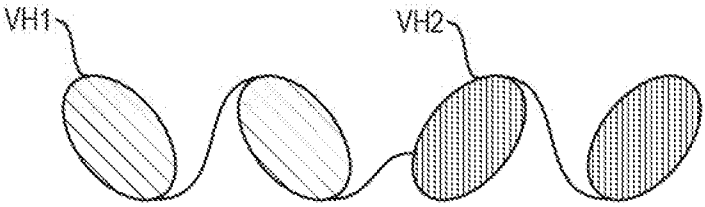

FIG. 14

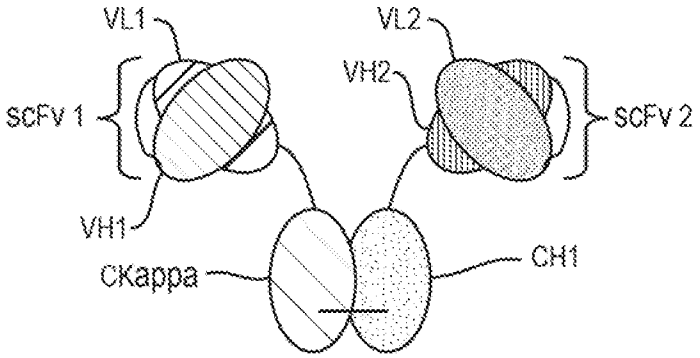

FIG. 15

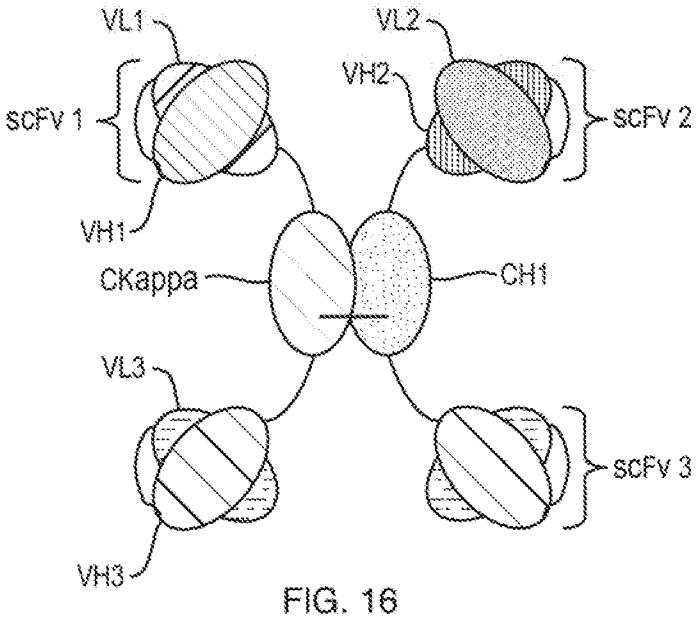

FIG. 16

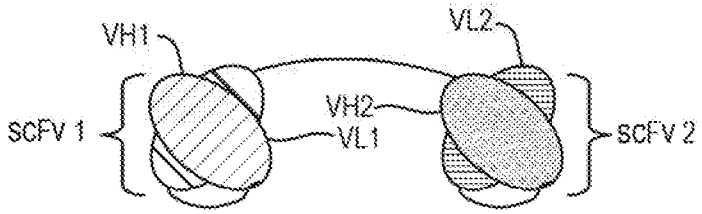

FIG. 17

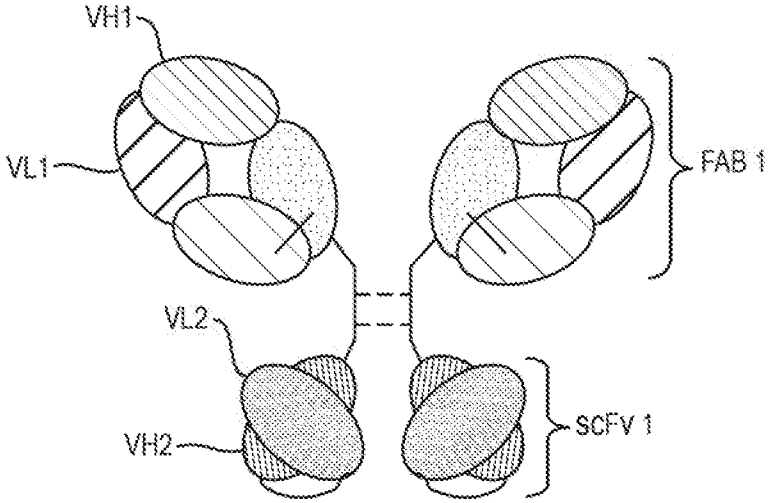

FIG. 18

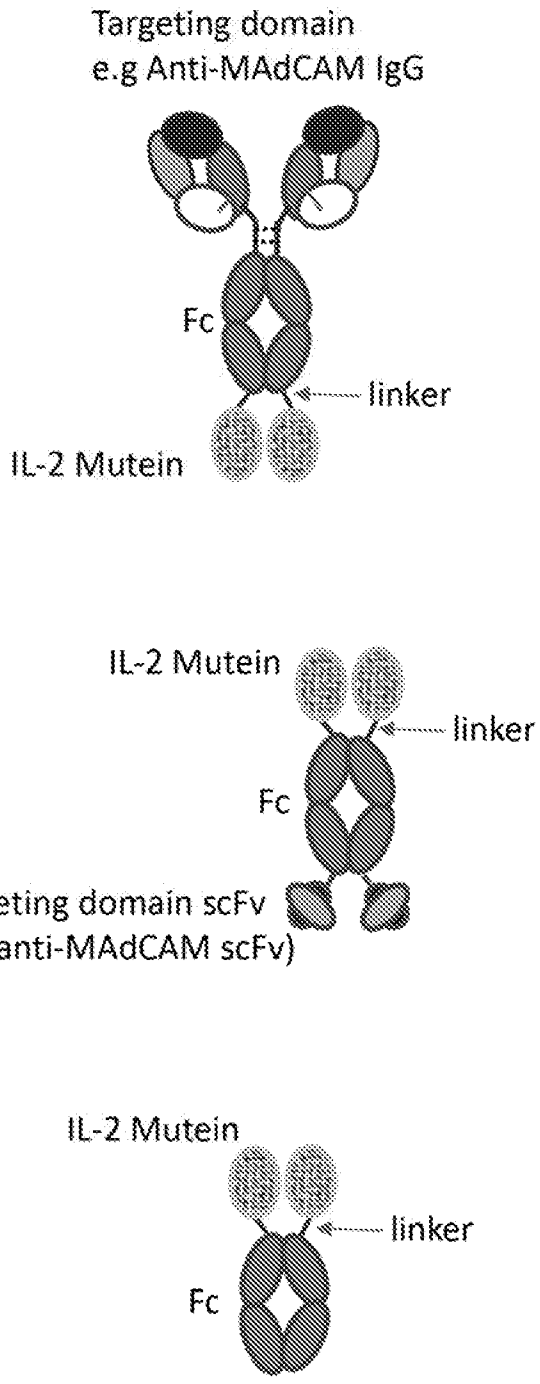
Targeting domain
e.g Anti-MAdCAM IgG
Fc
linker
IL-2 Mutein
IL-2 Mutein
linker
Fc
Targeting domain scFv
(e.g anti-MAdCAM scFv)
IL-2 Mutein
linker
Fc

FIG. 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/34334

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 38/20, 39/395; C07K 14/55 (2018.01)
CPC - A61K 38/20, 38/2013, 39/395, 39/39533; C07K 14/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2016/0175397 A1 (HOFFMANN - LA ROCHE INC) 23 June 2016; paragraphs [0017], [0239], [0240], [0261]	1-2, 4-6 --------- 3, 7
Y	US 2017/0051057 A1 (PFIZER INC et al.) 23 February 2017; paragraphs [0056], [0085]	3, 7
A	US 2006/0263857 A1 (LEFRANCOIS, L et al.) 23 November 2006; paragraphs [0025], [0195]	8-11, 13-15, 16/8-11, 16/13-15, 17/8-11, 17/13-15
A	US 2004/0132977 A1 (GANTIER, R et al.) 8 July 2004; paragraph [0520]	20
A	(SCHANZER, JM et al.) A human cytokine/single-chain antibody fusion protein for simultaneous delivery of GM-CSF and IL-2 to Ep-CAM overexpressing tumor cells. Cancer Immunity. 17 February 2006, Vol. 6; p. 4; page 2, 1st column, 2nd and 4th paragraphs	42-45

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 September 2018 (19.09.2018)	16 OCT 2018
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/34334

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 12
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 12 is indefinite for the following reason(s): the claim is not a proper dependent claim that, ultimately, depends from Claim 1. In particular, Claim 12 recites two antibodies in a construct having an R1 and an R2 joined by a linking moiety in contrast to Claim 1, wherein at least one moiety must be an IL-2 mutein. Since no such IL-2 mutein is included in Claim 12, the claim is not a properly constructed dependent claim (eventually) of Claim 1, and therefore has been considered to be unsearchable.

3. ☒ Claims Nos.: 21-41, 55-68a, 68b, and 69
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please see supplemental page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Groups I+, Claims 1-11, 13-20, 42-54; an antibody that binds to MadCAM (targeting moiety), SEQ ID NO: 6 with an L to I mutation at position 53 (L53I) (IL-2 mutein sequence), and a wild-type Fc domain (linker)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/34334

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-11, 13-20, 42-54, an antibody that binds to MadCAM, an IL-2 mutein encompassing SEQ ID NO: 6 with an L to I mutation at position 53 (L53I), and a wild-type Fc domain sequence are directed toward a polypeptide comprising a targeting moiety that binds to a target cell and an IL-2 mutein polypeptide.

The polypeptide will be searched to the extent it encompasses an antibody that binds to MadCAM (first exemplary targeting moiety) an IL-2 mutein encompassing SEQ ID NO: 6 with an L to I mutation at position 53 (L53I) (first exemplary IL-2 mutein sequence), and a wild-type Fc domain (first exemplary linker). Applicant is invited to elect additional targeting moiety(ies), and/or IL-2 mutein sequence(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), and/or linker region(s), with specified sequence thereof, or with substitution(s) at specified site(s) of a sequence, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), to be searched. Additional targeting moiety(ies) and/or IL-2 mutein sequence(s) and/or linker region(s) will be searched upon the payment of additional fees. It is believed that claims 1, 2, 3 (in-part), 4-11, 13-17, 20 (in-part), 42, 43, 44 (in-part), and 45 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass an antibody that binds to MadCAM (targeting moiety), SEQ ID NO: 6 with an L to I mutation at position 53 (L53I) (IL-2 mutein sequence), and a wild-type Fc domain (linker). Applicants must specify the claims that encompass any additionally elected targeting moity(ies), and/or IL-2 mutein sequence(s) and/or linker region sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a targeting moiety encompassing an antibody that binds to OAT1 (targeting moiety).

No technical features are shared between the targeting moieties and/or IL-2 mutein mutations and/or linker sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a polypeptide comprising a targeting moiety that binds to a target cell and an effector binding/modulating moiety, wherein the effector binding/modulating moiety is an IL-2 mutein polypeptide (IL-2 mutein); these shared technical features are previously disclosed by US 2016/0175397 A1 to Hoffman-La Roche Inc. (hereinafter 'Hoffman-La Roche').

Hoffman-La Roche discloses a polypeptide comprising a targeting moiety that binds to a target cell (a targeting moiety that binds to a target cell; paragraphs [0018], [0019]) and an effector binding/modulating moiety, wherein the effector binding/modulating moiety is an IL-2 mutein polypeptide (IL-2 mutein) (an effector binding/modulating moiety, wherein the effector binding/modulating moiety is an IL-2 mutein polypeptide (IL-2 mutein); paragraphs [0018], [0020]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Hoffman-La Roche reference, unity of invention is lacking.