HUMANIZED IL-10 RECEPTOR BINDING MOLECULES AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application No. 63/506,798, filed on June 7, 2023, and U.S. Provisional Patent Application No. 63/511,122, filed on June 29, 2023, the disclosures of which are incorporated herein by reference in their entirety for all purposes.

BACKGROUND OF THE DISCLOSURE

[0002] Cytokine and growth-factor ligands typically signal through the multimerization of cell surface receptor subunits. In some instances, cytokines act as multispecific (e.g., bispecific or trispecific) ligands that facilitate the association of such receptor subunits, bringing intracellular domains into proximity such that intracellular signaling may occur. The cytokine determines which receptor subunits are associated to form the cytokine receptor complex. Cytokines thus act to bridge the individual receptor subunits into a receptor complex that results in intracellular signaling.

[0003] One useful approach to cytokine engineering has been to use single-domain antibodies (sdAbs), such as single variable domain (sFv) antibodies and variable heavy domain (VHH) antibodies. VHHs are sdAbs comprising only the variable domains derived from heavy chain antibodies derived from camelid species.

[0004] Consequently, there is a need in the art to engineer and generate single-domain molecules that have activity similar to their surrogate or cognate cytokine or that engage with the cytokine receptor subunits by preferentially binding a particular subunit (i.e., “biased” signaling) and that do not have independent off-target activity, such as single-domain antibodies (sdAbs) and dimers of sdAbs having two independent binding domains (i.e., two sdAbs having similar or distinct binding specificity) that independently bind different receptors or receptor subunits.

SUMMARY

[0005] The present disclosure provides IL-10 agonist compounds and compositions comprising a humanized single-domain antibody (sdAb) that binds to IL-10Rα (such as an anti- IL-10Rα sdAb or VHH joined to a humanized single-domain antibody (sdAb) that binds to IL-10Rβ (such as an anti-IL-10Rα sdAb or VHH). Such compositions are useful in the pairing of cellular receptors to generate desirable effects useful in the treatment of disease in mammalian subjects.

[0006] The present disclosure provides IL-10 agonist compounds comprising at least a first domain (e.g., a first single-domain antibody polypetide) that specifically binds to a first receptor subunit (e.g., IL-10Rα) and a second domain (e.g., second single-domain antibody polypetide) that specifically binds to a second receptor subunit (e.g., IL-10Rβ). In some examples, contacting the IL-10 agonist compound with a cell expressing the first and second receptor subunits results in the functional association of the first and second receptor subunits, thereby triggering their interaction and resulting in downstream signaling. In some examples, the downstream signaling is different from the downstream signaling resulting from the native ligand binding to the native receptor subunits. In some examples, the first and second receptor subunits occur in proximity in response to the cognate ligand binding and are referred to herein as "natural" cytokine receptor pairs.

[0007] In some examples, the present disclosure provides IL-10 agonist compounds that comprise a first domain (e.g., a first single-domain antibody polypetide) that binds to IL-10Rα of the IL-10 receptor and a second domain (e.g., a second single-domain antibody polypetide) that binds to IL-10Rβ of the IL-10 receptor. In some examples, contacting the IL-10 agonist compound with a cell expressing IL-10Rα of the IL-10 receptor and IL-10Rβ of the IL-10 receptor results in the functional association of IL-10Rα and IL-10Rβ, thereby resulting in functional dimerization of the receptors and downstream signaling.

[0008] In one aspect, the present disclosure provides an IL-10 agonist compound comprising a first single-domain antibody polypeptide joined to a second single-domain antibody polypeptide, wherein

the first single-domain antibody polypeptide specifically binds to the α subunit of the IL10 receptor (IL10Rα) and comprises: a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:224-228; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:229-235; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:236; and

wherein the second single-domain antibody polypeptide specifically binds to the β subunit of the IL10 receptor (IL10Rβ) and comprises: a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:296; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:297; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:298.

[0008a] In some examples, the present disclosure provides IL-10 agonist compounds

comprising a first single-domain antibody polypeptide joined to (e.g., covalently linked, such as via a polypeptide linker or a chemical linker, or stably associated via a non-covalent linkage via an Fc construct, as described in more detail herein) a second single-domain antibody polypeptide, wherein the first single-domain antibody polypeptide that specifically binds to the α subunit of the IL-10 receptor (IL10Rα) comprises: a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:224-228; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:229-235; and a CDR3 comprising the amino acid sequence of of SEQ ID NO:236; and wherein the second single-domain antibody polypeptide that specifically binds to the β subunit of the IL-10 receptor (IL10Rβ) comprises: a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:296; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:297; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:298.

[0009] In some examples, the present disclosure provides IL-10 agonist compounds, wherein the first single-domain antibody and the second single-domain antibody are joined by a

linker. In some examples, the present disclosure provides IL-10 agonist compounds comprising a single polpeptide chain, wherein the IL-10 agonist compounds comprise a first single-domain antibody that binds to IL10Rα and a second single-domain antibody that binds that binds to IL10Rβ , optionally wherein the first single-domain antibody and the second single-domain antibody are joined by a polypeptide linker, wherein the polyptide linker comprises 1-50, alternatively 1-30, alternatively 1-20, alternatively 1-15, alternatively 1-12 alternatively 1-10, alternatively 1-8, alternatively 1-6, alternatively 1-5, alternatively 1-4, alternatively 1-3, alternatively 1-2, alternatively 1 amino acid(s).

[0010] In some examples, the present disclosure provides IL-10 agonist compounds comprising a first single-domain antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:25-48; and a second single-domain antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:49 and 50. In some examples, the present disclosure provides IL-10 agonist compounds comprising a first single-domain antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:25-48; and a second single-domain antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:49 and 50, optionally wherein the first single-domain antibody and the second single-domain antibody are joined by a polypeptide linker comprising 1-50, alternatively 1-30, alternatively 1-20, alternatively 1-15, alternatively 1-12 alternatively 1-10, alternatively 1-8, alternatively 1-6, alternatively 1-5, alternatively 1-4, alternatively 1-3, alternatively 1-2, alternatively 1 amino acid(s). In some examples, the present disclosure provides IL-10 agonist compounds comprising a first single-domain antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:25-48; and a second single-domain antibody comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:49 and 50 wherein the first single-domain antibody and the second single-domain antibody are joined by a polypeptide linker selected from the group consisting of SEQ ID NOS:416-439.

[0011] In some examples, the present disclosure provides IL-10 agonist compounds, wherein the C-terminus of the first single-domain antibody is joined to the N-terminus of the second single-domain antibody, optionally wherein the C-terminus of the first single-domain antibody is joined to the N-terminus of the second single-domain antibody via a linker.

[0012] In some examples, the present disclosure provides IL-10 agonist compounds, wherein the N-terminus of the first single-domain antibody is joined to the C-terminus of the second single-domain antibody, optionally wherein the N-terminus of the first single-domain antibody is joined to the C-terminus of the second single-domain antibody via a linker. In some

examples, the present disclosure provides IL-10 agonist compounds comprising a first single-domain antibody that binds to the extracellular domain of IL10Rα and second single-domain antibody that binds to the extracellular domain of IL10Rβ, wherein the N-terminus of the first single-domain antibody is joined to the C-terminus of the second single-domain antibody that binds to IL10Rβ, optionally wherein the N-terminus of the first single-domain antibody is joined to the C-terminus of the second single-domain antibody via a polypeptide linker comprising 1-50, alternatively 1-30, alternatively 1-20, alternatively 1-15, alternatively 1-12 alternatively 1-10, alternatively 1-8, alternatively 1-6, alternatively 1-5, alternatively 1-4, alternatively 1-3, alternatively 1-2, alternatively 1 amino acid(s). In some examples, the present disclosure provides IL-10 agonist compounds comprising a first single-domain antibody that binds to the extracellular domain of IL10Rβ and second single-domain antibody that binds to the extracellular domain of IL10Rα, wherein the N-terminus of the first single-domain antibody is joined to the C-terminus of the second single-domain antibody that binds to IL10Rβ, optionally wherein the N-terminus of the first single-domain antibody is joined to the C-terminus of the second single-domain antibody via a polypeptide linker comprising 1-50, alternatively 1-30, alternatively 1-20, alternatively 1-15, alternatively 1-12 alternatively 1-10, alternatively 1-8, alternatively 1-6, alternatively 1-5, alternatively 1-4, alternatively 1-3, alternatively 1-2, alternatively 1 amino acid(s).

[0013] In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide selected from the group consisting of: SEQ ID NOS:1-24 and 500-523.

[0014] In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95% amino acid sequence identity to an amino acid sequence selected from the group consisting of: SEQ ID NOS:1-24 and 500-523. In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino acid sequence selected from the group consisting of: SEQ ID NOS:1-24 and 500-523. In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino acid sequence selected from the group consisting of: SEQ ID NOS:1-24 and 500-523. In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino

acid of SEQ ID NO:1 (DR2463). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino acid of SEQ ID NO:2 (DR2485). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino acid of SEQ ID NO:3 (DR2519). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino acid of SEQ ID NO:4 (DR2520).

[0015] In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino acid of SEQ ID NO:500 (DR2463, non-his tagged). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino acid of SEQ ID NO:501 (DR2485, non-his tagged). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino acid of SEQ ID NO:502 (DR2519, non-his tagged). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising at least 95%, alternatively at least 96%, alternatively at least 97%, alternatively at least 98%, alternatively at least 99%, or alternatively 100% amino acid sequence identity to an amino acid of SEQ ID NO:503 (DR2520, non his-tagged).

[0016] In some examples, the present disclosure provides IL-10 agonist compounds, wherein the first single-domain antibody polypeptide comprises a combination of CDR1, CDR2, and CDR3 amino acid sequences selected from one of the rows of the following table:

IL-10Rα VHH CDRs		
CDR1 SEQ ID NO:	**CDR2 SEQ ID NO:**	**CDR3 SEQ ID NO:**
224	229	236
224	230	236

IL-10Rα VHH CDRs		
CDR1 SEQ ID NO:	**CDR2 SEQ ID NO:**	**CDR3 SEQ ID NO:**
224	231	236
225	229	236
226	229	236
224	232	236
224	233	236
224	234	236
227	229	236
224	235	236
227	235	236
228	229	236

and wherein the second single-domain antibody polypeptide comprises a combination of CDR1, CDR2, and CDR3 amino acid sequences selected from one of the rows of the following table:

IL-10Rβ VHH CDRs		
CDR1 SEQ ID NO:	**CDR2 SEQ ID NO:**	**CDR3 SEQ ID NO:**
296	297	298

[0017] In some examples, the present disclosure provides IL-10 agonist compounds comprising a first single-domain antibody polypeptide and a second single-domain antibody polypeptide, wherein the first single-domain antibody polypeptide binds to the extracellular domain of IL10Rα and comprises a CDR1, CDR2 and CDR3 wherein:

(a) CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:229 and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236; or

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:230, and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236; or

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:231 and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236;

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:231, and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236; or

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:232, and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236; or

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:233, and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236; or

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:234, and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236; or

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:225, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:229, and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236; or

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:225, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:235, and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236; or

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:226, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:230, and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236;

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:228, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:229, and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:236; and/or

(b) the second single-domain antibody polypeptide binds to the extracellular domain of IL10Rβ and comprises a CDR1, CDR2 and CDR3 wherein:

CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:296, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:297 and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:298,

optionally wherein the first single-domain antibody polypeptide that binds to the extracellular domain of IL-10Rα (IL-10Rα sdAb) is at least 80% humanized relative to UniProt V3-23 (UniProt No. P01764), and/or

optionally wherein the second single-domain antibody polypeptide that binds to IL-10Rβ (IL-10Rβ sdAb) is at least 89% humanized relative to Uniprot VH3-66 (UniProt A0A0C4DH42), and/or

further optionally wherein the N-terminus of the first single-domain antibody polypeptide is joined to the C-terminus of the second single-domain antibody polypeptide, or alternatively wherein the C-terminus of the first single-domain antibody polypeptide, is joined to the N-terminus of the second single-domain antibody polypeptide, via a polypeptide linker comprising 1-50, alternatively 1-30, alternatively 1-20, alternatively 1-15, alternatively 1-12 alternatively 1-10, alternatively 1-8, alternatively 1-6, alternatively 1-5, alternatively 1-4, alternatively 1-3, alternatively 1-2, alternatively 1 amino acid(s).

[0018] In some examples, the present disclosure provides and IL-10 agonist compound comprising a first single-domain antibody polypeptide and a second single-domain antibody polypeptide, wherein the first single-domain antibody polypeptide binds to the extracellular domain of IL10Rα and comprises a CDR1, CDR2 and CDR3 wherein CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:229 and CDR3 is a polypeptide comprising the amino acid sequence SEQ ID NO:236 and optionally wherein the first single domain antibody polypeptide that binds to the extracellular domain of IL-10Rα (IL-10Rα sdAb) is at least 80% humanized relative to UniProt V3-23 (UniProt No. P01764), and (b) the second single-domain antibody polypeptide binds to the extracellular domain of IL10Rβ and comprises a CDR1, CDR2 and CDR3 wherein: CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:296, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:297 and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:298, and optionally wherein the second single domain antibody polypeptide that binds to IL-10Rβ (IL-10Rβ sdAb) is at least 89% humanized relative to Uniprot VH3-66 (UniProt A0A0C4DH42) and further optionally wherein the C-terminus of the first single-domain antibody polypeptide is joined to the N-terminus of second single-domain antibody polypeptide via a polypeptide linker comprising 1-50, alternatively 1-30, alternatively 1-20, alternatively 1-15, alternatively 1-12 alternatively 1-10, alternatively 1-8, alternatively 1-6, alternatively 1-5, alternatively 1-4, alternatively 1-3, alternatively 1-2, alternatively 1 amino acid(s).

[0019] In some examples, the present disclosure provides and IL-10 agonist compound comprising a first single-domain antibody polypeptide and a second single-domain antibody polypeptide, wherein the first single-domain antibody polypeptide binds to the extracellular domain of IL10Rα and comprises a CDR1, CDR2 and CDR3 wherein CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:230 and CDR3 is a polypeptide comprising the amino acid sequence SEQ ID NO:236 and optionally wherein the first single domain antibody polypeptide that binds to the extracellular domain of IL-10Rα (IL-10Rα sdAb) is at least 80% humanized relative to UniProt V3-23 (UniProt No. P01764), and (b) the second single-domain antibody polypeptide binds to the extracellular domain of IL10Rβ and comprises a CDR1, CDR2 and CDR3 wherein: CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:296, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:297 and CDR3 is a polypeptide comprising the amino acid sequence of SEQ ID NO:298, and optionally wherein the second single domain antibody polypeptide that binds to IL-10Rβ (IL-10Rβ sdAb) is at least 89% humanized relative to Uniprot VH3-66 (UniProt A0A0C4DH42) and further optionally wherein the C-terminus of the first single-domain antibody polypeptide is joined to the N-terminus of second single-domain antibody polypeptide via a polypeptide linker comprising 1-50, alternatively 1-30, alternatively 1-20, alternatively 1-15, alternatively 1-12 alternatively 1-10, alternatively 1-8, alternatively 1-6, alternatively 1-5, alternatively 1-4, alternatively 1-3, alternatively 1-2, alternatively 1 amino acid(s).

[0020] In some examples, the present disclosure provides and IL-10 agonist compound comprising a first single-domain antibody polypeptide and a second single-domain antibody polypeptide, wherein the first single-domain antibody polypeptide binds to the extracellular domain of IL10Rα and comprises a CDR1, CDR2 and CDR3 wherein CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:224, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:231 and CDR3 is a polypeptide comprising the amino acid sequence SEQ ID NO:236 and optionally wherein the first single domain antibody polypeptide that binds to the extracellular domain of IL-10Rα (IL-10Rα sdAb) is at least 80% humanized relative to UniProt V3-23 (UniProt No. P01764), and (b) the second single-domain antibody polypeptide binds to the extracellular domain of IL10Rβ and comprises a CDR1, CDR2 and CDR3 wherein: CDR1 is a polypeptide comprising the amino acid sequence of SEQ ID NO:296, CDR2 is a polypeptide comprising the amino acid sequence of SEQ ID NO:297 and CDR3 is a polypeptide comprising the amino acid sequence SEQ ID NO:298, optionally wherein the second single domain antibody polypeptide that binds to IL-10Rβ (IL-10Rβ sdAb) is at least 89%

humanized relative to Uniprot VH3-66 (UniProt A0A0C4DH42) and further optionally wherein the C-terminus of the first single-domain antibody polypeptide is joined to the N-terminus of second single-domain antibody polypeptide via a polypeptide linker comprising 1-50, alternatively 1-30, alternatively 1-20, alternatively 1-15, alternatively 1-12 alternatively 1-10, alternatively 1-8, alternatively 1-6, alternatively 1-5, alternatively 1-4, alternatively 1-3, alternatively 1-2, alternatively 1 amino acid(s).

[0021] In some examples, the present disclosure provides IL-10 agonist compounds, wherein the first single-domain antibody polypeptide comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:224; a CDR2 comprising the amino acid sequence of SEQ ID NO:229; and a CDR3 comprising the amino acid sequence of SEQ ID NO:236; and wherein the second single-domain antibody polypeptide comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:296; a CDR2 comprising the amino acid sequence of SEQ ID NO:297; and a CDR3 comprising the amino acid sequence of SEQ ID NO:298.

[0022] In some examples, the present disclosure provides IL-10 agonist compounds, wherein the first single-domain antibody polypeptide comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:224; a CDR2 comprising the amino acid sequence of SEQ ID NO:230; and a CDR3 comprising the amino acid sequence of SEQ ID NO:236; and wherein the second single-domain antibody polypeptide comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:296; a CDR2 comprising the amino acid sequence of SEQ ID NO:297; and a CDR3 comprising the amino acid sequence of SEQ ID NO:298.

[0023] In some examples, the present disclosure provides IL-10 agonist compounds, wherein the first single-domain antibody polypeptide comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:224; a CDR2 comprising the amino acid sequence of SEQ ID NO:231; and a CDR3 comprising the amino acid sequence of SEQ ID NO:236; and wherein the second single-domain antibody polypeptide comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:296; a CDR2 comprising the amino acid sequence of SEQ ID NO:297; and a CDR3 comprising the amino acid sequence of SEQ ID NO:298.

[0024] In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:1-24 and 500-523.

[0025] In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:1 (DR2463). In some examples, the present disclosure provides IL-10 agonist compounds comprising a

polypeptide comprising the amino acid sequence of SEQ ID NO:2 (DR2485). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:3 (DR2519). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:4 (DR2520).

[0026] In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:500 (DR2463, non-his tagged). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:501 (DR2485, non-his tagged). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:502 (DR2519, non-his tagged). In some examples, the present disclosure provides IL-10 agonist compounds comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:4 (DR2520, non-his tagged).

[0027] In another aspect, the present disclosure provides an IL-10 agonist compound comprising an IL-10R binding molecule polypeptide of the following formula #1:

$$H2N\text{-}(huIL10\ VHH1)\text{–}(L1)_a(L1)_b\text{–}(huIL10\ VHH2)\text{-}COOH \qquad [\#1]$$

wherein:

"–" represents a covalent bond;

L1 is a linker;

a and b are independently selected from the integers 0 or 1;

"H2N" denotes the amino terminus; and

"COOH" denotes the carboxy terminus of the polypeptide.

[0027a] In another aspect, the present disclosure provides a pharmaceutically acceptable formulation of an IL-10 agonist compound disclosed herein.

[0027b] In some examples, the present disclosure provides pharmaceutically acceptable formulations of an IL-10 agonist compound disclosed herein.

[0028] In another aspect, the present disclosure provides a nucleic acid sequence encoding an IL-10 agonist compound disclosed herein.

[0028a] In another aspect, the present disclosure provides a recombinant vector comprising a nucleic acid disclosed herein.

[0028b] In some examples, the present disclosure provides nucleic acid sequences encoding an IL-10 agonist compound disclosed herein. In some examples, the present disclosure provides recombinant vectors comprising such nucleic acids.

[0029] In another aspect, the present disclosure provides an IL-10 agonist compound disclosed herein for use in therapy.

[0029a] In another aspect, the present disclosure provides a use of an IL-10 agonist compound disclosed herein in the manufacture of a medicament for treating a mammalian subject suffering from an autoimmune disease, infectious disease, or inflammatory disease, wherein a therapeutically effective amount of the IL-10 agonist compound is to be adminstered to the subject.

[0029b] The IL-10 agonist compounds (e.g., single-domain antibody polypeptides of the IL-10 agonist compounds) of the present disclosure are useful in the treatment or prevention of disease in mammalian subjects. In some examples, the present disclosure provides methods of treating a mammalian subject suffering from an autoimmune disease, infectious disease, or inflammatory disease by the administration of a therapeutically effective amount of an IL-10 agonist compound disclosed herein. In some examples, the present disclosure provides for the treatment or prevention of infectious disease, including viral and chronic viral infections, in a mammalian subject by the administration of a therapeutically effective amount of an IL-10 agonist compound of the present disclosure. In some examples, the present disclosure provides methods of treating a mammalian subject suffering from a neoplastic disease by the administration of a therapeutically effective amount of an IL-10 agonist compound disclosed herein.

[0030] In another aspect, the present disclosure provides a use of an IL-10 agonist compound disclosed herein in the manufacture of a medicament for treating a mammalian subject suffering from a neoplastic disease, wherein a therapeutically effective amount of the IL-10 agonist compound is to be adminstered to the subject

[0030a] In another example, the disclosure provides a method for treating neoplastic diseases, such as cancer in a subject in need thereof, comprising administering to the subject the IL-10 agonist protein described herein, wherein the IL-10 agonist protein binds to and activates $CD8^+$ T cells,

$CD4^+$ T cells, macrophages, and/or Treg cells. In some examples, the IL-10 agonist protein provides longer therapeutic efficacy than a pegylated IL-10. In some examples, the cancer is a solid tumor cancer.

[0031] In another aspect, the present disclosure provides a composition comprising: means for inducing intracellular signaling in a cell expressing IL-10Rα and IL-10Rβ, wherein the means comprises (i) a single-domain antibody that binds to IL-10Rα (IL-10Rα sdAb) and is humanized relative to UniProt V3-23 (UniProt No. P01764), joined to (ii) a single-domain antibody that binds to IL-10Rβ (IL-10Rβ sdAb) and that is humanized relative to Uniprot VH3-66 (UniProt A0A0C4DH42); and a pharmaceutically acceptable carrier.

[0031a] In some examples, the present disclosure provides means for inducing intracellular signaling in a cell expressing IL-10Rα and IL-10Rβ, wherein the means comprises (i) a single domain antibody polypeptide that binds to IL-10Rα (IL-10Rα sdAb) and is at least 80% humanized relative to UniProt V3-23 (UniProt No. P01764), joined to (ii) a single domain antibody polypeptide that binds to IL-10Rβ (IL-10Rβ sdAb) and that is at least 89% humanized relative to Uniprot VH3-66 (UniProt A0A0C4DH42); and a pharmaceutically acceptable carrier.

[0032] In some examples, the present disclosure provides compositions comprising IL-10 agonist compounds described herein, wherein the cell expressing IL-10Rα and IL-10Rβ is a monocyte.

[0033] In some examples, the present disclosure provides compositions, wherein dimerizing the extracellular domains of an IL-10Rα subunit and an IL-10Rβ subunit of the IL-10 receptor (IL-10R) on a cell induces pSTAT3 signaling.

[0034] In some examples, the present disclosure provides compositions wherein the intracellular pSTAT-3 signaling in the monocyte cell expressing IL10Rα and IL10R is greater than the pSTAT-3 signaling in a T-cell expressing IL10Rα and IL10Rβ.

[0035] In some examples, the present disclosure provides compositions wherein the IL-10 agonist compound (e.g., a single-domain antibody polypeptide of an IL-10 agonist compound) comprises a C-terminal amino acid modification that reduces immunogenicity resulting from pre-existing antibodies. In some examples, the first single-domain antibody polypeptide of the IL-10 agonist compound comprises the C-terminal modification. In some examples, the second single-domain antibody polypeptide of the IL-10 agonist

compound comprises the C-terminal modification. In some examples, the C-terminal polypeptide selected from the first single-domain antibody polypeptide and the second single-domain antibody polypeptide comprises the C-terminal modification.

[0036] In some examples, the present disclosure provides compositions, wherein the IL-10 agonist compound (e.g., a single-domain antibody polypeptide of an IL-10 agonist compound) comprises a C-terminal amino acid modification comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:474-499.

[0037] In some examples, the present disclosure provides compositions wherein the compound is selected from the group consisting of SEQ ID NOS:121-135, 138-141, 144-155, 158-175, 179, and 182-195, and 199.

[0038] In another example, the disclosure provides a method of treating a mammalian subject suffering from an autoimmune disease, infectious disease, or inflammatory disease by the administration of a therapeutically effective amount of an IL-10 agonist compound. In some examples, one sdAb of the binding molecule is an scFv and the other sdAb is a VHH.

[0039] In some examples, the first and second sdAbs are covalently bound via a chemical linkage.

[0040] In some examples, the first and second sdAbs are provided as single continuous polypeptide.

[0041] In some examples, the the IL-10 agonist compound (e.g., a single-domain antibody polypeptide of an IL-10 agonist compound) is provided as single continuous polypeptide.

[0042] The present invention provides IL-10 agonist compounds that are synthetic ligands of the IL-10 receptor.

[0043] In some examples, the invention provides and IL-10 agonist compound comprising an IL-10Rα sdAb having least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative to any one of SEQ ID NOS:25-48. In some examples, the IL-10Rα sdAb comprises a C-terminal amino acid modification that reduces immunogenicity resulting from pre-existing antibodies. In some examples, the IL-10Rα sdAb comprises a C-terminal amino acid modification comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:475-497.

[0044] In some examples, the invention provides and IL-10 agonist compound comprising an IL-10Rβ sdAb having least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity, or having 0, 1, 2, or 3 amino acid changes, optionally conservative amino acid changes relative to any one of SEQ ID NOS:49 and 50. In some examples, the IL-10Rβ sdAb comprises a C-terminal amino acid modification that reduces immunogenicity resulting from pre-existing antibodies. In some examples, the IL-10Rβ sdAb comprises a C-terminal amino acid modification comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:475-497.

[0045] In some examples, the present invention provides IL-10 agonist compounds having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the sequence of any one of the IL-10 agonist compounds (e.g., single-domain antibody polypeptides of the IL-10 agonist compounds) of SEQ ID NOS:1-24 and 500-523. In some examples, the present invention provides IL-10 agonist compounds substantially identical to any one of the IL-10 agonist compounds (e.g., single-domain antibody polypeptides of the IL-10 agonist compounds) of SEQ ID NOS:1-24 and 500-523. In some examples, the present invention provides IL-10 agonist compounds identical to a sequence of any one of the IL-10 agonist compounds (e.g., single-domain antibody polypeptides of the IL-10 agonist compounds) of SEQ ID NOS:1-24 and 500-523. In some examples, the IL-10 agonist compound comprises a C-terminal amino acid modification that reduces immunogenicity resulting from pre-existing antibodies. In some examples, the IL-10 agonist compound comprises a C-terminal amino acid modification comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:475-497.

[0046] In one example, the present disclosure provides an IL-10Rα binding molecule that preferentially activates T cells, such as CD8+ T cells, relative to monocytes. In one example, the present disclosure provides an IL-10Rα binding molecule of the formula (#1) wherein the affinity of the IL-10Rα sdAb has a higher affinity for the extracellular domain of IL-10Rα than the affinity of the IL-10Rβ sdAb for the extracellular domain of IL-10Rβ.

[0047] In some examples, the present disclosure provides an IL-10 agonist compound modified to provide prolonged duration of action in vivo in a mammalian subject and pharmaceutically acceptable formulations thereof. In some examples, the present invention provides a IL-10 agonist compounds that are PEGylated, wherein the PEG is conjugated to the IL-10 agonist compound (e.g., a single-domain antibody polypeptide of an IL-10 agonist compound). In some examples, the pegylation is at the C-terminal end of the IL-10R VHH2, and the PEG is a linear or branched PEG molecule having an average molecular weight from about 2,000 to about 80,000 daltons, alternatively about 2,000 to about 70,000 daltons, alternatively about 5,000 to about 50,000 daltons, alternatively about 10,000 to about 50,000 daltons, alternatively about 20,000 to about 50,000 daltons, alternatively about 30,000 to about 50,000 daltons, alternatively about 20,000 to about 40,000 daltons, or alternatively about 30,000 to about 40,000 daltons. In one example of the disclosure, the PEG is a 40kD branched PEG comprising two 20 kD arms.

[0048] The present disclosure further provides a pharmaceutically acceptable formulation of an IL-10 agonist compound for the administration to a mammalian subject. The present disclosure further provides a pharmaceutically acceptable composition for administration to a mammalian

subject the composition comprising a nucleic acid sequence encoding a polypeptide IL-10 agonist compound, a recombinant viral or non-viral vector encoding or polypeptide IL-10 agonist compound, or a recombinantly modified mammalian cell comprising a nucleic acid sequence encoding a polypeptide IL-10 agonist compound, in each case the nucleic acid sequence operably linked to one or more expression control elements functional in a mammalian cell.

[0049] The present disclosure provides nucleic acid sequences encoding polypeptide IL-10 agonist compounds. The present disclosure further provides a recombinant vector comprising a nucleic acid sequence encoding polypeptide IL-10 agonist compounds. The present disclosure further provides a recombinantly modified mammalian cell comprising a nucleic acid encoding a polypeptide IL-10 agonist compound. The present disclosure further provides methods for the recombinant production, isolation, purification and characterization of a polypeptide IL-10 agonist compound of recombinant vectors comprising a provides nucleic acid sequences encoding polypeptide IL-10 agonist compounds.

[0050] The disclosure also provides expression vectors comprising a nucleic acid encoding the bispecific IL-10 agonist compound operably linked to one or more expression control sequence. The disclosure also provides isolated host cells comprising the expression vector comprising a nucleic acid encoding the IL-10 agonist compound (e.g., a single-domain antibody polypeptide of an IL-10 agonist compound) operably linked to one or more expression control sequences functional in the host cell.

[0051] In another example, the disclosure provides a pharmaceutical composition comprising the IL-10 agonist compound (e.g., a single-domain antibody polypeptide of an IL-10 agonist compound) described herein, and a pharmaceutically acceptable carrier.

[0052] In another example, the disclosure provides a method of treating an autoimmune or inflammatory disease, disorder, or condition or a viral infection in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an IL-10 agonist compound described herein or a pharmaceutical composition described herein.

[0053] Several advantages flow from the binding molecules described herein. The natural ligand of the IL-10 receptor, IL-10, causes IL-10Rα and IL-10Rβ to come into proximity by their simultaneous binding of IL-10. However, when IL-10 is used as a therapeutic in mammalian, particularly human, subjects, it may also trigger a number of adverse and undesirable effects by a variety of mechanisms including the presence of IL-10Rα and IL-10Rβ on other cell types and the binding to IL-10Rα and IL-10Rβ on the other cell types may result in undesirable effects and/or undesired signaling on cells expressing IL-10Rα and IL-10Rβ. The present disclosure is directed

to methods and compositions that modulate the multiple undesirable adverse effects of IL-10 binding to IL-10Rα and IL-10Rβ so that desired therapeutic signaling occurs, particularly in a desired cellular or tissue subtype, while minimizing undesired activity and/or intracellular signaling.

[0054] In some examples, the IL-10 agonist compounds (e.g., single-domain antibody polypeptides of the IL-10 agonist compounds) described herein are partial agonists of the IL-10 receptor. In some examples, the binding molecules described herein are designed such that the binding molecules are full agonists. In some examples, the binding molecules described herein are designed such that the binding molecules are super agonists.

[0055] In some examples, the binding molecules provide the maximal desired IL-10 intracellular signaling from binding to IL-10Rα and IL-10Rβ on the desired cell types, while providing significantly less IL-10 signaling on other undesired cell types. This can be achieved, for example, by selection of binding molecules having differing affinities or causing different E_{max} for IL-10Rα and IL-10Rβ as compared to the affinity of IL-10 for IL-10Rα and IL-10Rβ.

[0056] Because different cell types respond to the binding of ligands to its cognate receptor with different sensitivity, by modulating the affinity of the dimeric ligand (or its individual binding moieties) for the IL-10 receptor relative to wild-type IL-10 binding facilitates the stimulation of desired activities while reducing undesired activities on non-target cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057] FIG. 1 of the attached drawings is a graph showing that the humanized IL-10Rα/IL-10Rβ VHH dimers DR1525 and DR2096 were less potent than the parental llama molecule DR841 in an LPS-induced monocyte secretion assay, as measured by inhibiting the production of IL1β.

[0058] FIG. 2 of the attached drawings is a graph showing that the humanized IL-10Rα/IL-10Rβ VHH dimers DR1525 and DR2096 were less potent than the parental llama molecule DR841 in an LPS-induced monocyte secretion assay, as measured by inhibiting the production of TNFα.

[0059] FIG. 3 of the attached drawings is a graph showing the humanized IL-10Rα/IL-10Rβ VHH dimer DR2503 has a comparable potency to the parental llama molecule DR841 in an LPS-induced monocyte secretion assay, as measured by inhibiting the production of IL1β.

[0060] FIG. 4 of the attached drawings is a graph showing the humanized IL-10Rα/IL-10Rβ VHH dimer DR2503 has a comparable potency to the parental llama molecule DR841 in an LPS-induced monocyte secretion assay, as measured by inhibiting the production of TNFα. The graph also shows functionality of IL10Rα/IL10Rβ VHH dimers DR2463 and DR2485.