MULTIVALENT AND MULTISPECIFIC OX40-BINDING FUSION PROTEINS

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/277,027, filed January 11, 2016; the contents of each of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] This invention relates generally to molecules that specifically engage OX40, a member of the TNF receptor superfamily (TNFRSF). More specifically this invention relates to multivalent and multispecific molecules that bind at least OX40.

BACKGROUND OF THE INVENTION

[0003] The tumor necrosis factor receptor superfamily consists of several structurally related cell surface receptors. Activation by multimeric ligands is a common feature of many of these receptors. Many members of the TNFRSF have therapeutic utility in numerous pathologies if activated properly. Agonism of this receptor family often requires higher order clustering and conventional bivalent antibodies are not ideal for this. Therefore, there exists a therapeutic need for more potent agonist molecules of the TNFRSF.

SUMMARY

[0004] The disclosure provides multivalent and multispecific TNF receptor superfamily (TNFRSF) binding fusion polypeptides that bind at least OX40 (also known as tumor necrosis factor receptor superfamily, member 4 (TNFRSF4) and/or CD134)). The use of the term "OX40" is intended to cover any variation thereof, such as, by way of non-limiting example, OX-40, and all variations are used herein interchangeably. These molecules that bind at least OX40 are referred to herein as "OX40-targeting molecules" or "OX40-targeting fusions" or "OX40-targeting proteins" or "OX40-targeting fusion polypeptides" or "OX40-targeting fusion proteins." In some embodiments, the OX40-

targeting molecule is a multivalent molecule, for example, a multivalent OX40-targeting fusion protein. In some embodiments, the OX40-targeting molecule is a multispecific molecule, for example, a multispecific OX40-targeting fusion protein. In some embodiments, the OX40-targeting molecule is a multivalent and multispecific molecule, for example, a multivalent and multispecific OX40-targeting fusion protein. As used herein, the term "fusion protein" or "fusion polypeptide" or "OX40-targeting fusion protein" or "OX40-targeting fusion polypeptide," unless otherwise specifically denoted, refers to any fusion protein embodiment of the disclosure, including, but not limited to, multivalent fusion proteins, multispecific fusion proteins, or multivalent and multispecific fusion proteins.

[0004a] In one aspect, there is provided an isolated polypeptide that binds OX40 and comprises a plurality of Tumor Necrosis Factor receptor superfamily (TNFRSF) binding domain (TBDs), wherein at least a first TBD (TBD1) binds OX40.

[0005] The disclosure also provides multivalent and multispecific fusion polypeptides that bind at least programmed death ligand 1 (PDL1), also known as PD-L1, CD274, B7 homolog 1 and/or B7-H1. The use of the term "PDL1" is intended to cover any variation thereof, such as, by way of non-limiting example, PD-L1 and/or PDL-1, all variations are used herein interchangeably. These molecules that bind at least PDL1 are referred to herein as "PDL1-targeting molecules" or "PDL1-targeting fusions" or "PDL1-targeting proteins" or "PDL1-targeting fusion polypeptides" or "PDL1-targeting fusion proteins." In some embodiments, the PDL1-targeting molecule is a multivalent molecule, for example, a multivalent PDL1-targeting fusion protein. In some embodiments, the PDL1-targeting molecule is a multispecific molecule, for example, a multispecific PDL1-targeting fusion protein. In some embodiments, the PDL1-targeting molecule is a multivalent and multispecific molecule, for example, a multivalent and multispecific PDL1-targeting fusion protein. As used herein, the term "fusion protein" or "fusion polypeptide" or "PDL1-targeting fusion protein" or "PDL1-targeting fusion polypeptide," unless otherwise specifically denoted, refers to any fusion protein embodiment of the disclosure, including, but not limited to, multivalent fusion proteins, multispecific fusion proteins, or multivalent and multispecific fusion proteins.

[0005a] In another aspect, there is provided an isolated polypeptide that binds PDL1 and comprises a plurality of polypeptide binding domains (BDs), wherein at least a first BD (BD1) binds PDL1.

[0006] The disclosure also provides multivalent and multispecific fusion polypeptides that bind at least PDL1 and OX40. These molecules that bind at least PDL1 are referred to herein as "PDL1xOX40-targeting molecules" or "PDL1xOX40-targeting fusions" or "PDL1xOX40-targeting proteins" or "PDL1xOX40-targeting fusion polypeptides" or "PDL1xOX40-targeting fusion proteins." In some embodiments, the PDL1xOX40-targeting molecule is a multivalent molecule, for example, a multivalent

PDL1xOX40-targeting fusion protein. In some embodiments, the PDL1xOX40-targeting molecule is a multispecific molecule, for example, a multispecific PDL1xOX40-targeting fusion protein. In some embodiments, the PDL1xOX40-targeting molecule is a multivalent and multispecific molecule, for example, a multivalent and multispecific PDL1-targeting fusion protein. As used herein, the term "fusion protein" or "fusion polypeptide" or "PDL1xOX40-targeting fusion protein" or "PDL1xOX40-targeting fusion polypeptide," unless otherwise specifically denoted, refers to any fusion protein embodiment of the disclosure, including, but not limited to, multivalent fusion proteins, multispecific fusion proteins, or multivalent and multispecific fusion proteins.

[0006a] In another aspect, there is provided an isolated polypeptide that binds PDL1 and OX40 and comprises a plurality of polypeptide binding domains (BDs) and a plurality of Tumor Necrosis Factor receptor superfamily (TNFRSF) binding domain (TBDs), wherein at least a first binding domain (BD1) binds PLD1 and at least a first TNFRSF binding domain (TBD1) binds OX40.

[0007] Conventional antibodies targeting members of the TNF receptor superfamily (TNFRSF) have been shown to require exogenous crosslinking to achieve sufficient agonist activity, as evidenced by the necessity for Fc-gamma Receptor (FcγRs) for the activity of antibodies to DR4, DR5, GITR and OX40 (Ichikawa *et al* 2001 al Nat. Med. 7, 954–960, Li *et al* 2008 Drug Dev. Res. 69, 69–82; Pukac et al 2005 Br. J. Cancer 92, 1430–1441; Yanda *et al* 2008 Ann. Oncol. 19, 1060–1067; Yang *et al* 2007 Cancer Lett. 251:146–157; Bulliard *et al* 2013 JEM 210(9): 1685; Bulliard *et al* 2014 Immunol and Cell Biol 92: 475-480). In addition to crosslinking via FcγRs other exogenous agents including addition of the oligomeric ligand or antibody binding entities (e.g. protein A and secondary antibodies) have been demonstrated to enhance anti-TNFRSF antibody clustering and downstream signaling. For example, the addition of the DR5 ligand TRAIL enhanced the apoptosis inducing ability of an anti-DR5 antibody (Graves *et al* 2014 Cancer Cell 26: 177-189). These findings suggest the need for clustering of TNFRSFs beyond a dimer.

[0008] The present disclosure provides multivalent TNFRSF binding fusion proteins, which comprise 2 or more TNFRSF binding domains (TBDs) where at least one TBD binds OX40. In some embodiments, the fusion proteins of the present disclosure have utility in treating neoplasms.

[0008a] In another aspect, there is provided use of the polypeptide as described herein in the manufacture of a medicament for treating neoplasms.

[0009] In some embodiments, the fusion protein contains two or more different TBDs, where each TBD binds OX40. In some embodiments, the fusion protein contains multiple copies of a TBD that binds OX40. For example, in some embodiments, the fusion protein contains at least two copies of a TBD that binds OX40. In some embodiments, the fusion protein contains at least three copies of a TBD that binds OX40. In some embodiments, the fusion protein contains at least four copies of a TBD that binds OX40. In some embodiments, the fusion protein contains at least five copies of a TBD that binds

OX40. In some embodiments, the fusion protein contains at least six copies of a TBD that binds OX40. In some embodiments, the fusion protein contains six or more copies of a TBD that binds OX40.

[0010] In other embodiments, the fusion proteins of the present disclosure bind OX40 and a second TNFRSF member such as, for example, GITR, CD137, CD27, TNFR2, and/or CD40. In these embodiments, the fusion proteins of the present disclosure modulate immune cells leading to enhanced tumor destruction. In other embodiments, the fusion proteins of the present disclosure have utility in treating inflammatory conditions. In these embodiments, the fusion proteins of the present disclosure modulate immune cells leading to dampening of the inflammatory insult. For example, specifically agonizing TNFR2 can enhance Treg proliferation leading to immune suppression.

[0010a] In another aspect, there is provided use of the polypeptide as described herein in the manufacture of a medicament for modulating immune cells to enhance tumor destruction. In another aspect, there is provided use of the polypeptide as described herein in the manufacture of a medicament for treating an inflammatory disorder.

[0011] The fusion proteins of the present disclosure are capable of enhanced clustering of TNFRSF members compared to non-cross-linked bivalent antibodies. The enhanced clustered of TNFRSF members mediated by the fusion proteins of the present disclosure induce enhanced TNFRSF-dependent signaling compared to non-cross-linked bivalent antibodies. In most embodiments, the fusion protein will incorporate more than 2 TBDs, for example, three, four, five, or six.

[0012] In some embodiments, the fusion proteins are multispecific containing a TBD and a binding domain directed toward a second antigen. In these, embodiments, the binding to the second antigen is capable of providing the additional crosslinking function and TNFRSF activation can be achieved with only one or two TBDs. In these embodiments, the TNFRSF signaling is enhanced and focused by the presence of the second antigen. These multispecific TBD containing fusion proteins are useful means to achieve conditional signaling of a given TNFRSF member.

[0013] The present disclosure provides isolated polypeptides that specifically bind OX40. In some embodiments, the isolated polypeptide is derived from antibodies or antibody fragments including scFv, Fabs, single domain antibodies (sdAb), V_{NAR}, or VHHs. In some embodiments, the isolated polypeptide is human or humanized sdAb. The sdAb fragments can be derived from VHH, V_{NAR}, engineered VH or VK domains. VHHs can be generated from camelid heavy chain only antibodies. V_{NAR}s can be generated from cartilaginous fish heavy chain only antibodies. Various methods have been implemented to generate monomeric sdAbs from conventionally heterodimeric VH and VK domains, including interface engineering and selection of specific germline families. In other

disclosure provides multispecific fusion proteins incorporating TBDs that bind GITR and OX40 or CD137 and OX40.

[0032] In some embodiments, all of the BDs of the fusion protein recognize the same epitope on PDL1. For example, the fusion proteins of present disclosure may incorporate 2, 3, 4, 5, or 6 BDs with identical specificity to PDL1. In other embodiments, the fusion protein incorporates BDs that recognize distinct epitopes on PDL1. For example, the fusion proteins of present disclosure may incorporate 2, 3, 4, 5, or 6 BDs with distinct recognition specificities toward various epitopes on PDL1. In these embodiments, the fusion proteins of the present disclosure with contain multiple BDs that target distinct regions of the PDL1. In some embodiments, the BDs may recognize different epitopes on PDL1.

[0033] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets OX40 and PDL1 and comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 387-394.

[0033a] In another aspect, there is provided an isolated polypeptide that binds OX40 and PDL1, wherein the polypeptide comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 387-394.

[0034] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets OX-40 and PDL1 and comprises an amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 387-394.

[0035] In some embodiments, the fusion protein of the present disclosure is composed of a single polypeptide. In other embodiments, the fusion protein of the present disclosure is composed of more than one polypeptide. For example, wherein a heterodimerization domain is incorporated into the fusion protein so as the construct an asymmetric fusion protein. For example, if an immunoglobulin Fc region is incorporated into the fusion protein the CH3 domain can be used as a homodimerization domain, or the CH3 dimer interface region can be mutated so as to enable heterodimerization.

[0036] In some embodiments, the fusion protein contains the BDs at opposite ends. For example, the BDs are located on both the amino-terminal (N-terminal) portion of the fusion protein and the carboxy-terminal (C-terminal) portion of the fusion protein. In other embodiments, all the TBDs reside on the same end of the fusion protein. For example, BDs reside on either the amino- or carboxy-terminal portions of the fusion protein.

[0037] In some embodiments, the linker polypeptide contains an immunoglobulin Fc region. In some embodiments, the immunoglobulin Fc region is an IgG isotype selected from the group consisting of IgG1 isotype, IgG2 isotype, IgG3 isotype, and IgG4 isotype.

42. The isolated polypeptide of any one of claims 1 to 3, wherein the polypeptide is hexavalent.

43. The isolated polypeptide of claim 42, wherein the polypeptide comprises the structure: VHH-Linker-VHH-Linker-VHH-Linker-Hinge-Fc, where the VHH is a humanized or fully human VHH sequence.

44. An isolated polypeptide that binds OX40 and PDL1, wherein the polypeptide comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 387-394.

45. Use of the polypeptide of any one of claims 1 to 44 in the manufacture of a medicament for treating neoplasms.

46. Use of the polypeptide of any one of claims 1 to 44 in the manufacture of a medicament for modulating immune cells to enhance tumor destruction.

47. Use of the polypeptide of any one of claims 1 to 44 in the manufacture of a medicament for treating an inflammatory disorder.