

MODULATORS OF CELL SURFACE PROTEIN INTERACTIONS AND METHODS AND COMPOSITIONS RELATED TO SAME

SEQUENCE LISTING

5 The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 27, 2020, is named 50474-182WO3_Sequence_Listing_3.27.20_ST25 and is 24,492 bytes in size.

FIELD OF THE INVENTION

10 Provided herein are methods for identifying modulators of cell surface protein interactions and activities, as well as modulators of cell surface protein interactions and activities.

BACKGROUND

Recent annotations predict that over 5,000 genes in the human genome encode plasma
15 membrane-expressed or secreted proteins, i.e., proteins acting at the cell surface. Classes of proteins present at the cell surface include cell surface receptors (e.g., single transmembrane (STM) receptors) and Immunoglobulin Superfamily (IgSF) proteins. Many of these proteins have been linked to cancer and other diseases, and are thus prime targets for development of therapeutic agents; however, the binding partners of many plasma membrane proteins (e.g., STM and IgSF proteins) have remained
20 uncharacterized. This discrepancy is due to the incompatibility of currently used proteomics technologies, e.g., yeast two-hybrid assays and affinity purification/mass spectrometry (AP/MS), for studying plasma membrane-expressed proteins. High-throughput screens for extracellular protein-protein interactions have recently been developed; however, therapeutic agents that modulate the majority of these interactions have not yet been identified.

25 Thus, there is an unmet need for methods and compositions for the identification of interactions between cell surface proteins, as well as novel modulators of such interactions and methods of using the same.

SUMMARY

30 The present disclosure provides methods for identifying modulators of cell surface protein interactions and activities, as well as modulators of cell surface protein interactions and activities.

In one example, the disclosure features a method of identifying an individual having a cancer who may benefit from a treatment comprising a PD-L1 axis binding antagonist, the method comprising determining an expression level of a first member and a second member of at least one of the gene pairs
35 of Table 15 in a sample from the individual, wherein an expression level of the first member of the gene pair that is above a first reference expression level and an expression level of the second member of the gene pair that is above a second reference expression level identifies the individual as one who may benefit from a treatment comprising a PD-L1 axis binding antagonist.

In another example, the disclosure features a method of selecting a therapy for an individual
40 having a cancer, the method comprising determining an expression level of a first member and a second member at least one of the gene pairs of Table 15 in a sample from the individual, wherein an expression level of

the first member of the gene pair that is above a first reference expression level and an expression level of the second member of the gene pair that is above a second reference expression level identifies the individual as one who may benefit from a treatment comprising a PD-L1 axis binding antagonist.

In some examples, the individual has an expression level of the first member of the gene pair that is above a first reference expression level and an expression level of the second member of the gene pair that is above a second reference expression level and the method further comprises administering to the individual an effective amount of a PD-L1 axis binding antagonist.

In another example, the disclosure features a method of treating an individual having a cancer, the method comprising (a) determining an expression level of a first member and a second member at least one of the gene pairs of Table 15 in a sample from the individual, wherein the expression level of the first member of the gene pair is above a first reference expression level and the expression level of the second member of the gene pair is above a second reference expression level; and (b) administering an effective amount of a PD-L1 axis binding antagonist to the individual.

In another example, the disclosure features a method of treating an individual having a cancer, the method comprising administering a PD-L1 axis binding antagonist to an individual who has been determined to have an expression level of a first member of a gene pair of Table 15 that is above a first reference expression level and an expression level of the second member of the gene pair that is above a second reference expression level.

In another example, the disclosure features a method of identifying an individual having a cancer who may benefit from a treatment other than or in addition to a PD-L1 axis binding antagonist, the method comprising determining an expression level of a first member and a second member of at least one of the gene pairs of Table 16 in a sample from the individual, wherein an expression level of the first member of the gene pair that is above a first reference expression level and an expression level of the second member of the gene pair that is above a second reference expression level identifies the individual as one who may benefit from a treatment other than or in addition to a PD-L1 axis binding antagonist.

In another example, the disclosure features a method of selecting a therapy for an individual having a cancer, the method comprising determining an expression level of a first member and a second member at least one of the gene pairs of Table 16 in a sample from the individual, wherein an expression level of the first member of the gene pair that is above a first reference expression level and an expression level of the second member of the gene pair that is above a second reference expression level identifies the individual as one who may benefit from a treatment other than or in addition to a PD-L1 axis binding antagonist.

In some examples, the individual has an expression level of the first member of the gene pair that is above a first reference expression level and an expression level of the second member of the gene pair that is above a second reference expression level and the method comprises administering to the individual an effective amount of a treatment other than or in addition to a PD-L1 axis binding antagonist.

In some examples, the first reference expression level is a pre-assigned expression level and the second reference expression level is a pre-assigned reference expression level.

In some examples, the sample from the individual is obtained from the individual prior to administration of an anti-cancer therapy. In some examples, the sample from the individual is obtained from the individual after administration of an anti-cancer therapy.

5 In some examples, the sample from the individual is a tumor tissue sample or a tumor fluid sample.

In some examples, the sample is a formalin-fixed and paraffin-embedded (FFPE) sample, an archival sample, a fresh sample, or a frozen sample. In some examples, the tumor tissue sample is a FFPE sample.

10 In some examples, the expression level of the first member and the second member of the gene pair in the sample is a protein expression level; or the expression level of the first member and the second member of the gene pair in the sample is an mRNA expression level. In some examples, the expression level of the first member and the second member of the gene pair in the sample is a mRNA expression level of the first member and the second member of the gene pair, respectively.

15 In some examples, the mRNA expression level of the first member and the second member of the gene pair is determined by *in situ* hybridization (ISH), RNA-seq, RT-qPCR, qPCR, multiplex qPCR or RT-qPCR, microarray analysis, SAGE, MassARRAY technique, FISH, or a combination thereof. In some examples, the first reference expression level is between about 0.25 to about 0.5 counts per million (CPM) and the second reference expression level is between about 0.25 to about 0.5 CPM. In some examples, the first reference expression level is 0.25 CPM and the second reference expression level is 0.25 CPM.

20 In some examples, the first reference expression level and the second reference expression level are expression levels of the first member and the second member of the gene pair, respectively, in a reference population of individuals having a cancer. In some examples, the cancer is a urinary tract cancer, e.g., a urinary tract carcinoma, e.g., a locally advanced urothelial carcinoma or a metastatic urothelial carcinoma (mUC).

25 In some examples, the benefit comprises an extension in the individual's overall survival (OS) as compared to treatment without the PD-L1 axis binding antagonist.

In some examples, the first member of the gene pair is SIGLEC6 and the second member of the gene pair is NCR1.

30 In some examples, the first member of the gene pair is BTN3A1 and the second member of the gene pair is LRRC4B.

In some examples, the first member of the gene pair is CD80 and the second member of the gene pair is CTLA4.

In some examples, the first member of the gene pair is BTN3A3 and the second member of the gene pair is LRRC4B.

35 In some examples, the first member of the gene pair is EFNB1 and the second member of the gene pair is TRHDE.

In some examples, the first member of the gene pair is CTLA4 and the second member of the gene pair is PCDHGB4.

40 In some examples, the first member of the gene pair is CTLA4 and the second member of the gene pair is FAM200A.

In some examples, the first member of the gene pair is CA12 and the second member of the gene pair is SIGLEC6.

In some examples, the first member of the gene pair is ILDR2 and the second member of the gene pair is CLEC12B.

5 In some examples, the first member of the gene pair is EFNB1 and the second member of the gene pair is ITLN1.

In some examples, the first member of the gene pair is CADM1 and the second member of the gene pair is CRTAM.

10 In some examples, the first member of the gene pair is CD79B and the second member of the gene pair is CD244.

In some examples, the first member of the gene pair is DAG1 and the second member of the gene pair is EFNB1.

In some examples, the first member of the gene pair is EFNB1 and the second member of the gene pair is EVC2.

15 In some examples, the first member of the gene pair is GPC4 and the second member of the gene pair is FGFR1.

In some examples, the first member of the gene pair is EFNB3 and the second member of the gene pair is EPHB4.

20 In some examples, the first member of the gene pair is PTPRD and the second member of the gene pair is LRFN4.

In some examples, the first member of the gene pair is EFNB1 and the second member of the gene pair is AQPEP.

In some examples, the first member of the gene pair is EFNB1 and the second member of the gene pair is DSG4.

25 In some examples, the first member of the gene pair is LDLR and the second member of the gene pair is LILRB5.

In some examples, the first member of the gene pair is EFNB3 and the second member of the gene pair is EPHB3.

30 In some examples, the first member of the gene pair is PLXNB3 and the second member of the gene pair is SEMA4G.

In some examples, the first member of the gene pair is EFNB1 and the second member of the gene pair is EPHB6.

In some examples, the first member of the gene pair is FLT4 and the second member of the gene pair is FLRT2.

35 In some examples, the first member of the gene pair is AXL1 and the second member of the gene pair is IL1RL1.

In some examples, the first member of the gene pair is CD320 and the second member of the gene pair is IGSF5.

40 In some examples, the first member of the gene pair is CD59 and the second member of the gene pair is STAB1.

In some examples, the first member of the gene pair is CNTN3 and the second member of the gene pair is PTPRG.

In some examples, the first member of the gene pair is EFNB1 and the second member of the gene pair is EPHA3.

5 In some examples, the first member of the gene pair is EFNB3 and the second member of the gene pair is EPHB2.

In some examples, the first member of the gene pair is EGF and the second member of the gene pair is TNFRSF11B.

10 In some examples, the first member of the gene pair is ENPEP and the second member of the gene pair is SLITRK1.

In some examples, the first member of the gene pair is FCGR3B and the second member of the gene pair is EDA2R.

In some examples, the first member of the gene pair is IL20RA and the second member of the gene pair is CLEC14A.

15 In some examples, the first member of the gene pair is IL6R and the second member of the gene pair is BTNL9.

In some examples, the first member of the gene pair is IZUMO1 and the second member of the gene pair is LILRA5.

20 In some examples, the first member of the gene pair is NGFR and the second member of the gene pair is LRRTM3.

In some examples, the first member of the gene pair is NTM and the second member of the gene pair is AMIGO2.

In some examples, the first member of the gene pair is PCDHB3 and the second member of the gene pair is IGSF11.

25 In some examples, the first member of the gene pair is PTGFRN and the second member of the gene pair is TMEM59L.

In some examples, the first member of the gene pair is TREM1 and the second member of the gene pair is VSIG8.

30 In some examples, the PD-L1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist, a PD-1 binding antagonist, and a PD-L2 binding antagonist.

In some examples, the PD-L1 axis binding antagonist is a PD-L1 binding antagonist.

In some examples, the PD-L1 binding antagonist inhibits the binding of PD-L1 to one or more of its ligand binding partners.

In some examples, the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1.

35 In some examples, the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1.

In some examples, the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1.

In some examples, the PD-L1 binding antagonist is an antibody or antigen-binding fragment thereof.

In some examples, the antibody is selected from the group consisting of atezolizumab, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab).

In some examples, the anti-PD-L1 antibody comprises the following hypervariable regions: (a) an HVR-H1 sequence of GFTFSDSWIH (SEQ ID NO: 19); (b) an HVR-H2 sequence of
5 AWISPYGGSTYYADSVKG (SEQ ID NO: 20); (c) an HVR-H3 sequence of RHWPGGFDY (SEQ ID NO: 21); (d) an HVR-L1 sequence of RASQDVSTAVA (SEQ ID NO: 22); (e) an HVR-L2 sequence of SASFLYS (SEQ ID NO: 23); and (f) an HVR-L3 sequence of QQYLYHPAT (SEQ ID NO: 24).

In some examples, the anti-PD-L1 antibody comprises: (a) a heavy chain variable (VH) domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of
10 SEQ ID NO: 3; (b) a light chain variable (VL) domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 4; or (c) a VH domain as in (a) and a VL domain as in (b).

In some examples, the anti-PD-L1 antibody comprises (a) a heavy chain variable (VH) domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of
15 SEQ ID NO: 3; (b) a light chain variable (VL) domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 4; or (c) a VH domain as in (a) and a VL domain as in (b).

In some examples, the anti-PD-L1 antibody comprises (a) a heavy chain variable (VH) domain comprising an amino acid sequence having at least 96% sequence identity to the amino acid sequence of
20 SEQ ID NO: 3; (b) a light chain variable (VL) domain comprising an amino acid sequence having at least 96% sequence identity to the amino acid sequence of SEQ ID NO: 4; or (c) a VH domain as in (a) and a VL domain as in (b).

In some examples, the anti-PD-L1 antibody comprises (a) a heavy chain variable (VH) domain comprising an amino acid sequence having at least 97% sequence identity to the amino acid sequence of
25 SEQ ID NO: 3; (b) a light chain variable (VL) domain comprising an amino acid sequence having at least 97% sequence identity to the amino acid sequence of SEQ ID NO: 4; or (c) a VH domain as in (a) and a VL domain as in (b).

In some examples, the anti-PD-L1 antibody comprises (a) a heavy chain variable (VH) domain comprising an amino acid sequence having at least 98% sequence identity to the amino acid sequence of
30 SEQ ID NO: 3; (b) a light chain variable (VL) domain comprising an amino acid sequence having at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 4; or (c) a VH domain as in (a) and a VL domain as in (b).

In some examples, the anti-PD-L1 antibody comprises (a) a heavy chain variable (VH) domain comprising an amino acid sequence having at least 99% sequence identity to the amino acid sequence of
35 SEQ ID NO: 3; (b) a light chain variable (VL) domain comprising an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 4; or (c) a VH domain as in (a) and a VL domain as in (b).

In some examples, the anti-PD-L1 antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 3; and (b) a VL domain comprising the amino acid sequence of SEQ ID
40 NO: 4.

In some examples, the anti-PD-L1 antibody is atezolizumab (MPDL3280A).

In some examples, the PD-L1 axis binding antagonist is a PD-1 binding antagonist. In some examples, the PD-1 binding antagonist inhibits the binding of PD-1 to one or more of its ligand binding partners.

In some examples, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1.

In some examples, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2.

In some examples, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2.

In some examples, the PD-1 binding antagonist is an antibody or antigen-binding fragment thereof.

In some examples, the antibody is selected from the group consisting of: MDX-1106 (nivolumab), MK-3475 (pembrolizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108.

In some examples, the PD-1 binding antagonist is an Fc-fusion protein.

In one aspect, the present disclosure provides use of an agonist of CD177 activity in the manufacture of a medicament for treating an individual having a cancer.

In another aspect, the present disclosure provides use of an agonist of CD177 activity in the manufacture of a medicament for treating an individual having a cancer, wherein the use comprises determining an expression level of podoplanin (PDPN) in a sample that has been obtained from the individual, wherein the expression level of PDPN in the sample is above a reference PDPN expression level, and wherein an effective amount of the agonist of CD177 is to be administered to the individual.

In yet another aspect, the present disclosure provides use of an agonist of CD177 activity in the manufacture of a medicament for treating an individual having a cancer, wherein an expression level of PDPN in a sample that has been obtained from the individual has been determined to be above a reference PDPN expression level.

In another example, the disclosure features a method of treating an individual having a cancer comprising administering to the individual an effective amount of an agonist of CD177 activity.

In another example, the disclosure features a method of identifying an individual having a cancer who may benefit from a treatment comprising an agonist of CD177 activity, the method comprising determining an expression level of podoplanin (PDPN) in a sample from the individual, wherein an expression level of PDPN in the sample that is above a reference PDPN expression level identifies the individual as one who may benefit from a treatment comprising an agonist of CD177 activity.

In one aspect, the present disclosure provides a method of identifying an individual having a cancer who may benefit from a treatment comprising an agonist of CD177 activity, the method comprising determining an expression level of PDPN in a sample that has been obtained from the individual, wherein an expression level of PDPN in the sample that is above a reference PDPN expression level identifies the individual as one who may benefit from the treatment comprising the agonist of CD177 activity.

In another example, the disclosure features a method of selecting a therapy for an individual having a cancer, the method comprising determining an expression level of PDPN in a sample from the individual, wherein an expression level of PDPN in the sample that is above a reference PDPN expression level identifies the individual as one who may benefit from a treatment comprising an agonist of CD177 activity.

In another aspect, the present disclosure provides a method of selecting a therapy for an individual having a cancer, the method comprising determining an expression level of PDPN in a sample that has been obtained from the individual, wherein an expression level of PDPN in the sample that is above a reference PDPN expression level identifies the individual as one who may benefit from a treatment comprising an agonist of CD177 activity.

In some examples, the individual has an expression level of PDPN in the sample that is above a reference PDPN expression level and the method further comprises administering to the individual an effective amount of an agonist of CD177 activity.

In another example, the disclosure features a method of treating an individual having a cancer, the method comprising (a) determining an expression level of PDPN in a sample from the individual, wherein the expression level of PDPN in the sample is above a reference PDPN expression level; and (b) administering to the individual an effective amount of an agonist of CD177 activity.

In another example, the disclosure features a method of treating an individual having a cancer, the method comprising administering to the individual an effective amount of an agonist of CD177 activity, wherein the expression level of PDPN in a sample from the individual has been determined to be above a reference PDPN expression level.

In some examples, the CD177 activity is inhibition of PDPN.

In some examples, the sample from the individual is a tumor tissue sample or a tumor fluid sample. In some examples, the tumor tissue sample is a formalin-fixed and paraffin-embedded (FFPE) sample, an archival sample, a fresh sample, or a frozen sample.

In some examples, the expression level of PDPN in the sample is a protein expression level of PDPN or an RNA expression level of PDPN. In some examples, the expression level of PDPN in the sample

is an RNA expression level of PDPN. In some examples, the RNA expression level of PDPN is determined by *in situ* hybridization (ISH), RNA-seq, RT-qPCR, qPCR, multiplex qPCR or RT-qPCR, microarray analysis, SAGE, MassARRAY technique, FISH, or a combination thereof.

5 In some examples, the reference PDPN expression level is an expression level of PDPN in a population of individuals having a cancer. In some examples, the cancer is a colorectal cancer (CRC), a squamous cell carcinoma of the head and neck, or a glioma.

In some examples, the reference PDPN expression level is the 50th percentile of expression levels in the population.

10 In some examples, the reference PDPN expression level is the 66th percentile of expression levels in the population.

In some examples, the reference PDPN expression level is a pre-assigned PDPN expression level.

In some examples, the cancer is a CRC, a squamous cell carcinoma of the head and neck, or a glioma. In some examples, the cancer is a CRC, e.g., a stage II CRC or a stage IV CRC.

15 In some examples, the benefit comprises an extension in the individual's recurrence-free survival (RFS) as compared to treatment without the agonist of CD177 activity.

In some examples, the agonist of CD177 activity results in an increase in the binding of PDPN and CD177 relative to binding of the two proteins in the absence of the agonist.

20 In some examples, the agonist of CD177 activity results in a change in a downstream activity of PDPN relative to the downstream activity in the absence of the agonist of CD177 activity.

In some examples, the change in the downstream activity is a decrease in tumor growth.

In some examples, the change in the downstream activity is a decrease in cancer-associated fibroblast (CAF) contractility.

25 In some examples, the agonist of CD177 activity is a small molecule, an antibody or antigen-binding fragment thereof, a peptide, or a mimic.

In some examples, the agonist of CD177 activity is a peptide. In some examples, the peptide is a CD177 peptide. In some examples, the CD177 peptide is an extracellular domain of CD177. In some examples, the peptide is multimerized, e.g., tetramerized, e.g., tetramerized using streptavidin.

30 In some examples, the agonist of CD177 activity is an antibody or antigen-binding fragment thereof.

In some examples, the antibody or antigen-binding fragment thereof binds PDPN. In some examples, the antibody or antigen-binding fragment thereof is an antagonist antibody or antigen-binding fragment thereof.

35 In some examples, the antibody or antigen-binding fragment thereof binds CD177. In some examples, the antibody or antigen-binding fragment thereof is an agonist antibody or antigen-binding fragment thereof.

In some examples, the antigen-binding fragment is a bis-Fab, an Fv, a Fab, a Fab'-SH, a F(ab')₂, a diabody, a linear antibody, an scFv, an ScFab, a VH domain, or a VHH domain.

In some examples, the individual is a human.

40 In another example, the disclosure features a collection of polypeptides, wherein each polypeptide comprises an extracellular domain, a tag, and an anchor, and wherein the collection of polypeptides comprises the extracellular domains of at least 81% of the proteins of Table 7.

In some examples, the collection of polypeptides comprises the extracellular domains of at least 85% of the proteins of Table 7. In some examples, the collection of polypeptides comprises at least 90% of the proteins of Table 7. In some examples, the collection of polypeptides comprises the extracellular domains of at least 95% of the proteins of Table 7. In some examples, the collection of polypeptides
5 comprises the extracellular domains of all of the proteins of Table 7.

In some examples, the anchor is capable of tethering the extracellular domain to the surface of a plasma membrane of a cell. In some examples, the anchor is a glycosylphosphatidylinositol (GPI) polypeptide.

In some examples, the tag can be directly or indirectly visualized. In some examples, the tag
10 comprises a moiety that can be detected using an antibody or an antibody fragment. In some examples, the tag is a glycoprotein D (gD) polypeptide. In some examples, the tag comprises a fluorescent protein.

In some examples, the extracellular domain has a native conformation. In some examples, the extracellular domain comprises a native post-translational modification.

In some examples, the cell is a mammalian cell. In some examples, the cell is a COS7 cell.

15 In some examples, the cell has been transiently transfected with a plasmid encoding the polypeptide.

In another example, the disclosure features a collection of vectors encoding the collection of polypeptides of any one of the above examples.

In another example, the disclosure features a collection of cells comprising the collection of vectors
20 vectors of the above example. In some examples, a plurality of the cells are capable of expressing at least one polypeptide of any one of the above examples, optionally wherein different cells express different polypeptides.

In some examples, each of the one or more of said polypeptides is immobilized to a distinct location on one or more solid surfaces.

25 In another example, the disclosure features a method for identifying a protein-protein interaction, the method comprising providing the collection of polypeptides of any one of the above examples, optionally wherein said polypeptides are immobilized on one or more solid surfaces; contacting the collection of step (a) with a multimerized query protein under conditions permitting the binding of the multimerized query protein and at least one of the extracellular domains of the polypeptides; and detecting
30 an interaction between the multimerized query protein and the at least one extracellular domain, thereby identifying a protein-protein interaction. In some examples, one or more of said polypeptides each is immobilized to a distinct location on said one or more solid surfaces. In some examples, the distinct location comprises a cell that displays the polypeptide.

In some examples, the cells are mammalian cells.

35 In some examples, the contacting step is semi-automated.

In some examples, detecting an interaction comprises detecting a signal, optionally a fluorescent signal, at a location on the solid surface that is above a threshold level. In some examples, the detecting is automated.

In some examples, the interaction is a transient interaction.

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In some examples, the interaction is a low-affinity interaction. In some examples, the low-affinity interaction is a micromolar-affinity interaction.

In some examples, the multimerized query protein is a dimerized, trimerized, tetramerized, or pentamerized query protein. In some examples, the multimerized query protein is a tetramerized query protein. In some examples, the multimerized query protein comprises an isolated extracellular domain of the query protein. In some examples, the isolated extracellular domain of the query protein has been biotinylated and conjugated to a fluorescent streptavidin to tetramerize the query protein.

In another example, the disclosure features a method of identifying a modulator of the interaction between a protein of Table 1 and a protein of Table 2, the method comprising (a) providing a candidate modulator; (b) contacting a protein of Table 1 with a protein of Table 2 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 1 to the protein of Table 2, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (c) measuring the binding of the protein of Table 1 to the protein of Table 2, wherein an increase or decrease in binding in the presence of the candidate modulator relative to binding in the absence of the candidate modulator identifies the candidate modulator as a modulator of the interaction between the protein of Table 1 and the protein of Table 2.

In another example, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 1, the method comprising (a) providing a candidate modulator; (b) contacting the protein of Table 1 with a protein of Table 2 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 1 to the protein of Table 2, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (c) measuring a downstream activity of the protein of Table 1, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 1.

In another example, the disclosure features a method of identifying a modulator of a downstream activity of a protein of Table 2, the method comprising (a) providing a candidate modulator; (b) contacting the protein of Table 2 with a protein of Table 1 in the presence or absence of the candidate modulator under conditions permitting the binding of the protein of Table 2 to the protein of Table 1, wherein the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (c) measuring a downstream activity of the protein of Table 2, wherein a change in the downstream activity in the presence of the candidate modulator relative to the downstream activity in the absence of the candidate modulator identifies the candidate modulator as a modulator of the downstream activity of the protein of Table 2.

In some examples, the increase or decrease in binding is at least 70%, as measured by surface plasmon resonance, biolayer interferometry, or an enzyme-linked immunosorbent assay (ELISA).

In some examples, the modulator is an inhibitor of the downstream activity of the protein of Table 1 or Table 2. In some examples, the modulator is an activator of the downstream activity of the protein of Table 1 or Table 2.

In some examples, the change in the downstream activity is a decrease in the amount, strength, or duration of the downstream activity. In some examples, the change in the downstream activity is an increase in the amount, strength, or duration of the downstream activity.

In some examples, the modulator is a small molecule, an antibody or antigen-binding fragment thereof, a peptide, a mimic, an antisense oligonucleotide, or a small interfering RNA (siRNA). In some examples, the antigen-binding fragment is a bis-Fab, an Fv, a Fab, a Fab'-SH, a F(ab')₂, a diabody, a linear antibody, an scFv, an ScFab, a VH domain, or a VHH domain. In some examples, the antibody or antigen-binding fragment thereof binds the protein of Table 1. In some examples, the antibody or antigen-binding fragment thereof binds the protein of Table 2.

In some examples, the protein of Table 1 is podoplanin (PDPN). In some examples, the protein of Table 2 is CD177.

In some examples, the downstream activity is cancer-associated fibroblast (CAF) contractility. In some examples, CAF contractility is decreased in the presence of the modulator. In some examples, CAF contractility is decreased by at least 20%, as measured in a gel contraction assay. In some examples, CAF contractility is decreased by at least 20%, as measured in a 3D gel elongation assay.

In some examples, the downstream activity is tumor growth. In some examples, tumor growth is decreased in the presence of the modulator. In some examples, tumor growth is decreased by at least 20%, as measured in a tumor growth assay.

In some examples, the modulator is an antibody or antigen-binding fragment thereof targeting PDPN. In some examples, the modulator is an antibody or antigen-binding fragment thereof targeting CD177.

In some examples, the protein of Table 1 is PD-L1 (CD274). In some examples, the protein of Table 2 is EPHA3.

In some examples, the protein of Table 1 is PD-L2 (PDCD1LG2). In some examples, the protein of Table 2 is CEACAM4, ICAM5, NECTIN3, PSG9, or TNFRSF11A. In some examples, the protein of Table 2 is CEACAM4.

In some examples, the downstream activity is immune checkpoint inhibition. In some examples, immune checkpoint inhibition is decreased in the presence of the modulator. In some examples, immune checkpoint inhibition is decreased by at least 30%, as measured in a cell-based assay.

In some examples, the protein of Table 1 is PTPRD. In some examples, the PTPRD comprises a G203E and K204E; R232C and R233C; P249L; G285E; E406K; S431L; R561Q; P666S; E755K; V892I; S912F; R995C; or R1088C amino acid substitution mutation or a ΔG61 ΔE106 amino acid deletion mutation. In some examples, the protein of Table 2 is BMP5, CEACAM3, IL1RAP, IL1RAPL2, LECT1, LRFN5, SIRPG, SLITRK3, SLITRK4, SLITRK6, or TGFA.

In some examples, the downstream activity is suppression of cell proliferation. In some examples, suppression of cell proliferation is increased in the presence of the modulator. In some examples, suppression of cell proliferation is increased by at least 30%, as measured in a colony formation assay. In some examples, the downstream activity is suppression of STAT3 phosphorylation. In some examples, suppression of STAT3 phosphorylation is increased in the presence of the modulator.

In some examples,

suppression of STAT3 phosphorylation is increased by at least 30%, as measured in a Western blot for phosphorylated STAT3.

In some examples, the protein of Table 1 is PTPRS. In some examples, the protein of Table 2 is C6orf25, IL1RAP, IL1RAPL1, IL1RAPL2, LRFN1, LRFN5, LRRC4B, NCAM1, SLITRK1, SLITRK2,
5 SLITRK3, SLITRK4, or SLITRK6.

In some examples, the protein of Table 1 is PTPRF. In some examples, the protein of Table 2 is CD177, IL1RAP, or LRFN5.

In some examples, the downstream activity is inhibition of cell migration. In some examples, inhibition of cell migration is increased in the presence of the modulator. In some examples, inhibition of
10 cell migration is increased by at least 20%.

In some examples, the downstream activity is phosphorylation of EGFR. In some examples, phosphorylation of EGFR is decreased in the presence of the modulator. In some examples, phosphorylation of EGFR is decreased by at least 30%, as measured in an assay for phosphorylation of EGFR.

15 In some examples, the protein of Table 1 is CHL1. In some examples, the protein of Table 2 is CNTN1, CNTN5, SIRPA, L1CAM, or TMEM132A.

In some examples, the downstream activity is suppression of tumor formation. In some examples, suppression of tumor formation is increased in the presence of the modulator. In some examples, suppression of tumor formation is increased by at least 20%.

20 In some examples, the protein of Table 1 is CNTN1. In some examples, the protein of Table 2 is CDH6, CHL1, FCGRT, PCDHB7, or SGCG.

In some examples, the downstream activity is cell proliferation or cell invasion. In some examples, cell proliferation or cell invasion is decreased in the presence of the modulator. In some examples, cell proliferation is decreased by at least 20%, as measured in a colony formation assay. In
25 some examples, cell invasion is decreased by at least 20%, as measured in a gel invasion assay.

In some examples, the protein of Table 1 is LILRB1. In some examples, the protein of Table 2 is CLEC6A, CXADR, EDAR, FLT4, IL6R, ILDR1, or LILRA5.

In some examples, the downstream activity is suppression of phagocytosis. In some examples, suppression of phagocytosis is decreased in the presence of the modulator. In some examples,
30 suppression of phagocytosis is decreased by at least 20%.

In some examples, the protein of Table 1 is LILRB2. In some examples, the protein of Table 2 is IGSF8 or MOG.

In some examples, the protein of Table 1 is LILRB3. In some examples, the protein of Table 2 is LRRC15 or LY6G6F.

35 In some examples, the protein of Table 1 is LILRB4. In some examples, the protein of Table 2 is CNTFR.

In some examples, the protein of Table 1 is LILRB5. In some examples, the protein of Table 2 is APLP2, CD177, CLEC10A, CLECSF13, LDLR, PILRA, or UNC5C. In some examples, the protein of Table 2 is LDLR.

In some examples, the downstream activity is osteoclast differentiation. In some examples, osteoclast differentiation is decreased by at least 20% in the presence of the modulator. In some examples, osteoclast differentiation is measured in an assay for TRAP+ multinucleated cells.

In some examples, the protein of Table 1 is AXL. In some examples, the protein of Table 2 is IL1RL1 or VSIG10L.

In some examples, the downstream activity is activation of the RAS/RAF/MAPK/ERK1/2 pathway, activation of the JAK/STAT pathway, activation of the PI3K signaling pathway, cell migration, formation of filopodia, or phosphorylation of AXL. In some examples, cell migration is decreased by at least 20% as measured in a gel invasion assay.

In some examples, the protein of Table 1 is LRRC4B. In some examples, the protein of Table 2 is BTN3A1 or BTN3A3

In some examples, expression of the protein of Table 1 or the protein of Table 2 is upregulated or downregulated in tumor tissue relative to healthy tissue.

In another example, the disclosure features an isolated modulator of the interaction between a protein of Table 1 and a protein of Table 2, wherein (a) the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (b) the modulator causes an increase or decrease in the binding of the protein of Table 1 to the protein of Table 2 relative to binding in the absence of the modulator.

In another example, the disclosure features an isolated modulator of the downstream activity of a protein of Table 1 or a protein of Table 2, wherein (a) the protein of Table 1 and the protein of Table 2 are reported to interact in Table 3; and (b) the modulator causes a change in the downstream activity of the protein of Table 1 or the protein of Table 2 relative to downstream activity in the absence of the modulator.

In some examples, the increase or decrease in binding is at least 70%, as measured by surface plasmon resonance, biolayer interferometry, or an enzyme-linked immunosorbent assay (ELISA).

In some examples, the modulator is an inhibitor of the downstream activity of the protein of Table 1 or Table 2. In some examples, the modulator is an activator of the downstream activity of the protein of Table 1 or Table 2.

In some examples, the change in the downstream activity is a decrease in the amount, strength, or duration of the downstream activity. In some examples, the change in the downstream activity is an increase in the amount, strength, or duration of the downstream activity.

In some examples, the modulator is a small molecule, an antibody or antigen-binding fragment thereof, a peptide, or a mimic. In some examples, the antigen-binding fragment is a bis-Fab, an Fv, a Fab, a Fab'-SH, a F(ab')₂, a diabody, a linear antibody, an scFv, an ScFab, a VH domain, or a VHH domain. In some examples, the antibody or antigen-binding fragment thereof binds the protein of Table 1. In some examples, the antibody or antigen-binding fragment thereof binds the protein of Table 2.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A is a pair of graphs showing the occurrence of main protein domains and motifs according to UniProt annotations in the set of immunoglobulin superfamily (IgSF) members and other non-IgSF

WHAT IS CLAIMED IS:

1. Use of an agonist of CD177 activity in the manufacture of a medicament for treating an individual having a cancer.
2. Use of an agonist of CD177 activity in the manufacture of a medicament for treating an individual having a cancer, wherein the use comprises determining an expression level of podoplanin (PDPN) in a sample that has been obtained from the individual, wherein the expression level of PDPN in the sample is above a reference PDPN expression level, and wherein an effective amount of the agonist of CD177 is to be administered to the individual.
3. Use of an agonist of CD177 activity in the manufacture of a medicament for treating an individual having a cancer, wherein an expression level of PDPN in a sample that has been obtained from the individual has been determined to be above a reference PDPN expression level.
4. A method of identifying an individual having a cancer who may benefit from a treatment comprising an agonist of CD177 activity, the method comprising determining an expression level of PDPN in a sample that has been obtained from the individual, wherein an expression level of PDPN in the sample that is above a reference PDPN expression level identifies the individual as one who may benefit from the treatment comprising the agonist of CD177 activity.
5. A method of selecting a therapy for an individual having a cancer, the method comprising determining an expression level of PDPN in a sample that has been obtained from the individual, wherein an expression level of PDPN in the sample that is above a reference PDPN expression level identifies the individual as one who may benefit from a treatment comprising an agonist of CD177 activity.
6. The method of claim 4 or 5, wherein the individual has an expression level of PDPN in the sample that is above the reference PDPN expression level and wherein an effective amount of the agonist of CD177 activity is to be administered to the individual.
7. The use of any one of claims 1-3 or the method of any one of claims 4-6, wherein the CD177 activity is inhibition of PDPN.
8. The use of claim 2 or 3, the method of any one of claims 4-6 or the use or method of claim 7, wherein the sample that has been obtained from the individual is a tumor tissue sample or a tumor fluid sample.
9. The use or method of claim 8, wherein the tumor tissue sample is a formalin-fixed and paraffin-embedded (FFPE) sample, an archival sample, a fresh sample, or a frozen sample.

10. The use of claim 2 or 3, the method of any one of claims 4-6, or the use or method of any one of claims 7-9, wherein the expression level of PDPN in the sample is a protein expression level of PDPN or an RNA expression level of PDPN.

11. The use or method of claim 10, wherein the expression level of PDPN in the sample is an RNA expression level of PDPN.

12. The use or method of claim 11, wherein the RNA expression level of PDPN is determined by *in situ* hybridization (ISH), RNA-seq, RT-qPCR, qPCR, multiplex qPCR or RT-qPCR, microarray analysis, SAGE, MassARRAY technique, FISH, or a combination thereof.

13. The use of claim 2 or 3, the method of any one of claims 4-6, or the use or method of any one of claims 7-12, wherein the reference PDPN expression level is an expression level of PDPN in a population of individuals having a cancer.

14. The use or method of claim 13, wherein the cancer is a colorectal cancer (CRC), a squamous cell carcinoma of the head and neck, or a glioma.

15. The use or method of claim 13 or 14, wherein the reference PDPN expression level is the 50th percentile of expression levels in the population.

16. The use or method of claim 13 or 14, wherein the reference PDPN expression level is the 66th percentile of expression levels in the population.

17. The use of claim 2 or 3, the method of any one of claims 4-6, or the use or method of any one of claims 7-16, wherein the reference PDPN expression level is a pre-assigned PDPN expression level.

18. The use of any one of claims 1-3 or the method of any one of claims 4-6, or the use or method of any one of claims 7-17, wherein the cancer is a CRC, a squamous cell carcinoma of the head and neck, or a glioma.

19. The use or method of claim 18, wherein the cancer is a CRC.

20. The use or method of claim 19, wherein the CRC is a stage II CRC.

21. The use or method of claim 19, wherein the CRC is a stage IV CRC.

22. The method of any one of claims 4-21, wherein the benefit comprises an extension in the individual's recurrence-free survival (RFS) as compared to treatment without the agonist of CD177 activity.

23. The use or method of any one of claims 1-22, wherein the agonist of CD177 activity results in an increase in the binding of PDPN and CD177 relative to binding of the two proteins in the absence of the agonist.

24. The use or method of any one of claims 1-23, wherein the agonist of CD177 activity results in a change in a downstream activity of PDPN relative to the downstream activity in the absence of the agonist of CD177 activity.

25. The use or method of claim 24, wherein the change in the downstream activity is a decrease in tumor growth.

26. The use or method of claim 24, wherein the change in the downstream activity is a decrease in cancer-associated fibroblast (CAF) contractility.

27. The use or method of any one of claims 1-26, wherein the agonist of CD177 activity is a small molecule, an antibody or antigen-binding fragment thereof, a peptide, or a mimic.

28. The use or method of claim 27, wherein the agonist of CD177 activity is a peptide.

29. The use or method of claim 28, wherein the peptide is a CD177 peptide.

30. The use or method of claim 29, wherein the CD177 peptide is an extracellular domain of CD177.

31. The use or method of claim 30, wherein the peptide is multimerized.

32. The use or method of claim 31, wherein the peptide is tetramerized.

33. The use or method of claim 32, wherein the peptide is tetramerized using streptavidin.

34. The use or method of claim 27, wherein the agonist of CD177 activity is an antibody or antigen-binding fragment thereof.

35. The use or method of claim 34, wherein the antibody or antigen-binding fragment thereof binds PDPN.

36. The use or method of claim 35, wherein the antibody or antigen-binding fragment thereof is an antagonist antibody or antigen-binding fragment thereof.

37. The use or method of claim 34, wherein the antibody or antigen-binding fragment thereof binds CD177.

38. The use or method of claim 37, wherein the antibody or antigen-binding fragment thereof is an agonist antibody or antigen-binding fragment thereof.

39. The use or method of any one of claims 27 and 34-38, wherein the antigen-binding fragment is a bis-Fab, an Fv, a Fab, a Fab'-SH, a F(ab')₂, a diabody, a linear antibody, an scFv, an ScFab, a VH domain, or a VHH domain.

40. The use or method of any one of claims 1-39, wherein the individual is a human.