

chronic proliferated /activated T-cells (Chen *et al.*, (2004) Blood, 104(10): 3233-3242; Huang *et al.*, (2005) Eur J Immunol, 35(7): 2239-2249). Neihulizumab (AbGn-168H), a humanized IgG4κ monoclonal antibody (mAb) directed against PSGL-1, preferentially induces apoptosis (programmed cell death) of late stage activated T cells upon binding to PSGL-1. Neihulizumab has been tested in GVHD with encouraging clinical outcomes.

**[0009]** Several studies identified that PSGL-1 can be a potential target for immunotherapy treatment of several hematological malignancies. For example, gene expression profiling revealed that PSGL-1 is strongly expressed by the cells of multiple myeloma and anaplastic large T-cell lymphoma, and further evaluation of the effects elicited by in vitro treatment also indicated a promising candidate for humoral immunotherapy in these malignancies (Tripodo *et al.*, (2009) Curr Cancer Drug Targets, 9(5):617-625; Azab *et al.*, (2012) Blood, 119(6):1468-1478; Belmonte *et al.*, (2021) Cancers, 13(12):2958-2973).

**[0010]** There remains a need for more effective treatments for T-cell mediated inflammatory diseases and cancers.

**[0011]** All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

## SUMMARY

**[0012]** To meet this need, provided herein are methods for using anti-PSGL-1 antibodies in combination with JAK inhibitors to treat or prevent T-cell mediated inflammatory diseases or cancers. These methods are based at least in part on the demonstration herein that anti-PSGL-1 antibodies were found to act synergistically in combination with JAK inhibitors to promote induction of apoptosis in activated T cells and improve outcomes in patients with GVHD.

**[0013]** Accordingly, in one example, provided herein is a method of treating or preventing a T-cell mediated inflammatory disease, the method comprising administering to a subject in need thereof a therapeutically effective amount of an antibody that specifically binds to human PSGL-1 in combination with a Janus kinase (JAK) inhibitor. In another example, provided herein is a method of treating or preventing a cancer (*e.g.*, a T-cell mediated cancer such as T-cell leukemia or T-cell lymphoma), the method comprising administering

to a subject in need thereof a therapeutically effective amount of an antibody that specifically binds to human PSGL-1 in combination with a Janus kinase (JAK) inhibitor. In some examples, the antibody that specifically binds to human PSGL-1 comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain. In some examples, the VH domain comprises a CDR-H1 comprising the amino acid sequence SFGMH (SEQ ID NO:8), a CDR-H2 comprising the amino acid sequence YINGGSSTIFYANAVKG (SEQ ID NO:9), and a CDR-H3 comprising the amino acid sequence YASYGGGAMDY (SEQ ID NO:10); and the VL domain comprises a CDR-L1 comprising the amino acid sequence RSSQSIVHNDGNTYFE (SEQ ID NO:5), a CDR-L2 comprising the amino acid sequence KVSNRFS (SEQ ID NO:6), and a CDR-L3 comprising the amino acid sequence FQGSYVPLT (SEQ ID NO:7). In some examples, the VH domain comprises a CDR-H1 comprising the amino acid sequence AYYIH (SEQ ID NO:24), a CDR-H2 comprising the amino acid sequence RVNPNTGGTSYNPKFKG (SEQ ID NO:25), and a CDR-H3 comprising the amino acid sequence SGSPYYRYDD (SEQ ID NO:26); and the VL domain comprises a CDR-L1 comprising the amino acid sequence RASSTVNSTYLH (SEQ ID NO:28), a CDR-L2 comprising the amino acid sequence GSSNLAS (SEQ ID NO:29), and a CDR-L3 comprising the amino acid sequence QQYSGYPLT (SEQ ID NO:30). In some examples, the VH domain comprises a CDR-H1 comprising the amino acid sequence TNAMN (SEQ ID NO:32), a CDR-H2 comprising the amino acid sequence RIRSKSNNYATYYADSVKD (SEQ ID NO:33), and a CDR-H3 comprising the amino acid sequence GGSYWYFDV (SEQ ID NO:34); and the VL domain comprises a CDR-L1 comprising the amino acid sequence RSSQSIVNSNGNTYLE (SEQ ID NO:36), a CDR-L2 comprising the amino acid sequence KVSNRFS (SEQ ID NO:37), and a CDR-L3 comprising the amino acid sequence FQGSHVPWT (SEQ ID NO:38).

**[0013a]** In one aspect, the present disclosure refers to use of an antibody that specifically binds to human PSGL-1 in the manufacture of a medicament for use in combinatorial therapy for treating or preventing (a) a T-cell mediated inflammatory disease or (b) cancer in a subject in need thereof, wherein said medicament is to be administered in combination with a Janus kinase (JAK) inhibitor.

**[0014]** In some examples, the JAK inhibitor inhibits JAK1 and/or JAK2. In some examples, the JAK inhibitor inhibits JAK1 and/or JAK3. In some examples, the JAK inhibitor is ruxolitinib or tofacitinib. In some examples, the antibody is a humanized antibody. In some examples, the VH domain comprises the amino acid sequence EVQLVESGGGLVQPGGSLRLSCAASGFTFSSFGMHWVRQAPGKGLEWVAYINGGSSTIFYANAVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARYASYGGGAMDYWGQGTLLTVSS

(SEQ ID NO:4). In some examples, the VL domain comprises the amino acid sequence DIQMTQSPSSLSASVGDRVTITCRSSQSI VHNDGNTYFEWYQQKPGKAPKLLIYKVS NRFS GVPSRFSGSGSGTHFTLTIS SLQPEDFATYYCFQGSYVPLTFTGQGTKVEIK (SEQ ID NO:3). In some examples, the heavy chain further comprises an antibody constant domain. In some examples, the constant domain is a human IgG4 constant domain. In some examples, the constant domain is a human IgG4 constant domain comprising an S228P amino acid substitution at position 228, wherein the numbering is according to EU numbering. In some examples, the light chain is a human kappa or lambda light chain. In some examples, the heavy chain comprises the amino acid sequence of SEQ ID NO:2. In some examples, the VH domain comprises a VH domain sequence of the amino acid sequence of SEQ ID NO:27 and/or the VL domain comprises a VL domain sequence of the amino acid sequence of SEQ ID NO:31. In some examples, the heavy chain comprises the amino acid sequence of SEQ ID NO:27 and/or the light chain comprises the amino acid sequence of SEQ ID NO:31. In some examples, the VH domain comprises a VH domain sequence of the amino acid sequence of SEQ ID NO:35 and/or the VL domain comprises a VL domain sequence of the amino acid sequence of SEQ ID NO:39. In some examples, the heavy chain comprises the amino acid sequence of SEQ ID NO:35 and/or the light chain comprises the amino acid sequence of SEQ ID NO:39. In some examples, the antibody that specifically binds to human PSGL-1 comprises the amino acid sequence of SEQ ID NO:23. In some examples, the light chain comprises the amino acid sequence of SEQ ID NO:1.

**[0015]** In some examples, the T-cell mediated inflammatory disease is graft-versus-host disease (GVHD). In some examples, the T-cell mediated inflammatory disease is acute GVHD or chronic GVHD. In some examples, the T-cell mediated inflammatory disease is steroid-refractory acute GVHD (SR-aGVHD) or treatment-refractory acute GVHD (TR-aGVHD). In some examples, the T-cell mediated inflammatory disease is selected from the group consisting of: a skin disorder, multiple sclerosis, rheumatoid arthritis, juvenile arthritis, type I diabetes, lupus, inflammatory bowel disease, Crohn's disease, myasthenia gravis, immunoglobulin nephropathies, myocarditis, and autoimmune thyroid disorder. In some examples, the cancer is a T-cell mediated cancer. In some examples, the cancer is a T-cell leukemia or T-cell lymphoma. In some examples, human PSGL-1 comprises the amino acid sequence of SEQ ID NO:11 or SEQ ID NO:22. In some examples, the subject is a human. In some examples, the antibody is

administered by intravenous infusion. In some examples, the JAK inhibitor is administered orally. In some examples, the antibody is a monoclonal antibody. In some examples, the JAK inhibitor is administered to the subject before, after, or simultaneous with administration of the antibody. In some examples, prior to administration of the antibody and the JAK inhibitor, the subject has been treated with a corticosteroid. In some examples, administration of the antibody and the JAK inhibitor results in a reduction in one or more symptoms of the T-cell mediated inflammatory disease in the subject.

**[0016]** In another example, provided herein is an antibody that specifically binds to human PSGL-1 for use in a method of treating or preventing a T-cell mediated inflammatory disease or cancer in a subject, *e.g.*, according to any one of the above examples. In some examples, the method comprises administering to the subject a therapeutically effective amount of the antibody in combination with a Janus kinase (JAK) inhibitor. In some examples, the antibody comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain; wherein the VH domain comprises a CDR-H1 comprising the amino acid sequence SFGMH (SEQ ID NO:8), a CDR-H2 comprising the amino acid sequence YINGGSSTIFYANAVKG (SEQ ID NO:9), and a CDR-H3 comprising the amino acid sequence YASYGGGAMDY (SEQ ID NO:10); and wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence RSSQSIVHNDGNTYFE (SEQ ID NO:5), a CDR-L2 comprising the amino acid sequence KVSNRFS (SEQ ID NO:6), and a CDR-L3 comprising the amino acid sequence FQGSYVPLT (SEQ ID NO:7). In some examples, the antibody comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain; wherein VH domain comprises a CDR-H1 comprising the amino acid sequence AYYIH (SEQ ID NO:24), a CDR-H2 comprising the amino acid sequence RVNPNTGGTSYNPKFKG (SEQ ID NO:25), and a CDR-H3 comprising the amino acid sequence SGSPYYRYDD (SEQ ID NO:26); and the VL domain comprises a CDR-L1 comprising the amino acid sequence RASSTVNSTYLH (SEQ ID NO:28), a CDR-L2 comprising the amino acid sequence GSSNLAS (SEQ ID NO:29), and a CDR-L3 comprising the amino acid sequence QQYSGYPLT (SEQ ID NO:30). In some examples, the antibody comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain; wherein the

VH domain comprises a CDR-H1 comprising the amino acid sequence TNAMN (SEQ ID NO:32), a CDR-H2 comprising the amino acid sequence RIRSKSNNYATYYADSVKD (SEQ ID NO:33), and a CDR-H3 comprising the amino acid sequence GGSYWYFDV (SEQ ID NO:34); and the VL domain comprises a CDR-L1 comprising the amino acid sequence RSSQSIVNSNGNTYLE (SEQ ID NO:36), a CDR-L2 comprising the amino acid sequence KVSNRFS (SEQ ID NO:37), and a CDR-L3 comprising the amino acid sequence FQGSHVPWT (SEQ ID NO:38).

**[0017]** In another example, provided herein is the use of an antibody that specifically binds to human PSGL-1 in the manufacture of a medicament for treating or preventing a T-cell mediated inflammatory disease or cancer; wherein the antibody is to be administered in combination with a Janus kinase (JAK) inhibitor. In another example, provided herein is the use of an antibody that specifically binds to human PSGL-1 and a Janus kinase (JAK) inhibitor in the manufacture of a medicament for treating or preventing a T-cell mediated inflammatory disease or cancer. In some examples, the antibody comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain; wherein the VH domain comprises a CDR-H1 comprising the amino acid sequence SFGMH (SEQ ID NO:8), a CDR-H2 comprising the amino acid sequence YINGGSSTIFYANAVKG (SEQ ID NO:9), and a CDR-H3 comprising the amino acid sequence YASYGGGAMDY (SEQ ID NO:10); and wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence RSSQSIVHNDGNTYFE (SEQ ID NO:5), a CDR-L2 comprising the amino acid sequence KVSNRFS (SEQ ID NO:6), and a CDR-L3 comprising the amino acid sequence FQGSYVPLT (SEQ ID NO:7). In some examples, the antibody comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain; wherein VH domain comprises a CDR-H1 comprising the amino acid sequence AYYIH (SEQ ID NO:24), a CDR-H2 comprising the amino acid sequence RVNPNTGGTSYNPKFKG (SEQ ID NO:25), and a CDR-H3 comprising the amino acid sequence SGSPYYRYDD (SEQ ID NO:26); and the VL domain comprises a CDR-L1 comprising the amino acid sequence RASSTVNSTYLH (SEQ ID NO:28), a CDR-L2 comprising the amino acid sequence GSSNLAS (SEQ ID NO:29), and a CDR-L3 comprising the amino acid sequence QQYSGYPLT (SEQ ID NO:30). In some examples, the antibody comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain;

wherein the VH domain comprises a CDR-H1 comprising the amino acid sequence TNAMN (SEQ ID NO:32), a CDR-H2 comprising the amino acid sequence RIRSKSNNYATYYADSVKD (SEQ ID NO:33), and a CDR-H3 comprising the amino acid sequence GGSYWYFDV (SEQ ID NO:34); and the VL domain comprises a CDR-L1 comprising the amino acid sequence RSSQSIVNSNGNTYLE (SEQ ID NO:36), a CDR-L2 comprising the amino acid sequence KVSNRFS (SEQ ID NO:37), and a CDR-L3 comprising the amino acid sequence FQGSHVPWT (SEQ ID NO:38).

**[0018]** In another example, provided herein is a kit or article of manufacture comprising an antibody that specifically binds to human PSGL-1 and a package insert comprising instructions for using the antibody in combination with a Janus kinase (JAK) inhibitor for treating or preventing a T-cell mediated inflammatory disease or cancer in a subject. In some examples, the antibody comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain; wherein the VH domain comprises a CDR-H1 comprising the amino acid sequence SFGMH (SEQ ID NO:8), a CDR-H2 comprising the amino acid sequence YINGGSSTIFYANAVKG (SEQ ID NO:9), and a CDR-H3 comprising the amino acid sequence YASYGGGAMDY (SEQ ID NO:10); and wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence RSSQSIVHNDGNTYFE (SEQ ID NO:5), a CDR-L2 comprising the amino acid sequence KVSNRFS (SEQ ID NO:6), and a CDR-L3 comprising the amino acid sequence FQGSYVPLT (SEQ ID NO:7). In some examples, the antibody comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain; wherein VH domain comprises a CDR-H1 comprising the amino acid sequence AYYIH (SEQ ID NO:24), a CDR-H2 comprising the amino acid sequence RVNPNTGGTSYNPKFKG (SEQ ID NO:25), and a CDR-H3 comprising the amino acid sequence SGSPYYRYDD (SEQ ID NO:26); and the VL domain comprises a CDR-L1 comprising the amino acid sequence RASSTVNSTYLH (SEQ ID NO:28), a CDR-L2 comprising the amino acid sequence GSSNLAS (SEQ ID NO:29), and a CDR-L3 comprising the amino acid sequence QQYSGYPLT (SEQ ID NO:30). In some examples, the antibody comprises a heavy chain comprising a heavy chain variable (VH) domain and a light chain comprising a light chain variable (VL) domain; wherein the VH domain comprises a CDR-H1 comprising the amino acid sequence TNAMN (SEQ ID NO:32), a CDR-H2 comprising the amino acid sequence RIRSKSNNYATYYADSVKD (SEQ

ID NO:33), and a CDR-H3 comprising the amino acid sequence GGSYWYFDV (SEQ ID NO:34); and the VL domain comprises a CDR-L1 comprising the amino acid sequence RSSQSIVNSNGNTYLE (SEQ ID NO:36), a CDR-L2 comprising the amino acid sequence KVSNRFS (SEQ ID NO:37), and a CDR-L3 comprising the amino acid sequence FQGSHVPWT (SEQ ID NO:38). In some examples, the kit or article of manufacture further comprises the JAK inhibitor.

**[0018a]** In one aspect, the present disclosure refers to a kit comprising an antibody that specifically binds to human PSGL-1 and a package insert comprising instructions for using the antibody in combination with a Janus kinase (JAK) inhibitor for treating or preventing a T-cell mediated inflammatory disease in a subject.

**[0018b]** In one aspect, the present disclosure refers to a kit comprising an antibody that specifically binds to human PSGL-1 and a package insert comprising instructions for using the antibody in combination with a Janus kinase (JAK) inhibitor for treating or preventing cancer in a subject.

**[0019]** It is to be understood that one, some, or all of the properties of the various examples described herein may be combined to form other examples of the present disclosure. These and other examples of the disclosure will become apparent to one of skill in the art.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0020]** **FIGS. 1A-1C** provide results of T cell apoptosis assays that tested the synergistic effects of ruxolitinib (“RUXO”) in combination with anti-PSGL-1 antibody 15A7H. Ruxolitinib and antibody 15A7H were tested alone or in combination at the indicated concentrations (RUXO: 10  $\mu$ M, 3.3  $\mu$ M, 1.1  $\mu$ M, 0.367  $\mu$ M, and 0.122  $\mu$ M; antibody 15A7H: 0.03  $\mu$ g/mL, 0.3  $\mu$ g/mL, and 3  $\mu$ g/mL) in activated T cells derived from peripheral blood mononuclear cells (PBMCs) from three donors (Donor 1, **FIG. 1A**; Donor 2; **FIG. 1B**; Donor 3, **FIG. 1C**). An isotype control antibody (“Isotype ctrl”) alone or in combination with ruxolitinib was also tested at a concentration of 3  $\mu$ g/mL control antibody. The level of cell apoptosis, as shown on the y-axis, was assessed as described in Example 1 herein.

**[0021]** **FIG. 2** provides results of experiments testing the combinatorial effect of ruxolitinib (“RUXO”) in combination with anti-PSGL-1 antibody 15A7H on activated T cell apoptosis, assessed using Chou–Talalay median effect analysis as described in Example 1 herein. Ruxolitinib and antibody 15A7H were tested at the indicated fixed ratios (3.3:1, 1.1:1, and 0.37:1) for their effect on activated T cell apoptosis in cells derived from the indicated donors (N089, N084, N102, and N103). The x-axis shows the observed apoptosis fractions as compared to untreated control cells, and the y-axis shows the corresponding combination index (CI). Combinatorial effects were defined as “synergistic”, “additive” or “antagonistic” when the CI was < 1, 1 and >1, respectively.