

selectin, expressed on activated platelets and endothelial cells, also binds to ligands on most leukocytes.

[0005] P-selectin glycoprotein ligand-1 ("PSGL-1"), also known as SELPLG or CD162 (cluster of differentiation 162) is a human mucin-type glycoprotein ligand for all three selectins (Constantin, Gabriela, 2004, Drug News Perspect., 17(9): 579-585; McEver et al., 1997, J. Clin. Invest., 100 (3): 485-492). PSGL-1 is a disulfide-bonded homodimer with two 120-kD subunits and is expressed on the surface of monocytes, lymphocytes, granulocytes, and in some CD34⁺ stem cells. PSGL-1 is likely to contribute to pathological leukocyte recruitment in many inflammatory disorders since it facilitates the adhesive interactions of selectins. In addition, PSGL-1 is shown to have a unique regulatory role in T cells. Mice deficient in PSGL-1 show enhanced proliferative responses and autoimmunity, suggesting that PSGL-1 plays an important role in down-regulating T cell responses (Krystle M. et al. J. Immunol. 2012; 188:1638-1646. Urzainqui et al. Ann Rheum Dis 2013;71:650; Pérez-Frías A, et al. Arthritis Rheumatol. 2014 Nov;66(11):3178-89.; Angiari et al. J Immunol. 2013;191(11):5489-500).

[0006] Several anti-PSGL-1 antibodies have been developed (see, e.g., International Application Pub. Nos. WO 2005/110475, WO 2003/013603, and WO 2012/174001; Constantin, Gabriela, 2004, Drug News Perspect., 17(9): 579-585, Chen et al. Blood. 2004;104(10):3233-42, Huang et al, Eur J Immunol. 2005;35(7):2239-49; and U.S. Patent No. 7,604,800). Some of the existing agonistic PSGL-1 antibodies preferentially induce apoptosis of late-stage activated T cells but not other PSGL-1-expressing cells; such antibodies may therefore be useful as anti-inflammatory therapeutics, or for use in transplantations and/or transfusions. However, a need exists for improved anti-PSGL-1 antibodies with greater *in vivo* efficacy than existing antibodies.

[0007] All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

SUMMARY

[0008] To meet this need, provided herein are tetravalent antibodies that specifically bind to human PSGL-1, as well as polynucleotides, vectors, host cells, methods, pharmaceutical compositions, kits, and uses related thereto. The present disclosure demonstrates that tetravalent antibodies that specifically bind to human PSGL-1 have greater potency and

efficacy than conventional (*e.g.*, bivalent) anti-PSGL-1 antibodies. As such, these tetravalent antibodies may find use, *inter alia*, in diagnostic and/or therapeutic methods, uses, and compositions related to T-cell function, such as in treating T-cell mediated inflammatory diseases, transfusions, and/or transplantations.

[0009] Accordingly, in one example, provided herein is a tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises a single-chain polypeptide comprising: (a) two light chain variable (VL) domains, wherein each of the two VL domains comprises a CDR-L1, a CDR-L2, and a CDR-L3; (b) two heavy chain variable (VH) domains, wherein each of the two VH domains comprises a CDR-H1, a CDR-H2, and a CDR-H3; and (c) an antibody Fc domain, wherein each of the two VL domains forms a VH-VL binding unit with a corresponding VH domain of the two VH domains, and wherein each of the two VH-VL binding units is specific for human PSGL-1. In some examples, at least one of the two VH domains comprises: (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19. In some examples, each of the two VH domains comprises: (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19. In some examples, one or both of the two VH domains comprises the amino acid sequence of SEQ ID NO:23, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:23. In some examples, one or both of the two VH domains comprises the amino acid sequence of SEQ ID NO:29, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:29. In some examples, at least one of the two VL domains comprises: (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22. In some examples, each of the two VL domains comprises: (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22. In some examples, one or both of the two VL domains comprises the amino acid sequence of SEQ ID NO:24, or an amino acid sequence having at least 90%, at least 95%, or at least

99% sequence identity to SEQ ID NO:24. In some examples, one or both of the two VL domains comprises the amino acid sequence of SEQ ID NO:30, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:30. In some examples, each of the two single-chain polypeptides comprises, from N-terminus to C-terminus: (a) a first VL domain of the two VL domains; (b) a first linker sequence; (c) a first VH domain of the two VH domains; (d) a second linker sequence; (e) a second VL domain of the two VL domains; (f) a third linker sequence; (g) a second VH domain of the two VH domains; (h) a fourth linker sequence; and (i) the antibody Fc domain. In some examples, the first, second and third linker sequences each comprise two or more repeats of the amino acid sequence of SEQ ID NO:25. In some examples, the first and the third linker sequences have the same sequence and comprise two repeats of SEQ ID NO:25. In some examples, the second linker sequence comprises five repeats of SEQ ID NO:25. In some examples, the fourth linker sequence comprises the amino acid sequence of SEQ ID NO:26. In some examples, each of the two single-chain polypeptides comprises the amino acid sequence of SEQ ID NO:1, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:1. In some examples, each of the two single-chain polypeptides is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2. In some examples, each of the two single-chain polypeptides comprises, from N-terminus to C-terminus: (a) a first VH domain of the two VH domains; (b) a first linker sequence; (c) a first VL domain of the two VL domains; (d) a second linker sequence; (e) a second VL domain of the two VL domains; (f) a third linker sequence; (g) a second VH domain of the two VH domains; (h) a fourth linker sequence; and (i) the antibody Fc domain. In some examples, each of the two single-chain polypeptides comprises, from N-terminus to C-terminus: (a) a first VL domain of the two VL domains; (b) a first linker sequence; (c) a first VH domain of the two VH domains; (d) a second linker sequence; (e) a second VH domain of the two VH domains; (f) a third linker sequence; (g) a second VL domain of the two VL domains; (h) a fourth linker sequence; and (i) the antibody Fc domain. In some examples, the first, second or third linker sequence comprises two or more repeats of the amino acid sequence of SEQ IN NO:25. In some examples, the first, second or third linker sequence comprises the amino acid sequence of SEQ ID NO:33, 34, 35, or 36. In some examples, the first and the third linker sequences have the same sequence comprising five repeats of SEQ ID NO:25. In some examples, the second linker sequence comprises the amino acid sequence of SEQ ID NO:27. In some examples, the

fourth linker sequence comprises the amino acid sequence of SEQ ID NO:26. In some examples, each of the two single-chain polypeptides comprises the amino acid sequence of SEQ ID NO:3, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:3. In some examples, each of the two single-chain polypeptides is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:4. In some examples, each of the two single-chain polypeptides comprises the amino acid sequence of SEQ ID NO:5. In some examples, each of the two single-chain polypeptides is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:6.

[0009a] In a first aspect, the present disclosure refers to a tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

- (a) a first light chain variable (VL) domain;
 - (b) a first linker sequence, wherein the first linker sequence has 5-12 amino acids;
 - (c) a first heavy chain variable (VH) domain;
 - (d) a second linker sequence, wherein the second linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (e) a second VL domain;
 - (f) a third linker sequence, wherein the third linker sequence has 5-12 amino acids;
 - (g) a second VH domain;
 - (h) a fourth linker sequence, wherein the fourth linker sequence has 5-12 amino acids;
- and
- (i) an antibody Fc domain,

wherein each of the first and the second VL domains comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22; wherein each of the first and the second VH domains comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19; wherein the first VL domain forms a VH-VL binding unit with the second VH domain, and the first VH domain forms a VH-VL binding unit with the second VL domain; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

[0009b] In a second aspect, the present disclosure refers to a tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

- (a) a first heavy chain variable (VH) domain;
 - (b) a first linker sequence, wherein the first linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (c) a first light chain variable (VL) domain;
 - (d) a second linker sequence, wherein the second linker sequence has 5-12 amino acids;
 - (e) a second VL domain;
 - (f) a third linker sequence, wherein the third linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (g) a second VH domain;
 - (h) a fourth linker sequence, wherein the fourth linker sequence has 5-12 amino acids;
- and
- (i) an antibody Fc domain,

wherein each of the first and the second VL domains comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of

SEQ ID NO:22; wherein each of the first and the second VH domains comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19; wherein each of the first and the second VL domains forms a VH-VL binding unit with a corresponding VH domain of the first and the second VH domains; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

[0009c] In a third aspect, the present disclosure refers to a tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

- (a) a first light chain variable (VL) domain;
 - (b) a first linker sequence, wherein the first linker sequence has 5-12 amino acids;
 - (c) a first heavy chain variable (VH) domain;
 - (d) a second linker sequence, wherein the second linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (e) a second VL domain;
 - (f) a third linker sequence, wherein the third linker sequence has 5-12 amino acids;
 - (g) a second VH domain;
 - (h) a fourth linker sequence, wherein the fourth linker sequence has 5-12 amino acids;
- and
- (i) an antibody Fc domain,

wherein each of the first and the second VL domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:24 or SEQ ID NO:30; wherein each of the first and the second VH domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:23 or SEQ ID NO:29; wherein the first VL domain forms a VH-VL binding unit with the second VH domain, and the first VH domain forms a VH-VL binding unit with the second VL domain; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

[0009d] In a fourth aspect, the present disclosure refers to a tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

- (a) a first heavy chain variable (VH) domain;
 - (b) a first linker sequence, wherein the first linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (c) a first light chain variable (VL) domain;
 - (d) a second linker sequence, wherein the second linker sequence has 5-12 amino acids;
 - (e) a second VL domain;
 - (f) a third linker sequence, wherein the third linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (g) a second VH domain;
 - (h) a fourth linker sequence, wherein the fourth linker sequence has 5-12 amino acids;
- and
- (i) an antibody Fc domain,

wherein each of the first and the second VL domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:24 or SEQ ID NO:30; wherein each of the first and the second VH domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:23 or SEQ ID NO:29; wherein each of the first and the second VL domains forms a VH-VL binding unit with a corresponding VH domain of the first and the second VH domains; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

[0010] In another example, provided herein is a tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises an antibody heavy chain and an antibody light chain; wherein the antibody light chain comprises: (i) two light chain variable (VL) domains, wherein each of the two VL domains comprises a CDR-L1, a CDR-L2, and a CDR-L3, (ii) a first heavy chain variable (VH) domain, and (iii) a light chain

constant (CL) domain; wherein the antibody heavy chain comprises: (i) a second heavy chain variable (VH) domain, and (ii) a heavy chain constant region comprising a first heavy chain constant region (CH1) domain, an antibody hinge region, an second heavy chain constant region (CH2) domain, and a third heavy chain constant region (CH3) domain; wherein the first and the second VH domains each comprise a CDR-H1, a CDR-H2, and a CDR-H3, wherein each of the two VL domains forms a VH-VL binding unit with a corresponding VH domain of the first and the second VH domains, and wherein each of the two VH-VL binding units is specific for human PSGL-1. In some examples, at least one of the first and the second VH domains comprises: (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19. In some examples, the first and the second VH domains each comprise: (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19. In some examples, the first and/or the second VH domains comprise the amino acid sequence of SEQ ID NO:23, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:23. In some examples, the first and/or the second VH domains comprise the amino acid sequence of SEQ ID NO:29, or an

amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:29. In some examples, at least one of the first and the second VL domains comprises: (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22. In some examples, the first and the second VL domains each comprise: (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22. In some examples, the first and/or the second VL domains comprise the amino acid sequence of SEQ ID NO:24, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:24. In some examples, the first and/or the second VL domains comprise the amino acid sequence of SEQ ID NO:30, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:30. In some examples, the antibody light chain comprises, from N-terminus to C-terminus: (a) the first VH domain; (b) a first linker sequence; (c) a first VL domain of the two or more VL domains; (d) a second linker sequence; (e) a second VL domain of the two or more VL domains; and (f) the CL domain. In some examples, the CL domain is a kappa CL domain. In some examples, the first linker sequence comprises five repeats of SEQ ID NO:25. In some examples, the second linker sequence comprises the amino acid sequence of SEQ ID NO:28. In some examples, the antibody light chain comprises the amino acid sequence of SEQ ID NO:7, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:7. In some examples, the antibody light chain is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:8. In some examples, the antibody light chain comprises, from N-terminus to C-terminus: (a) a first VL domain of the two VL domains; (b) the CL domain; (c) a first linker sequence; (d) the first VH domain; (e) a second linker sequence; and (f) a second VL domain of the two VL domains. In some examples, the CL domain is a kappa CL domain. In some examples, the first linker sequence comprises two repeats of SEQ ID NO:25. In some examples, the second linker sequence comprises five repeats of SEQ ID NO:25. In some examples, the antibody light chain comprises the amino acid sequence of SEQ ID NO:9. In some examples, the antibody light chain is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:10. In some examples, the antibody heavy chain comprises, from N-terminus to C-terminus: (a) the second VH domain; and (b) a heavy chain constant

region comprising a first heavy chain constant region (CH1) domain, an antibody hinge region, an second heavy chain constant region (CH2) domain, and a third heavy chain constant region (CH3) domain. In some examples, the antibody heavy chain comprises the amino acid sequence of SEQ ID NO:11, or an amino acid sequence having at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:11. In some examples, the antibody heavy chain is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:12.

[0011] In another example, provided herein is a tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises an antibody heavy chain and an antibody light chain; wherein the antibody light chain comprises: (i) a first heavy chain variable (VH) domain, (ii) a first light chain variable (VL) domain, and (iii) a light chain constant (CL) domain; wherein the antibody heavy chain comprises: (i) a second heavy chain variable (VH) domain, (ii) a second light chain variable (VL) domain, and (iii) a heavy chain constant domain comprising a first heavy chain constant region (CH1) domain, an antibody hinge region, an second heavy chain constant region (CH2) domain, and a third heavy chain constant region (CH3) domain; wherein each of the first and second VL domains comprises a CDR-L1, a CDR-L2, and a CDR-L3; wherein each of the first and second VH domains comprises a CDR-H1, a CDR-H2, and a CDR-H3; wherein each of the first and second VL domains forms a VH-VL binding unit with a corresponding VH domain of the first and second VH domains; and wherein each of the two VH-VL binding units is specific for human PSGL-1. In some examples, at least one of the first and second VH domains comprises: (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19. In some examples, the first and the second VH domains each comprise: (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19. In some examples, the first and/or the second VH domains comprise the amino acid sequence of SEQ ID NO:23. In some examples, the first and/or the second VH domains comprise the amino acid sequence of SEQ ID NO:29. In some examples, at least one of the first and second VL domains comprises: (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence

of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22. In some examples, the first and the second VL domains each comprise: (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22. In some examples, the first and/or the second VL domains comprise the amino acid sequence of SEQ ID NO:24. In some examples, the first and/or the second VL domains comprise the amino acid sequence of SEQ ID NO:30. In some examples, the antibody light chain comprises, from N-terminus to C-terminus: (a) the first VH domain; (b) a first linker sequence; (c) the first VL domain; and (d) the CL domain. In some examples, the CL domain is a kappa CL domain. In some examples, the first linker sequence comprises five repeats of SEQ ID NO:25. In some examples, the antibody light chain comprises the amino acid sequence of SEQ ID NO:13. In some examples, the antibody light chain is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:14. In some examples, the antibody heavy chain comprises, from N-terminus to C-terminus: (a) the second VH domain; (b) a second linker sequence; (c) the second VL domain; and (d) the heavy chain constant region comprising the first heavy chain constant region (CH1) domain, the antibody hinge region, the second heavy chain constant region (CH2) domain, and the third heavy chain constant region (CH3) domain. In some examples, the second linker sequence comprises five repeats of SEQ ID NO:25. In some examples, the antibody heavy chain comprises the amino acid sequence of SEQ ID NO:15. In some examples, the antibody heavy chain is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:16.

[0012] In some examples of any of the above examples, the antibody Fc domain is a human antibody Fc domain. In some examples, the antibody Fc domain is a human IgG4 Fc domain. In some examples, the human IgG4 Fc domain comprises a hinge region sequence comprising one or more amino acid substitutions that result in reduced IgG4 shuffling, as compared to an IgG4 hinge region lacking the one or more amino acid substitutions. In some examples, the human IgG4 Fc domain comprises a hinge region sequence comprising a serine to proline substitution at amino acid 228, numbering according to EU index. In some examples of any of the above examples, the antibody hinge region comprises a serine to proline substitution at amino acid 228, numbering according to EU index. In some examples, a tetravalent antibody of the

present disclosure displays enhanced induction of apoptosis in a target cell (*e.g.*, a cell expressing human PSGL-1 or an epitope thereof) as compared to a conventional (*e.g.*, bivalent) antibody having one or more VH or VL domains in common with the tetravalent antibody. In some examples, a tetravalent antibody of the present disclosure displays enhanced inhibition of DTH (*e.g.*, in a *trans vivo* animal model) as compared to a conventional (*e.g.*, bivalent) antibody having one or more VH or VL domains in common with the tetravalent antibody.

[0012a] In a fifth aspect, the present disclosure refers to a tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises an antibody heavy chain and an antibody light chain;

wherein the antibody light chain comprises, from N-terminus to C-terminus:

- (i) a first heavy chain variable (VH) domain,
- (ii) a first linker sequence, wherein the first linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25),
- (iii) a first light chain variable (VL) domain,
- (iv) a second linker sequence, wherein the second linker sequence has 5-12 amino acids,
- (v) a second VL domain, and
- (vi) a light chain constant (CL) domain;

wherein the antibody heavy chain comprises:

- (i) a second VH domain, and
- (ii) a heavy chain constant region comprising a first heavy chain constant region (CH1) domain, an antibody hinge region, an second heavy chain constant region (CH2) domain, and a third heavy chain constant region (CH3) domain;

wherein each of the first and the second VL domains comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22; wherein each of the first and the second VH domains comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19; wherein each of the first and the second VL domains forms a VH-VL binding unit with a corresponding VH domain of the first and the second VH domains; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

[0012b] In a sixth aspect, the present disclosure refers to a tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises an antibody heavy chain and an antibody light chain;

wherein the antibody light chain comprises, from N-terminus to C-terminus:

- (i) a first heavy chain variable (VH) domain,
- (ii) a first linker sequence, wherein the first linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25),
- (iii) a first light chain variable (VL) domain,
- (iv) a second linker sequence, wherein the second linker sequence has 5-12 amino acids,
- (v) a second VL domain, and
- (vi) a light chain constant (CL) domain;

wherein the antibody heavy chain comprises:

- (i) a second VH domain, and
- (ii) a heavy chain constant region comprising a first heavy chain constant region (CH1) domain, an antibody hinge region, an second heavy chain constant region (CH2) domain, and a third heavy chain constant region (CH3) domain;

wherein each of the first and the second VL domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:24 or SEQ ID NO:30;

wherein each of the first and the second VH domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:23 or SEQ ID NO:29; wherein each of the first and the second VL domains forms a VH-VL binding unit with a corresponding VH domain of the first and the second VH domains; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

[0013] In a seventh aspect, the present disclosure refers to an isolated polynucleotide encoding any one of the tetravalent antibodies as described herein. In an eighth aspect, the present disclosure refers to an isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NO:8. In a ninth aspect, the present disclosure refers to an isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NO:12. In a tenth aspect, the present disclosure refers to an isolated polynucleotide encoding the tetravalent antibody of the fifth aspect, wherein the isolated polynucleotide comprises the polynucleotide sequence of SEQ ID NO:8 and the polynucleotide sequence of SEQ ID NO:12. In some examples, the isolated polynucleotide comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, and 16. In an eleventh aspect, the present disclosure refers to a vector comprising any one of the isolated polynucleotides as described herein. In another example, provided herein is a host cell comprising the polynucleotide of any of the above examples and/or the vector of any of the above examples. In a twelfth aspect, the present disclosure refers to an isolated host cell comprising any one of the polynucleotides described herein. In a thirteenth aspect, the present disclosure refers to a method of producing a tetravalent antibody comprising culturing any one of the host cells as described herein so that the tetravalent antibody is produced. In some examples, the method further comprises recovering the tetravalent antibody from the host cell.

[0014] In a fourteenth aspect, the present disclosure refers to a pharmaceutical composition comprising any one of the tetravalent antibodies as described herein and a pharmaceutically acceptable carrier. In a fifteenth aspect, the present disclosure refers to a kit comprising any one of the tetravalent antibodies as described herein and an optional pharmaceutically acceptable carrier. In some

examples, the kit further comprises a package insert comprising instructions for administration of the tetravalent antibody to treat a T-cell mediated inflammatory disease or condition. In some examples, the kit further comprises a package insert comprising instructions for administration of the tetravalent antibody before, concurrently with, and/or after a transfusion or transplantation. In another example, provided herein is the tetravalent antibody of any one of the above examples for use in treating a T-cell mediated inflammatory disease or condition. In another example, provided herein is the tetravalent antibody of any one of the above examples for use in treating an individual in need of a transfusion or transplantation. In another example, provided herein is a use of the tetravalent antibody of any one of the above examples in the manufacture

of a medicament for treating a T-cell mediated inflammatory disease or condition. In a sixteenth aspect, the present disclosure refers to use of a therapeutically effective amount of any one of the tetravalent antibodies as described herein, in the manufacture of a medicament for treating a T-cell mediated inflammatory disease in a subject in need thereof. In another example, provided herein is a use of the tetravalent antibody of any one of the above examples in the manufacture of a medicament for treating an individual in need of a transfusion or transplantation. In a seventeenth aspect, the present disclosure refers to use of a therapeutically effective amount of any one of the tetravalent antibodies as described herein, in the manufacture of a medicament for treating an individual in need of a transfusion or transplantation, wherein the medicament is to be administered concurrently with or after the transfusion or transplantation. In another example, provided herein is a method of treating a T-cell mediated inflammatory disease or condition, the method comprising administering to a subject in need thereof a therapeutically effective amount of the tetravalent antibody of any one of the above examples. In another example, provided herein is a method for treating an individual in need of a transfusion or transplantation, comprising administering to the individual a therapeutically effective amount of the tetravalent antibody of any one of the above examples before, concurrently with, and/or after the transfusion or transplantation. In some examples, the T-cell mediated inflammatory disease is an autoimmune disease. In some examples, the T-cell mediated inflammatory disease is selected from the group consisting of psoriasis, psoriatic arthritis, rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, type I diabetes, ulcerative colitis, multiple sclerosis, and graft versus host disease (GVHD). In some examples, the psoriasis is plaque psoriasis, chronic plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, or erythrodermic psoriasis. In some examples, the transplantation is a transplantation of a tissue selected from the group consisting of bone marrow, kidney, heart, liver, neuronal tissue, lung, pancreas, skin, and intestine. In some examples, the transfusion is a transfusion comprising one or more of white blood cells, red blood cells, and platelets.

[0015] 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 aspects of the disclosure will become apparent to one of skill in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **FIGS. 1A & 1B** provide schematics illustrating exemplary tetravalent antibodies in accordance with some embodiments. **FIG. 1A** illustrates the following exemplary formats: (1) a dimer composed of two single-chain diabodies fused to an Fc domain (scDb₂-Fc), showing linker sequences: (GGGGS)₅ (SEQ ID NO:33), GGGGSAAA (SEQ ID NO:26) and (GGGGS)₂ (SEQ ID NO:34)/(GGGGS)₂G (SEQ ID NO:35)/(GGGGS)₂GG (SEQ ID NO:36); (2) two different formats, each having a dimer of two tandem single-

CLAIMS

What is claimed is:

Claim 1: A tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

- (a) a first light chain variable (VL) domain;
 - (b) a first linker sequence, wherein the first linker sequence has 5-12 amino acids;
 - (c) a first heavy chain variable (VH) domain;
 - (d) a second linker sequence, wherein the second linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (e) a second VL domain;
 - (f) a third linker sequence, wherein the third linker sequence has 5-12 amino acids;
 - (g) a second VH domain;
 - (h) a fourth linker sequence, wherein the fourth linker sequence has 5-12 amino acids;
- and
- (i) an antibody Fc domain,

wherein each of the first and the second VL domains comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22; wherein each of the first and the second VH domains comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19; wherein the first VL domain forms a VH-VL binding unit with the second VH domain, and the first VH domain forms a VH-VL binding unit with the second VL domain; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

Claim 2: The tetravalent antibody of claim 1, wherein each of the two VH domains comprises the amino acid sequence of SEQ ID NO:23 or SEQ ID NO:29.

Claim 3: The tetravalent antibody of claim 1, wherein each of the two VL domains comprises the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:30.

Claim 4: The tetravalent antibody of claim 1, wherein the first and the third linker sequences both have two repeats of SEQ ID NO:25.

Claim 5: The tetravalent antibody of claim 1, wherein the second linker sequence has five repeats of SEQ ID NO:25.

Claim 6: The tetravalent antibody of claim 1, wherein the fourth linker sequence has the amino acid sequence of SEQ ID NO:26.

Claim 7: The tetravalent antibody of claim 1, wherein each of the two single-chain polypeptides comprises the amino acid sequence of SEQ ID NO:1.

Claim 8: The tetravalent antibody of claim 7, wherein each of the two single-chain polypeptides is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2.

Claim 9: A tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

- (a) a first heavy chain variable (VH) domain;
 - (b) a first linker sequence, wherein the first linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (c) a first light chain variable (VL) domain;
 - (d) a second linker sequence, wherein the second linker sequence has 5-12 amino acids;
 - (e) a second VL domain;
 - (f) a third linker sequence, wherein the third linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (g) a second VH domain;
 - (h) a fourth linker sequence, wherein the fourth linker sequence has 5-12 amino acids;
- and
- (i) an antibody Fc domain,

wherein each of the first and the second VL domains comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22; wherein each of the first and the second VH domains comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19; wherein each of the first and the second VL domains forms a VH-VL binding unit with a corresponding VH domain of the first and the second VH domains; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

Claim 10: The tetravalent antibody of claim 9, wherein each of the two VH domains comprises the amino acid sequence of SEQ ID NO:23 or SEQ ID NO:29.

Claim 11: The tetravalent antibody of claim 9, wherein each of the two VL domains comprises the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:30.

Claim 12: The tetravalent antibody of claim 9, wherein the first and the third linker sequences both have five repeats of SEQ ID NO:25.

Claim 13: The tetravalent antibody of claim 9, wherein the second linker sequence has the amino acid sequence of SEQ ID NO:27.

Claim 14: The tetravalent antibody of claim 9, wherein the fourth linker sequence has the amino acid sequence of SEQ ID NO:26.

Claim 15: The tetravalent antibody of claim 9, wherein each of the two single-chain polypeptides comprises the amino acid sequence of SEQ ID NO:3.

Claim 16: The tetravalent antibody of claim 15, wherein each of the two single-chain polypeptides is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:4.

Claim 17: A tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises an antibody heavy chain and an antibody light chain;

wherein the antibody light chain comprises, from N-terminus to C-terminus:

- (i) a first heavy chain variable (VH) domain,
- (ii) a first linker sequence, wherein the first linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25),
- (iii) a first light chain variable (VL) domain,
- (iv) a second linker sequence, wherein the second linker sequence has 5-12 amino acids,
- (v) a second VL domain, and
- (vi) a light chain constant (CL) domain;

wherein the antibody heavy chain comprises:

- (i) a second VH domain, and
- (ii) a heavy chain constant region comprising a first heavy chain constant region (CH1) domain, an antibody hinge region, a second heavy chain constant region (CH2) domain, and a third heavy chain constant region (CH3) domain;

wherein each of the first and the second VL domains comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22; wherein each of the first and the second VH domains comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19; wherein each of the first and the second VL domains forms a VH-VL binding unit with a corresponding VH domain of the first and the second VH domains; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

Claim 18: The tetravalent antibody of claim 17, wherein the first and the second VH domains each comprise the amino acid sequence of SEQ ID NO:23 or SEQ ID NO:29.

Claim 19: The tetravalent antibody of claim 17, wherein the first and the second VL domains each comprise the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:30.

Claim 20: The tetravalent antibody of claim 17, wherein the CL domain is a kappa CL domain.

Claim 21: The tetravalent antibody of claim 17, wherein the first linker sequence has five repeats of SEQ ID NO:25.

Claim 22: The tetravalent antibody of claim 17, wherein the second linker sequence has the amino acid sequence of SEQ ID NO:28.

Claim 23: The tetravalent antibody of claim 17, wherein the antibody light chain comprises the amino acid sequence of SEQ ID NO:7.

Claim 24: The tetravalent antibody of claim 23, wherein the antibody light chain is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:8.

Claim 25: The tetravalent antibody of claim 17, wherein the antibody heavy chain comprises the amino acid sequence of SEQ ID NO:11.

Claim 26: The tetravalent antibody of claim 25, wherein the antibody heavy chain is encoded by a polynucleotide comprising the polynucleotide sequence of SEQ ID NO:12.

Claim 27: The tetravalent antibody of claim 1, wherein the antibody Fc domain is a human antibody Fc domain.

Claim 28: The tetravalent antibody of claim 27, wherein the antibody Fc domain is a human IgG4 Fc domain.

Claim 29: The tetravalent antibody of claim 28, wherein the human IgG4 Fc domain comprises a hinge region sequence comprising one or more amino acid substitutions that result in reduced IgG4 shuffling, as compared to an IgG4 hinge region lacking the one or more amino acid substitutions.

Claim 30: The tetravalent antibody of claim 28, wherein the human IgG4 Fc domain comprises a hinge region sequence comprising a serine to proline substitution at amino acid 228, numbering according to EU index.

Claim 31: An isolated polynucleotide encoding the tetravalent antibody of claim 1.

Claim 32: The isolated polynucleotide of claim 31, wherein the isolated polynucleotide comprises the polynucleotide sequence of SEQ ID NO:2.

Claim 33: The tetravalent antibody of claim 9, wherein the antibody Fc domain is a human IgG4 Fc domain.

Claim 34: The tetravalent antibody of claim 33, wherein the human IgG4 Fc domain comprises a hinge region sequence comprising a serine to proline substitution at amino acid 228, numbering according to EU index.

Claim 35: An isolated polynucleotide encoding the tetravalent antibody of claim 9.

Claim 36: The isolated polynucleotide of claim 35, wherein the isolated polynucleotide comprises the polynucleotide sequence of SEQ ID NO:4.

Claim 37: A vector comprising the isolated polynucleotide of claim 31 or 35.

Claim 38: An isolated host cell comprising the polynucleotide of claim 31 or 35.

Claim 39: A method of producing a tetravalent antibody comprising culturing the host cell of claim 38 so that the tetravalent antibody is produced.

Claim 40: The method of claim 39, further comprising recovering the tetravalent antibody from the host cell.

Claim 41: A pharmaceutical composition comprising the tetravalent antibody of any one of claims 1, 9 and 17, and a pharmaceutically acceptable carrier.

Claim 42: A kit comprising the tetravalent antibody of any one of claims 1, 9 and 17, and an optional pharmaceutically acceptable carrier.

Claim 43: Use of a therapeutically effective amount of the tetravalent antibody of any one of claims 1, 9 and 17, in the manufacture of a medicament for treating a T-cell mediated inflammatory disease in a subject in need thereof.

Claim 44: Use of a therapeutically effective amount of the tetravalent antibody of any one of claims 1, 9 and 17, in the manufacture of a medicament for treating an individual in need of a transfusion or transplantation, wherein the medicament is to be administered concurrently with or after the transfusion or transplantation.

Claim 45: The use of claim 43, wherein the T-cell mediated inflammatory disease is an autoimmune disease.

Claim 46: The use of claim 43, wherein the T-cell mediated inflammatory disease is selected from the group consisting of psoriasis, psoriatic arthritis, rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, type I diabetes, ulcerative colitis, multiple sclerosis, allergy, atopic dermatitis, asthma, and graft versus host disease (GVHD).

Claim 47: The use of claim 46, wherein the psoriasis is plaque psoriasis, chronic plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, or erythrodermic psoriasis.

Claim 48: The use of claim 44, wherein the transplantation is a transplantation of a tissue selected from the group consisting of bone marrow, kidney, heart, liver, neuronal tissue, lung, pancreas, skin, and intestine.

Claim 49: The use of claim 44, wherein the transfusion is a transfusion comprising one or more of white blood cells, red blood cells, and platelets.

Claim 50: The tetravalent antibody of claim 17, wherein the heavy chain constant region comprises a human IgG4 Fc domain.

Claim 51: The tetravalent antibody of claim 50, wherein the human IgG4 Fc domain comprises a hinge region sequence comprising a serine to proline substitution at amino acid 228, numbering according to EU index.

Claim 52: An isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NO:8.

Claim 53: An isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NO:12.

Claim 54: An isolated polynucleotide encoding the tetravalent antibody of claim 17, wherein the isolated polynucleotide comprises the polynucleotide sequence of SEQ ID NO:8 and the polynucleotide sequence of SEQ ID NO:12.

Claim 55: A vector comprising the isolated polynucleotide of claim 54.

Claim 56: An isolated host cell comprising the polynucleotide of claim 54.

Claim 57: A method of producing a tetravalent antibody comprising culturing the host cell of claim 56 so that the tetravalent antibody is produced.

Claim 58: The method of claim 57, further comprising recovering the tetravalent antibody from the host cell.

Claim 59: A tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

- (a) a first light chain variable (VL) domain;
 - (b) a first linker sequence, wherein the first linker sequence has 5-12 amino acids;
 - (c) a first heavy chain variable (VH) domain;
 - (d) a second linker sequence, wherein the second linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
 - (e) a second VL domain;
 - (f) a third linker sequence, wherein the third linker sequence has 5-12 amino acids;
 - (g) a second VH domain;
 - (h) a fourth linker sequence, wherein the fourth linker sequence has 5-12 amino acids;
- and
- (i) an antibody Fc domain,

wherein each of the first and the second VL domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:24 or SEQ ID NO:30; wherein each of the first and the second VH domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:23 or SEQ ID NO:29; wherein the first VL domain forms a VH-VL binding unit with the second VH domain, and the first VH domain forms a VH-VL binding unit with the second VL domain; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

Claim 60: The tetravalent antibody of claim 59, wherein the first and the second VL domains each comprise (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22.

Claim 61: The tetravalent antibody of claim 59, wherein the first and the second VH domains each comprise (i) a CDR-H1 comprising the amino acid sequence of SEQ ID

NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19.

Claim 62: The tetravalent antibody of claim 59, wherein the first and the third linker sequences both have two repeats of SEQ ID NO:25.

Claim 63: The tetravalent antibody of claim 59, wherein the second linker sequence has five repeats of SEQ ID NO:25.

Claim 64: The tetravalent antibody of claim 59, wherein the fourth linker sequence has the amino acid sequence of SEQ ID NO:26.

Claim 65: The tetravalent antibody of claim 59, wherein the antibody Fc domain is a human antibody Fc domain.

Claim 66: The tetravalent antibody of claim 65, wherein the antibody Fc domain is a human IgG4 Fc domain.

Claim 67: The tetravalent antibody of claim 66, wherein the human IgG4 Fc domain comprises a hinge region sequence comprising (a) one or more amino acid substitutions that result in reduced IgG4 shuffling, as compared to an IgG4 hinge region lacking the one or more amino acid substitutions, or (b) a serine to proline substitution at amino acid 228, numbering according to EU index.

Claim 68: A tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

- (a) a first heavy chain variable (VH) domain;
- (b) a first linker sequence, wherein the first linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
- (c) a first light chain variable (VL) domain;
- (d) a second linker sequence, wherein the second linker sequence has 5-12 amino acids;
- (e) a second VL domain;

- (f) a third linker sequence, wherein the third linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25);
- (g) a second VH domain;
- (h) a fourth linker sequence, wherein the fourth linker sequence has 5-12 amino acids;
- and
- (i) an antibody Fc domain,

wherein each of the first and the second VL domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:24 or SEQ ID NO:30; wherein each of the first and the second VH domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:23 or SEQ ID NO:29; wherein each of the first and the second VL domains forms a VH-VL binding unit with a corresponding VH domain of the first and the second VH domains; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

Claim 69: The tetravalent antibody of claim 68, wherein the first and the second VL domains each comprise (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22.

Claim 70: The tetravalent antibody of claim 68, wherein the first and the second VH domains each comprise (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19.

Claim 71: The tetravalent antibody of claim 68, wherein the first and the third linker sequences both have five repeats of SEQ ID NO:25.

Claim 72: The tetravalent antibody of claim 68, wherein the second linker sequence has the amino acid sequence of SEQ ID NO:27.

Claim 73: The tetravalent antibody of claim 68, wherein the fourth linker sequence has the amino acid sequence of SEQ ID NO:26.

Claim 74: The tetravalent antibody of claim 68, wherein the antibody Fc domain is a human antibody Fc domain.

Claim 75: The tetravalent antibody of claim 74, wherein the antibody Fc domain is a human IgG4 Fc domain.

Claim 76: The tetravalent antibody of claim 75, wherein the human IgG4 Fc domain comprises a hinge region sequence comprising (a) one or more amino acid substitutions that result in reduced IgG4 shuffling, as compared to an IgG4 hinge region lacking the one or more amino acid substitutions, or (b) a serine to proline substitution at amino acid 228, numbering according to EU index.

Claim 77: A tetravalent antibody that specifically binds to human PSGL-1, the tetravalent antibody comprising a dimer of two monomers, wherein each monomer of the dimer comprises an antibody heavy chain and an antibody light chain;

wherein the antibody light chain comprises, from N-terminus to C-terminus:

- (i) a first heavy chain variable (VH) domain,
- (ii) a first linker sequence, wherein the first linker sequence has two, three, four, or five repeats of the amino acid sequence of GGGGS (SEQ ID NO:25),
- (iii) a first light chain variable (VL) domain,
- (iv) a second linker sequence, wherein the second linker sequence has 5-12 amino acids,
- (v) a second VL domain, and
- (vi) a light chain constant (CL) domain;

wherein the antibody heavy chain comprises:

- (i) a second VH domain, and
- (ii) a heavy chain constant region comprising a first heavy chain constant region (CH1) domain, an antibody hinge region, a second heavy chain constant region (CH2) domain, and a third heavy chain constant region (CH3) domain;

wherein each of the first and the second VL domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:24 or SEQ ID NO:30; wherein each of the first and the second VH domains comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:23 or SEQ ID NO:29; wherein each of the first and the second VL domains forms a VH-VL binding unit with a corresponding VH

domain of the first and the second VH domains; and wherein each of the two VH-VL binding units is specific for human PSGL-1.

Claim 78: The tetravalent antibody of claim 77, wherein the first and the second VL domains each comprise (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:21; and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:22.

Claim 79: The tetravalent antibody of claim 77, wherein the first and the second VH domains each comprise (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:17; (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:18; and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:19.

Claim 80: The tetravalent antibody of claim 77, wherein the CL domain is a kappa CL domain.

Claim 81: The tetravalent antibody of claim 77, wherein the first linker sequence has five repeats of SEQ ID NO:25.

Claim 82: The tetravalent antibody of claim 77, wherein the second linker sequence has the amino acid sequence of SEQ ID NO:28.

Claim 83: The tetravalent antibody of claim 77, wherein the antibody Fc domain is a human antibody Fc domain.

Claim 84: The tetravalent antibody of claim 83, wherein the antibody Fc domain is a human IgG4 Fc domain.

Claim 85: The tetravalent antibody of claim 84, wherein the human IgG4 Fc domain comprises a hinge region sequence comprising (a) one or more amino acid substitutions that result in reduced IgG4 shuffling, as compared to an IgG4 hinge region lacking the one or more amino acid substitutions, or (b) a serine to proline substitution at amino acid 228, numbering according to EU index.

Claim 86: An isolated polynucleotide encoding the tetravalent antibody of any one of claims 59, 68 and 77.

Claim 87: A vector comprising the isolated polynucleotide of claim 86.

Claim 88: An isolated host cell comprising the polynucleotide of claim 86.

Claim 89: A method of producing a tetravalent antibody comprising culturing the host cell of claim 88 so that the tetravalent antibody is produced.

Claim 90: A pharmaceutical composition comprising the tetravalent antibody of any one of claims 59, 68 and 77, and a pharmaceutically acceptable carrier.

Claim 91: Use of a therapeutically effective amount of the tetravalent antibody of any one of claims 59, 68 and 77, in the manufacture of a medicament for treating a T-cell mediated inflammatory disease in a subject in need thereof.

Claim 92: The use of claim 91, wherein the T-cell mediated inflammatory disease is an autoimmune disease.

Claim 93: The use of claim 91, wherein the T-cell mediated inflammatory disease is selected from the group consisting of psoriasis, psoriatic arthritis, rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, type I diabetes, ulcerative colitis, multiple sclerosis, allergy, atopic dermatitis, asthma, and graft versus host disease (GVHD).

Claim 94: Use of a therapeutically effective amount of the tetravalent antibody of any one of claims 59, 68 and 77, in the manufacture of a medicament for treating an individual in need of a transfusion or transplantation, wherein the medicament is to be administered concurrently with or after the transfusion or transplantation.

Claim 95: The use of claim 94, wherein the transplantation is a transplantation of a tissue selected from the group consisting of bone marrow, kidney, heart, liver, neuronal tissue, lung, pancreas, skin, and intestine.

Claim 96: The use of claim 94, wherein the transfusion is a transfusion comprising one or more of white blood cells, red blood cells, and platelets.

Claim 97: The tetravalent antibody of claim 9, wherein one of the first and the second VH domains comprises the amino acid sequence of SEQ ID NO:23, and the other of the first and the second VH domains comprises the amino acid sequence of SEQ ID NO:29.

Claim 98: The tetravalent antibody of claim 9, wherein one of the first and the second VL domains comprises the amino acid sequence of SEQ ID NO:24, and the other of the first and the second VL domains comprises the amino acid sequence of SEQ ID NO:30.

Claim 99: The tetravalent antibody of claim 17, wherein one of the first and the second VH domains comprises the amino acid sequence of SEQ ID NO:23, and the other of the first and the second VH domains comprises the amino acid sequence of SEQ ID NO:29.

Claim 100: The tetravalent antibody of claim 17, wherein one of the first and the second VL domains comprises the amino acid sequence of SEQ ID NO:24, and the other of the first and the second VL domains comprises the amino acid sequence of SEQ ID NO:30.