

ANTI-E-SELECTIN ANTIBODIES, COMPOSITIONS AND METHODS OF USE**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Provisional Patent Application No.

5 62/965,688, filed January 24, 2020, U.S. Provisional Patent Application No. 63/104,213 filed October 22, 2020, and U.S. Provisional Patent Application No. 63/121,467 filed December 4, 2020, the contents of each of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

10 **[0002]** 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 January 6, 2021, is named PC072498A_Sequence_Listing_ST25.txt and is 1,048,576 bytes in size.

FIELD OF THE INVENTION

15 **[0003]** The present invention related to antibodies, and antigen-binding fragments thereof, that specifically bind E-selectin, and compositions, methods and uses thereof, including use of antibodies of the disclosure to treat Sickle Cell disease (SCD) including treatment and prevention of vaso-occlusive crisis (VOC) associated with SCD.

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BACKGROUND

[0004] Sickle cell disease (SCD) is a severe, rare genetic disorder affecting over 100,000 people in the United States (US) alone (Center for Disease Control and Prevention). It is a chronic condition with substantial morbidity and mortality in a population with high
25 unmet medical need. Individuals with SCD suffer from progressive organ damage and markedly shortened life expectancy, with a median survival of approximately 56 years (Gardner et al., Blood 2016; 128(10):1436-38).

[0005] SCD is characterized by the presence of an abnormal form of hemoglobin (Hb) – sickle hemoglobin (HbS). A single nucleotide substitution in the β -globin gene (HBB) results
30 in a one amino acid substitution (valine for glutamic acid) at residue 6 (HBS allele). Individuals homozygous for HBS have the most common and most severe form of sickle cell disease (SCD-SS). Variant forms of SCD arise when an individual has one copy of HBS and one copy of a mutation in another HBB gene. Individuals with 1 copy of the HBS allele and 1 copy of the hemoglobin C allele (HBC) have SC disease (SCD-SC). When an individual has
35 1 copy of HBS and one copy of a β -thalassemia allele the severity of the SCD is dependent on the severity of the β -thalassemia allele with Hb β^0 -thalassemia deletion (SCD-S β^0 -thal)

often more severe than Hb β^+ -thalassemia allele (SCD-S β^+ -thal), or another interacting HB variant (SCD-SVariant) (Frenett & Atweh J. Clin. Invest. 2007; 117:850-858).

[0006] The primary event in the molecular pathogenesis of SCD is the tendency of HbS to polymerize under conditions of low oxygen tension causing red blood cells (RBCs) to become rigid and sickle shaped (Fabry & Nagel Blood 1982; 60(6):1370-77). Hypoxia in the microcapillary venous bed leads to inflammation of the endothelium and adherence of neutrophils, and a decrease in neutrophil rolling and flow velocity. These cell aggregates become trapped in the vasculature through interactions with endothelial cells. The adhesive interactions of the sickled RBC, leukocytes, and endothelial cells obstruct the vasculature leading to vaso-occlusion (Zhang et al., Blood 2016:127:801-809; Okpala, 2006; Frenette & Atweh, J. Clin. Invest. 2007; 117:850-858). Dysregulated nitric oxide homeostasis contributes to vascular dysfunction in SCD (Aslan & Freeman, 2007). Blood cell aggregates lead to episodes of vascular obstruction, organ infarction and ischemia which manifest clinically as episodes of severe pain. Anemia is consequent upon a shortened red cell life span due to hemolysis and vascular occlusion that is precipitated by interactions between the vascular endothelium and sickled RBCs, leukocytes and platelets (Rees et al., Lancet 2010; 376:2018-31).

[0007] Vaso-occlusive crisis (VOC) is the most common clinical manifestation of SCD and is the major cause of morbidity in SCD with an interruption of daily functioning (Ballas & Lusardi, Am. J. Hematol. 2005; 79:17-25; Piel et al., New Engl. J. Med. 2017; 376:1561-1573; Darbari et al., PloS One 2013; 8(11):e79923). VOC is initiated by interaction between sickled RBCs and vascular endothelium in post-capillary venules, where oxygen tension is at its lowest (Manwani & Frenette, Blood 2013; 122(24):3892-8). This leads to endothelial damage that triggers an inflammatory response and causes leukocytes, platelets and additional RBCs to be recruited to the site of inflammation (Zhang et al., Blood 2016; 127(7):801). These cellular aggregates lead to vascular obstruction (Turhan et al., Proc. Natl. Acad. Sci. 2002; 99(5):3047-51) and slowing of blood flow in post-capillary venules which causes local tissue hypoxia, and further tissue inflammation. This results in more deoxygenation and sickling of RBCs, and propagation of the occlusion, sometimes called secondary recruitment of sickled cells and occluded vessels (Stuart & Nagel, Lancet 2004; 364(9942):1343-60).

[0008] VOC can manifest in patients with SCD as early as 6 months of age, although they are considerably less common in young infants than in older children or adults (Benjamin et al., Amer. Pain Soc. 1994; vol.1, 94pp). Approximately 60% of patients with homozygous SCD have at least 1 severe VOC episode per year, but a proportion of patients have many more episodes (Platt et al., N. Engl. J. Med. 1991; 325(1):11-6). In this same study 5.2% of patients with SCD genotype had 3-10 severe VOC episodes per year and a

small proportion (>1%) of patients had 10 or more episodes per year.

[0009] Pain is the clinical manifestation of initial and ongoing vascular occlusion and ischemia (Ballas, *Hematol. Oncol. Clin. North Am.* 2005; 19(5):785-802), which may be particularly severe for patients with the SCD-SS genotype, who have also been observed to suffer from higher mortality than other genotypes (Platt et al., *N. Engl. J. Med.* 1994; 330(23):1639-44).

[0010] Recruitment of leukocytes to areas of vascular endothelial damage involves the selectin family of adhesion molecules: E-selectin (also known as CD62E); P-selectin (also known as CD62P); and L-selectin (also known as CD62L), all of which are all regulated as part of an inflammatory response (Ernst & Magnani *Nat. Rev. Drug Discov.* 2009; 8(8):661-77; Morikis et al., *Blood* 2017; 130(19):2101-10). While similar in structure, each selectin exhibits a different distribution, ligand binding kinetics and diversity in both pathological and physiological functions.

[0011] The selectin family of adhesion molecules and their ligands are part of a proinflammatory response in SCD promoted by alteration in the sickled red blood cells and the activated endothelial cells. The selectins also play a critical role in regulating the initial contact of cell-cell adhesion, leukocyte rolling on the endothelium and integrin activation and transmigration of cells. Adhesion of leukocytes to inflamed endothelium and circulating cellular aggregates are a hallmark event in SCD.

[0012] The selectin family of carbohydrate binding proteins share a similar structure, with each having an N-terminal carbohydrate-recognition domain characteristic of Ca^{2+} -dependent (C type) lectins, followed by an epidermal growth factor (EGF)-like domain, a series of short consensus repeats with homology to complement regulatory domains, a transmembrane domain, and a short cytoplasmic tail (McEver & Zhu, 2010). The selectins and their ligands mediate the recruitment of platelets and leukocytes from the blood to the vascular endothelium contributing to creation of a chronic pro-inflammatory environment.

[0013] The pathophysiology of SCD is complex and heterogeneous. Symptoms include pain crises, chronic anemia, acute chest syndrome, stroke, splenic sequestration, vaso-occlusive acute pain events or crises, renal dysfunction, and susceptibility to bacterial infections (Ashley-Koch et al., *Am. J. Epidemiol.* 2000; 151:839-845; Steinberg, *New Engl. J. Med.* 1999; 340:1021-1030; Piel et al., *New Engl. J. Med.* 2017; 376:1561-1573). Acute end organ complications associated with SCD can include acute chest syndrome, acute stroke priapism, hepatobiliary complications, splenic sequestration and acute renal failure. Chronic complications from the cumulative insult of SCD include avascular necrosis, pulmonary hypertension, renal complications, ophthalmologic complications, leg ulcers and recurrent priapism (Yawn et al., *JAMA* 2014; 312:1033-48).

[0014] Hydroxyurea is approved for prophylactic therapy of SCD. Mechanistically,

hydroxyurea increases fetal hemoglobin (HbF) concentrations and reduces the number of pain crises (Charache et al., New Engl. J. Med. 1995; 332:1317-1322). Although hydroxyurea is considered the standard of care in the prevention of VOC, it has a failure rate of ~30-35% and is ineffective in treating symptoms during an acute VOC. ENDARI (L-glutamine) was recently approved for prophylactic SCD treatment, however, the mechanism of action is uncertain and clinical benefit is modest (Quinn, Blood 2018; 132:689-693). Current treatments for acute VOC episodes are largely supportive with opioid analgesics, hydration, oxygen, and transfusion. In addition, most patients treat a VOC at home and do not seek direct medical intervention (Smith et al., Ann. Intern. Med. 2008; 148:94-101; Callaghan et al., Blood 2017; 130:973).

[0015] There remains a significant need for the prophylaxis and treatment of SCD and, in particular, to address the underlying pathophysiology (e.g., reduction of inflammation and cellular aggregation) of recurring and debilitating VOC in patients. The present invention provides novel therapeutic antibodies that specifically bind to E-selectin and are capable of neutralizing E-selectin functional activity. These antibodies may be used advantageously to prevent, or reduce the occurrence of, VOC when utilized as a prophylactic treatment for SCD, and to treat acute VOC in patients with SCD by decreasing the duration (e.g., a reduction in the time to resolve a VOC), intensity and/or severity of the VOC.

SUMMARY

[0016] The invention provides antibodies, and antigen-binding fragments thereof, that specifically bind to E-selectin, as well as uses, and associated methods. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following embodiments (E).

E1. An isolated antibody or antigen-binding fragment thereof that specifically binds to E-selectin (e.g., human and/or cynomolgus monkey E-selectin).

E2. The antibody, or antigen-binding fragment thereof, of E1, comprising the HCDR-1, HCDR-2, and HCDR-3 sequences selected from the group consisting of SEQ ID NO: 8, 23, 52, 63, 77, 92, 111, 125, 9, 24, 29, 38, 41, 44, 53, 64, 78, 93, 112, 126, 10, 54, 65, 79, 94, 113 and 127.

E3. The antibody, or antigen-binding fragment thereof, of any one of E1-E2, comprising the LCDR-1, LCDR-2, and LCDR-3 sequences selected from the group consisting of SEQ ID NO: 2, 18, 47, 68, 82, 97, 106, 9, 24, 29, 38, 41, 44, 53, 64, 78, 93, 112, 126, 10, 54, 65, 79, 94, 113 and 127.

E4. The antibody, or antigen binding fragment thereof, of any one of E1-E3 comprising

one or more of (a)-(f)

- (a) LCDR-1 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:2, 18, 47, 68, 82, 97 and 106;
- (b) a LCDR-2 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:3, 19, 48, 69, 83, 98, 107 and 120;
- (c) a LCDR-3 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:4, 20, 49, 70, 84, 99, 108 and 121;
- (d) a HCDR-1 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:8, 23, 52, 63, 77, 92, 111 and 125;
- (e) a HCDR-2 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:9, 24, 29, 38, 41, 44, 53, 64, 78, 93, 112 and 126; and
- (f) a HCDR-3 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:10, 54, 65, 79, 94, 113 and 127.

E5. The antibody, or antigen binding fragment thereof, of any one of E1-E4 comprising one or more of the following:

- a LCDR-1 comprising the amino acid sequence of SEQ ID NO:2,
- a LCDR-2 comprising the amino acid sequence of SEQ ID NO:3,
- a LCDR-3 comprising the amino acid sequence of SEQ ID NO:4,
- a HCDR-1 comprising the amino acid sequence of SEQ ID NO:8,
- a HCDR-2 comprising the amino acid sequence of SEQ ID NO:9, and
- a HCDR-3 comprising the amino acid sequence of SEQ ID NO:10.

E6. The antibody, or antigen-binding fragment thereof, of any one of E1-E5, comprising the HCDR-1, HCDR-2, and HCDR-3 sequences of at least one sequence selected from the group consisting of SEQ ID NO:11, 25, 30, 35, 39, 42, 45, 55, 60, 66, 75, 80, 90, 95, 104, 114, 118, and 128.

E7. The antibody, or antigen-binding fragment thereof, of any one of E1-E6, comprising the LCDR-1, LCDR-2, and LCDR-3 sequences of at least one sequence selected from the group consisting of SEQ ID NO:5, 21, 27, 32, 50, 57, 71, 73, 85, 87, 100, 102, 109, 116, and 122.

E8. The antibody, or antigen-binding fragment thereof, of any one of E1-E7, comprising the HCDR-1, HCDR-2, and HCDR-3 sequences of at least one sequence selected from the group consisting of SEQ ID NO:7, 13, 22, 28, 34, 37, 40, 43, 51, 59, 62, 74, 76, 89, 91, 103, 110, 117 and 124.

E9. The antibody, or antigen-binding fragment thereof, of any one of E1-E8, comprising the LCDR-1, LCDR-2, and LCDR-3 sequences of at least one sequence selected from the group consisting of SEQ ID NO:1, 17, 26, 31, 46, 56, 67, 72, 81, 86, 96, 101, 105, 115 and 119.

- E10. The antibody, or antigen-binding fragment thereof, of any one of E1-E9, comprising the HCDR-1, HCDR-2, and HCDR-3 sequences of SEQ ID NO:11.
- E11. The antibody, or antigen-binding fragment thereof, of any one of E1-E10, comprising the LCDR-1, LCDR-2, and LCDR-3 sequences of SEQ ID NO:5.
- 5 E12. The antibody, or antigen-binding fragment thereof, of any one of E1-E11, comprising the HCDR-1, HCDR-2, and HCDR-3 sequences of SEQ ID NO:7 or 13.
- E13. The antibody, or antigen-binding fragment thereof, of any one of E1-E12, comprising the LCDR-1, LCDR-2, and LCDR-3 sequences of SEQ ID NO:1.
- E14. The antibody, or antigen-binding fragment thereof, of any one of E1-E13, comprising
10 a LCDR-1 comprising the amino acid sequence of SEQ ID NO:2, a LCDR-2 comprising the amino acid sequence of SEQ ID NO:3, a LCDR-3 comprising the amino acid sequence of SEQ ID NO:4, a HCDR-1 comprising the amino acid sequence of SEQ ID NO:8, a HCDR-2 comprising the amino acid sequence of SEQ ID NO:9, and a HCDR-3 comprising the amino acid sequence of SEQ ID NO:10.
- 15 E15. The antibody, or antigen-binding fragment thereof, of any one of E1-E4, comprising a LCDR-1 comprising the amino acid sequence of SEQ ID NO:2, a LCDR-2 comprising the amino acid sequence of SEQ ID NO:3, a LCDR-3 comprising the amino acid sequence of SEQ ID NO:4
- E16. The antibody, or antigen-binding fragment thereof, of any one of E1-E15, comprising
20 a HCDR-1 comprising the amino acid sequence of SEQ ID NO:8, a HCDR-2 comprising the amino acid sequence of SEQ ID NO:9, and a HCDR-3 comprising the amino acid sequence of SEQ ID NO:10.
- E17. The antibody, or antigen-binding fragment thereof, of any one of E1-E16, comprising a VL framework sequence derived from a human germline VL sequence selected from the
25 group consisting of IGKV1-12*01, IGKV1-13*02, IGKV1-33*01, IGKV1-39*01, IGKV1-5*01, IGKV3-11*01, IGKV3-15*01, IGKV3-20*01, IGKV3D-20*02, and IGKV4-1*01.
- E18. The antibody, or antigen-binding fragment thereof, of any one of E1-E17, comprising a VH framework sequence derived from a human germline VH sequence selected from the group consisting of IGHV1-2*02, IGHV1-3*01, IGHV1-46*01, IGHV1-69*01, IGHV1-69*02,
30 IGHV1-8*01, IGHV3-7*01, IGHV3-13*01, IGHV3-23*01, IGHV3-23*04, IGHV3-30*01, IGHV3-30*18, IGHV5-10-1*01, IGHV5-10-1*04, and IGHV5-51*01.
- E19. The antibody, or antigen-binding fragment thereof, of any one of E1-E18, comprising an IGHV1-39*01 VL framework sequence.
- E20. The antibody, or antigen-binding fragment thereof, of any one of E1-E19, comprising
35 an IGHV3-07*01 VH framework sequence.
- E21. The antibody, or antigen-binding fragment thereof, of any one of E1-E20, comprising a VL framework sequence and a VH framework sequence, and wherein the VL framework

sequence is at least 72% identical to the human germline sequence from which it was derived.

E22. The antibody, or antigen-binding fragment thereof, of any one of E1-E21, comprising a VL framework sequence and a VH framework sequence, and wherein the VL framework
5 sequence is at least 72%, 74%, 75%, 77%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the human germline sequence from which it was derived.

E23. The antibody, or antigen-binding fragment thereof, of any one of E1-E22, comprising a VL framework sequence and a VH framework sequence, and wherein the VH framework
10 sequence is at least 53% identical to the human germline sequence from which it was derived.

E24. The antibody, or antigen-binding fragment thereof, of any one of E1-E23, comprising a VL framework sequence and a VH framework sequence, and wherein the VH framework
15 sequence is at least 53%, 58%, 60%, 63%, 71%, 72%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the human germline sequence from which it was derived.

E25. The antibody, or antigen-binding fragment thereof, of any one of E1-E24, comprising a VH domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:11.

E26. The antibody, or antigen-binding fragment thereof, of any one of E1-E25, comprising a VH domain comprising an amino acid sequence at least 91%, 92%, 93%, 94%, 95%, 96%,
20 97%, 98%, or 99% identical to SEQ ID NO:11.

E27. The antibody, or antigen-binding fragment thereof, of any one of E1-E26, comprising a VH domain comprising, or consisting of, the amino acid sequence of SEQ ID NO:11.

E28. The antibody, or antigen-binding fragment thereof, of any one of E1-E27, comprising a VL domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:5.

25 E29. The antibody, or antigen-binding fragment thereof, of any one of E1-E28, comprising a VL domain comprising an amino acid sequence at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identical to SEQ ID NO:5.

E30. The antibody, or antigen-binding fragment thereof, of any one of E1-E29, comprising, or consisting of, a VL domain comprising the amino acid sequence of SEQ ID NO:5.

30 E31. The antibody, or antigen-binding fragment thereof, of any one of E1-E30, comprising a VH domain comprising, or consisting of, the amino acid sequence of SEQ ID NO:11 and a VL domain comprising, or consisting of, the amino acid sequence of SEQ ID NO:5.

E32. The antibody, or antigen-binding fragment thereof, of any one of E1-E31, comprising an Fc domain.

35 E33. The antibody, or antigen-binding fragment thereof, of E32, wherein the Fc domain is the Fc domain of an IgA (for example IgA₁ or IgA₂), IgD, IgE, IgM, or IgG (for example IgG₁, IgG₂, IgG₃, or IgG₄).

- E34. The antibody, or antigen-binding fragment thereof, of E33 wherein the Fc domain is the Fc domain of an IgG.
- E35. The antibody, or antigen-binding fragment thereof, of E34, wherein the IgG is selected from the group consisting of IgG₁, IgG₂, IgG₃, and IgG₄.
- 5 E36. The antibody, or antigen-binding fragment thereof, of E35 wherein the IgG is IgG₁.
- E37. The antibody, or antigen-binding fragment thereof, of any one of E1-E36, comprising a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO:7 or SEQ ID NO:13.
- E38. The antibody, or antigen-binding fragment thereof, of any one of E1-E37, comprising
10 a heavy chain comprising an amino acid sequence at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identical to SEQ ID NO:7 or SEQ ID NO:13.
- E39. The antibody, or antigen-binding fragment thereof, of any one of E1-E38, comprising a heavy chain comprising, or consisting of, the amino acid sequence of SEQ ID NO:7 or SEQ ID NO:13.
- 15 E40. The antibody, or antigen-binding fragment thereof, of any one of E1-E39, comprising a heavy chain comprising an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identical to any one of SEQ ID NO:22, 28, 34, 37, 40, 43, 51, 59, 62, 74, 76, 89, 91, 103, 110, 117 and 124.
- E41. The antibody, or antigen-binding fragment thereof, of any one of E1-E40, comprising
20 a LC comprising an amino acid sequence at least 90% identical to SEQ ID NO:1.
- E42. The antibody, or antigen-binding fragment thereof, of any one of E1-E41, comprising a LC comprising an amino acid sequence at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identical to SEQ ID NO:1.
- E43. The antibody, or antigen-binding fragment thereof, of any one of E1-E42, comprising
25 a LC comprising, or consisting of, the amino acid sequence of SEQ ID NO:1.
- E44. An antibody, or antigen-binding fragment thereof, of any one of E1-E43, comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:7 or SEQ ID NO:13 and a light chain comprising the amino acid sequence of SEQ ID NO:1.
- E45. The antibody, or antigen-binding fragment thereof, of any one of E1-E45, comprising
30 a light chain comprising an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identical to any one of SEQ ID NO:17, 26, 31, 1, 46, 56, 67, 72, 81, 86, 96, 101, 105, 115 and 119.
- E46. The antibody, or antigen-binding fragment thereof, of any one of E1-E45, comprising the HCDR-1, HCDR-2 and HCDR-3 encoded by the insert of the plasmid deposited at the
35 ATCC and having ATCC Accession No. PTA-126529.
- E47. The antibody, or antigen-binding fragment thereof, of any one of E1-E46, comprising the LCDR-1, LCDR-2 and LCDR-3 encoded by the insert of the plasmid deposited at the

ATCC and having ATCC Accession No. PTA-126530.

E48. The antibody, or antigen-binding fragment thereof, of any one of E1-E47, comprising a VH domain encoded by the insert in the plasmid deposited at the ATCC and having ATCC Accession No. PTA-126529.

- 5 E49. The antibody, or antigen-binding fragment thereof, of any one of E1-E48, comprising a VL domain encoded by the insert in the plasmid deposited at the ATCC and having ATCC Accession No. PTA-126530.

- E50. The antibody, or antigen-binding fragment thereof, of any one of E1-E49, comprising the HC amino acid sequence encoded by the insert in the plasmid deposited at the ATCC
10 and having ATCC Accession No. PTA-126529 and the LC amino acid sequence encoded by the insert in the plasmid deposited at the ATCC and having ATCC Accession No. PTA-126530.

- E51. The antibody, or antigen-binding fragment thereof, of any one of E1-E50, wherein the antibody or antigen-binding fragment is an Fc fusion protein, a monobody, a maxibody, a
15 bifunctional antibody, an scFab, an scFv, a peptibody.

- E52. The antibody, or antigen-binding fragment thereof, of any one of E1-E52, wherein the antibody, or antigen binding fragment thereof, binds human E-selectin with a K_D about or less than a value selected from the group consisting of about 800 nM, 700 nM, 600 nM, 500
20 nM, 400 nM, 300 nM, 200 nM, 175 nM, 150 nM, 125 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 900pM, 800pM, 700pM, 600pM and 500pM.

- E53. The antibody, or antigen-binding fragment thereof, of any one of E1-E52, wherein the antibody, or antigen binding fragment thereof, binds cynomolgus monkey E-selectin with a K_D about or less than a value selected from the group consisting of about 800 nM, 700 nM,
25 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, 175 nM, 150 nM, 125 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 900pM, 800pM, 700pM, 600pM and 500pM.

- E54. The antibody, or antigen-binding fragment thereof, of any one of E1-E53, wherein the antibody, or antigen binding fragment thereof, binds human E-selectin with a K_D of about 10
30 nM to about 200 nM.

E55. The antibody, or antigen-binding fragment thereof, of any one of E1-E54, wherein the antibody, or antigen binding fragment thereof, binds human E-selectin with a K_D of about 68.4 +/- 3.18 nM.

- E56. The antibody, or antigen-binding fragment thereof, of any one of E1-E55, wherein the
35 antibody, or antigen binding fragment thereof, binds cynomolgus monkey E-selectin with a K_D of about 10 nM to about 200 nM.

E57. The antibody, or antigen-binding fragment thereof, of any one of E1-E56, wherein the

antibody, or antigen binding fragment thereof, binds cynomolgus monkey E-selectin with a K_D of about 64.9 +/- 1.13 nM.

5 E58. The antibody, or antigen-binding fragment thereof, of any one of E1-E57, wherein the anti-E-selectin antibody binds human E-selectin with a K_D selected from the group consisting of about 92.85 nM, about 70.3 nM, about 65.2 nM, about 61.8 nM, about 60.5 nM, about 68.0 nM, about 21.6 nM, about 324 nM, about 54.4 nM, about 628.5 nM and 2940 nM.

E59. The antibody, or antigen-binding fragment thereof, of any one of E1-E58, wherein the anti-E-selectin antibody binds cynomolgus monkey E-selectin with a K_D selected from the group of about 138.5 nM, about 78.3 nM, about 76.5 nM, about 81.5 nM, about 67.8 nM, 10 about 45.8 nM, about 243.5 nM, about 45.4 nM, about 492 nM and 3145 nM.

E60. The antibody, or antigen-binding fragment thereof, of any one of E1-E59, wherein the mean half-life in cynomolgus monkeys is at least about 14.4 days (345 hours) following IV administration at a dose of 10 mg/kg.

15 E61. The antibody, or antigen-binding fragment thereof, of any one of E1-E60, wherein the mean half-life in cynomolgus monkeys is at least about 12 days (287 hours) following IV administration at a dose of 3 mg/kg.

E62. The antibody, or antigen-binding fragment thereof, of any one of E1-E61, wherein the mean half-life in cynomolgus monkeys is about 21.5 days (518 hours) following SC administration at a dose of 3 mg/kg.

20 E63. The antibody, or antigen-binding fragment thereof, of any one of E1-E62, wherein the antibody, or antigen-binding fragment thereof, does not induce anti-drug antibodies.

E64. The antibody, or antigen-binding fragment thereof, of any one of E1-E63, wherein the predicted immunogenic potential of the antibody, as indicated by the t-regitope (T-Reg) adjusted score, is less than about -30.

25 E65. The antibody, or antigen-binding fragment thereof, of any one of E1-E64, wherein the predicted immunogenic potential of the antibody, as indicated by the t-regitope (T-Reg) adjusted score, is less than about -45 and there are 0 non-germline T cell epitopes.

E66. The antibody, or antigen-binding fragment thereof, of any one of E1-E65, wherein the predicted immunogenic potential of the antibody, as indicated by the T-Reg adjusted score, 30 is less than the T-Reg adjusted score selected from the group consisting of about -24, -26, -27, -30, -32, -33, -34, -35, -36, -37, -38, -39, -40, -41, -42, -43, -44, -45, -46, -47 and -48.

E67. The antibody, or antigen-binding fragment thereof, of any one of E1-E66, wherein the predicted immunogenic potential of the antibody, as indicated by T-Reg adjusted score, is about -45 or -46.

35 E68. The anti-E-selectin antibody, or antigen-binding fragment thereof, of any one of E1-E67, wherein the antibody, or antigen-binding fragment thereof is at low risk for polyreactivity, as measured by, for example an AC-SINS assay, a DNA binding assay and/or

an insulin binding assay.

E69. The antibody, or antigen-binding fragment thereof, of any one of E1-E68, wherein the antibody or antigen-binding fragment has a viscosity selected from the group consisting of about 7.97 +/- 1.83 cP at a concentration of about 23 mg/mL, about 12.38 +/- 5.28 cP at a concentration of about 48 mg/mL, about 4.26 +/- 0.6 cP at a concentration of about 90 mg/mL, about 5.58 +/- 0.99 cP at a concentration of about 102 mg/mL, about 8.44 +/- 1.54 cP at a concentration of about 121 mg/mL, about 9.78 +/- 2.32 cP at a concentration of about 140 mg/mL, about 17.47 +/- 3.24 cP at a concentration of about 158 mg/mL and about 37.99 +/- 7.03 cP at a concentration of about 188 mg/mL, when measured at 25°C by, for example, dynamic light scattering (DLS).

E70. The antibody, or antigen-binding fragment thereof, of any one of E1-E69, wherein the antibody or antigen-binding fragment has a viscosity of about 15 cP to 40 cP at a concentration of about 150 mg/mL to about 190 mg/mL when measured at 25°C by, for example DLS.

E71. The antibody, or antigen-binding fragment thereof, of any one of E1-E70, wherein the antibody or antigen-binding fragment has a viscosity of 33.4 cP at 185.7 mg/mL when measured at 25°C by, for example an Anton Parr method.

E72. The antibody, or antigen-binding fragment thereof, of any one of E1-E71, wherein the antibody, or antigen-binding fragment thereof, binds to at least one of three epitopes of human E-selectin as determined by, for example a competition assay using, for example an Octet biosensor.

E73. The antibody, or antigen-binding fragment thereof, of E72, wherein at least 2 of the epitopes are overlapping.

E74. The antibody, or antigen-binding fragment thereof, of any one of E1-E73, wherein the antibody, or antigen-binding fragment thereof, interacts with at least one amino acid residue of human E-selectin selected from the group consisting of T7, E8, A9, M10, T11, P46, S47, Y48, N82, N83, Q85, E88, E92, Y94, R97, N105, E107, R108, S110, K111, K112, K113, and a combination thereof.

E75. The antibody, or antigen-binding fragment thereof, of any one of E1-E74, wherein the antibody, or antigen-binding fragment thereof, interacts with at least one amino acid residue of human E-selectin within 3.8 Å selected from the group consisting of T7, E8, A9, T11, P46, S47, Y48, N82, N83, Q85, E92, Y94, N105, E107, R108, S110, K111, K112 and a combination thereof.

E76. The antibody, or antigen-binding fragment thereof, of any one of E1-E75, wherein the antibody, or antigen-binding fragment thereof, interacts with at least one amino acid residue of human E-selectin with a buried surface area (Å²) of >5 Å² selected from the group consisting of T7, E8, A9, T11, P46, S47, Y48, N82, N83, Q85, E88, E92, Y94, R97, E107,

R108, S110, K111, K112, K113 and a combination thereof.

E77. The antibody, or antigen-binding fragment thereof, of any one of E1-E76, wherein the antibody, or antigen-binding fragment thereof interacts with at least one amino acid residue of human E-selectin via a hydrogen bond selected from the group consisting of E8, S47,

5 N82, N83, E88, E92, Y94, N105, E107, R108, S110, K112, and a combination thereof.

E78. The antibody, or antigen-binding fragment thereof, of any one of E1-E77, wherein the antibody, or antigen-binding fragment thereof interacts with at least one amino acid residue of human E-selectin via a salt bridge selected from the group consisting of K111, K112, and a combination thereof.

10 E79. The antibody, or antigen-binding fragment thereof, of any one of E1-E78, wherein the antibody, or antigen-binding fragment thereof interacts with at least one amino acid residue of human E-selectin via a water-mediated hydrogen bond selected from the group consisting of R97, K112, and a combination thereof.

E80. The antibody, or antigen-binding fragment thereof, of any one of E1-E79, wherein the
15 antibody, or antigen-binding fragment thereof, interacts with at least one amino acid residue of human E-selectin which also interacts within 3.8Å of an sLex amino acid residue selected from the group consisting of Y48, N82, N83, E92, Y94, R97, N105, E107 and a combination thereof.

E81. The antibody, or antigen-binding fragment thereof, of any one of E1-E80, wherein the
20 percentage of HMMS and/or the percentage LMMS is less than 5% following storage at 40°C for 4 weeks in a solution selected from the group consisting of 20 mM Tris at pH 7.5, 20 mM histidine at pH 5.8 and 20 mM glutamic acid at pH 4.5, and wherein optionally, the analysis is performed by aSEC.

E82. The antibody, or antigen-binding fragment thereof, of any one of E1-E81, wherein the
25 percentage of HMMS is less than 5% following storage at 4°C or 25°C for up to 6 weeks in a solution selected from the group consisting of 20 mM Tris, 8.5% sucrose at pH 7.5, 20 mM histidine, 8.5% sucrose, 0.005% EDTA at pH 5.8 and 20 mM glutamic acid, 8.5% trehalose at pH 4.5; wherein the antibody is at a concentration of about 150 mg/ml; and wherein optionally, the analysis is performed by aSEC.

30 E83. The antibody, or antigen-binding fragment thereof, of any one of E1-E82, wherein the antibody, or antigen-binding fragment thereof, has a thermal stability with a melting temperature (T_m), or the temperature at which the C_H2 of the antibody is 50% unfolded, of about 65°C or greater, as measured by Differential Scanning Calorimetry.

E84. The antibody, or antigen-binding fragment thereof, of any one of E1-E83, wherein the
35 antibody, or antigen-binding fragment thereof, has a thermal stability with a melting temperature (T_m), or the temperature at which the C_H2 of the antibody is 50% unfolded, between 65°C and 72°C, as measured by Differential Scanning Calorimetry.

E85. The antibody, or antigen-binding fragment thereof, of any one of E1-E84, wherein the antibody, or antigen-binding fragment thereof, has a thermal stability with a melting temperature (T_m1), or the temperature at which the C_H2 of the antibody is 50% unfolded, of about 71.7°C, as measured by Differential Scanning Calorimetry.

- 5 E86. The antibody, or antigen-binding fragment thereof, of any one of E1-E85, wherein the antibody, or antigen-binding fragment thereof, has a thermal stability with a melting temperature (T_m2), or the temperature at which the Fab of the antibody is 50% unfolded, of about 74°C or greater, as measured by Differential Scanning Calorimetry.

- 10 E87. The antibody, or antigen-binding fragment thereof, of any one of E1-E86, wherein the antibody, or antigen-binding fragment thereof, has a thermal stability with a melting temperature (T_m2), or the temperature at which the Fab of the antibody is 50% unfolded, between 74°C and 78°C, as measured by Differential Scanning Calorimetry.

- 15 E88. The antibody, or antigen-binding fragment thereof, of any one of E1-E87, wherein the antibody, or antigen-binding fragment thereof, has a thermal stability with a melting temperature (T_m2), or the temperature at which the Fab of the antibody is 50% unfolded, of about 78.2°C, as measured by Differential Scanning Calorimetry.

- 20 E89. The antibody, or antigen-binding fragment thereof, of any one of E1-E88, wherein the antibody, or antigen-binding fragment thereof, has a thermal stability with a melting temperature (T_m3), or the temperature at which the C_H3 of the antibody is 50% unfolded, of about 82°C or greater, as measured by Differential Scanning Calorimetry.

- E90. The antibody, or antigen-binding fragment thereof, of any one of E1-E89, wherein the antibody, or antigen-binding fragment thereof, has a thermal stability with a melting temperature (T_m3), or the temperature at which the C_H3 of the antibody is 50% unfolded, between 82°C and 86°C, as measured by Differential Scanning Calorimetry.

- 25 E91. The antibody, or antigen-binding fragment thereof, of any one of E1-E90 wherein the antibody, or antigen-binding fragment thereof, has a thermal stability with a melting temperature (T_m3), or the temperature at which the C_H3 of the antibody is 50% unfolded, of about 84.3°C, as measured by Differential Scanning Calorimetry.

- 30 E92. The antibody, or antigen-binding fragment thereof, of any one of E1-E91, wherein the antibody, or antigen-binding fragment thereof, has a binding affinity, expressed as EC₅₀, for cell-surface expressed human E-selectin, that is less than or equal to 50 nM, for example, less than or equal to 48 nM, 45 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.75 nM, 0.5 nM, 0.25 nM or 0.1 nM as measured, for example, by FACS.

- 35 E93. The antibody, or antigen-binding fragment thereof, of any one of E1-E92, wherein the antibody, or antigen-binding fragment thereof, has a binding affinity, expressed as EC₅₀, for cell-surface expressed human E-selectin that is about 0.66 nM, as measured, for example, by FACS.

E94. The antibody, or antigen-binding fragment thereof, of any one of E1-E93, wherein the antibody, or antigen-binding fragment thereof, has a binding affinity, expressed as EC_{50} , for cell-surface expressed cynomolgus E-selectin that is less than or equal to 50 nM, for example, less than or equal to 48 nM, 45 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2

5 nM, 1 nM, 0.75 nM, 0.5 nM, 0.25 nM or 0.1 nM as measured, for example, by FACS.

E95. The antibody, or antigen-binding fragment thereof, of any one of E1-E94, wherein the antibody, or antigen-binding fragment thereof, has a binding affinity, expressed as EC_{50} , for cell-surface expressed human P-selectin that is greater than or equal to 350 nM, for example, greater than or equal to 400 nM, 450 nM, 500 nM, 550 nM, 600 nM, 650 nM or

10 more, as measured, for example, by FACS.

E96. The antibody, or antigen-binding fragment thereof, of any one of E1-E95, wherein the antibody, or antigen-binding fragment thereof, has weak or no binding to soluble rat, mouse or rabbit E-selectin or to soluble human L- or P-selectin.

E97. The antibody, or antigen-binding fragment thereof, of any one of E1-E96, wherein the

15 antibody, or antigen-binding fragment thereof, has weak or no binding to cell-surface expressed human P-selectin.

E98. The antibody, or antigen-binding fragment thereof, of any one of E1-E97, wherein the antibody, or antigen-binding fragment thereof, demonstrates no binding to rat, mouse or rabbit E-selectin or to soluble human L- or P-selectin up to 405 nM as measured, for

20 example by SPR.

E99. The antibody, or antigen-binding fragment thereof, of any one of E1-E98, wherein the antibody, or antigen-binding fragment thereof, demonstrates weak-nonsaturable binding to soluble mouse or rat E-selectin that is about >100 x lower than binding to human or rat E-selectin as measured, for example, by direct binding ELISA.

25 E100. The antibody, or antigen-binding fragment thereof, of any one of E1-E99, wherein the antibody, or antigen-binding fragment thereof, demonstrates weak-nonsaturable binding to soluble mouse or rat E-selectin up to 133.3 nM as measured, for example, by direct binding ELISA.

E101. The antibody, or antigen-binding fragment thereof, of any one of E1-E100, wherein

30 the antibody, or antigen-binding fragment thereof, binds to soluble human E-selectin with an EC_{50} of less than or equal to 2 nM, for example, less than or equal to 0.010 nM, 0.015 nM, 0.020 nM, 0.025 nM, 0.030 nM, 0.035 nM, 0.040 nM, 0.045 nM, 0.05 nM, 0.055 nM, 0.06 nM, 0.065 nM, 0.070 nM, 0.075 nM, 0.080 nM, 0.085 nM, 0.090 nM, 0.10 nM, 0.12 nM, 0.15 nM, 0.2 nM, 0.5 nM, 0.9 nM, 0.95 nM, 1.0 nM, 1.5 nM, 18 nM or 1.9 nM.

35 E102. The antibody, or antigen-binding fragment thereof, of any one of E1-E101, wherein the antibody, or antigen-binding fragment thereof, binds to soluble human E-selectin with an EC_{50} of about 0.085 nM to about 0.12 nM as measured, for example, by direct binding

ELISA.

E103. The antibody, or antigen-binding fragment thereof, of any one of E1-E102, wherein the antibody, or antigen-binding fragment thereof, binds to soluble cynomolgus E-selectin with an EC_{50} of less than or equal to 1 nM, for example, less than or equal to 0.010 nM,

5 0.015 nM, 0.020 nM, 0.025 nM, 0.030 nM, 0.035 nM, 0.040 nM, 0.045 nM, 0.05 nM, 0.055 nM, 0.06 nM, 0.065 nM, 0.070 nM, 0.075 nM, 0.080 nM, 0.085 nM, 0.090 nM, 0.10 nM, 0.12 nM, 0.15 nM, 0.2 nM, 0.5 nM, 0.9 nM or 0.95 nM.

E104. The antibody, or antigen-binding fragment thereof, of any one of E1-E103, wherein the antibody, or antigen-binding fragment thereof, binds to soluble cynomolgus E-selectin

10 with an EC_{50} of 0.071 nM to 0.093 nM as measured, for example, by direct binding ELISA.

E105. The antibody, or antigen-binding fragment thereof, of any one of E1-E104, wherein the antibody, or antigen-binding fragment thereof, binds free soluble human E-selectin in human serum with an IC_{50} of about 1 nM to about 3 nM, and preferably with an IC_{50} of about 1.2 nM.

15 E106. The antibody, or antigen-binding fragment thereof, of any one of E1-E105, wherein the antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to soluble human E-selectin with an IC_{50} of less than or equal to 100 nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM or 1 nM as measured, for example, by competition ELISA under static
20 conditions.

E107. The antibody, or antigen-binding fragment thereof, of any one of E1-E106, wherein the antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to soluble human E-selectin with an IC_{50} of about 2.87 nM to about 3.01 nM as measured, for example, by competition ELISA under static conditions.

25 E108. The antibody, or antigen-binding fragment thereof, of any one of E1-107, wherein the antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to soluble cynomolgus E-selectin with an IC_{50} of less than or equal to 100 nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM or 1 nM as measured, for example, by competition ELISA under static
30 conditions.

E109. The antibody, or antigen-binding fragment thereof, of any one of E1-E108, wherein the antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to soluble cynomolgus E-selectin with an IC_{50} of about 2.39 nM to about 2.91 nM as measured, for example, by competition ELISA under static conditions.

35 E110. The antibody, or antigen-binding fragment thereof, of any one of E1-E109, wherein the antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to cell-surface expressed human E-selectin with an IC_{50} of less than or equal to 100

nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM or 1 nM as measured, for example, by competition ELISA under static conditions.

5 E111. The antibody, or antigen-binding fragment thereof, of any one of E1-E110, wherein the antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to cell-surface expressed human E-selectin with an IC_{50} of about 1.88 nM to about 2.89 nM as measured, for example, by competition ELISA under static conditions.

E112. The antibody, or antigen-binding fragment thereof, of any one of E1-E111, wherein the antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A
10 ligand to cell-surface expressed cynomolgus E-selectin with an IC_{50} of less than or equal to 100 nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM or 1 nM as measured, for example, by competition ELISA under static conditions.

E113. The antibody, or antigen-binding fragment thereof, of any one of E1-E112, wherein
15 the antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to cell-surface expressed cynomolgus E-selectin with an IC_{50} of about 1.47 nM to about 2.65 nM as measured, for example, by competition ELISA under static conditions.

E114. The antibody, or antigen-binding fragment thereof, of any one of E1-E113, wherein the antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-
20 selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed human E-selectin with an IC_{50} of less than or equal to 100 nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM or 1 nM as measured, for example, under static conditions.

E115. The antibody, or antigen-binding fragment thereof, of any one of E1-E114, wherein
25 the antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed human E-selectin with an IC_{50} of about 3.36 nM to about 4.7 nM as measured, for example, under static conditions.

E116. The antibody, or antigen-binding fragment thereof according to E1-E115, wherein the
30 antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed cynomolgus E-selectin with an IC_{50} of about 3.84 nM as measured, for example, under static conditions.

E117. The antibody, or antigen-binding fragment thereof, of any one of E1-E116, wherein
35 the antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed human E-selectin with an IC_{50} of less than or equal to 100 nM, for example, less

than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM or 2 nM as measured, for example, under physiological flow conditions.

E118. The antibody, or antigen-binding fragment thereof, of any one of E1-E117, wherein the antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface
5 expressed human E-selectin with an IC_{50} of about 4.25 nM to about 4.56 nM as measured, for example, under physiological flow conditions.

E119. The antibody, or antigen-binding fragment thereof, of any one of E1-E118, wherein the antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface
10 expressed cynomolgus E-selectin with an IC_{50} of about 4.32 nM to about 4.35 nM as measured, for example, under physiological flow conditions.

E120. The antibody, or antigen-binding fragment thereof, of any one of E1-E119, wherein the antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to soluble
15 human E-selectin with an IC_{50} of less than or equal to 300 nM, for example, less than or equal to 290 nM, 280 nM, 270 nM, 260 nM, 250 nM, 150 nM, 100 nM, 90 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 20 nM, 20 nM, 5 nM, 2 nM, or 1 nM as measured, for example, under physiologic flow conditions.

E121. The antibody, or antigen-binding fragment thereof, of any one of E1-E120, wherein the antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to soluble
20 human E-selectin with an IC_{50} of about 13.28 nM to about 15.94 nM as measured, for example, under physiologic flow conditions.

E122. The antibody, or antigen-binding fragment thereof, of any one of E1-E121, wherein the antibody, or antigen-binding fragment thereof, inhibits adhesion of activated human neutrophils (e.g., TNF- α activated) to cell-surface expressed cynomolgus E-selectin with an
25 IC_{50} of about 9.45 nM to about 16.33 nM as measured, for example, under physiologic flow conditions.

E123. The antibody, or antigen-binding fragment thereof, of any one of E1-E122, wherein the antibody, or antigen-binding fragment thereof, inhibits adhesion of activated human neutrophils (e.g., TNF- α activated) to cell-surface expressed human E-selectin with an IC_{50}
30 of about 2.87 nM to about 4.65 nM as measured, for example, under physiologic flow conditions.

E124. The antibody, or antigen-binding fragment thereof, of any one of E1-E123, wherein the antibody, or antigen-binding fragment thereof, inhibits adhesion of blood cells from SCD patients to soluble human E-selectin with an IC_{50} of about 6.17 nM to about 18.66 nM as
35

measured, for example, under physiologic flow conditions.

E125. The antibody, or antigen-binding fragment thereof, of any one of E1-E124, wherein the antibody, or antigen-binding fragment thereof, inhibits adhesion of blood cells from SCD patients to soluble human E-selectin with an IC₅₀ of about 12.4 nM as measured, for

5 example, under physiologic flow conditions.

E126. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding the antibody, or antigen binding fragment thereof, of any one of E1-E125.

E127. An isolated nucleic acid molecule comprising at least one nucleic acid sequence encoding the antibody, or antigen binding fragment thereof, of any one of E1-E125.

10 E128. An isolated nucleic acid molecule encoding a VL, VH, or both, of an antibody, or an antigen-binding fragment thereof, that specifically binds human E-selectin, wherein said nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO:136, the nucleic acid sequence of SEQ ID NO:137, or both.

E129. An isolated nucleic acid molecule comprising, or consisting of, the nucleic acid
15 sequence of SEQ ID NO:136, the nucleic acid sequence of SEQ ID NO:137, or both.

E130. An isolated nucleic acid molecule comprising, or consisting of, the nucleic acid sequence as set forth as SEQ ID NO:136.

E131. An isolated nucleic acid molecule comprising, or consisting of, the nucleic acid sequence as set forth as SEQ ID NO:137.

20 E132. An isolated nucleic acid molecule encoding the VH of an antibody, or antigen-binding fragment thereof, that specifically binds human E-selectin, comprising at least one nucleic acid sequence selected from the group consisting of SEQ ID NO:136, 144, 146, 148, 150, 151, 152, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171 and 173.

E133. An isolated nucleic acid molecule encoding the VH of an antibody, or antigen-binding
25 fragment thereof, that specifically binds human E-selectin, comprising a nucleic acid at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:136, 144, 146, 148, 150, 151, 152, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171 and 173.

E134. An isolated nucleic acid molecule encoding the VL of an antibody, or antigen-binding
30 fragment thereof, that specifically binds human E-selectin, comprising at least one nucleic acid sequence selected from the group consisting of SEQ ID NO:137, 145, 147, 149, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172 and 174.

E135. An isolated nucleic acid molecule encoding the VL of an antibody, or antigen-binding
35 fragment thereof, that specifically binds human E-selectin, comprising a nucleic acid at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 137, 145, 147, 149, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172 and 174.

- E136. An isolated nucleic acid molecule encoding a light chain, heavy chain, or both, of an antibody, or an antigen-binding fragment thereof, that specifically binds human E-selectin, wherein said nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO:206 or 138; the nucleic acid sequence of SEQ ID NO:139; or both.
- 5 E137. An isolated nucleic acid molecule comprising, or consisting of, the nucleic acid sequence of SEQ ID NO:206 or 138; the nucleic acid sequence of SEQ ID NO:139; or both.
- E138. An isolated nucleic acid molecule comprising, or consisting of, the nucleic acid sequence of SEQ ID NO:206 or 138.
- E139. An isolated nucleic acid molecule comprising, or consisting of, the nucleic acid
10 sequence of SEQ ID NO:139.
- E140. An isolated nucleic acid molecule encoding an antibody, or an antigen-binding fragment thereof, that specifically binds human E-selectin, wherein said nucleic acid molecule comprises the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having the Accession Number PTA-126529.
- 15 E141. An isolated nucleic acid molecule encoding an antibody, or an antigen-binding fragment thereof, that specifically binds human E-selectin, wherein said nucleic acid molecule comprises the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having the Accession Number PTA-126530.
- E142. An isolated nucleic acid molecule encoding an antibody, or an antigen-binding
20 fragment thereof, that specifically binds human E-selectin, wherein said nucleic acid comprises the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having the Accession Number PTA-126529 and the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having the Accession Number PTA-126530.
- E143. An isolated nucleic acid molecule comprising the nucleic acid sequence of the insert
25 of the plasmid deposited with the ATCC and having the Accession Number PTA-126529.
- E144. An isolated nucleic acid molecule comprising the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having the Accession Number PTA-126530.
- E145. An isolated nucleic acid molecule comprising the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having the Accession Number PTA-126529,
30 and the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having the Accession Number PTA-126530.
- E146. A vector comprising the nucleic acid molecule of any one of E126-E145.
- E147. A host cell comprising the nucleic acid molecule of any one of E126-E145, or the vector of E146.
- 35 E148. The host cell of E147, wherein said cell is a mammalian cell.
- E149. The host cell of E147 or E148, wherein said host cell is a CHO cell, a HEK-293 cell, an NS0 cell, a PER.C6® cell, or an Sp2.0 cell.

E150. A method of making an antibody or antigen-binding fragment thereof, comprising culturing the host cell of any one of E147-E149, under a condition wherein said antibody or antigen-binding fragment is expressed by said host cell.

5 E151. The method of E150, further comprising isolating said antibody or antigen-binding fragment thereof.

E152. A pharmaceutical composition comprising an antibody or antigen-binding fragment thereof of any one of E1-E125 and E221, and a pharmaceutically acceptable carrier or excipient.

10 E153. The pharmaceutical composition of E152, wherein the composition comprises 1.12 mg/mL L-histidine, 2.67 mg/mL L-histidine hydrochloride monohydrate, 85 mg/mL sucrose, 0.05 mg/mL edetate disodium dihydrate, 0.2 mg/mL polysorbate 80 at pH 5.8.

E154. The pharmaceutical composition of E152 or E153, wherein the composition comprises 20 mM histidine, 8.5% sucrose, and 0.02% polysorbate 80, 0.005% EDTA at pH 5.8.

15 E155. The pharmaceutical composition of any one of E152-E154, wherein the composition comprises about 25 mg/mL, 50 mg/mL, 75 mg/mL, 100 mg/mL, 125 mg/mL, 150 mg/mL antibody, or antigen binding fragment thereof.

E156. The pharmaceutical composition of E155, wherein the composition comprises about 100 mg/mL antibody, or antigen binding fragment thereof.

20 E157. The pharmaceutical composition of any one of E152-E156, wherein the dose is a 1 mL dose.

E158. The pharmaceutical composition of E152-E157, wherein the composition is suitable for SC and/or IV administration.

25 E159. The pharmaceutical composition of any one of E152-E158 comprising an antibody, or antigen-binding fragment thereof, comprising i) HCDR-1, HCDR-2, and HCDR-3 sequences of SEQ ID NO:11 and LCDR-1, LCDR-2, and LCDR-3 sequences of SEQ ID NO:5; ii) a VH domain comprising the amino acid sequence of SEQ ID NO:11 and a VL domain comprising the amino acid sequence of SEQ ID NO:5; or iii) a HC comprising the amino acid sequence of SEQ ID NO:7 or 13 and a LC comprising the amino acid sequence of SEQ ID NO:1.

30 E160. The pharmaceutical composition of any one of E152-E159 comprising an additional therapeutically active compound selected from the group consisting of L-glutamine (e.g., ENDARI), an anti-P-selectin antibody (e.g., crizanlizumab (ADAKVEO)), a compound that modulates HbS so as to maintain it in its R state (i.e., oxygenated), for example, a 2-aminoquinoline and those described in WO 2020/109994 (incorporated herein by reference),
35 a compound that modulates oxygen affinity of HbS (e.g., voxelotor (OXBRYTA)), a compound that targets HbS polymerization by modulating generation of 2,3-disphosphoglyceric acid, a compound that targets HbS polymerization by inducing

expression of fetal hemoglobin (e.g., hydroxyurea, e.g., DROXIA, HYDREA), a compound that targets dysfunctional cellular adhesion, vascular dysfunction and/or inflammation (e.g., a phosphodiesterase-9 inhibitor), a compound that increases levels of nitric oxide in the blood (e.g., a soluble guanylate cyclase stimulator, e.g., IW-1701, riociguat (ADEMPAS)),
5 intravenous IG, a compound that targets hypercoagulability (e.g., riociguat (ADEMPAS), apixaban (ELIQUIS), rivaroxaban (XARELTO)), a compound that blocks NMDA receptor binding (e.g., memantine (NAMENDA)) and a combination thereof.

E161. The pharmaceutical composition of any one of E152-E159 comprising an additional therapeutically active compound selected from the group consisting of penicillin prophylaxis
10 to prevent pneumococcal infection, hydroxyurea (e.g., DROXIA, HYDREA), L-glutamine (e.g., ENDARI), crizanlizumab (ADAKVEO), voxelotor (OXBRYTA), apixaban (ELIQUIS), rivaroxaban (XARELTO), a non-steroidal anti-inflammatory drug, an analgesic generally, an opioid analgesic, IW-1701, riociguat (ADEMPAS), ticagrelor (BRILINTA), memantine (NAMENDA) and a combination thereof.

15 E162. A method of reducing or inhibiting E-selectin activity, comprising administering to a subject in need thereof a therapeutically effective amount of the antibody, or antigen-binding fragment thereof, of any one of E1-E125, E221, or the pharmaceutical composition of any one of E152-E161, and comparing the activity of E-selectin before administration with the level of E-selectin activity after administration of the antibody, thereby reducing the activity of
20 E-selectin.

E163. The method of E162, wherein reducing or inhibiting E-selectin activity treats a disease, disorder or condition which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of E-selectin activity.

E164. The method of any one of E162-E163, wherein the activity of E-selectin is selected
25 from the group consisting of:

- (a) leukocyte tethering to endothelial cells;
- (b) activation of stable adhesion to endothelial cells;
- (c) slow rolling of leukocytes to arrest;
- (d) efficient trans-endothelial migration of leukocytes;
- 30 (e) affinity and avidity of CD18 integrins;
- (f) trafficking of leukocytes to sites of acute inflammation;
- (g) increase in cytosolic calcium;
- (h) increase in tyrosine phosphorylation that activates p38 MAP kinase and Syk
kinase;
- 35 (i) recruitment of platelets and leukocytes from the blood to the vascular endothelium;
and
- (j) creation of a pro-inflammatory environment.

- E165. A method of reducing the level of free E-selectin in a subject in need thereof, the method comprising administering to a subject a therapeutically effective amount of the antibody, or antigen-binding fragment thereof, of any one of E1-E125, or the pharmaceutical composition of any one of E152-E161.
- 5 E166. A method of treating and/or preventing a disease, disorder and/or condition associated with, or mediated by, E-selectin expression and/or E-selectin binding to a ligand, comprising administering to a subject in need thereof a therapeutically effective amount of the antibody, or antigen-binding fragment thereof, of any one of E1-E125, or the pharmaceutical composition of any one of E152-E161.
- 10 E167. The method of E166, wherein the disease, disorder and/or condition is at least one selected from the group consisting of SCD, vaso-occlusive crisis (VOC), pain, organ infarction, ischemia, stroke, end organ dysfunction, acute chest and vascular obstruction, skin diseases (e.g., psoriasis), inflammatory diseases (e.g., rheumatoid arthritis) and complications of diabetes.
- 15 E168. The method of any one of E167, wherein the VOC is associated with SCD.
- E169. The method of any one of E166-E168, comprising a prophylactic treatment for SCD by preventing or reducing the occurrence of a VOC.
- E170. The method of any one of E167-E169, comprising an acute treatment for SCD by decreasing the duration (e.g., reduction in the time to resolve a VOC) and intensity of a
- 20 VOC.
- E171. The method of any one of E166-E170, wherein the treatment is a prophylactic treatment.
- E172. The method of any one of E166-E171, that treats, prevents and/or ameliorates at least one sign and/or symptom of SCD, for example, those affecting the cardiothoracic
- 25 system (e.g., chronic restrictive lung disease, left ventricular diastolic disease, pulmonary hypertension, acute chest syndrome, dysrhythmias, sudden death, vaso-occlusive crisis), the nervous system (e.g., hemorrhagic stroke, venous sinus thrombosis, silent cerebral infarction of the brain, chronic pain, acute ischemic stroke of the brain, proliferative retinopathy, orbital infarction, cognitive impairment) the reticuloendothelial system (e.g., splenic sequestration, functional hyposplenism, anemia, hemolysis), the musculoskeletal
- 30 system (e.g., avascular necrosis, skin ulcerations), the urogenital system (e.g., papillary necrosis, proteinuria, renal failure, hematuria, nocturnal enuresis, priapism) and the gastrointestinal system (e.g., cholelithiasis, cholangiopathy, hepatopathy, mesenteric vaso-occlusion).
- 35 E173. The method of any one of E166-E172, wherein the subject is a human.
- E174 The method of any one of E166-E173, wherein the subject is a patient with SCD.
- E175. The method of any one of E166-E174, wherein the subject has a HBSS, HBSC,

HBS/ β° thal, HBS/ β^{+} thal or HBS-variant genotype.

E176. The method of any one of E166-E175, wherein the antibody or antigen-binding fragment thereof, or pharmaceutical composition, is administered subcutaneously.

5 E177. The method of any one of E166-E176, wherein the antibody or antigen-binding fragment thereof, or pharmaceutical composition, is administered intravenously.

E178. The method of any one of E166-E177, wherein the antibody or antigen-binding fragment thereof, or pharmaceutical composition, is administered about twice a week, once a week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once
10 every nine weeks, once every ten weeks, twice a month, once a month, once every two months, once every three months, once every four months, once every five months, once every six months, once every seven months, once every eight months, once every nine months, once every ten months, once every eleven months or once every twelve months.

E179. The method of any one of E166-E177, wherein the antibody or antigen-binding
15 fragment thereof, or pharmaceutical composition, is administered once a month.

E180. The method of any one of E166-E179, wherein the antibody, or antigen-binding fragment thereof, or pharmaceutical composition, is administered once a week.

E181. The method of any one of E166-E180, wherein the therapeutically effective amount comprises a dose of about 1 mg to about 800 mg of the anti-E-selectin antibody, or antigen
20 binding fragment thereof.

E182. The method of E181, wherein the dose is an initial fixed dose.

E183. The method of any one of E181-E182, wherein the dose is about 1 mg to about 10 mg, about 10 mg to about 20 mg, about 20 mg to about 30 mg, about 30 mg to about 40 mg, about 40 mg to about 50 mg, about 50 mg to about 60 mg, about 60 mg to about 70 mg,
25 about 70 mg to about 80 mg, about 80 mg to about 90 mg, about 90 mg to about 100 mg, about 100 mg to about 150 mg, about 150 mg to about 200 mg, about 200 mg to about 300 mg, about 300 mg to about 400 mg, about 400 mg to about 500 mg, about 500 mg to about 600 mg, about 600 mg to about 700 mg or about 700 mg to about 800 mg of the anti-E-selectin antibody, or antigen binding fragment thereof.

30 E184. The method of any one of E181-E183, wherein the dose is about 15 mg, 40 mg, 100 mg, 150 mg, 300 mg, 500 mg or 600 mg of an anti-E-selectin antibody, or antigen binding fragment thereof.

E185. The method of any one of E181-E184, wherein the dose is about 150 mg of the anti-E-selectin antibody, or antigen binding fragment thereof.

35 E186. The method of any one of E181-E185, comprising administering the dose once a week, once every 2 weeks, once a month, once every two months, or a combination thereof.

E187. The method of any one of E162-E186, wherein the antibody is antibody 1444 and the

antigen-binding fragment is a fragment of antibody 1444.

E188. The method of any one of E162-E187, wherein the administration is subcutaneous or intravenous administration.

E189. The method of any one of E162-E188 comprising administering an antibody, or antigen-binding fragment thereof, comprising i) HCDR-1, HCDR-2, and HCDR-3 sequences of SEQ ID NO:11 and LCDR-1, LCDR-2, and LCDR-3 sequences of SEQ ID NO:5; ii) a VH domain comprising the amino acid sequence of SEQ ID NO:11 and a VL domain comprising the amino acid sequence of SEQ ID NO:5; or iii) a HC comprising the amino acid sequence of SEQ ID NO:7 or 13 and a LC comprising the amino acid sequence of SEQ ID NO:1.

E190. A method of any one of E162-E188 comprising administering a pharmaceutical composition comprising an antibody, or antigen-binding fragment thereof, comprising i) HCDR-1, HCDR-2, and HCDR-3 sequences of SEQ ID NO:11 and LCDR-1, LCDR-2, and LCDR-3 sequences of SEQ ID NO:5; ii) a VH domain comprising the amino acid sequence of SEQ ID NO:11 and a VL domain comprising the amino acid sequence of SEQ ID NO:5; or iii) a HC comprising the amino acid sequence of SEQ ID NO:7 or 13 and a LC comprising the amino acid sequence of SEQ ID NO:1.

E191. The method of any one of E162-E190, wherein the subject is a patient with SCD.

E192. A method of treating SCD, comprising administering to a subject in need thereof a therapeutically effective amount an anti-E-selectin antibody, or antigen-binding fragment thereof, comprising i) HCDR-1, HCDR-2, and HCDR-3 sequences of SEQ ID NO:11 and LCDR-1, LCDR-2, and LCDR-3 sequences of SEQ ID NO:5; ii) a VH domain comprising the amino acid sequence of SEQ ID NO:11 and a VL domain comprising the amino acid sequence of SEQ ID NO:5; or iii) a HC comprising the amino acid sequence of SEQ ID NO:7 or 13 and a LC comprising the amino acid sequence of SEQ ID NO:1, and a pharmaceutically acceptable carrier or excipient.

E193. The method of E192, wherein treating SCD includes treating at least one symptom of SCD including VOC.

E194. The method of any one of E192-E193, wherein the subject is a patient with SCD.

E195. The method of any one of E192-E194, comprising administering the antibody or antigen-binding fragment thereof, subcutaneously and/or intravenously.

E196. The method of any one of E192-E195, wherein said antibody or antigen-binding fragment thereof, is administered about twice a week, once a week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, twice a month, once a month, once every two months, once every three months, once every four months, once every five months, once every six months, once every seven months, once every eight months, once every nine months, once every ten

months, once every eleven months or once every twelve months.

E197. The method of any one of E192-E196, wherein the antibody or antigen-binding fragment thereof, or pharmaceutical composition, is administered once a week, once every 2 weeks, once every 3 weeks, once a month, or a combination thereof.

5 E198. The method of any one of E192-E197, wherein the antibody or antigen-binding fragment thereof is administered at a dose between about 1 mg to about 800 mg.

E199. The method of any one of E192-198, wherein said antibody or antigen-binding fragment thereof, or pharmaceutical composition, is administered at a dose selected from the group consisting of about 15 mg, about 40 mg, about 100 mg, about 150 mg, about 300
10 mg, about 500 mg, and about 600 mg.

E200. The method of any one of E192-E199, wherein the antibody, or antigen-binding fragment thereof is administered in combination with a therapeutically effective amount of one or more additional therapeutically active compounds or treatment modalities effective in treating and/or preventing at least one sign and/or symptom of SCD.

15 E201. The method of any one of E192-E200, wherein the amount of the anti-E-selectin antibody, or antigen-binding fragment thereof, and the amount of the therapeutically active compound or treatment modality effective in treating and/or preventing at least one sign and/or symptom of SCD, are administered in amounts that together achieve synergistic effects in the treatment and/or prevention of at least one sign and/or symptom of SCD.

20 E202. The method of any one of E192-E201, wherein the amount of the anti-E-selectin antibody, or antigen-binding fragment thereof, and/or the amount of the therapeutically active compound or treatment modality effective in treating and/or preventing at least one sign and/or symptom of SCD, are each administered at a dosage that is lower than would be administered if not in combination.

25 E203. The method of any one of E192-E202, wherein the additional therapeutically active compound is selected from the group consisting of penicillin prophylaxis to prevent pneumococcal infection, hydroxyurea (e.g., DROXIA, HYDREA), L-glutamine (e.g., ENDARI), crizanlizumab (ADAKVEO), voxelotor (OXBRYTA), apixaban (ELIQUIS), rivaroxaban (XARELTO), a non-steroidal anti-inflammatory drug, an analgesic generally, an
30 opioid analgesic, IW-1701, riociguat (ADEMPAS), ticagrelor (BRILINTA), memantine (NAMENDA) and a combination thereof.

E204. The method of any one of E192-E203, wherein the additional therapeutically active compound is selected from the group consisting of L-glutamine (e.g., ENDARI), an anti-P-selectin antibody (e.g., crizanlizumab (ADAKVEO)), a compound that modulates HbS so as
35 to maintain it in its R state (i.e., oxygenated), for example, a 2-aminoquinoline and those described in WO 2020/109994 (incorporated herein by reference), a compound that modulates oxygen affinity of HbS (e.g., voxelotor (OXBRYTA)), a compound that targets

- HbS polymerization by modulating generation of 2,3-disphosphoglyceric acid, a compound that targets HbS polymerization by inducing expression of fetal hemoglobin (HbF) (e.g., hydroxyurea, e.g., DROXIA, HYDREA), a compound that targets dysfunctional cellular adhesion, vascular dysfunction and/or inflammation (e.g., phosphodiesterase-9 inhibitors), a compound that increases levels of nitric oxide in the blood (e.g., soluble guanylate cyclase stimulators, e.g., IW-1701, riociguat (ADEMPAS)), intravenous IG, a compound that targets hypercoagulability (e.g., riociguat (ADEMPAS), apixaban (ELIQUIS), rivaroxaban (XARELTO)), a compound that blocks NMDA receptor binding (e.g., memantine (NAMENDA)) and a combination thereof.
- 5
- 10 E205. The method of any one of E192-204, wherein the therapeutically active treatment modality useful for the treatment and/or prevention of at least one sign and/or symptom of SCD is selected from the group consisting of supplemental oxygen, blood transfusion, optionally with iron chelation, bone marrow transplant, gene therapy (e.g., LentiGlobin®), a gene editing therapy by CRISPR (e.g., CTX001) or a zinc finger technique and a combination thereof.
- 15
- E206. The method of any one of E200-E205 wherein the anti-E-selectin antibody, or antigen-binding fragment thereof and the therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and or symptom of SCD, are co-administered.
- 20
- E207. The method of any one of E200-E206, wherein the combination therapies are administered according to the the same dosing regimen (e.g., both therapies are administered daily) or according to different dosing regimens (e.g., one therapy is administered daily, the other therapy is administered weekly).
- E208. The method of any one of E200-E207, wherein the combination therapies are
- 25
- administered to a subject by the same or different routes of administration.
- E209. Use of the pharmaceutical composition of any one of E152-E161 in the manufacture of a medicament for treating a disease, disorder or condition mediated by E-selectin (e.g., SCD).
- E210. Use of an antibody, or antigen-binding fragment thereof, of any one of E1-E125 or
- 30
- E221 in the manufacture of a medicament for treating and/or preventing a disease, disorder or condition associated with, or mediated by, E-selectin expression and/or E-selectin binding to a ligand.
- E211. Use of a pharmaceutical composition of any one of E152-E161 in the manufacture of a medicament for treating and/or preventing a disease, disorder or condition associated with, or mediated by, E-selectin expression and/or E-selectin binding to a ligand.
- 35
- E212. An antibody, or antigen-binding fragment thereof, of any one of E1-E125 or E221, or the pharmaceutical composition of E152-E161, for use as a medicament.

E213. An antibody, or antigen binding fragment thereof, of any one of E1-E125 or E221, or the pharmaceutical composition of any one of E152-E161, for use in the treatment and/or prevention of at least one sign and/or symptom of SCD.

5 E214. The antibody, or antigen binding fragment thereof, or the pharmaceutical composition, for use of E213, wherein the symptom of SCD is VOC.

E215. The antibody, or antigen binding fragment thereof, or the pharmaceutical composition, for use of any one of E213-214, wherein the treatment and/or prevention further comprises an additional therapeutic agent, such as, but not limited to at least one other therapeutically active compound or treatment modality which is effective in treating
10 and/or preventing at least one sign and/or symptom of SCD.

E216. The antibody, or antigen binding fragment thereof, or the pharmaceutical composition, for use of E215, wherein the additional therapeutic agent is an agent that is standard of care for the prevention and/or treatment of at least one sign and/or symptom of SCD (e.g., L-glutamine, hydroxyurea, a blood transfusion and any other therapy known in
15 the art).

E217. The antibody, or antigen binding fragment thereof, or the pharmaceutical composition, for use of E215-216, wherein the treatment and/or prevention comprises i) a synergistic, therapeutically effective amount of the anti-E-selectin antibody or antigen-binding fragment thereof, and ii) a synergistic, therapeutically effective amount of the
20 additional therapeutic agent.

E218. The antibody, or antigen binding fragment thereof, or the pharmaceutical composition, for use of E215 or E216, wherein the treatment and/or prevention comprises i) a synergistic, therapeutically effective amount of the anti-E-selectin antibody, or antigen-binding fragment thereof, and ii) a synergistic, therapeutically effective amount of the
25 treatment modality.

E219. The pharmaceutical composition of any one of E152-E161, wherein the appropriate amount of each compound, as used in the combination for administration to a patient with SCD is determined by taking into account at least one factor selected from the group consisting of age, weight, general health, the compound administered, the route of
30 administration, the nature and advancement of the treatment of SCD, and the presence of other medications.

E220. A pharmaceutical composition of any one of E152-E161, formulated for use as a medicament for treating a disease, disorder or condition mediated by E-selectin (e.g., SCD).

35 E221. An isolated monoclonal antibody that specifically binds E-selectin, wherein the antibody is antibody 0039, 0164, 0158, 0159, 0170, 0180, 0841, 1282 1284, 1444 or 1448.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

- [0017]** **FIG. 1** depicts an exemplary crystal structure of Fab of anti-E-selectin antibody 0164 co-crystallized with truncated human E-selectin. E-selectin is shown as a gray surface (molecule on the right). The 0164 Fab (molecule on the left) is shown as ribbons with the VL in light gray and the VH in dark gray.
- [0018]** **FIG. 2** depicts exemplary data from a three-part mass spectroscopy analysis of optimized anti-E-selectin antibody 0841 at time 0 (T0) and following storage at 40°C for 4 weeks in Tris buffer (TrisT4), histidine buffer (HisT4) or glutamic acid (Glu4) buffer.
- [0019]** **FIG. 3** depicts an exemplary competition ELISA analysis of antibody 841 (also known as 0841) following forced degradation. Antibody 841 was analyzed at time 0 (0wk) and following incubation in Tris, His or Glu buffer for 2 weeks (2wk) or 4 weeks (4wk) at 40°C.
- [0020]** **FIG. 4** depicts predicted non-germline T-cell epitopes for the VH and VL regions of humanized antibody 0841. Amino acid residues predicted by Epivax/ISPRI or IEDB to encompass T-cell epitopes not found in human germlines are underlined.
- [0021]** **FIG. 5** depicts exemplary neutralization of sialyl Lewis A ligand adhesion to Chinese hamster ovary (CHO) cells expressing human E-selectin. Antibodies tested included anti-E-selectin antibodies 164 (also known as 0164), 1282, 1284, 1444 and 1448 and an IgG isotype control.
- [0022]** **FIG. 6** depicts exemplary neutralization of sialyl Lewis X ligand adhesion to CHO cells expressing cynomolgus monkey E-selectin. Antibodies tested included anti-E-selectin antibodies 164 (also known as 0164), 1282, 1284, 1444, 1448 and an IgG isotype control.
- [0023]** **FIG. 7** depicts exemplary neutralization of HL-60 cell adhesion to CHO cells expressing human E-selectin by antibodies 1282, 1284, 1444, 1448 and 164 (also known as 0164).
- [0024]** **FIG. 8** depicts exemplary data from a three-part mass spectroscopy analysis of optimized anti-E-selectin antibody 1444 at time 0 (top panel) and following storage at 40°C for 4 weeks in Tris, His or Glu buffer.
- [0025]** **FIG. 9A-9D** depicts exemplary competition ELISA analysis of antibodies 1282 (FIG. 9A), 1284 (FIG. 9B), 1444 (FIG. 9C) and 1448 (FIG. 9D) following forced degradation. Antibodies were analyzed at time 1 (T0) and following incubation in Tris, His or Glu buffer for 2 weeks (T2) or 4 weeks (T4) at 40°C.
- [0026]** **FIG. 10A-10D** depicts aSEC analysis of high concentration samples of optimized anti-E-selectin antibody samples. Antibodies at a concentration of 150 mg/mL were analyzed at time 0 (0) and following storage at 4°C or 25°C in Tris/Suc, His/Suc or Glu/Tre buffer for 1, 2, 4, 6 and 7 weeks. The percentage of high molecular mass species (% HMMS) was quantified. FIG. 10A depicts results for antibody 1282; FIG. 10B depicts results for antibody

1284; FIG. 10C depicts results for antibody 1444; FIG. 10D depicts results for antibody 1448.

[0027] FIG. 11 depicts exemplary viscosity curves of optimized anti-E-selectin antibodies. The viscosity of optimized antibodies 1282, 1284, 1444 and 1448 was measured using a DLS bead-based method. Additional single data points are provided for each using an Anton Paar cone and plate method.

[0028] FIG. 12 depicts exemplary SPR analysis of optimized antibody 1444 binding to human E-selectin (rhE-Selectin), homologs P-selectin and L-selectin, and species homologs of rabbit, rat, mouse (muE-Selectin), and cynomolgus monkey (rcyE-Selectin).

[0029] FIG. 13 depicts exemplary binding of anti-E-selectin antibodies (0841, 1282, 1284, 1444 and 1448) to immobilized human L-selectin and human P-selectin. An anti-L-selectin control antibody and an anti-P-selectin control antibody bound human L-selectin and human P-selectin, respectively.

[0030] FIG. 14 depicts exemplary neutralization of adhesion of SCD patient cells (Donor 11253 and Donor 22358) to recombinant E-selectin under physiological flow.

DETAILED DESCRIPTION

[0031] The present disclosure provides antibodies, and antigen-binding fragments thereof, that specifically bind to E-selectin and reduce or inhibit E-selectin activity, including but not limited to, the ability of E-selectin to interact (e.g., bind) with ligands comprising carbohydrate structures with a sialyl Lewis (sLex) determinant on glycoproteins or glycolipids. The disclosure also provides processes for making, preparing, or producing anti-E-selectin antibodies. Antibodies of the disclosure are useful in the diagnosis, prophylaxis, and/or treatment of disorders or conditions mediated by, or associated with, E-selectin activity (e.g., binding), including, but not limited to, SCD, vaso-occlusive crisis, pain, organ infarction, ischemia, stroke and vascular obstruction. The disclosure further encompasses expression of antibodies, and preparation and manufacture of compositions comprising antibodies of the disclosure, or antigen-binding fragments thereof, such as medicaments for the use of the antibodies.

[0032] Polynucleotides encoding antibodies that bind E-selectin, or antigen-binding fragments thereof, are provided. Polynucleotides encoding antibody heavy chains or light chains, or both are also provided. Host cells that express anti-E-selectin antibodies are provided. Methods of treatment using antibodies to E-selectin are provided. Such methods include, but are not limited to, methods of treating and/or preventing diseases associated with or mediated by E-selectin expression and/or E-selectin binding to sLex ligands, including, but not limited to, SCD, vaso-occlusive crisis, pain, organ infarction, ischemia, stroke, end organ dysfunction, acute chest and vascular obstruction.

- [0033]** Without wishing to be bound by any particular theory, the selectin family of adhesion molecules (e.g., E-selectin) and their ligands play a critical role in regulating the initial contact of cell-cell adhesion, leukocyte rolling on the endothelium, integrin activation and transmigration of cells, all part of a pro-inflammatory response in SCD which manifests clinically as episodes of severe pain or vaso-occlusive crisis (including, e.g., vascular obstruction, organ infarction and ischemia). Thus, antibodies, and antigen-binding fragments thereof, of the disclosure enable selective antagonism of E-selectin activity and binding in the vascular system, thus reducing vaso-occlusion in a subject. In some embodiments disclosed in the Examples herein, antibodies, and antigen-binding fragments thereof, against E-selectin have been shown to inhibit, neutralize or reduce binding of E-selectin to its ligands when the E-selectin is in solution or expressed on cells (e.g., Chinese hamster ovary (CHO) cells, neutrophils and/or blood cells from patients with SCD) and its ligand(s) are in solution or expressed on cells (e.g., HL-60 cells, human umbilical vein endothelial cells (HUVEC), cynomolgus monkey lung microvascular endothelial cells (CLMEC)).
- [0034]** An anti-E-selectin antibody, or antigen-binding fragment thereof, including a humanized antibody, can be used, alone or in combination with a second therapy, in the prevention, treatment, and/or amelioration of at least one sign and/or symptom of SCD including vaso-occlusive crisis, pain, organ infarction, ischemia, stroke and vascular obstruction.
- [0035]** The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.
- [0036]** All references cited herein, including patent applications, patent publications, UniProtKB accession numbers are herein incorporated by reference, as if each individual reference were specifically and individually indicated to be incorporated by reference in its entirety.
- [0037]** The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al, *Molecular Cloning: A Laboratory Manual* 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY* (F. M. Ausubel, et al. eds., (2003)); the series *METHODS IN ENZYMOLOGY* (Academic Press, Inc.): *PCR 2: A PRACTICAL APPROACH* (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *ANTIBODIES, A LABORATORY MANUAL*, and *ANIMAL CELL CULTURE* (R. I. Freshney, ed. (1987)); *Oligonucleotide Synthesis* (M. J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J. E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R. I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J. P.

Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al, eds., 1994); Current Protocols in Immunology (J. E. Coligan et al, eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: A Practical Approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal Antibodies: A Practical Approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using Antibodies: A Laboratory Manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999)); The Antibodies (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and updated versions thereof.

[0038] The present disclosure may be understood more readily by reference to the following detailed description of exemplary embodiments of the invention and the Examples included therein.

[0039] Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

Definitions

[0040] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present specification, including definitions, will control.

[0041] Further, unless otherwise required by context or expressly indicated, singular terms shall include pluralities and plural terms shall include the singular.

[0042] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Moreover, all ranges disclosed herein are to be understood to encompass any and all subranges subsumed therein. For example, a stated range of "1 to 10" should be considered to include any and all subranges between (and inclusive of) the minimum value of 1 and the maximum value of 10; that is, all subranges

beginning with a minimum value of 1 or more, e.g. 1 to 6.1, and ending with a maximum value of 10 or less, e.g., 5.5 to 10.

[0043] As used herein, the singular form “a”, “an”, and “the” includes plural references unless indicated otherwise.

- 5 **[0044]** As used herein, the term “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative, “or”.

- [0045]** As used herein, the term “about,” or “approximately” refers to a measurable value such as an amount of the biological activity, length of a polynucleotide or polypeptide
10 sequence, content of G and C nucleotides, codon adaptation index, number of CpG dinucleotides, dose, time, temperature, and the like, and is meant to encompass variations of 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% 1%, 0.5% or even 0.1%, in either direction (greater than or less than) of the specified amount unless otherwise stated, otherwise evident from the context, or except
15 where such number would exceed 100% of a possible value.

- [0046]** As used herein, the term “ameliorate” means a detectable or measurable improvement in a subject’s disease, disorder or condition, (e.g., SCD) or symptom thereof (e.g., vaso-occlusive crisis), or an underlying cellular response. A detectable or measurable improvement includes a subjective or objective decrease, reduction, inhibition, suppression,
20 limit or control in the occurrence, frequency, severity, progression or duration of, complication caused by or associated with, improvement in a symptom of, or a reversal of the disease, disorder or condition.

- [0047]** As used herein “another” may mean at least a second or more. Unless otherwise defined herein, scientific and technical terms used in connection with the present invention
25 shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

- [0048]** As used herein, the term “associated with” refers to with one another, if the presence, level and/or form of one is correlated with that of the other. For example, a
30 particular entity (e.g., polypeptide, genetic signature, metabolite, microbe, etc.) is considered to be associated with a particular disease, disorder, or condition, if its presence, level and/or form correlates with incidence of and/or susceptibility to the disease, disorder, or condition (e.g., across a relevant population). In some embodiments, two or more entities are physically “associated” with one another if they interact, directly or indirectly, so that they are
35 and/or remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another; in some embodiments, two or more entities that are physically associated with one another

are not covalently linked to one another but are non-covalently associated, for example, by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and a combination thereof.

[0049] As used herein, the term “coding sequence” refers to a sequence which encodes a particular protein or “encoding nucleic acid,” denotes a nucleic acid sequence which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vitro* or *in vivo* when placed under the control of (operably linked to) appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and even synthetic DNA sequences.

[0050] Throughout this specification and claims, the word “comprise,” or variations such as “comprises” or “comprising,” and the words “having/including” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Any example(s) following the term “e.g.” or “for example” is not meant to be exhaustive or limiting. It is understood that wherever embodiments are described herein with the language “comprising,” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided.

[0051] As used herein, the term “conservative substitution” refers to replacement of one amino acid by a biologically, chemically or structurally similar residue. Biologically similar means that the substitution does not destroy a biological activity. Structurally similar means that the amino acids have side chains with similar length, such as alanine, glycine and serine or a similar size. Chemical similarity means that the residues have the same charge or are both hydrophilic or hydrophobic. Particular examples include the substitution of a hydrophobic residue, such as isoleucine, valine, leucine or methionine with another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic acid for aspartic acid or glutamine for asparagine, serine for threonine, and the like. Particular examples of conservative substitutions include the substitution of a hydrophobic residue such as isoleucine, valine, leucine or methionine for one another, the substitution of a polar residue for another, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like. Conservative amino acid substitutions typically include, for example, substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. A “conservative substitution”

also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid.

5 [0052] As used herein, the term “expression control sequence” means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

10 [0053] As used herein, the term an “effective dosage” or “effective amount” of drug, compound, or pharmaceutical composition is an amount sufficient to affect any one or more beneficial or desired results. In more specific aspects, an effective amount prevents, alleviates and/or ameliorates at least one sign and/or symptom of a disease, e.g., SCD. For prophylactic use, beneficial or desired results include eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histological and/or a behavioral symptom of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, 15 beneficial or desired results include clinical results such as reducing at least one sign and/or symptom of an E-selectin-mediated disease, disorder or condition, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication, and/or delaying the progression of the disease of patients. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective 20 dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective dosage” may be considered 25 in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

30 [0054] As used herein, the term “functional” refers to a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized. A biological molecule may have two functions (i.e., bifunctional) or many functions (i.e., multifunctional).

[0055] As used herein, the term “glycosylation pattern” means the pattern of carbohydrate units that are covalently attached to a protein (e.g., the glycoform) as well as to the site(s) to which the glycoform(s) are covalently attached to the peptide backbone of a protein, more specifically to an immunoglobulin protein.

35 [0056] As used herein, the term “homologous” or “homology” refer to two or more reference entities (e.g., nucleotide or polypeptide sequences) that share at least partial identity over a given region or fragment. For example, when an amino acid position in two

peptides is occupied by identical amino acids, the peptides are homologous at that position. Notably, a homologous peptide will retain activity or function associated with the unmodified or reference peptide and the modified peptide will generally have an amino acid sequence "substantially homologous" with the amino acid sequence of the unmodified sequence.

- 5 When referring to a polypeptide, nucleic acid or fragment thereof, "substantial homology" or "substantial similarity," means that when optimally aligned with appropriate insertions or deletions with another polypeptide, nucleic acid (or its complementary strand) or fragment thereof, there is sequence identity in at least about 95% to 99% of the sequence. The extent of homology (identity) between two sequences can be ascertained using computer program
- 10 or mathematical algorithm. Such algorithms that calculate percent sequence homology (or identity) generally account for sequence gaps and mismatches over the comparison region or area. Exemplary programs and algorithms are provided below.

- [0057]** As used herein, the terms "host cell," "host cell line," and "host cell culture" are used interchangeable and mean an individual cell or cell culture that can be or has been a
- 15 recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include "transformants," "transformed cells," and "transduced cells," which include the primary transformed or transduced cell and progeny derived therefrom without regard to the number of passages. Host cell progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or
- 20 deliberate mutation. A host cell includes cells transfected and/or transformed *in vivo* with a polynucleotide of this invention (e.g., a polynucleotide encoding an amino acid sequence of an anti-E-selectin antibody).

- [0058]** As used herein, the term "identity" or "identical to" refers to the overall relatedness between polymeric molecules, e.g., between nucleic acid molecules (e.g., DNA
- 25 molecules and/or RNA molecules) and/or between polypeptide molecules. "Identity" measures the percent of identical matches between two or more sequences with gap alignments addressed by a particular mathematical model of computer programs (i.e. "algorithms").

- [0059]** In some embodiments, polymeric molecules are considered to be "substantially identical" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%,
- 30 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical.

- [0060]** Calculation of the percent identity of two nucleic acid or polypeptide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second sequence for
- 35 optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least

80%, at least 90%, at least 95%, or 100% of the length of a reference sequence. The nucleotides at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (e.g., nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm.

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10 **[0061]** To determine percent identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST/. Other alignment programs include MegAlign® program in the Lasergene® suite of bioinformatics software (DNASTAR®, Inc., Madison, WI). Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wis., USA. Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc. Of particular interest are alignment programs that permit gaps in the sequence. Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See Meth. Mol. Biol. 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See J. Mol. Biol. 48: 443-453 (1970).

15 **[0062]** Also, of interest is the BestFit program using the local homology algorithm of Smith and Waterman (1981, Advances in Applied Mathematics 2: 482-489) to determine sequence identity. The gap generation penalty will generally range from 1 to 5, usually 2 to 4 and in some embodiments will be 3. The gap extension penalty will generally range from about 0.01 to 0.20 and in some instances will be 0.10. The program has default parameters determined by the sequences inputted to be compared. Preferably, the sequence identity is determined using the default parameters determined by the program. This program is available also from Genetics Computing Group (GCG) package, from Madison, WI, USA.

30 **[0063]** Another program of interest is the FastDB algorithm. FastDB is described in Current Methods in Sequence Comparison and Analysis, Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp. 127-149, 1988, Alan R. Liss, Inc. Percent sequence identity is calculated by FastDB based upon the following parameters: Mismatch Penalty: 1.00; Gap Penalty: 1.00; Gap Size Penalty: 0.33; and Joining Penalty: 30.0.

35 **[0064]** As used herein, the terms "increase," "improve," "decrease" or "reduce" indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of treatment described herein, or a measurement in a control

individual (or multiple control individuals) in the absence of the treatment described herein. In some embodiments, a "control individual" is an individual afflicted with the same form of disease or injury as an individual being treated. In some embodiments, a "control individual" is an individual that is not afflicted with the same form of disease or injury as an individual being treated.

5 **[0065]** As used herein, the term "isolated molecule" (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody or antigen-binding fragment thereof) means a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the cell from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

20 **[0066]** As used herein, the terms "leader peptide" or "leader sequence" or "leader signal sequence" or "signal sequence", (used interchangeably herein) mean any nucleic acid sequence, or amino acid sequence encoded thereby, that may be present on the 5' end of a nucleic acid molecule and/or at or near the N-terminus of a polypeptide, that when present may mediate the transport of the polypeptide to an organelle of destination, including, but not limited to, the secretion of the polypeptide from a cell. Such leader sequences include, but are not limited to, nucleic acid sequences comprising, e.g.,

ATGGAATGGAGCTGGGTCTTCTCTTCTTCCTGTCAGTAACTACAGGTGTCCACTCC
(SEQ ID NO: 140) and

30 ATGGGATGGAGCTGTATCATCCTCTTCTTGGTGGCAACAGCTACAGGCGTGCACTCC
(SEQ ID NO:141), and amino acid sequences encoded thereby, such as, but not limited to, MGWSCILFLVATATGVHS (SEQ ID NO:129) and MEWSWVFLFFLSVTTGVHS (SEQ ID NO:130) or other leader sequences such as MGWSCILFLVATATGAHS (SEQ ID NO:131). The invention encompasses these and any other leader signals (nucleic and amino acid sequences) known in the art or to be identified which can result in the transport of a polypeptide to the desired organelle, e.g., the endoplasmic reticulum, and/or secreted from the cell. Generally, the signal peptide is removed from and/or is not present in the mature polypeptide.

[0067] As used herein, the term "residue" means a position in a protein and its associated amino acid identity. For example, asparagine 297 (also referred to as Asn297, also referred to as N297) is a residue in a human antibody IgG1.

[0068] The term "similarity" is a related concept, but in contrast to "identity," refers to a measure of similarity which includes both identical matches and conservative substitution matches. Since conservative substitutions apply to polypeptides and not nucleic acid molecules, similarity only applies to polypeptide sequence comparisons. If two polypeptide sequences have, for example, 10 out of 20 identical amino acids, and the remainder are all nonconservative substitutions, then the percent identity and similarity would both be 50%. If in the same example, there are 5 more positions where there are conservative substitutions, then the percent identity remains 50%, but the percent similarity would be 75% (15 out of 20). Therefore, in cases where there are conservative substitutions, the degree of similarity between two polypeptide sequences will be higher than the percent identity between those two sequences.

[0069] As used herein, the term "subject" means a mammal, more preferably, a human. Mammals also include, but are not limited to, farm animals (e.g., cows, pigs, horses, chickens, etc.), pets, primates, horses, dogs, cats, mice and rats. In some embodiments, a subject is a patient. In some embodiments, a subject is at risk for a disease, disorder or condition mediated by or associated with E-selectin binding to its ligand. In some embodiments, a subject is a patient who has a disease, disorder or condition as described herein, e.g., SCD. In some embodiments, a subject (e.g., a patient) has SCD, a variant of SCD or SC disease. In some embodiments, a subject (e.g., a patient) has a HBSS, HBSC, HBS/ β° thal, HBS/ β^{+} thal, HBS/HPHP, HBSE or HBS-variant genotype.

[0070] As used herein, the term "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species (e.g., a glycoprotein, including an antibody or receptor) comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species. In certain embodiments, a substantially pure material is at least 50% pure (i.e., free from contaminants), more preferably, at least 90% pure, more preferably, at least 95% pure, yet more preferably, at least 98% pure, and most preferably, at least 99% pure.

[0071] Polypeptide or antibody “fragments” or “portions” according to the invention may be made by truncation, e.g. by removal of one or more amino acids from the N and/or C-terminal ends of a polypeptide. One, 2, 3, 4, 5, up to 10, up to 20, up to 30, up to 40 or more amino acids may be removed from the N and/or C terminus in this way. Fragments may also be generated by one or more internal deletions.

[0072] As used herein, the terms “nucleic acid sequence” and “nucleotide sequence,” refer interchangeably to any molecule composed of or comprising monomeric nucleotides. A nucleic acid may be an oligonucleotide or a polynucleotide. A nucleotide sequence may be a DNA or RNA (e.g., genomic DNA, cDNA, antisense DNA, mRNA, tRNA, rRNA, etc.). A nucleotide sequence may be chemically modified or artificial. Nucleotide sequences include peptide nucleic acids (PNA), morpholinos and locked nucleic acids (LNA), as well as glycol nucleic acids (GNA) and threose nucleic acids (TNA). Each of these sequences is distinguished from naturally occurring DNA or RNA by changes to the backbone of the molecule. Also, phosphorothioate nucleotides may be used. Other deoxynucleotide analogs include methylphosphonates, phosphoramidates, phosphorodithioates, N3'-P5'-phosphoramidates, and oligoribonucleotide phosphorothioates and their 2'-O-allyl analogs and 2'-O-methylribonucleotide methylphosphonates which may be used in a nucleotide sequence of the disclosure.

[0073] As used here, the term “nucleic acid construct,” refers to a non-naturally occurring nucleic acid molecule resulting from the use of recombinant DNA technology (e.g., a recombinant nucleic acid). A nucleic acid construct is a nucleic acid molecule, either single or double stranded, which has been modified to contain segments of nucleic acid sequences, which are combined and arranged in a manner not found in nature. A nucleic acid construct may be a “vector” (e.g., a plasmid), that is, a nucleic acid molecule designed to deliver exogenously created DNA into a host cell.

[0074] As used herein, the term “operably linked” refers to a linkage of polynucleotide (or polypeptide) elements in a functional relationship. A nucleic acid is operably linked when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or other transcription regulatory sequence (e.g., an enhancer) is operably linked to a coding sequence if it affects the transcription of the coding sequence. In some embodiments, operably linked means that the nucleic acid sequences being linked are contiguous. In some embodiments, operably linked does not mean that the nucleic acid sequences are contiguously linked, rather intervening sequences are between those nucleic acid sequences that are linked.

[0075] As used herein, the term “polynucleotide” (also referred to herein as a “nucleic acid molecule”) refers to a sequence of nucleotides connected by phosphodiester linkages. Polynucleotides are presented herein in the direction from the 5' to the 3' direction. A

polynucleotide of the present disclosure can be a deoxyribonucleic acid (DNA) molecule or ribonucleic acid (RNA) molecule and refers to all forms of a nucleic acid such as, double stranded molecules, single stranded molecules, small or short hairpin RNA (shRNA), micro RNA, small or short interfering RNA (siRNA), trans-splicing RNA, antisense RNA. Where a polynucleotide is a DNA molecule, that molecule can be a gene, a cDNA, an antisense molecule or a fragment of any of the foregoing molecules. Nucleotide bases are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C), inosine (I) and uracil (U). A polynucleotide of the present disclosure can be prepared using standard techniques well known to one of skill in the art.

10 **[0076]** As used herein, the terms "polypeptide," "protein" and "peptide" encoded by a polynucleotide (nucleic acid sequence or nucleotide sequence) refer to full-length native sequences, as with naturally occurring proteins, as well as functional subsequences, modified forms or sequence variants so long as the subsequence, modified form or variant retains some degree of functionality of the native full-length protein. In methods and uses of
15 the disclosure, such polypeptides, proteins and peptides encoded by the polynucleotide sequences can be but are not required to be identical to the endogenous protein that is defective, or whose expression is insufficient, or deficient in a subject treated with gene therapy.

[0077] As used herein, the term "prevent" or "prevention" refers to delay of onset, and/or
20 reduction in frequency and/or severity of at least one sign and/or symptom (e.g., vaso-occlusive crisis, pain) of a particular disease, disorder or condition (e.g., SCD). In some embodiments, prevention is assessed on a population basis such that an agent is considered to "prevent" a particular disease, disorder or condition if a statistically significant decrease in the development, frequency and/or intensity of one or more symptoms of the
25 disease, disorder or condition is observed in a population susceptible to the disease, disorder or condition. Prevention may be considered complete when onset of disease, disorder or condition has been delayed for a predefined period of time.

[0078] As used herein, the term "recombinant," refers to a vector, polynucleotide, polypeptide or cell that is the product of various combinations of cloning, restriction or
30 ligation steps (e.g. relating to a polynucleotide or polypeptide comprised therein), and/or other procedures that result in a construct that is distinct from a product found in nature.

[0079] As used herein, the terms "treat" or "treatment" means to administer a therapy that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features and causes of a
35 particular disease, disorder and/or condition (e.g., SCD). For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: improved survival rate (reduced mortality), reduction in the amount of tissue

fibrosis, decreased extent of damage from the disease, decreased duration of the disease, and/or reduction in the number, extent, or duration of a symptom related to the disease. The term includes the administration of a compound or agent of the present invention to prevent or delay the onset of a symptom, complication, or biochemical indicia of a disease, alleviating a symptom or arresting or inhibiting further development of a disease, condition, or disorder. Treatment may be prophylactic (to prevent or delay the onset of the disease, or to prevent the manifestation of a clinical or subclinical symptom thereof) or therapeutic suppression or alleviation of a symptom after the manifestation of the disease. In some embodiments, the disease, condition or disorder is SCD.

10

Antibodies

- [0080]** An “antibody” or “Ab” is an immunoglobulin molecule capable of recognizing and binding to a specific target or antigen (Ag), such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody” can encompass any type of antibody, including but not limited to monoclonal antibodies, polyclonal antibodies, antigen-binding fragments (or portion), of intact antibodies that retain the ability to specifically bind to a given antigen (e.g., E-selectin), and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site.
- [0081]** An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or subclass thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains (HC), immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂. In some embodiments, an anti-E-selectin antibody of the present disclosure is an IgG1 antibody. The heavy chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.
- [0082]** Antibodies may be derived from any mammal, including, but not limited to, humans, monkeys, pigs, horses, rabbits, dogs, cats, mice, rats (e.g., a Sprague Dawley rat) etc., or other animals such as birds (e.g. chickens), fish (e.g., sharks) and camelids (e.g., llamas).
- [0083]** The term “antigen” refers to the molecular entity used for immunization of an immunocompetent vertebrate to produce the antibody that recognizes the antigen or to screen an expression library (e.g., phage, yeast or ribosome display library, among others). Herein, antigen is termed more broadly and is generally intended to include target molecules

that are specifically recognized by the antibody, thus including fragments or mimics of the molecule used in an immunization process for raising the antibody or in library screening for selecting the antibody. Thus, for antibodies of the invention binding to E-selectin, full-length E-selectin from mammalian species (e.g., human, monkey (including cynomolgus monkey), mouse, rabbit and rat), including monomers and multimers, such as dimers, trimers, etc. thereof, truncated and other variants of E-selectin (e.g., extracellular domain), as well as soluble E-selectin and cell-surface expressed E-selectin, are referred to herein as an antigen.

[0084] An “*antigen-binding fragment*” of an antibody refers to a one or more fragments of a full-length antibody that retains the ability to specifically bind to an antigen (preferably with substantially the same binding affinity). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term “antigen-binding fragment” of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., Nature 1989; 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR), disulfide-linked Fvs (dsFv), and anti-idiotypic (anti-Id) antibodies and intrabodies. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv)); see e.g., Bird et al., Science 1988; 242:423-426 and Huston et al., Proc. Natl. Acad. Sci. USA 1988 85:5879-5883. Other forms of single chain antibodies, such as diabodies are also encompassed. Such single chain antibodies are also intended to be encompassed within the term “antigen-binding fragment” of an antibody. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen-binding sites (see e.g., Holliger et al, Proc. Natl. Acad. Sci. USA 1993; 90:6444-6448; Poljak et al., Structure 1994; 2:1121-1123).

[0085] An antibody “variable domain” refers to the variable region of the antibody light chain (VL) or the variable region of the antibody heavy chain (VH), either alone or in combination. As known in the art, the variable regions of the heavy and light chains each consist of four framework regions (FR) connected by three “complementarity determining regions” (CDRs) and contribute to the formation of the antigen-binding site of antibodies. If

variants of a subject variable region are desired, particularly with substitution in amino acid residues outside of a CDR region (i.e., in the framework region), appropriate amino acid substitution, preferably, conservative amino acid substitution, can be identified by comparing the subject variable region to the variable regions of other antibodies which contain CDR1 and CDR2 sequences in the same canonical class as the subject variable region (Chothia and Lesk, J. Mol. Biol. 1987; 196(4): 901-917).

[0086] Residues in a variable domain are typically numbered according Kabat, which provides a numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies. See, Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence. Various algorithms for assigning Kabat numbering are available. For example, the algorithm implemented in the version 2.3.3 release of Abysis (www.abysis.org) can be used to assign Kabat numbering to variable regions LCDR-1, LCDR-2, LCDR-3, HCDR-2, and HCDR-3, and the AbM definition can then be used for HCDR-1.

[0087] In certain embodiments, definitive delineation of a CDR and identification of residues comprising the binding site of an antibody is accomplished by solving the structure of the antibody and/or solving the structure of the antibody-ligand complex. In certain embodiments, that can be accomplished by any of a variety of techniques known to those skilled in the art, such as X-ray crystallography. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. Examples of such methods include, but are not limited to, the Kabat definition, the Chothia definition, the AbM definition, the contact definition, and the conformational definition.

[0088] "Complementarity Determining Regions" (CDRs) can be identified according to the definitions of Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, North, and/or conformational definitions or any method of CDR determination well known in the art. See, e.g., Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th ed. (hypervariable regions); Chothia et al., Nature 1989; 342:877-883 (structural loop structures). The identity of the amino acid residues in a particular antibody that make up a CDR can be determined using methods well known in the art. The AbM definition of CDRs is a compromise between Kabat and Chothia and uses Oxford Molecular AbM antibody

modeling software (Accelrys®).

[0089] The “contact” definition of CDRs is based on observed antigen contacts, set forth in MacCallum et al., J. Mol. Biol. 1996; 262:732-745. The “conformational” definition of CDRs is based on residues that make enthalpic contributions to antigen binding (see, e.g., Makabe et al., J. Biol. Chem., 2008; 283:1156-1166). North has identified canonical CDR conformations using a different preferred set of CDR definitions (North et al., J. Mol. Biol. 2011; 406: 228-256). In another approach, referred to herein as the “conformational definition” of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding (Makabe et al., J. Biol. Chem. 2008, 283:1156-1166). Still other CDR boundary definitions may not strictly follow one of the above approaches but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs (or other residue of the antibody) may be defined in accordance with any of Kabat, Chothia, North, extended, AbM, contact, and/or conformational definitions.

[0090] “Contact residue” as used herein with respect to an antibody or the antigen specifically bound thereby, refers to an amino acid residue present on an antibody/antigen comprising at least one heavy atom (i.e., not hydrogen) that is within 4 Å or less of a heavy atom of an amino acid residue present on the cognate antibody/antigen.

[0091] “Framework” (FR) residues are antibody variable domain residues other than the CDR residues. A VH or VL domain framework comprises four framework sub-regions, FR1, FR2, FR3 and FR4, interspersed with CDRs in the following structure: FR1 – CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4.

[0092] As known in the art, a “constant region” of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

[0093] The terms “IgG Fc region,” “Fc region,” “Fc domain” and “Fc,” as interchangeably used herein, refer to the portion of an immunoglobulin (Ig) molecule that correlates to a crystallizable fragment obtained by papain digestion of an Ig molecule. As used herein, the terms relate to the constant region of an antibody excluding the first constant region immunoglobulin domain and further relates to portions of that region. Thus, Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains, or portions thereof. For IgA and IgM, Fc may include the J chain.

[0094] For IgG, Fc comprises immunoglobulin domains C γ 2 and C γ 3 (C gamma 2 and C gamma 3) and the hinge between C γ 1 (C gamma 1) and C γ 2 (C gamma 2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index of Edelman et al., Proc. Natl. Acad. Sci. USA 1969; 63(1):78-85 and as described in Kabat et al., 1991. Typically, the Fc domain comprises from about amino acid residue 236 to about 447 of the human IgG1 constant domain. An exemplary human wild type IgG1 Fc domain amino acid sequence is set forth in SEQ ID NO: 16 and SEQ ID NO: 15 (including an optional terminal lysine (K) residue). Fc polypeptide may refer to this region in isolation, or this region in the context of an antibody, or an antigen-binding fragment thereof, or Fc fusion protein.

[0095] The heavy chain constant domain comprises the Fc region and further comprises the CH1 domain and hinge as well as the CH2 and CH3 (and, optionally, CH4 of IgA and IgE) domains of the IgG heavy chain.

[0096] A "functional Fc region" possesses at least one effector function of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down-regulation of cell surface receptors (e.g., B cell receptor), B cell activation, etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain or antigen-binding fragment thereof) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

[0097] As used herein, "Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. In some embodiments, an Fc $\square$ R is a native human FcR. In some embodiments, an FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the Fc $\square$ RI, Fc $\square$ RII, and Fc $\square$ RIII subclasses, including allelic variants and alternatively spliced forms of those receptors. Fc $\square$ RII receptors include Fc $\square$ RIIA (an "activating receptor") and Fc γ RIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor Fc γ RIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor Fc γ RIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain, (see, e.g., Daeron, Annu. Rev. Immunol. 1997; 15:203-234). FcRs are reviewed, for example, in Ravetch and Kinet, Annu. Rev. Immunol 1991; 9:457-92; Capel et al., Immunomethods 1994; 4:25-34; and de Haas et al., J. Lab. Clin. Med. 1995; 126:330-41. Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

[0098] The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which

is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 1976; 117:587 and Kim et al., J. Immunol. 1994; 24:249) and regulation of homeostasis of immunoglobulins. Methods of measuring binding to FcRn are known (see, e.g., Ghetie and Ward., Immunol. Today 1997; 18(12):592-598; Ghetie et al., Nature Biotechnology, 1997; 15(7):637- 640; Hinton et al., J. Biol. Chem. 2004; 279(8):6213-6216; WO 2004/92219).

[0099] "Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. In certain embodiments, the cells express at least FcγRIII and perform ADCC effector function(s). Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, macrophages, cytotoxic T cells, and neutrophils. The effector cells may be isolated from a native source, e.g., from blood.

[0100] As used herein, "antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., NK cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII, and FcγRIII. To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. Nos. 5,500,362, 5,821,337 or 6,737,056, may be performed. Useful effector cells for such assays include PBMC and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes et al., Proc. Natl. Acad. Sci. (USA) 1998; 95:652-656. Additional antibodies with altered Fc region amino acid sequences and increased or decreased ADCC activity are described, e.g., in U.S. Pat. No. 7,923,538, and U.S. Pat. No. 7,994,290.

[0101] An antibody having an "enhanced ADCC activity" refers to an antibody that is more effective at mediating ADCC *in vitro* or *in vivo* compared to the parent antibody, wherein the antibody and the parent antibody differ in at least one structural aspect, and when the amounts of such antibody and parent antibody used in the assay are essentially the same. In some embodiments, the antibody and the parent antibody have the same amino acid sequence, but the antibody is afucosylated while the parent antibody is fucosylated. In some embodiments, ADCC activity will be determined using the *in vitro* ADCC assay as herein disclosed, but other assays or methods for determining ADCC activity, e.g. in an animal model etc., are contemplated. In some embodiments, an antibody with enhanced ADCC activity has enhanced affinity for Fc gamma RIIIA.

[0102] An antibody with "altered" FcR binding affinity or ADCC activity is one which has either enhanced or diminished FcR binding activity and/or ADCC activity compared to a parent antibody, wherein the antibody and the parent antibody differ in at least one structural

aspect. An antibody that “displays increased binding” to an FcR binds at least one FcR with better affinity than the parent antibody. An antibody that “displays decreased binding” to an FcR, binds at least one FcR with lower affinity than a parent antibody. Such antibodies that display decreased binding to an FcR may possess little or no appreciable binding to an FcR, e.g., 0-20 percent binding to the FcR compared to a native sequence IgG Fc region.

[0103] “Enhanced affinity for FcγRIIIA” refers to an antibody that has greater affinity for FcγRIIIA than a parent antibody, wherein the antibody and the parent antibody differ in at least one structural aspect.

[0104] “Complement dependent cytotoxicity” or “CDC” refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass), which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al., J. Immunol. Methods 1996; 202: 163, may be performed. Antibodies with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described, e.g., in U.S. Pat. No. 6,194,551, U.S. Pat. No. 7,923,538, U.S. Pat. No. 7,994,290 and WO 1999/51642.

[0105] A heavy chain constant domain comprises a Fc region and further comprises the CH1 domain and hinge as well as the CH2 and CH3 (and, optionally, CH4 of IgA and IgE) domains of the IgG heavy chain.

[0106] In some embodiments, where an anti-E-selectin antibody comprises a C-terminal lysine (K) amino acid residue on a heavy chain polypeptide (e.g., human IgG1 heavy chain comprises a terminal lysine), one skilled in the art would understand that the lysine residue may be clipped resulting in an antibody with a heavy chain lacking the C-terminal lysine residue. Additionally, the antibody heavy chain may be produced using a nucleic acid that does not encode the lysine. Thus, in some embodiments, an anti-E-selectin antibody comprises a heavy chain where the terminal lysine otherwise present is not present.

[0107] In certain embodiments, the antibody, or antigen-binding fragment thereof, described herein comprises an Fc domain. The Fc domain can be derived from IgA (e.g., IgA₁ or IgA₂), IgD, IgE, IgM, or IgG (e.g., IgG₁, IgG₂, IgG₃, or IgG₄). In some embodiments, an anti-E-selectin antibody is an IgG antibody. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444) is an IgG₁ antibody.

[0108] An “Fc fusion” protein is a protein wherein one or more polypeptides are operably linked to an Fc polypeptide. An Fc fusion combines the Fc region of an immunoglobulin with a fusion partner.

[0109] A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at

least one amino acid modification yet retains at least one effector function of the native sequence Fc region. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably, at least about 90% sequence identity therewith, more preferably, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity therewith.

[0110] An "epitope" refers to the area or region of an antigen to which an antibody specifically binds, e.g., an area or region comprising residues that interact with the antibody, as determined by any method well known in the art, for example, by conventional immunoassays or as described in Example 9 and 10 of the present disclosure. There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, *Using Antibodies*, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999. In an additional example, epitope mapping can be used to determine the sequence to which an anti-E-selectin antibody binds. Epitope mapping is commercially available from various sources, for example, Pepscan Systems (Edelhertweg 15, 8219 PH Lelystad, The Netherlands). Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition and cross-competition studies to find antibodies that compete or cross-compete with one another for binding to E-selectin, e.g., the antibodies compete for binding to the antigen.

[0111] In addition, the epitope to which the anti-E-selectin antibody binds can be determined in a systematic screening by using overlapping peptides derived from the E-selectin (e.g., a human E-selectin sequence) and determining binding by the antibody. According to the gene fragment expression assays, the open reading frame encoding E-selectin can be fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of E-selectin with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein *in vitro*, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled E-selectin fragments is then

determined by immunoprecipitation and gel electrophoresis.

[0112] Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries) or yeast (yeast display). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. In an additional example, mutagenesis of an antigen, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or necessary for epitope binding.

[0113] At its most detailed level, the epitope for the interaction between the antigen and the antibody can be defined by the spatial coordinates defining the atomic contacts present in the antigen-antibody interaction, as well as information about their relative contributions to the binding thermodynamics. At a less detailed level, the epitope can be characterized by the spatial coordinates defining the atomic contacts between the antigen and antibody. At a further less detailed level the epitope can be characterized by the amino acid residues that it comprises as defined by a specific criterion, e.g., by distance between atoms (e.g., heavy, i.e., non-hydrogen atoms) in the antibody and the antigen. At a further less detailed level the epitope can be characterized through function, e.g., by competition binding with other antibodies. The epitope can also be defined more generically as comprising amino acid residues for which substitution by another amino acid will alter the characteristics of the interaction between the antibody and antigen (e.g. using alanine scanning).

[0114] From the fact that descriptions and definitions of epitopes, dependent on the epitope mapping method used, are obtained at different levels of detail, it follows that comparison of epitopes for different antibodies on the same antigen can similarly be conducted at different levels of detail.

[0115] Epitopes described at the amino acid level, e.g., determined from an X-ray crystallography, Nuclear Magnetic Resonance (NMR) spectroscopy, hydrogen/deuterium exchange Mass Spectrometry (H/D-MS), are said to be identical if they contain the same set of amino acid residues. Epitopes are said to overlap if at least one amino acid is shared by the epitopes. Epitopes are said to be separate (unique) if no amino acid residue is shared by the epitopes.

[0116] Yet another method which can be used to characterize an anti-E-selectin antibody is to use competition assays (e.g., as described in Example 9 of the present disclosure) with other antibodies known to bind to the same antigen, to determine if an anti-E-selectin antibody binds to the same epitope as other antibodies. Competition assays are well known to those of skill in the art. Epitopes characterized by competition binding are said to be overlapping if the binding of the corresponding antibodies are mutually exclusive, i.e., binding of one antibody excludes simultaneous or consecutive binding of the other antibody. The epitopes are said to be separate (unique) if the antigen is able to accommodate binding

of both corresponding antibodies simultaneously.

[0117] Epitopes can be linear or conformational. In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. A “nonlinear epitope” or
5 “conformational epitope” comprises noncontiguous polypeptides (or amino acids) within the antigenic protein to which an antibody specific to the epitope binds.

[0118] The binding affinity of an antibody can be expressed as K_D value, which refers to the dissociation rate of a particular antigen-antibody interaction. K_D is the ratio of the rate of dissociation, also called the “off-rate (k_{off}),” or “ k_d ” to the association rate, also called the “on-
10 rate (k_{on})” or “ k_a .” Thus, K_D equals k_{off} / k_{on} (or k_d/k_a) and is expressed as a molar concentration (M), and the smaller the K_D , the stronger the affinity of binding. K_D values for antibodies can be determined using methods well established in the art. One exemplary method for measuring K_D is surface plasmon resonance (SPR), typically using a biosensor system such as a BIAcore® system. BIAcore kinetic analysis comprises analyzing the
15 binding and dissociation of an antigen from chips with immobilized molecules (e.g. molecules comprising epitope binding domains), on their surface. Another method for determining the K_D of an antibody is by using Bio-Layer Interferometry, typically using OCTET technology (Octet QKe system, ForteBio). Alternatively, or in addition, a KinExA (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, ID) can also
20 be used.

[0119] An antibody that “preferentially binds” or “specifically binds” (used interchangeably herein) to an epitope is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule (e.g., a protein, a nucleic acid, an antibody, and the like) is said to exhibit “specific binding”
25 or “preferential binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody “specifically binds” or “preferentially binds” to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds
30 to an E-selectin epitope is an antibody that binds a particular epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other E-selectin epitopes or non-E-selectin epitopes, including P-selectin and/or L-selectin epitopes. Thus, under designated assay conditions, the specified binding moiety (e.g., an antibody or an antigen-binding fragment thereof or a receptor or a ligand binding fragment thereof) binds
35 preferentially to a particular target molecule and does not bind in a significant amount to other components present in a test sample. Generally, but not necessarily, reference to binding means preferential binding.

[0120] A variety of assay formats may be used to select an antibody or peptide that specifically binds a molecule of interest. For example, solid-phase ELISA immunoassay (including a competition binding ELIA), AlphaLISA® immunoassay (Perkin-Elmer), immunoprecipitation, BIAcore™ (GE Healthcare, Piscataway, NJ), fluorescence-activated cell sorting (FACS), Octet™ (FortéBio, Inc., Menlo Park, CA) and Western blot analysis are among many assays that may be used to identify an antibody that specifically reacts with an antigen or a receptor, or ligand binding fragment thereof, that specifically binds with a cognate ligand or binding partner. Typically, a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 times background, more than 50 times background, more than 1000 times background or more. An antibody is said to “specifically bind” an antigen when the equilibrium dissociation constant (K_D) is $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$ or $\leq 100 \text{ pM}$. In some embodiments, an anti-E-selectin antibody binds E-selectin (e.g., human E-selectin) with a K_D of $<70 \text{ nM}$ (e.g., $68.4 \pm 3.18 \text{ nM}$). In some embodiments, an anti-E-selectin antibody binds E-selectin (e.g., cynomolgus monkey E-selectin) with a K_D of $<68 \text{ nM}$ (e.g., $64.9 \pm 1.13 \text{ nM}$).

[0121] In some embodiments, an anti-E-selectin antibody binds human E-selectin with a K_D selected from the group consisting of about 92.85 nM , about 70.3 nM , about 65.2 nM , about 61.8 nM , about 60.5 nM , about 68.0 nM , about 21.6 nM , about 324 nM , about 54.4 nM , about 628.5 nM and 2940 nM . In some embodiments, an anti-E-selectin antibody binds cynomolgus monkey E-selectin with a K_D selected from the group of about 138.5 nM , about 78.3 nM , about 76.5 nM , about 81.5 nM , about 67.8 nM , about 45.8 nM , about 243.5 nM , about 45.4 nM , about 492 nM and 3145 nM .

[0122] The term “compete,” as used herein with regard to an antibody, means that binding of a first antibody, or an antigen-binding fragment thereof, to an antigen reduces the subsequent binding of the same antigen by a second antibody or an antigen-binding fragment thereof. The alternative, where the binding of the second antibody to an antigen is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to an antigen without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to “cross-compete” with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or fragment thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed

and can be useful for the methods disclosed herein.

[0123] Standard competition assays may be used to determine whether two antibodies compete with each other. One suitable assay for antibody competition involves the use of the Biacore technology, which can measure the extent of interactions using surface plasmon resonance (SPR) technology, typically using a biosensor system (such as a BIACORE system). For example, SPR can be used in an *in vitro* competitive binding inhibition assay to determine the ability of one antibody to inhibit the binding of a second antibody. Another assay for measuring antibody competition uses an ELISA-based approach.

[0124] Furthermore, a high throughput process for “binning” antibodies based upon their competition is described in International Patent Application No. WO2003/48731. Competition is present if one antibody (or fragment) reduces the binding of another antibody (or fragment) to E-selectin. For example, a sequential binding competition assay may be used, with different antibodies being added sequentially. The first antibody may be added to reach binding that is close to saturation. Then, the second antibody is added. If the binding of second antibody to E-selectin is not detected, or is significantly reduced (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% reduction) as compared to a parallel assay in the absence of the first antibody (which value can be set as 100%), the two antibodies are considered as competing with each other.

[0125] The definition of the term “paratope” is derived from the above definition of “epitope” by reversing the perspective. Thus, the term “paratope” refers to the area or region on the antibody which specifically binds an antigen, i.e., the amino acid residues on the antibody which make contact with the antigen (E-selectin, or a fragment thereof) as “contact” is defined elsewhere herein. The paratope for a given antibody/antigen pair may be identified by routine methods. For example, the antibody and target molecule may be combined, and the antibody/antigen complex may be crystallized. The crystal structure of the complex may be determined and used to identify specific sites of interaction between the antibody and its target.

[0126] In some embodiments, an antibody is a “variant antibody”. A variant antibody may comprise 1, 2, 3, 4, 5, up to 10, up to 20, up to 30 or more amino acid substitutions and/or deletions and/or insertions from the specific sequences and fragments disclosed herein, and in particular in Table 2. “Deletion” variants may comprise the deletion of individual amino acids, deletion of small groups of amino acids such as 1, 2, 3, 4 or 5 amino acids, or deletion of larger amino acid regions, such as the deletion of specific amino acid domains or other features. “Insertion” variants may comprise the insertion of individual amino acids, insertion of small groups of amino acids such as 1, 2, 3, 4 or 5 amino acids, or insertion of larger amino acid regions, such as the insertion of specific amino acid domains

or other features. "Substitution" variants preferably involve the replacement of one or more amino acids with the same number of amino acids and making conservative amino acid substitutions. For example, an amino acid may be substituted with an alternative amino acid having similar properties, for example, another basic amino acid, another acidic amino acid, another neutral amino acid, another charged amino acid, another hydrophilic amino acid, another hydrophobic amino acid, another polar amino acid, another aromatic amino acid or another aliphatic amino acid.

[0127] Substitution variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but framework alterations are also contemplated. Conservative substitutions are shown in Table 1. If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" shown below, or as further described below in reference to amino acid classes, may be introduced and the products screened.

TABLE 1
Amino Acids and Substitutions

Original Residue	Conservative Substitutions	Exemplary Substitutions
alanine Ala (A)	Val	Val; Leu; Ile
arginine Arg (R)	Lys	Lys; Gln; Asn
asparagine Asn (N)	Gln	Gln; His; Asp, Lys; Arg
aspartic Asp (D)	Glu	Glu; Asn
cysteine Cys (C)	Ser	Ser; Ala
glutamine Gln (Q)	Asn	Asn; Glu
glutamic Glu (E)	Asp	Asp; Gln
glycine Gly (G)	Ala	Ala
histidine His (H)	Arg	Asn; Gln; Lys; Arg
isoleucine Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
leucine Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
lysine Lys (K)	Arg	Arg; Gln; Asn
methionine Met (M)	Leu	Leu; Phe; Ile
phenylalanine Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
proline Pro (P)	Ala	Ala

Original Residue	Conservative Substitutions	Exemplary Substitutions
serine Ser (S)	Thr	Thr
threonine Thr (T)	Ser	Ser
tryptophan Trp (W)	Tyr	Tyr; Phe
tyrosine Tyr (Y)	Phe	Trp; Phe; Thr; Ser
valine Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

[0128] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta-sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- i. Non-polar: Norleucine, Met, Ala, Val, Leu, Ile;
- ii. Polar without charge: Cys, Ser, Thr, Asn, Gln;
- iii. Acidic (negatively charged): Asp, Glu;
- iv. Basic (positively charged): Lys, Arg;
- v. Residues that influence chain orientation: Gly, Pro; and
- vi. Aromatic: Trp, Tyr, Phe, His.

[0129] Non-conservative substitutions are made by exchanging a member of one of these classes for another class.

[0130] One type of substitution, for example, that may be made is to change one or more cysteines in the antibody, which may be chemically reactive, to another residue, such as, without limitation, alanine or serine. For example, there can be a substitution of a non-canonical cysteine. The substitution can be made in a CDR or framework region of a variable domain or in the constant region of an antibody. In some embodiments, the cysteine is canonical. Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant cross-linking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability, particularly where the antibody is an antibody fragment such as an Fv fragment.

[0131] In a process known as "germlining," certain amino acids in the VH and VL sequences can be mutated to match those found naturally in germline VH and VL sequences. In particular, the amino acid sequences of the framework regions in the VH and

VL sequences can be mutated to match the germline sequences to reduce the risk of immunogenicity when the antibody is administered. As used herein, the term "germline" refers to the nucleotide sequences and amino acid sequences of the antibody genes and gene segments as they are passed from parents to offspring via the germ cells. This

5 germline sequence is distinguished from the nucleotide sequences encoding antibodies in mature B cells which have been altered by recombination and hypermutation events during the course of B cell maturation. An antibody that "utilizes" a particular germline has a nucleotide or amino acid sequence that most closely aligns with that germline nucleotide sequence or with the amino acid sequence that it specifies. Such antibodies frequently are

10 mutated compared with the germline sequence. Germline DNA sequences for human VH and VL genes are known in the art (see e.g., the "Vbase" human germline sequence database; see also Kabat, E. A., et al., 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson et al., J. Mol. Biol. 1992; 227:776-798; and Cox et al., Eur. J. Immunol.

15 1994; 24:827-836).

Antibodies to E-selectin

[0132] The disclosure provides antibodies, and antigen-binding fragments thereof, that bind to E-selectin. E-selectin is also known as CD62 antigen-like family member E (CD62E),

20 endothelial-leukocyte adhesion molecule 1 (ELAM-1) or leukocyte-endothelial cell adhesion molecule 2 (LECAM2).

[0133] As used herein, the term "E-selectin" includes variants, isoforms, homologs, orthologs and paralogs of human E-selectin. In some embodiments, an antibody, or antigen-binding fragment thereof, disclosed herein cross-reacts with E-selectin from species other

25 than human, such as E-selectin of cynomolgus monkey, as well as different forms of E-selectin. In some embodiments, an antibody, or antigen binding fragment thereof, may be completely specific for human E-selectin and may not exhibit species cross-reactivity (e.g., does not bind mouse E-selectin) or other types of cross-reactivity (e.g., does not bind P-selectin and/or L-selectin). As used herein the term E-selectin refers to naturally occurring

30 human E-selectin unless contextually dictated otherwise. Therefore, an "E-selectin antibody, or antigen-binding fragment thereof," "anti- E-selectin antibody, or antigen-binding fragment thereof" or other similar designation means any antibody, or antigen-binding fragment thereof, (as defined herein) that specifically and/or preferentially associates, binds or reacts with E-selectin, an isoform, fragment or derivative thereof. The full length, mature form of

35 human E-selectin, as represented by UniProtKB/Swiss-Prot accession number P16581 (amino acids 22-610) is herein provided as SEQ ID NO:132. The full length, mature form of mouse E-selectin, as represented by UniProtKB/Swiss-Prot accession number Q00690

(amino acids 22-612) is herein provided as SEQ ID NO:134. The full length, mature form of cynomolgus E-selectin, as represented by UniProtKB/Swiss-Prot accession number G8F370 (amino acids 22-610) is herein provided as SEQ ID NO:201.

[0134] E-selectin is expressed on endothelial cells, L-selectin is constitutively expressed on leukocyte microvilli, and P-selectin is stored in the α -granules of platelets and Weibel-Palade bodies of endothelial cells (Tedder et al., FASEB J. 1995; 9:866-873; Kanas et al., Blood 1996; 88:3259-3287). They all bind to carbohydrate structures with a sialyl Lewis (sLex) determinant on glycoproteins or glycolipids (Chase et al., Ann. Biomed. Eng. 2012; 40(4):849-885). P- and L-selectins also require sulfation on the ligand for optimal binding, while E-selectin binding to ligands is more permissive requiring only sialyl Lewis determinants. The P-selectin glycoprotein ligand-1 (PSGL-1) is expressed on leukocytes and binds to all of the selectins. E-selectin also interacts with ligands including L-selectin ligand, CD44 and E-selectin ligand-1 (ESL-1) (Chase et al., Ann. Biomed. Eng. 2012; 40(4):849-885; Hidalgo et al., Immunity 2007; 26(4):477-489).

[0135] Expression of E-selectin by endothelial cells requires new protein synthesis in response to hypoxia or an inflammatory stimulus. E-selectin is critical for neutrophil adherence to the endothelium and generation of waves of activating signals on the endothelium that produce a polarized expression of activated $\alpha M\beta 2$ integrin (Hidalgo et al., Nat. Med. 2009; 15:384-391; Pruenster et al., Nat. Commun. 2015; 6:6915; Manwani, & Frenette, Blood 2014; 122:3892-3898). E-selectin-deficient mice do not exhibit an obvious phenotype distinguishable from wild-type mice (Labow et al., Immunity 1994; 1:709-720).

[0136] In addition to endothelial cell E-selectin, soluble E-selectin (sE-selectin) is found in circulation. The mechanism for sE-selectin release into the circulation may be enzymatic cleavage or result from shedding of damaged or activated endothelial cells; however, the precise mechanism is not known (Roldán et al., Thromb. Haemost. 2003; 90:1007-1020). The concentration of sE-selectin appears to correlate with its expression on the surface of endothelial cells, thus plasma sE-selectin concentration might be a marker of endothelial cell damage or activation (Leeuwenberg et al., Immunology 1992; 77(4):543-549; Roldán et al., Thromb. Haemost. 2003; 90:1007-1020). Increased levels of circulating soluble E-selectin levels have been found in a number of disease states including hypertension, diabetes and hyperlipidemia (Roldán et al., Thromb. Haemost. 2003; 90:1007-1020). Kato et al. studied 160 SCD patients and 41 control subjects and found that soluble E-selectin was significantly elevated in the plasma of SCD patients as compared to controls (median 74.6 . 41.5 ng/mL, $p < 0.001$) (British J. Haem. 2005; 130:943-953). Mild and moderate levels of pulmonary hypertension were significantly associated with the linear concentration of sE-selectin and had an increased relative risk for early mortality in SCD patients (RR of 4.2; 95% CI 2.0,8.9) (Kato et al.).

[0137] Preferably, antibodies, and antigen binding fragments thereof, of the present disclosure bind to E-selectin but do not bind, or bind at a lower affinity, to other selectins (e.g., P-selectin, L-selectin). In some embodiments, antibodies, or antigen-binding fragments thereof, of the present disclosure specifically bind E-selectin, and more preferably, specifically bind human and/or cynomolgus monkey E-selectin. The disclosure also provides for compositions comprising such antibodies, and antigen-binding fragments thereof, as well as uses for such antibodies, including therapeutic and pharmaceutical uses.

[0138] An anti-E-selectin antibody, preferably, a high affinity antibody (e.g., a specific antibody), may be effective in the vascular system and multiple tissue compartments, where E-selectin is expressed on the surface of endothelial cells, or found in a soluble form, and is thought to interact with target cells (e.g., neutrophils, monocytes, eosinophils, memory-effector T-like lymphocytes, natural killer cells, myeloid cells) expressing E-selectin ligands. Antibodies, and antigen-binding fragments thereof, of the disclosure have the potential to inhibit binding of E-selectin to ligands including, for example, glycoproteins or glycolipids with a sialyl Lewis (sLex) determinant (e.g., $\alpha 2,3$ sialylated and $\alpha 1,3$ or $\alpha 1,4$ fucosylated tetrasaccharide sialyl Lewis x), sialyl Lewis A determinant, E-selectin ligand-1 (ESL-1), L-selectin, CD44, P-selectin glycoprotein ligand-1 (PSGL-1), lysosomal-associated membrane protein 1 (LAMP1), lysosomal-associated membrane protein 2 (LAMP2), death receptor-3 (DR3) and $\alpha M\beta 2$ integrin (CD11b/CD18; Mac-1) (Chase et al., *Annals Biomed. Engin.* 2012; 40(4): 849-859). Without wishing to be bound by any particular theory, blockade of the E-selectin cellular ligand interaction inhibits leukocyte (e.g., neutrophil) adherence to the endothelium preventing cellular aggregates from blocking blood flow and leading to VOC.

[0139] A neutralizing or "blocking" antibody refers to an antibody whose binding to E-selectin (i) interferes with, limits, or inhibits the interaction between E-selectin, or an E-selectin fragment, and a E-selectin ligand, such as a sLex determinant; and/or (ii) results in inhibition of at least one biological function of E-selectin binding. Assays to determine neutralization by an antibody of the disclosure are described elsewhere herein and are well-known in the art.

[0140] "Biological function" or "biological activity" of E-selectin is meant to include leukocyte tethering, slow rolling and activation of stable adhesion of leukocytes to endothelial cells, selective and efficient extravasation signaling at sites of inflammation. "Biological function" or "biological activity" of E-selectin includes mediating an increase in: affinity and avidity of CD18 integrins to support PMN deceleration and trafficking to sites of acute inflammation, cytosolic calcium, tyrosine phosphorylation to activate p38 MAP kinase and Syk kinase, among others now known in the art or later identified. The biological function or biological activity of E-selectin can, but need not be, mediated by the interaction between E-selectin and its ligands.

[0141] The disclosure includes an antibody, or antigen-binding fragment thereof, that can modulate a biological activity of E-selectin. That is, the invention includes an isolated antibody, or antigen-binding fragment thereof, that specifically binds E-selectin and modulates at least one detectable E-selectin activity such that the antibody: (a) decreases leukocyte tethering to endothelial cells; (b) decreases activation of stable adhesion to endothelial cells; (c) reduces slow rolling of leukocytes to arrest; (d) reduces efficient trans-endothelial migration of leukocytes; (e) decreases affinity and avidity of CD18 integrins; (f) reduces trafficking of leukocytes to sites of acute inflammation; (g) decreases cytosolic calcium; (h) decreases tyrosine phosphorylation that activates p38 MAP kinase and Syk kinase; (i) reduces recruitment of platelets and leukocytes from the blood to the vascular endothelium; and/or (j) does not create a pro-inflammatory environment.

[0142] The biological activity of E-selectin can be assessed in an *in vitro* static neutralization binding assays using E-selectin (e.g., soluble E-selectin or CHO cells expressing E-selectin) and a ligand (e.g., soluble sialyl Lewis ligand or cell surface expressed ligand (e.g., on HL-60 cells)). Binding of E-selectin can also be assessed using soluble or cell surface expressed proteins in physiological flow assays known in the art and set forth in the Examples section of the present disclosure. The ability of neutralizing antibodies to prevent E-selectin binding can also be assessed by incubating cells expressing E-selectin (e.g., human, cynomolgus monkey) with a soluble (sialyl Lewis antigen) or cell surface expressed (e.g., on HL-60 cells, HUVEC, CLMEC) E-selectin ligand in the absence or presence of increasing concentrations of the anti-E-selectin antibody, or antigen-binding fragment thereof.

[0143] In some embodiments, an anti-E-selectin antibody of the disclosure encompasses an antibody that competes for binding to human E-selectin with, and/or binds the same epitope as, an antibody, or antigen-binding fragment thereof, having the amino acid sequence of a heavy chain variable region set forth as SEQ ID NO:11 and the amino acid sequence of a light chain variable region set forth as SEQ ID NO:5.

[0144] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, of the disclosure encompasses an antibody, or antigen-binding fragment thereof, that inhibits or reduces binding of E-selectin to at least one E-selectin ligand (e.g., glycoproteins or glycolipids with a sialyl Lewis (sLex) determinant (e.g., α 2,3 sialylated and α 1,3 or α 1,4 fucosylated tetrasaccharide sialyl Lewis x), sialyl Lewis A determinant, ESL-1, L-selectin, CD44, PSGL-1, LAMP1, LAMP2, DR3 and α M β 2 integrin (CD11b/CD18; Mac-1)).

[0145] In some embodiments, the disclosure encompasses an antibody, or antigen-binding fragment thereof, that competes with an antibody, or antigen-binding fragment thereof, having the amino acid sequence of a heavy chain variable region set forth as SEQ ID NO:11 and the amino acid sequence of a light chain variable region set forth as SEQ ID NO:5, in

inhibiting the binding of E-selectin with a ligand.

[0146] In some embodiments, an antibody, or antigen-binding fragment thereof of the disclosure, includes an IgG1 heavy chain constant region, for example an anti-E-selectin heavy chain set forth as SEQ ID NO:7, or SEQ ID NO:13 (without C-terminal lysine). In

5 some embodiments, an antibody, or antigen-binding fragment thereof, includes a kappa light chain constant region, for example an anti-E-selectin light chain set forth as SEQ ID NO:1.

[0147] Anti-E-selectin antibodies of the present disclosure can encompass monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')₂, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, heteroconjugate antibodies, single chain (ScFv),

10 mutants thereof, fusion proteins comprising an antibody fragment (e.g., a domain antibody), humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The antibodies may be murine, rat, human, or any other origin (including
15 chimeric or humanized antibodies). In some embodiments, an anti-E-selectin antibody is a monoclonal antibody. In some embodiments, an anti-E-selectin antibody is a human or humanized antibody. In some embodiments, an anti-E-selectin antibody is a chimeric antibody.

[0148] Table 2 provides amino acid and nucleotide sequences for the chimeric and
20 humanized anti-E-selectin antibodies as described herein. Generally, unless specifically indicated, anti-E-selectin antibodies of the disclosure can include any combination of one or more CDRs. In some embodiments, anti-E-selectin antibodies of the disclosure can include any combination of one or more VH and/or VL sequences as set forth in Table 2, with particular antibodies defined by SEQ ID NO: in Table 3. The CDRs of the anti-E-selectin VHs
25 and VLs were defined using the Kabat definition with the extended H1. For HCDR-1, the last residue includes any insert before the H36 position (i.e. H35a, H35b, H35c, etc.). The CDRs were defined as follows: HCDR-1 (H26 to H35c), HCDR-2 (H50 to H65), HCDR-3 (H95 to H102), LCDR-1 (L24 to L34), LCDR-2 (L50 to L56), and LCDR-3 (L89 to L87).

[0149] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment
30 thereof, comprises a HC, LC, VL domain, and/or VH domain comprising an amino acid sequence at least 80%, 85%, 90%, 91%, 925, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence of Table 2. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a HC, LC, VL domain, and/or VH domain encoded by a nucleic acid sequence at least 80%, 85%, 90%, 91%, 925, 93%, 94%,
35 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence of Table 2.

[0150] **Table 2.** Sequences of E-selectin peptides, anti-E-selectin antibodies and

fragments thereof.

SEQ	Description	Sequence
1	1444_optimized_L (LC)	DIQMTQSPSS LSASVGDRVT ITCKTSQNIE RYLNWYQQKP GKAPKLLIYA ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYFCLQ DNAWPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK
2	1444_optimized_L CDR_L1	KTSQNIERYL N
3	1444_optimized_L CDR_L2	AASSLQS
4	1444_optimized_L CDR_L3	LQDNAWPLT
5	1444_optimized_L FV_L (VL)	DIQMTQSPSS LSASVGDRVT ITCKTSQNIE RYLNWYQQKP GKAPKLLIYA ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYFCLQ DNAWPLTFGQ GTKVEIK
6	1444_optimized _L FW_L4	FGQGKTKVEIK
7	1444_optimized _H (HC) (with C-terminal lysine(K))	EVQLVESGGG LVQPGGSLRL SCAASGYAIR SAYMHWVRQA PGKGLEWVAR IDPANGNTIY VDSVTGRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTGPS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNATK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLD S DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK
8	1444_optimized _H CDR_H1	GYAIRSAYMH
9	1444_optimized _H CDR_H2	RIDPANGNTI YVDSVTG
10	1444_optimized _H CDR_H3	DLYSTSEY
11	1444_optimized _H FV_H (FV)	EVQLVESGGG LVQPGGSLRL SCAASGYAIR SAYMHWVRQA PGKGLEWVAR IDPANGNTIY VDSVTGRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSS
12	1444_optimized _H FW_H4	WGQGTILTVS S
13	1444_optimized _H (HC) (without C-terminal lysine(K))	EVQLVESGGG LVQPGGSLRL SCAASGYAIR SAYMHWVRQA PGKGLEWVAR IDPANGNTIY VDSVTGRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF

		PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPSS SSLGTQTYIC NVNHNKPSNTK VDKKVEPKSC DKTHTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPFPVLDL DGSFFLYSKL TVDKSRWQQG NVFSCSVSMHE ALHNHYTQKS LSLSPG
14	1444_optimized _L CL	RTVAAPSVFIF FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLTKADYE KHKVYACEVT HQLGLSPVTK SFNRGEC
15	1444_optimized _H CH (with C-terminal lysine (K))	ASTKGPSVFPP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHNKPS NTKVDKKVEP KSCDKHTTCP PCPAPEAAGA PSVFLFPPKP KDTLMISRTPEVT CVVVDVSHED HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSGSSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
16	1444_optimized _H CH (without C-terminal lysine (K))	ASTKGPSVFPP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHNKPS NTKVDKKVEP KSCDKHTTCP PCPAPEAAGA PSVFLFPPKP KDTLMISRTPEVT CVVVDVSHED HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSGSSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
17	0841_humanized1_L	DIQMTQSPSS LSASVGDRVT ITCKTSQNIN RYLNWYQQKP GKAPKLLIYN ANSLQTGVPS RFGSGSGTD FTLTISSLQP EDFATYFCLQ DNSWPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
18	0841_humanized1_L CDR_L1	KTSQNINRYL N
19	0841_humanized1_L CDR_L2	NANSLQT
20	0841_humanized1_L CDR_L3	LQDNSWPLT
21	0841_humanized1_L FV_L	DIQMTQSPSS LSASVGDRVT ITCKTSQNIN RYLNWYQQKP GKAPKLLIYN ANSLQTGVPS RFGSGSGTD FTLTISSLQP EDFATYFCLQ DNSWPLTFGQ GTKVEIK

22	0841_humanized1_H	EVQLVESGGG LVQPGGSLRL SCAASGYNIR SSYMHWRQA PGKGLEWVAR IDPANGNTIY AEKFKIRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPSS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKHTTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDSDGSFFLYSKL TVDKSRWQQG NVFSCSVSMHE ALHNHYTQKS LSLSPG
23	0841_humanized1_H CDR_H1	GYNIRSSYMH
24	0841_humanized1_H CDR_H2	RIDPANGNTI YAEKFKI
25	0841_humanized1_H FV_H	EVQLVESGGG LVQPGGSLRL SCAASGYNIR SSYMHWRQA PGKGLEWVAR IDPANGNTIY AEKFKIRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSS
26	0978_humanized2_L	DIQMTQSPSS LSASVGDRVT ITCKTSQNIN RYLNWYQQKP GKAPKLLIYA ASSLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYFCLQ DNSWPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK
27	0978_humanized2_L FV_L	DIQMTQSPSS LSASVGDRVT ITCKTSQNIN RYLNWYQQKP GKAPKLLIYA ASSLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYFCLQ DNSWPLTFGQ GTKVEIK
28	0978_humanized2_H	EVQLVESGGG LVQPGGSLRL SCAASGYNIR SSYMHWRQA PGKGLEWVAR IDPANGNTIY VDSVKGRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPSS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKHTTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDSDGSFFLYSKL TVDKSRWQQG NVFSCSVSMHE ALHNHYTQKS LSLSPG
29	0978_humanized2_H CDR_H2	RIDPANGNTI YVDSVKG
30	0978_humanized2_H FV_H	EVQLVESGGG LVQPGGSLRL SCAASGYNIR SSYMHWRQA PGKGLEWVAR IDPANGNTIY

		VDSVKGRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSS
31	0164_chimera_L	DIQMTQSPSF LSASVGDRV INCKTSQNIN RYLNWYQQKL GEAPKLLIYN ANSLQTGIPS RFSASGSGTD FTLTINSLQP EDVATYFCLQ DNSWPLTFGS GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE
32	0164_chimera_L FV_L	DIQMTQSPSF LSASVGDRV INCKTSQNIN RYLNWYQQKL GEAPKLLIYN ANSLQTGIPS RFSASGSGTD FTLTINSLQP EDVATYFCLQ DNSWPLTFGS GTKLEIK
33	0164_chimera_L FW_L4	FGSGTKLEIK
34	0164_chimera_H	EVQLQQSGAE FGKPGTSVKL SCKVSGYNIR SSYMHVWNQR PGKGLEWIGR IDPANGNTIY AEKFKIKAIL TADSSSNTAY MQLSQLKSDD TAIYFCAMD YSTSEYWGQG VMVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTWNS GALTSGVHTF PAVLQSSGLY SLSSVTVPS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKHTCPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNATK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLD DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK
35	0164_chimera_H FV_H	EVQLQQSGAE FGKPGTSVKL SCKVSGYNIR SSYMHVWNQR PGKGLEWIGR IDPANGNTIY AEKFKIKAIL TADSSSNTAY MQLSQLKSDD TAIYFCAMD YSTSEYWGQG VMVTVSS
36	0164_chimera_H FW_H4	WGQGVMTVS S
37	1448_optimized_H	EVQLVESGGG LVQPGGSLRL SCAASGYAIR SAYMHVVRQA PGKGLEWVAR IDPANGNTIY VDSVKERFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTWNS GALTSGVHTF PAVLQSSGLY SLSSVTVPS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKHTCPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNATK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLD DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPG
38	1448_optimized_H CDR_H2	RIDPANGNTI YVDSVKE
39	1448_optimized_H FV_H	EVQLVESGGG LVQPGGSLRL SCAASGYAIR SAYMHVVRQA PGKGLEWVAR IDPANGNTIY

		VDSVKERFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSS
40	1284_optimized_H	EVQLVESGGG LVQPGGSLRL SCAASGYAIR SAYMHWVRQA PGKGLEWVAR IDPANGNTIY VESVEGRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPSSSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDSDGSFFLYSKL TVDKSRWQQG NVFSCSVSMHE ALHNHYTQKS LSLSPG
41	1284_optimized_H CDR_H2	RIDPANGNTI YVESVEG
42	1284_optimized_H FV_H	EVQLVESGGG LVQPGGSLRL SCAASGYAIR SAYMHWVRQA PGKGLEWVAR IDPANGNTIY VESVEGRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSS
43	1282_optimized_H	EVQLVESGGG LVQPGGSLRL SCAASGYAIR SAYMHWVRQA PGKGLEWVAR IDPANGNTIY VDSVEGRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPSSSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDSDGSFFLYSKL TVDKSRWQQG NVFSCSVSMHE ALHNHYTQKS LSLSPG
44	1282_optimized_H CDR_H2	RIDPANGNTI YVDSVEG
45	1282_optimized_H FV_H	EVQLVESGGG LVQPGGSLRL SCAASGYAIR SAYMHWVRQA PGKGLEWVAR IDPANGNTIY VDSVEGRFTI SADNAKNSAY LQMNSLRAED TAVYYCAMDL YSTSEYWGQG TLVTVSS
46	0525_humanized_L	DIQMTQSPSS LSASVGDRVT ITCKASQTVG INVDWYQQKP GKAPKLLIYG ASNRHTGVPS RFGSGSGSGTD FTLTISSLQP EDFATYCCLO YGSIPHTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
47	0525_humanized_L CDR_L1	KASQTVGINV D

48	0525_humanized_L CDR_L2	GASNRHT
49	0525_humanized_L CDR_L3	LQYGSIPHT
50	0525_humanized_L FV_L	DIQMTQSPSS LSASVGDRVT ITCKASQTVG INVDWYQQKP GKAPKLLIYG ASNRHTGVPS RFGSGSGSTD FTLTISSLQP EDFATYCCLQ YGSIPHTFGQ GTKVEIK
51	0525_humanized_H	EVQLVESGGG LVQPGGSLRL SCAASGFSLT GYMQWVRQA PGKGLEWMGF IRSSGSTEYN SEFKSRFTIS RDNAKNSVYL QMNSLRAEDT AVYYCARCPY KYSSFVYVGV MDAWGQGTLV TVSSASTKGP SVFPLAPSSK STSGGTAALG CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSL SVVTVPSSSL GTQTYICNVN HKPSNTKVDK KVEPKSCDKT HTCPCPAPE AAGAPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNATKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPG
52	0525_humanized_H CDR_H1	GFSLTGYMQ
53	0525_humanized_H CDR_H2	FIRSSGSTEY NSEFKS
54	0525_humanized_H CDR_H3	CPYKYSSFVY VGVMDA
55	0525_humanized_H FV_H	EVQLVESGGG LVQPGGSLRL SCAASGFSLT GYMQWVRQA PGKGLEWMGF IRSSGSTEYN SEFKSRFTIS RDNAKNSVYL QMNSLRAEDT AVYYCARCPY KYSSFVYVGV MDAWGQGTLV TVSS
56	0039_chimera_L	EIVMTQSPTS MSTSIGERTV LNCKASQTVG INVDWYQQTP GQPPKLLIYG ASNRHTGVDP RFTGSGFGRD FTLTISNVEA EDLAVYCCLQ YGSIPHTFGP GTKLELKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
57	0039_chimera_L FV_L	EIVMTQSPTS MSTSIGERTV LNCKASQTVG INVDWYQQTP GQPPKLLIYG ASNRHTGVDP RFTGSGFGRD FTLTISNVEA EDLAVYCCLQ YGSIPHTFGP GTKLELK
58	0039_chimera_L FW_L4	FGPGTKLELK
59	0039_chimera_H	QVQLKETGPG LVQPTQTLSI TCTVSGFSLT GYMQWVRQT PGKGLEWMGF IRSSGSTEYN SEFKSRLSIS RDTSKNQVFL KMNSLKTEDT GVYYCARCPY KYSSFVYVGV MDAWGQGAPV TVSSASTKGP SVFPLAPSSK STSGGTAALG CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSL SVVTVPSSSL GTQTYICNVN

		HKPSNTKVDK KVEPKSCDKT HTCPCCPAPE AAGAPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNATKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGDS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
60	0039_chimera_H FV_H	QVQLKETGPG LVQPTQTLSI TCTVSGFSLT GYMQWVRQT PGKGLEWMGF IRSSGSTEYN SEFKSRLSIS RDTSKNQVFL KMNSLKTEDT GVYYCARCPY KYSSFVYVGV MDAWGQGAPV TVSS
61	0039_chimera_H FW_H4	WGQGAPVTVS S
62	0265_0254_humanized_ H	EVQLVESGGG LVQPGGSLRL SCAVSGFSIS TYNVHWRQA PGKGLEWMGM MWSGGSPDYN SALKSRFTIS RDTAKNSVYL QMNSLRAEDT AVYYCARWGG GFDYWGQGT VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPCCPAP EAAGAPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNATKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPVLDSG SFFLYSKLTV DKSRWQQGNV FSCVMHEAL HNHYTQKSLS LSPG
63	0265_0254_humanized_ H CDR_H1	GFSISTYNVH
64	0265_0254_humanized_ H CDR_H2	MMWSGGSPDY NSALKS
65	0265_0254_humanized_ H CDR_H3	WGGGFDY
66	0265_0254_humanized_ H FV_H	EVQLVESGGG LVQPGGSLRL SCAVSGFSIS TYNVHWRQA PGKGLEWMGM MWSGGSPDYN SALKSRFTIS RDTAKNSVYL QMNSLRAEDT AVYYCARWGG GFDYWGQGT VTVSS
67	0265_0254_humanized_ L	DIQLTQSPSS LSASVGDRVT ITCRASHSIG TNLHWYQQKPK GKAPKLLIYF TSQSISGVPS RFGSGSGTD FTLTISSLQP EDFATYYCQQ TQSWPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
68	0265_0254_humanized_ L CDR_L1	RASHSIGTNL H
69	0265_0254_humanized_ L CDR_L2	FTSQSIS
70	0265_0254_humanized_ L CDR_L3	QQTQSWPLT

71	0265_0254_humanized_ L FV_L	DIQLTQSPSS LSASVGDRVT ITCRASHSIG TNLHWYQQKP GKAPKLLIYF TSQSISGVPS RFSASGSGTD FTLTISSLQP EDFATYYCQQ TQSWPLTFGQ GTKVEIK
72	0158_chimera_L	DIVLTQSPTT LSVTPGETVS LSCRASHSIG TNLHWYQQKT NESPRLLIKF TSQSISGIPS RFSASGSGTD FTLNINNVEF DDVSSYFCQQ TQSWPLTFGS GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY BREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK
73	0158_chimera_L FV_L	DIVLTQSPTT LSVTPGETVS LSCRASHSIG TNLHWYQQKT NESPRLLIKF TSQSISGIPS RFSASGSGTD FTLNINNVEF DDVSSYFCQQ TQSWPLTFGS GTKLEIK
74	0158_chimera_H	QVQLKESGPG LVQPSETLSL TCTVSGFSIS TYNVHWLRQP PGKGLEWMGM MWSGGSPDYN SALKSRLSIS RDTSKNQVFL KMNSLQSED TTYCARWGG GFDYWGQGVMTVTSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYS SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPCPAP EAAGAPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRLWQQGNV FSCSVMEAL HNHYTQKSLS LSPGK
75	0158_chimera_H FV_H	QVQLKESGPG LVQPSETLSL TCTVSGFSIS TYNVHWLRQP PGKGLEWMGM MWSGGSPDYN SALKSRLSIS RDTSKNQVFL KMNSLQSED TTYCARWGG GFDYWGQGVMTVTSS
76	0929_0548_humanized_ H	QVQLVQSGAE VKKPGASVKV SCKVSGYNIR STYMHVVRQA PGQGLEWMGR IDPANGNTIY AEKFKRRVTL TRDTSTSTAY MELSSLRSED TAVYYCAMEV RVSFEYWGQG TLVTSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPSSS LGTQTYIC NVNHKPSNTK VDKKVEPKSCDKTHTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDNLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDSDG SFFLYSKL TVDKSRWQQG NVFSCSVMEAL HNHYTQKS LSLSPG
77	0929_0548_humanized_ H CDR_H1	GYNIRSTYMH
78	0929_0548_humanized_ H CDR_H2	RIDPANGNTI YAEKFKR

79	0929_0548_humanized_ H CDR_H3	EV RV SF EY
80	0929_0548_humanized_ H FV_H	QVQLVQSGAE VKKPGASVKV SCKVSGYNIR STYMHVWRQA PGQGLEWMGR IDPANGNTIY AEKFKRRVTL TRDTSTSTAY MELSSLRSED TAVYYCAMEV RV SF EY WGQG TLVTVSS
81	0929_0548_humanized_ L	DIQMTQSPSS LSASVGDRVT ITCKASQNIN KYLDWYQQKP GKAPKLLIYY TNNLHTGVPS RFGSGSGSGTD FTFTISLQP EDIATYYCLQ HDSGYTFGQG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC
82	0929_0548_humanized_ L CDR_L1	KASQNINKYL D
83	0929_0548_humanized_ L CDR_L2	YTNNLHT
84	0929_0548_humanized_ L CDR_L3	LQHDSGYT
85	0929_0548_humanized_ L FV_L	DIQMTQSPSS LSASVGDRVT ITCKASQNIN KYLDWYQQKP GKAPKLLIYY TNNLHTGVPS RFGSGSGSGTD FTFTISLQP EDIATYYCLQ HDSGYTFGQG TKVEIK
86	0159_chimera_L	DIQMTQSPSF LSASVGDRVT INCKASQNIN KYLDWYQQKL GEGPKLLIYY TNNLHTGIPS RFGSGSGSGTD FTLTISLQP EDVATYFCLQ HDSGYTFGAG TKLELKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC
87	0159_chimera_L FV_L	DIQMTQSPSF LSASVGDRVT INCKASQNIN KYLDWYQQKL GEGPKLLIYY TNNLHTGIPS RFGSGSGSGTD FTLTISLQP EDVATYFCLQ HDSGYTFGAG TKLELK
88	0159_chimera_L FW_L4	FGAGTKLELK
89	0159_chimera_H	EVQLQQSGAE LGKPGTSVKL SCKVSGYNIR STYMHVWSQR PGKGLEWIGR IDPANGNTIY AEKFKRKATL TADTSSNTAY MQLSQLKSDD RAIYFCAMEV RV SF EY WGQG VMVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPSSSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTFPPVLDSDGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK
90	0159_chimera_H FV_H	EVQLQQSGAE LGKPGTSVKL SCKVSGYNIR STYMHVWSQR PGKGLEWIGR IDPANGNTIY

		AEKFKRKATL TADTSSNTAY MQLSQLKSDD RAIYFCAMEV RVSFEYWGQG VMVTVSS
91	0955_0300_humanized_ H	QVQLVQSGAE VKKPGSSVKV SCKVSGYSIR STYMHWVRQA PGQGLEWMGR IDPANGNTIY AERFKNRVTL TADTSTSTAY MELSSLRSED TAVYYCAVEI LGIFDYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPSS SLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKHTCTPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDSD DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPG
92	0955_0300_humanized_ H CDR_H1	GYSIRSTYMH
93	0955_0300_humanized_ H CDR_H2	RIDPANGNTI YAERFKN
94	0955_0300_humanized_ H CDR_H3	EILGIFDY
95	0955_0300_humanized_ H FV_H	QVQLVQSGAE VKKPGSSVKV SCKVSGYSIR STYMHWVRQA PGQGLEWMGR IDPANGNTIY AERFKNRVTL TADTSTSTAY MELSSLRSED TAVYYCAVEI LGIFDYWGQG TLVTVSS
96	0955_0300_humanized_ L	DIQMTQSPSS LSASVGDRVT ITCKASQNID KYLDWYQQKP GKAPKLLMYN TNSLHTGVPS RFGSGSGTD FTLTISSLQP EDFATYFCLQ HNSGYTFGQG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYF REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC
97	0955_0300_humanized_ L CDR_L1	KASQNIDKYL D
98	0955_0300_humanized_ L CDR_L2	NTNSLHT
99	0955_0300_humanized_ L CDR_L3	LQHNSGYT
100	0955_0300_humanized_ L FV_L	DIQMTQSPSS LSASVGDRVT ITCKASQNID KYLDWYQQKP GKAPKLLMYN TNSLHTGVPS RFGSGSGTD FTLTISSLQP EDFATYFCLQ HNSGYTFGQG TKVEIK
101	0170_chimera_L	DIQMTQSPSF LSASVGDRVT INCKASQNID KYLDWYQQKL GEAPKLLMYN TNSLHTGIPS RFGSGSGTD FTLTISSLQP EDVATYFCLQ HNSGYTFGAG TKLELKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYF REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC
102	0170_chimera_L FV_L	DIQMTQSPSF LSASVGDRVT INCKASQNID KYLDWYQQKL GEAPKLLMYN TNSLHTGIPS

		RFSGSGSGTD FTLTISSLQP EDVATYFCLQ HNSGYTFGAG TKLELK
103	0170_chimera_H	EVQLQQSGAE LGKPGTSVKL SCKVSGYSIR STYMHVWVNR PGKGLEWVGR IDPANGNTIY AERFKNKATL TADTSSNTAY MQLSQLKSDD TAIFYCAVEI LGIFDYWGQG VMVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTGPS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDSD DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK
104	0170_chimera_H FV_H	EVQLQQSGAE LGKPGTSVKL SCKVSGYSIR STYMHVWVNR PGKGLEWVGR IDPANGNTIY AERFKNKATL TADTSSNTAY MQLSQLKSDD TAIFYCAVEI LGIFDYWGQG VMVTVSS
105	0564_humanized_L	DIQMTQSPSS LSASVGDRVT ITCKASQHIN RYLNWYQQKP GKAPKLLIYD ANNLQTGVPS RFSGSGSGTD FTLTISSLQP EDFATYFCLQ HNSWPNTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE
106	0564_humanized_L CDR_L1	KASQHINRYL N
107	0564_humanized_L CDR_L2	DANNLQT
108	0564_humanized_L CDR_L3	LQHNSWPNT
109	0564_humanized_L FV_L	DIQMTQSPSS LSASVGDRVT ITCKASQHIN RYLNWYQQKP GKAPKLLIYD ANNLQTGVPS RFSGSGSGTD FTLTISSLQP EDFATYFCLQ HNSWPNTFGQ GTKVEIK
110	0564_humanized_H	EVQLVESGGG LVQPGGSLRL SCAVSGYKIR SSYMHVWRQA PGKGLEWIGR IDPANGNTIY GDKFKSRFTL SSDTAKNSAY LQMNSLRAED TAVYYCAIDI GTTFDYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTGPS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDSD DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPG

111	0564_humanized_H CDR_H1	GYKIRSSYMH
112	0564_humanized_H CDR_H2	RIDPANGNTI YGDKFKS
113	0564_humanized_H CDR_H3	DIGTTFDY
114	0564_humanized_H FV_H	EVQLVESGGG LVQPGGSLRL SCAVSGYKIR SSYMHWVRQA PGKGLEWIGR IDPANGNTIY GDKFKSRFTL SSDTAKNSAY LQMNSLRAED TAVYYCAIDI GTTFDYWGQG TLVTVSS
115	0180_chimera_L	DIQMTQSPSF LSASVGDRVT INCKASQHIN RYLNWYQQKL GEAPKLLIYD ANNLQTGIPS RFGSGSGGTD FTLTISLQP EDVATYFCLQ HNSWPNTFGA GTKLELKRIV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ ESVTEQDSK STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE
116	0180_chimera_L FV_L	DIQMTQSPSF LSASVGDRVT INCKASQHIN RYLNWYQQKL GEAPKLLIYD ANNLQTGIPS RFGSGSGGTD FTLTISLQP EDVATYFCLQ HNSWPNTFGA GTKLEL
117	0180_chimera_H	EVQLQQSGAE LGKPGTSVKL SCKVSGYKIR SSYMHWVNQR PGKGLEWIGR IDPANGNTIY GDKFKSKATL TSDTSSNTAY IQLSQLKSDD TAIFYCAIDI GTTFDYWGQG VMVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVTVPS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKHTCPCP APEAAGAPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLD SDFSFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK
118	0180_chimera_H FV_H	EVQLQQSGAE LGKPGTSVKL SCKVSGYKIR SSYMHWVNQR PGKGLEWIGR IDPANGNTIY GDKFKSKATL TSDTSSNTAY IQLSQLKSDD TAIFYCAIDI GTTFDYWGQG VMVTVSS
119	0027_chimera_L	DIQMTQSPSF LSASVGDRIT INCKTSQNIN RYLNWFQQKL GEPPKLLIYN ANSLQADIPS RFGSGSGGTD FTLTITSLQP EDVATYFCLQ HHFWPYTFGA GTKLELRRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ ESVTEQDSK STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE
120	0027_chimera_L CDR_L2	NANSLQA
121	0027_chimera_L CDR_L3	LQHHFWPYT
122	0027_chimera_L FV_L	DIQMTQSPSF LSASVGDRIT INCKTSQNIN RYLNWFQQKL GEPPKLLIYN ANSLQADIPS

		RFSGSGSGTD FTLTITSLQP EDVATYFCLQ HHFWPYTFGA GTKLELR
123	0027_chimera_L FW_L4	FGAGTKLELR
124	0027_chimera_H	EVHLHQSGPE LGRPGSSVKI SCKASGYTFT DYVMNWVRQS PGQGLEWIGW INPEDYSFDS GEKFLERATL TAATSSNTVY IQLSGLTSDD TATYFCVRGG LPGDWFAYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY ICNVNHNKPSN TKVDKKVEPK SCDKTHTCPP CPAPEAAGAP SVFLFPPKPK DTLNISRTPE VTCVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
125	0027_chimera_H CDR_H1	GYTFTDYVMN
126	0027_chimera_H CDR_H2	WINPEDYSFD SGEKFLE
127	0027_chimera_H CDR_H3	GGLPGDWFAY
128	0027_chimera_H FV_H	EVHLHQSGPE LGRPGSSVKI SCKASGYTFT DYVMNWVRQS PGQGLEWIGW INPEDYSFDS GEKFLERATL TAATSSNTVY IQLSGLTSDD TATYFCVRGG LPGDWFAYWG QGTLVTVSS
129	1444_L Leader Sequence	MGWSCIIILFL VATATGVHS
130	1444_H Leader Sequence	MEWSWVFLFF LSVTTGVHS
131	0164_chimera_L Leader Sequence	MGWSCIIILFL VATATGAHS
132	Human E-selectin (amino acid residues 22-610 of UniProtKB sequence P16581)	WSYNTSTEAM TYDEASAYCQ QRYTHLVAIQ NKEEIEYLNS ILSYSPSYW IGIRKVNNVW VWVGTVQKPLT EEAKNWAPGE PNNRQKDEDC VEIYIKREKD VGMWNDERCS KKKLALCYTA ACTNTSCSGH GECVETINNY TCKCDPGFSG LKCEQIVNCT ALESPEHGSL VCSHPLGNFS YNSSCSISCD RGYLPSSMET MQCMSSGEWS APIPACNVVE CDAVTNPANG FVECFQNP GS FPWNTTCTFD CEEGFELMGA QSLQCTSSGN WDNEKPTCKA VTCRAVRQPQ NGSVRCSHSP AGEFTFKSSC NFTCEEGFML QGPAQVECTT QGQWTQQIPV CEAFQCTALS NPERGYMNCL PSASGSFRYG SSCEFSCEQG FVLKGSKRLQ CGPTGEWDNE KPTCEAVRCD AVHQPPKGLV RCAHSPIGEF TYKSSCAFC SC EGFELHGST QLECTSQGQW TEEVPSCQV KCSSLAVPGK INMCSGEPV FGTVCVFACP EGWTLNGSAA RTCGATGHWS GLLPTCEAPT ESNIPLVAGL SAAGLSLLTL APFLLWLRKC LRKAKKFVPA SSCQSLESDG SYQKPSYIL

133	Human E-selectin extracellular domain	WSYNTSTEAM TYDEASAYCQ QRYTHLVAIQ NKEEIEYLNS ILSYSPSYW IGIRKVNNVW VWVGTQKPLT EEAKNWAPGE PNNRQKDEDC VEIYIKREKD VGMWNDERCS KKKLALCYTA ACTNTSCSGH GECVETINNY TCKCDPGFSG LKCEQIVNCT ALESPEHGSL VCSHPLGNFS YNSSCSISCD RGYLPSSMET MQCMSSGEWS APIPACNVVE CDAVTNPANG FVECFQNP GS FPWNTTCTFD CEEGFELMGA QSLQCTSSGN WDNEKPTCKA VTCRAVRQPQ NGSVRCSHSP AGEFTFKSSC NFTCEEGFML QGPAQVECTT QGQWTQQIPV CEAFQCTALS NPERGYMNCL PSASGSFRYG SSCEFSCEQG FVLKGSKRLQ CGPTGEWDNE KPTCEAVRCD AVHQPPKGLV RCAHSPIGEF TYKSSCAFS CEEGFELHGST QLECTSQGQW TEEVPSCQV KCSSLAVPGK INMSCSGEPV FGTVCKFACP EGWTLNGSAA RTCGATGHWS GLLPTCEAPT ESNIP
134	Mouse E-selectin (amino acid residues 22-612 of UniProtKB sequence Q00690)	WYYNASSELM TYDEASAYCQ RDYTHLVAIQ NKEEINY LNS NLKHSPSYW IGIRKVNNVW IWVGTGKPLT EEAQNWAPGE PNNKQRNEDC VEIYIQR TKD SGMWNDERCN KKKLALCYTA SCTNASCSGH GECIETINSY TCKCHPGFLG PNCEQAVTCK PQEHPDYGSL NCSHPFGPFS YNSSCSFGCK RGYLPSSMET TVRCTSSGEW SAPAPACHVV ECEALTHPAH GIRKCSSNPG SYPWNTTCTF DCVEGYRRVG AQNLQCTSSG IWDNETP SCK AVTCD AIPQP QNGFVSCSHS TAGELAFKSS CNFTCEQSFT LQGPAQVECS AQGQWTPQIP VCKAVQCEAL SAPQQGNMKC LPSASGPFQ N GSSCEFSCEE GFELKGSRR L QCGPRGEWDS KKPTCSAVKC DDVPRPQNGV MECAHATTGE FTYKSSCAFQ CNEGFSLHGS AQLECTSQGK WTQEVPSQV VQCPSLDVPG KMNMSCSGTA VFGTVCEFTC PDDWTLNGSA VLTCGATGRW SGMPPTCEAP VSPTRPLVVA LSAAGTSLLT SSSLLYLLMR YFRKKAKKFV PASSCQSLQS FENYHVPSYN V
135	Mouse E-selectin extracellular Domain	WYYNASSELM TYDEASAYCQ RDYTHLVAIQ NKEEINY LNS NLKHSPSYW IGIRKVNNVW IWVGTGKPLT EEAQNWAPGE PNNKQRNEDC VEIYIQR TKD SGMWNDERCN KKKLALCYTA SCTNASCSGH GECIETINSY TCKCHPGFLG PNCEQAVTCK PQEHPDYGSL NCSHPFGPFS YNSSCSFGCK RGYLPSSMET TVRCTSSGEW SAPAPACHVV ECEALTHPAH GIRKCSSNPG SYPWNTTCTF DCVEGYRRVG AQNLQCTSSG IWDNETP SCK AVTCD AIPQP QNGFVSCSHS TAGELAFKSS CNFTCEQSFT LQGPAQVECS AQGQWTPQIP VCKAVQCEAL SAPQQGNMKC LPSASGPFQ N GSSCEFSCEE GFELKGSRR L QCGPRGEWDS KKPTCSAVKC DDVPRPQNGV MECAHATTGE FTYKSSCAFQ CNEGFSLHGS AQLECTSQGK WTQEVPSQV VQCPSLDVPG

		KMNMSCSGTA VFGTVCEFTC PDDWTLNGSA VLTCGATGRW SGMPPTCEAP VSPTRP
136	1444_H FV_H	GAAGTGCAGC TTGTGGAATC CGGGGGCGGC TTGGTCCAAC CGGGTGGCAG CCTCCGTCTG TCGTGCGCGG CTTCTGGGCTA TGCCATCCGT TCTGCCTACA TGCACTGGGT TCGCCAGGCG CCTGGGAAGG GCCTGGAATG GGTGGCCAGG ATTGATCCTG CAAACGGAAA TACTATATAT GTGGACTCCG TGACCGGCCG CTTTACAATC AGCGCCGACA ACGCTAAGAA TTCCGCCTAC CTGCAAATGA ATAGCCTGCG GGCAGAGGAT ACCGCGGTGT ACTATTGTGC CATGGATTTA TATTCCACGT CTGAATATTG GGGCCAAGGA ACCCTGGTAA CCGGTGTCGTC G
137	1444_L FV_L	GATATTCAGA TGACGCAGTC CCCATCTTCC CTTTCAGCAT CTGTGGGTGA CCGGGTTACA ATCACTTGTA AAACATCCCA GAACATTGAG CGTTATTTAA ATTGGTATCA GCAGAAACCG GGTAAAGCCC CGAAACTATT GATTTATGCC GCGTCCTCGC TGCAATCCGG CGTGCCGAGT CGTTTTAGCG GCTCCGGGAG CGGCACCGAT TTACTCTTA CCATTTTCGAG TCTGCAGCCG GAAGACTTTG CCACTTATTT CTGTCTCCAG GATAACGCCT GGCCATTAAC CTTCGGTCAG GGTACCAAAG TTGAAATTAA A
138	1444_HC (with C-terminal lysine (K))	GAAGTGCAGC TTGTGGAATC CGGGGGCGGC TTGGTCCAAC CGGGTGGCAG CCTCCGTCTG TCGTGCGCGG CTTCTGGGCTA TGCCATCCGT TCTGCCTACA TGCACTGGGT TCGCCAGGCG CCTGGGAAGG GCCTGGAATG GGTGGCCAGG ATTGATCCTG CAAACGGAAA TACTATATAT GTGGACTCCG TGACCGGCCG CTTTACAATC AGCGCCGACA ACGCTAAGAA TTCCGCCTAC CTGCAAATGA ATAGCCTGCG GGCAGAGGAT ACCGCGGTGT ACTATTGTGC CATGGATTTA TATTCCACGT CTGAATATTG GGGCCAAGGA ACCCTGGTAA CCGGTGTCGTC GGCCTCGACC AAGGGCCCAT CCGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG GCCCTGGGCT GCCTGGTCAA GGACTIONTTC CCCGAACCGG TGACGGTGTC GTGGAACCTCA GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAG CTACATCTGC AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT GACAAAACCTC ACACATGCCC ACCGTGCCCA GCACCTGAAG CCGCTGGGGC ACCGTCAGTC TTCTCTTCC CCCCCAACCC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC

		CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGAGGA GATGACCAAG AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC GACGGCTCCT TCTTCCTCTA TAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC CTCTCCCTGT CCCC GGAAA A
139	1444_LC	GATATTCAGA TGACGCAGTC CCCATCTTCC CTTTCAGCAT CTGTGGGTGA CCGGGTTACA ATCACTTGTA AAACATCCCA GAACATTGAG CGTTATTTAA ATTGGTATCA GCAGAAACCG GGTAAAGCCC CGAAACTATT GATTTATGCC GCGTCCTCGC TGCAATCCGG CGTGCCGAGT CGTTTTAGCG GCTCCGGGAG CGGCACCGAT TTTACTCTTA CCATTTTCGAG TCTGCAGCCG GAAGACTTTG CCACTTATTT CTGTCTCCAG GATAACGCCT GGCCATTAAC CTTCGGTCAG GGTACCAAAG TTGAAATTAA ACGTACGGTG GCTGCACCAT CTGTCTTCAT CTTCCCGCCA TCTGATGAGC AGTTGAAATC TGGAAGTCC TCTGTTGTGT GCCTGCTGAA TAACTTCTAT CCCAGAGAGG CCAAAGTACA GTGGAAGGTG GATAACGCCC TCCAATCGGG TAACTCCAG GAGAGTGTC CAGAGCAGGA CAGCAAGGAC AGCACCTACA GCCTCAGCAG CACCCTGACG CTGAGCAAAG CAGACTACGA GAAACACAAA GTCTACGCCT GCGAAGTCAC CCATCAGGGC CTGAGCTCGC CCGTCACAAA GAGCTTCAAC AGGGGAGAGT GT
140	1444_H Leader Sequence	ATGGAATGGA GCTGGGTCTT TCTCTTCTTC CTGTCAGTAA CTACAGGTGT CCACTCC
141	1444_L Leader Sequence	ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTGGCAACAG CTACAGGCGT GCACTCC
142	1444_H CH (with C- terminal lysine (K))	GCGTCGACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT CCTCCAAGAG CACCTCTGGG GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCAGT GACGGTGTG TGGAAGTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC AAATCTTGTG ACAAACACTCA CACATGCCCA CCGTGCCCAG CACCTGAAGC CGCTGGGGCA CCGTGAGTCT TCCTCTTCCC CCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT

		GAGGTCACAT GCGTG GTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAAC TACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT CTTCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC CCCGGGAAAA
143	1444_L CL	CGTACGGTGG CTGCACCATC TGTCTTCATC TTCCC GCCAT CTGATGAGCA GTTGAAATCT GGAAGTGCCT CTGTTGTGTG CCTGCTGAAT AACTTCTATC CCAGAGAGGC CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG AGAGTGTAC AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC ACCCTGACGC TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG CGAAGTCACC CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA GGGGAGAGTG T
144	0841_humanized1_H FV_H	GAAGTGCAGC TTGTGGAATC CGGGGGCGGC TTGGTCCAAC CGGGTGGCAG CCTCCGTCTG TCGTGCGCGG CTTCCGGGCTA TAACATCCGT TCTAGCTACA TGCACTGGGT TCGCCAGGCG CCTGGGAAGG GCCTGGAATG GGTGGCCAGG ATTGATCCTG CAAACGGAAA TACTATATAT GCTGAGAAAGT TCAAAATCCG CTTTACAATC AGCGCCGACA ACGCTAAGAA TTCCGCCTAC CTGCAAATGA ATAGCCTGCG GGCAGAGGAT ACCGCGGTGT ACTATTGTGC CATGGATTTA TATTCCACGT CTGAATATTG GGGCCAAGGA ACCCTGGTAA CGGTGTCGTC G
145	0841_humanized1_L FV_L	GATATTGAGA TGACGCAGTC CCCATCTTCC CTTTCAGCAT CTGTGGGTGA CCGGGTTACA ATCACTTGTA AAACATCCCA GAACATTAAC CGTTATTTAA ATTGGTATCA GCAGAAACCG GGTAAAGCCC CGAAACTATT GATTTATAAC GCGAACTCGC TGCAAACTGG CGTGCCGAGT CGTTTTAGCG GCTCCGGGAG CGGCACCGAT TTTACTCTTA CCATTTTCGAG TCTGCAGCCG GAAGACTTTG CCACTTATTT CTGTCTCCAG GATAACTCCT GGCCATTAAC CTTCCGTCAG GGTACCAAAG TTGAAATTAA A
146	0978_humanized2_H FV_H	GAAGTGCAGC TTGTGGAATC CGGGGGCGGC TTGGTCCAAC CGGGTGGCAG CCTCCGTCTG

		TCGTGCGCGG CTTGCGGCTA TAACATCCGT TCTAGCTACA TGCACTGGGT TCGCCAGGCG CCTGGGAAGG GCCTGGAATG GGTGGCCAGG ATTGATCCTG CAAACGGAAA TACTATATAT GTGGAATCCG TGAAAGGCCG CTTTACAATC AGCGCCGACA ACGCTAAGAA TTCCGCCTAC CTGCAAATGA ATAGCCTGCG GGCAGAGGAT ACCGCGGTGT ACTATTGTGC CATGGATTTA TATTCCACGT CTGAATATTG GGGCCAAGGA ACCCTGGTAA CGGTGTCGTC G
147	0978_humanized2_L FV_L	GATATTCAGA TGACGCAGTC CCCATCTTCC CTTTCAGCAT CTGTGGGTGA CCGGGTTACA ATCACTTGTA AAACATCCCA GAACATTAAC CGTTATTTAA ATTGGTATCA GCAGAAACCG GGTAAAGCCC CGAAACTATT GATTTATGCC GCGTCCTCGC TGCAATCCGG CGTGCCGAGT CGTTTTAGCG GCTCCGGGAG CGGCACCGAT TTTACTCTTA CCATTTTCGAG TCTGCAGCCG GAAGACTTTG CCACTTATTT CTGTCTCCAG GATAACTCCT GGCCATTAAC CTTGCGTCAG GGTACCAAAG TTGAAATTAA A
148	0164_chimera_H FV_H	GAAGTCCAGC TGCAGCAGTC TGGGGCTGAG TTTGGGAAAC CTGGGACCTC AGTCAAGTTG TCTTGCAAGG TTTCTGGGTA TAACATTAGG AGTTCATACA TGCACTGGGT GAATCAGAGG CCTGGAAAGG GCCTGGAATG GATAGGAAGG ATTGATCCTG CAAACGGAAA TACTATATAT GCTGAGAAGT TCAAAATCAA GGCCATTCTG ACTGCAGATT CATCGTCCAA CACAGCCTAC ATGCAACTCA GCCAACTGAA ATCTGACGAC ACAGCAATCT ATTTTTGTGC TATGGACCTC TACAGTACCT CTGAATACTG GGGCCAAGGA GTCATGGTCA CAGTCTCCTC A
149	0164_chimera_L FV_L	GACATCCAGA TGACCCAGTC TCCTTCATTC CTGTCTGCAT CTGTGGGAGA CAGAGTCACT ATCAACTGCA AAACGAGTCA GAATATTAAC AGGTACTTAA ACTGGTACCA GCAAAAGCTT GGAGAAGCTC CCAAACTCCT GATATATAAT GCAAAACAGTT TGCAAACGGG CATCCCATCA CGGTTCAGTG CCAGTGGATC CGGTACTGAT TTCACACTCA CCATCAACAG CCTGCAGCCT GAAGATGTTG CCACATATTT TTGCTTGCAG GATAATAGTT GGCCGCTCAC GTTCGGTTCT GGGACCAAGC TGGAGATCAA A
150	1448_optimized_H FV_H	GAAGTGCAGC TTGTGGAATC CGGGGGCGGC TTGGTCCAAC CGGGTGGCAG CCTCCGCTCTG TCGTGCGCGG CTTGCGGCTA TGCCATCCGT TCTGCCTACA TGCACTGGGT TCGCCAGGCG CCTGGGAAGG GCCTGGAATG GGTGGCCAGG ATTGATCCTG CAAACGGAAA TACTATATAT GTGGAATCCG TGAAAGAGCG CTTTACAATC AGCGCCGACA ACGCTAAGAA TTCCGCCTAC CTGCAAATGA ATAGCCTGCG GGCAGAGGAT ACCGCGGTGT ACTATTGTGC CATGGATTTA

		TATTCCACGT CTGAATATTG GGGCCAAGGA ACCCTGGTAA CCGTGTCGTC G
151	1284_optimized_H FV_H	GAAGTGCAGC TTGTGGAATC CGGGGGCGGC TTGGTCCAAC CGGGTGGCAG CCTCCGTCTG TCGTGCGCGG CTTCTGGGCTA TGCCATCCGT TCTGCCTACA TGCACTGGGT TCGCCAGGCG CCTGGGAAGG GCCTGGAATG GGTGGCCAGG ATTGATCCTG CAAACGGAAA TACTATATAT GTGGAGTCCG TGGAGGGCCG CTTTACAATC AGCGCCGACA ACGCTAAGAA TTCCGCCTAC CTGCAAATGA ATAGCCTGCG GGCAGAGGAT ACCGCGGTGT ACTATTGTGC CATGGATTTA TATTCCACGT CTGAATATTG GGGCCAAGGA ACCCTGGTAA CCGTGTCGTC G
152	1282_optimized_H FV_H	GAAGTGCAGC TTGTGGAATC CGGGGGCGGC TTGGTCCAAC CGGGTGGCAG CCTCCGTCTG TCGTGCGCGG CTTCTGGGCTA TGCCATCCGT TCTGCCTACA TGCACTGGGT TCGCCAGGCG CCTGGGAAGG GCCTGGAATG GGTGGCCAGG ATTGATCCTG CAAACGGAAA TACTATATAT GTGGACTCCG TGGAGGGCCG CTTTACAATC AGCGCCGACA ACGCTAAGAA TTCCGCCTAC CTGCAAATGA ATAGCCTGCG GGCAGAGGAT ACCGCGGTGT ACTATTGTGC CATGGATTTA TATTCCACGT CTGAATATTG GGGCCAAGGA ACCCTGGTAA CCGTGTCGTC G
153	0525_humanized_H FV_H	GAGGTACAGT TGGTGAATC TGGCGGCGGC CTGGTCCAGC CGGGCGGGTC TTTGCGCCTG AGTTGTGCAG CGAGTGGGTT TAGCCTGACG GGCTACTACA TGCAATGGGT CCGTCAGGCG CCGGGCAAAG GTCTGGAATG GATGGGTTTT ATACGGAGTA GTGGAAGCAC AGAGTATAAT TCAGAGTTCA AATCCCGTTT TACCATCTCT CGCGATAACG CGAAAAACAG CGTGTATCTG CAGATGAATA GCCTGCGCGC CGAAGATACC GCCGTGTACT ACTGCGCGCG TTGCCCCGTAT AAATATAGTT CATTTGTATA TGTGGGTGTC ATGGATGCGT GGGGCCAGGG TACACTGGTT ACCGTGAGCT CG
154	0525_humanized_L FV_L	GATATCCAAA TGACGCAATC GCCTAGCAGC TTATCCGCGT CAGTTGGCGA TCGCGTGACC ATCACTTGCA AAGCGTCGCA AACCGTCGGA ATCAACGTGG ATTGGTACCA ACAGAAACCG GGCAAGGCGC CGAAACTGCT GATCTATGGA GCCAGCAATC GCCACACAGG AGTGCCGTCC CGTTTTAGCG GCAGCGGGAG CCGTACGGAT TTTACCCTGA CGATTTCTTC ACTCCAACCC GAAGACTTTG CAACCTATTG CTGCTTGCAA TATGGTTCAA TCCCGCATAC TTTCGGCCAG GGTACAAAAG TGGAATTAA A
155	0039_chimera_H FV_H	CAGGTGCAGC TGAAGGAGAC AGGACCTGGC CTGGTGCAAC CAACACAGAC CCTGTCCATC ACATGTACTG TTTCTGGGTT CTCATTAACC GGCTATTATA TGCACTGGGT TCGCCAGACT CCAGGAAAGG GGCTAGAATG GATGGGATTT

		ATACGGAGTA GTGGAAGCAC AGAGTATAAT TCAGAGTTCA AATCCCGACT TAGCATCAGC AGGGACACCT CCAAGAACCA AGTTTTCTTA AAAATGAACA GTCTGAAAAC AGAAGATACA GGCGTGTATT ACTGTGCCAG ATGCCCTTAT AAGTATAGCA GCTTTGTCTA CGTAGGGGTT ATGGATGCCT GGGGTCAAGG AGCTCCAGTC ACTGTCTCCT CA
156	0039_chimera_L FV_L	GAAATTGTGA TGACCCAGTC TCCCACATCC ATGTCCACAT CAATAGGAGA GAGGGTCACC CTGAACTGCA AGGCCAGTCA GACTGTGGGT ATTAATGTTG ACTGGTACCA ACAGACACCA GGGCAGCCTC CTAAACTACT GATATATGGG GCATCCAACC GACACACTGG GGTCCCTGAT CGCTTCACAG GCAGTGGATT TGGGAGAGAT TTCACCTCTCA CCATCAGCAA CGTGGAGGCT GAAGACCTAG CTGTTTATTG CTGTCTGCAA TATGGCTCCA TTCCTCACAC GTTTGGACCT GGGACCAAGC TGGAGCTGAA A
157	0265_0254_humanized_ H FV_H	GAAGTGCAGT TAGTGGAAAG TGGCGGTGGC CTGGTGCAAC CGGGAGGATC CTTACGTTTA AGCTGCGCCG TGTCCGGGTT TAGTATCAGC ACCTATAATG TAACTGGCT GCGTCAAGCC CCGGGCAAAG GGTTAGAATG GATGGGAATG ATGTGGAGTG GTGGAAGCCC AGATTATAAT TCAGCTCTCA AATCCCGATT CACTATTAGT CGCGATAACG CAAAAAACTC CGTGTACCTT CAGATGAACT CTCTTCGCGC AGAGGATACG GCGGTTTACT ACTGTGCTCG CTGGGGCGGC GGGTTTGATT ACTGGGGCCA GGGAACGCTG GTAACGGTTT CCACT
158	0265_0254_humanized_ L FV_L	GACATTCAAC TGACCCAGAG CCCGTCCAGC TTATCTGCGA GTGTTGGGGA CCGGGTCACG ATTACCTGCC GGGCTAGTCA CAGCATTGGG ACGAACTTGC ATTGGTACCA GCAGAAACCT GGCAAAGCTC CGAAACTGCT GATTTATTTT ACATCCCCAA GCATCAGCGG TGTCCCCTCC CGATTTTCCG GGTCCGGATC CGGTACCGAT TTACTTTTAA CGATCAGCAG TCTGCAGCCA GAGGATTTTCG CCACCTACTA TTGTCAGCAA ACTCAGTCTT GGCCCTGAC CTTTGGCCAA GGGACCAAGG TAGAAATCAA G
159	0158_chimera_H FV_H	CAGGTGCAGC TGAAGGAGTC AGGACCTGGC CTGGTGACGC CCTCAGAGAC CCTGTCCCTC ACCTGCACTG TCTCTGGGTT CTCAATAAGC ACCTATAACG TAACTGGCT TCGACAGCCT CCAGGAAAAG GTCTGGAGTG GATGGGAATG ATGTGGAGTG GTGGAAGCCC AGATTATAAT TCAGCTCTCA AATCCCGACT GAGCATCAGC AGGGACACCT CCAAGAACCA AGTTTTCTTA AAAATGAACA GTCTGCAAAG TGAAGACACA ACCACTTACT ACTGTGCCAG ATGGGGGGGG GGCTTTGATT ACTGGGGCCA AGGAGTCATG GTCACAGTCT CCTCA

160	0158_chimera_L FV_L	GACATCGTGC CTGTCTGTGA CTCTCCTGCA ACAAATCTAC AATGAGTCTC ACTTCCCAGT AGGTTTCAGT TTTACTCTCA GATGATGTCT ACTCAAAGCT GGGACCAAGC	TGACTCAGTC CTCCAGGAGA GGGCTAGCCA ACTGGTATCA CAAGGCTTCT CCATCTCTGG CCAGTGGATC ACATCAACAA CAAGTTATTT GGCCCCCTCAC TGGAGATCAA	TCCAACCACC GACAGTCAGT TAGTATTGGC ACAAAAAACA CATCAAGTTT GATCCCCTCC AGGGACAGAT TGTGGAGTTT TTGTCAACAG GTTTCGGTTCT A
161	0929_0548_humanized_H FV_H	CAAGTACAAC GTGAAAAAAC AGCTGTAAAG AGCACCTATA CCGGGCCAGG ATTGATCCTG GCTGAGAAGT ACCCGCGACA ATGGAGCTGT ACTGCAGTTT AGAGTTAGCT ACATTGGTCA	TGGTGCAGAG CCGGCGCTAG TGTCCGGTTA TGCATTGGGT GCTTAGAGTG CAAATGGAAA TCAAAAGGAG CGTCCACCTC CTTCTTTACG ACTATTGTGC TCGAATATTG CGGTCAAGGC CGGTCAGCAG	TGGGGCCGAA CGTGAAAGTC TAATATTAGA GCGTCAAGCG GATGGGTAGG TACTATTTAT AGTTACGCTG GACGGCCTAT CTCAGAGGAC CATGGAAGTT GGGTCAAGGC T
162	0929_0548_humanized_L FV_L	GATATCCAGA CTTTTCGGCGT ATCACTTGTA AAATATCTGG GGCAAAGCCC ACAAATAACC CGCTTTTCAG TTTACGTTTA GAAGACATTG CATGACAGTG ACTAAGGTGG	TGACTCAATC CAGTGGGTGA AGGCCTCCCA ACTGGTACCA CAAAGTTACT TACACACAGG GTAGCGGAAG CGATCTCCAG CCACTTATTA GCTATACCTT AAATCAAG	TCCATCGAGC TCGTGTTACC AAACATTAAT GCAGAAACCG GATCTACTAT TGTTCCATCA CGGGACCGAC CTTGCAACCA TTGTCTCCAG TGGACAGGGT
163	0159_chimera_H FV_H	GAAGTCCAGC CTAGGGAAAC TCTTGCAAGG AGTACCTACA CCTGGAAAGG ATTGATCCTG GCTGAGAAGT ACTGCAGATA ATGCAACTCA AGAGCAATCT CGGGTGTCTT GTCATGGTCA	TGCAGCAGTC CTGGGACCTC TTTCTGGCTA TGCACTGGGT GCCTGGAATG CAAATGGAAA TCAAAAGGAA CATCGTCCAA GCCAACTGAA ATTTTGTGC TTGAGTACTG CCGTCTCCTC	TGGGGCTGAG AGTCAAGTTG TAACATTAGG GAGTCAGAGG GATAGGAAGG TACTATTTAT GGCCACACTG CACAGCCTAC ATCTGACGAC TATGGAAGTA GGGCCAGGGA A
164	0159_chimera_L FV_L	GACATCCAGA CTGTCTGCAT ATCAACTGCA AAGTACTTAG GGTGAAGGTC ACAAACAATT AGGTTTCAGT TTCACACTTA GAAGATGTTG	TGACCCAGTC CTGTGGGAGA AAGCAAGTCA ACTGGTATCA CCAAACTCCT TACATACAGG GCAGTGGGTC CCATCAGCAG CCACATATTT	TCCTTCATT CAGAGTCACT GAATATTAAC GCAAAAGCTT GATATATTAT AATCCCATCA TGGTACTGAT CCTGCAGCCT CTGCCTTCAG

		CATGACAGTG GGTACACGTT TGGAGCTGGG ACCAAGCTGG AACTGAAA
165	0955_0300_humanized_ H FV_H	CAAGTGCAGC TGGTACAGTC TGGTGCCGAG GTTAAAAAGC CGGGTAGTAG CGTGAAAGTA AGCTGCAAAG TGAGTGGTTA TAGCATTTCGT TCAACCTATA TGCACCTGGGT TCGTCAGGCG CCAGGCCAAG GTCTCGAGTG GATGGGAAGG ATTGATCCTG CAAATGGAAA TACAATATAT GCTGAGAGGT TCAAAAACCG CGTGACGCTG ACCGCAGATA CCAGCACTTC CACGGCGTAC ATGGAAGTGT CCTCCCTGCG GTCCGAAGAT ACCGCAGTAT ATTATTGCGC CGTAGAAATC CTAGGCATTT TTGATTATTG GGGGCAGGGC ACACTGGTCA CCGTATCGAG C
166	0955_0300_humanized_ L FV_L	GATATACAAA TGACACAGAG TCCGAGTTCC CTATCAGCGA GCGTGGGAGA CAGGGTTACC ATAACGTGTA AAGCATCGCA GAATATTGAC AAATATCTCG ACTGGTATCA ACAGAAGCCG GGCAAAGCAC CAAAACCTCT TATGTATAAC ACCAACTCTT TACATACTGG CGTCCCAAGT CGTTTTTCGG GGTCTGGCAG CGGCACAGAT TTTACGCTCA CCATTAGTTC GCTGCAGCCA GAAGACTTTG CTACCTACTT CTGTCTGCAA CATAATAGCG GCTACACCTT CGGTCAGGGG ACTAAAGTTG AAATAAAA
167	0170_chimera_H FV_H	GAAGTCCAGC TGCAGCAGTC CGGGGCTGAG CTTGGGAAAC CTGGGACCTC AGTCAAGTTG TCTTGCAAGG TTTCTGGCTA TAGTATTAGG AGTACCTACA TGCACCTGGGT GAATCAGAGG CCTGGAAAGG GCCTGGAATG GGTAGGAAGG ATTGATCCTG CAAATGGAAA TACAATATAT GCTGAGAGGT TCAAAAACAA GGCCACACTG ACTGCAGATA CATCGTCCAA CACAGCCTAC ATGCAACTCA GCCAACTGAA ATCTGACGAC ACAGCAATCT ATTTTGTGTC TGTGGAGATC CTTGGGATCT TTGATTACTG GGGCCAAGGA GTCATGGTCA CAGTCTCCTC A
168	0170_chimera_L FV_L	GACATCCAGA TGACCCAGTC TCCTTCATTC CTGTCTGCAT CTGTGGGAGA CAGAGTCACT ATCAACTGCA AAGCAAGTCA GAATATTGAC AAGTACTTAG ACTGGTATCA GCAAAAGCTT GGTGAAGCTC CCAAACCTCT GATGTATAAT ACAAACAGTT TGCATACAGG AATTCCATCA AGGTTCAGTG GCAGTGGATC TGGTACTGAT TTCACACTTA CCATCAGCAG CCTGCAGCCT GAAGATGTTG CCACATATTT CTGCCTTCAG CATAACAGTG GGTACACGTT TGGAGCTGGG ACCAAGCTGG AACTGAAA
169	0564_humanized_H FV_H	GAGGTACAGC TGGTTGAATC GGGTGGTGGT CTGGTTCAGC CGGGTGGCTC ATTAAGACTG TCATGCGCCG TGTCTGGTTA TAAAATCCGC AGCAGTTATA TGCATTGGGT TCGTCAAGCT CCGGGTAAAG GTTTAGAATG GATCGGGAGG ATTGATCCTG CAAATGGAAA TACTATATAC GGTGACAAGT TCAAAAGTCG GTTTACTCTG

		TCATCCGATA CCGCGAAAAA CTCAGCCTAT CTGCAAAATGA ATTCCCTGCG CGCGGAAGAC ACTGCTGTCT ATTATTGCGC AATTGATATC GGTACCACGT TTGATTATTG GGGCCAGGGT ACGTTGGTGA CGGTTAGCTC C
170	0564_humanized_L FV_L	GACATCCAAA TGACCCAATC TCCGAGTTCT CTGTCTGCTT CCGTGGGCGA CCGAGTCACC ATAACCTGTA AGGCTTCGCA ACACATCAAC CGTTATTTGA ACTGGTATCA ACAGAAACCG GGGAAAGCGC CGAAATTGCT GATTTATGAT GCTAACAACC TGCAGACAGG CGTACCATCG CGATTTAGCG GCTCCGGAAG CGGGACGGAT TTTACTCTCA CCATCAGCTC TCTGCAGCCG GAAGACTTTG CAACCTATTT CTGTTTACAG CATAATTCCT GGCCGAATAC CTTTGGCCAG GGGACAAAGG TGGAAATCAA A
171	0180_chimera_H FV_H	GAGGTCCAGC TGCAGCAGTC TGGGGCTGAG CTTGGGAAAC CTGGGACCTC AGTCAAGTTG TCTTGCAAGG TTTCTGGCTA TAAGATTAGG AGTTCCTACA TGCACTGGGT GAATCAGAGG CCTGGAAAGG GCCTGGAATG GATAGGAAGG ATTGATCCTG CAAATGGAAA TACTATATAC GGTGACAAGT TCAAAAGTAA GGCCACACTG ACTTCAGATA CATCGTCCAA CACAGCCTAC ATCCAACCTCA GCCAACTGAA ATCTGACGAC ACAGCAATCT ATTTTTGTGC TATAGATATA GGTACAACCT TTGATTATTG GGGCCAAGGA GTCATGGTCA CAGTCTCCTC A
172	0180_chimera_L FV_L	GACATCCAGA TGACCCAGTC TCCTTCATT CTGTCTGCAT CTGTGGGAGA CAGAGTCACT ATCAACTGCA AAGCAAGTCA GCATATTAAT AGGTACTTAA ACTGGTACCA GCAAAAGCTT GGAGAAGCTC CCAAACCTCT GATATATGAT GCAAACAATT TGCAAACGGG CATCCCATCA CGGTTCAAGT GCAGTGGATC TGGTACTGAT TTCACACTCA CCATCAGCAG CCTGCAGCCT GAAGATGTTG CCACATATTT CTGCTTGCAG CATAATAGTT GGCCGAACAC GTTTGGGGCT GGGACCAAGC TGGAATTGAA A
173	0027_chimera_H FV_H	GAAGTCCACC TGCACCAGTC TGGGCCTGAG CTTGGGAGGC CTGGGTCTC AGTCAAGATT TCTTGCAAGG CTTCTGGCTA CACCTTTACA GATTACGTTA TGAAGTGGGT GAGGCAGAGT CCTGGACAGG GGCTGGAATG GATAGGATGG ATCAATCCTG AAGATTATAG TTTTGATTCT GGTGAGAAGT TCCTAGAGAG GGCCACACTG ACTGCAGCTA CGTCCTCCAA CACAGTCTAC ATCCAGCTTA GCGGCCTGAC ATCTGACGAC ACAGCCACCT ATTTTTGTGT TAGAGGGGGA CTACCCGGGG ATTGGTTTGC TTAGGGGGC CAAGGCACTC TGGTCACTGT CTCTTCA
174	0027_chimera_L FV_L	GACATCCAGA TGACCCAGTC TCCTTCATT CTGTCTGCAT CTGTGGGAGA CAGAATCACT ATCAACTGCA AGACAAGTCA GAATATTAAC AGGTACTTAA ACTGGTTCCA GCAAAAGCTT

		GGAGAACCTC CCAAACCTCCT GATATATAAT GCAAAACAGTT TGCAAGCGGA CATTCCATCA CGGTTCACTG GCAGTGGATC TGGTACTGAT TTCACACTCA CCATCACCAG CCTGCAGCCT GAAGATGTTG CCACATATTT CTGCTTGCAG CATCATTTCT GGCCGTACAC GTTTGGAGCT GGGACCAAGC TGGAAGTGAG A
175	truncated cynomolgus monkey E-selectin (amino acid residues 22-556 of UniProtKB sequence G8F370)	WSYNTSTEAMTYDEASAYCQORYTHLVAIQNKEE IEYLNSILSYSPSYWIGIRKVNNVWVWGTQKP LTEEAKNWAPGEPNNRQKDEDCVEIYIKRDKDVG MWNDERCSKKKLALCYTAACNTNTSCSGHGECVET INNYTCKCDPGFSGLECEQIVNCTALESPEHGS VCSHPLGNFSYSSSCSVSCDRGYLPSSVETTQCM SSGEWSVIPACKVVECDVATNPANGFVECFQNP GSFPWNTTCTFDCEEGLMGAQSLQCTSSGNWD NEKPTCKAVTCRAIRQPQNGSVRCSHSPAGEFTF KSSCNFTCEEGLMGAQSLQCTSSGNWD CEAFQCTALSNPERGYMNCLPSASGSFRNGSSCE FSCEQGFVLKGSKRLQCGPTGEWDNEKPTCEAVR CDAVHQFQRLVRCASPIGEFTYKSSCAFSCEE GFELHGSTQLECTSQGWTEEVPSQVVKCSSLA VLEKINMSSCGEPVFGTVCNFACPEGWRLNGSAA MTCGATGHWSGMLPTCEAPTESNTP
176	site of NG deamidation (underlined) in HC CDR2	IDPANGNTIYAEK
177	site of NR deamidation (underlined) in LC CDR1	TSQNINRYLNWYQQKPGK
178	H27 predicted non- germline T-cell epitope	YNIRSSYM
179	H63 predicted non- germline T-cell epitope	FKIRFTISA
180	H65 predicted non- germline T-cell epitope	IRFTISADN
181	L29 predicted non- germline T-cell epitope	INRYLNWYQ
182	L46 predicted non- germline T-cell epitope	LLIYNANSL
183	L47 predicted non- germline T-cell epitope	LIYNANSLQ
184	L48 predicted non- germline T-cell epitope	IYNANSLQT
185	L49 predicted non- germline T-cell epitope	YNANSLQTG

186	DP-54 sequence of H59-H65	YVDSVKG
187	H63 predicted T-cell epitope	VKGRFTISA
188	H27 predicted T-cell epitope	YAIRSAYMH
189	H63 predicted T-cell epitope	VEGRFTISA
190	H63 predicted T-cell epitope	VTGRFTISA
191	H63 predicted T-cell epitope	VKERFTISA
192	L29 predicted T-cell epitope	IERYLNWYQ
193	tryptic peptide for E-selectin	CSSLAVLEK
194	1444 HC CDR2 region	IDPANGNTIYVDSVTGR
195	1444 LC CDR1 region	TSQNIERYLNWYQQKPGK
196	human E-selectin UniProtKB P16581 (leader sequence is underlined)	<u>MIASQFLSALTILVLLIKESGAWSYNTSTEAMTYD</u> <u>EASAYCQQR</u>YTHLVAIQNKEEIEYLNSILSYSPS YYWIGIRKVNNVWVWVGTKPLTEEAKNWAPGEP NNRQKDEDCVEIYIKREKDVGMWNDERCSKKLA LCYTAACTNTSCSGHGECVETINNYTCKCDPGFS GLKCEQIVNCTALESPEHGSLVCSHPLGNFSYNS SCSISCDRGYLPSSMETMQCMSSGEWSAPIACN VVECDAVTNPANGFVECFQNPGSFPWNTTCTFDC EEGFELMGAQSLQCTSSGNWDNEKPTCKAVTCRA VRQPQNGSVRCSHSPAGEFTFKSSCNFTCEEGFM LQGPAQVECTTQGWTOQIPVCEAFQCTALSNPE RGYMNCLPSASGSFRYGSSCEFSCEQGFVLKGSK RLQCGPTGEWDNEKPTCEAVRCDAVHQPPKGLVR CAHSPIGFTYKSSCAFSCEEGFELHGSTQLECT SQGQWTEVPSCQVVKCSSLAVPGKINMSCSGEP VFGTVCKFACPEGWTLNLSAARTCGATGHWSGLL PTCEAPTESNIPLVAGLSAAGLSLLTLAPFLLWL RKCLRKAKKFVPASSCQSLES DGSYQKPSYIL
197	truncated human E-selectin (amino acid residues 22-178 of UniProtKB sequence P16581)	WSYNTSTEAMTYDEASAYCQQR~~YTHLVAIQNKEE~~ IEYLNSILSYSPSYWIGIRKVNNVWVWVGTKP LTEEAKNWAPGEPNNRQKDEDCVEIYIKREKDV GMWNDERCSKKKLALCYTAACTNTSCSGHGECVET INNYTCKCDPGFSGLKCEQIV
198	mouse E-selectin UniProtKB Q00690 (leader sequence is underlined)	<u>MNASRFLSALVFVLLAGESTAWYYNASSELMTYD</u> <u>EASAYCQR</u>DYTHLVAIQNKEEINYLNSNLKHSPS YYWIGIRKVNNVWVWVGTKPLTEEAQNWAPGEP NNKQRNEDCVEIYIQRTKDSGMWNDERCNKKLA LCYTASCTNASCSGHGECIETINSYTCKCHPGFL GPNCEQAVTCKPQEHDPYDGLNCSHPFGPFSYNS SCSFGCKRGYLPSSMETTVRCTSSGEWSAPAPAC HVVECEALTHPAHGIRKCSSNPGSYPWNTTCTFD CVEGYRRVGAQNLQCTSSGIWDNETPSCKAVTCD AIPQPQNGFVSCSHSTAGELAFKSSCNFTCEQSF TLQGPAQVECSAQGWTPQIPVCKAVQCEALSAP QQGNMKCLPSASGPFQNGSSCEFSCEEGFELKGS RRLQCGPRGEWDSKKPTCSAVKCDDVPRPQNGVM ECAHATTGEFTYKSSCAFQCNEGFS LHGSAQLEK

		TSQGKWTQEVPSQVQVQCPSLDVP GKMMNSCSGT AVFGTVCEFTCPDDWTLNGSAVLTCGATGRWSGM PPTCEAPVSPTRPLVVALSAAGTSLTSSSLLYL LMRYFRKKAKKFVPASSCQSLQSFENYHVPSYNV
199	truncated mouse E-selectin (amino acid residues 22-178 of UniProtKB sequence Q00690)	WYNNASSELMTYDEASAYCQRDYTHLVAIQNKEE INYLNSNLKHSPSYWIGIRKVNNVWVGTGKP LTEEAQNWAPGEPNNKQRNEDCVEIYIQRTKDSG MWNDERCNKKKLALCYTASCTNASCSGHGECIET INSYTCKCHPGFLGPNCQAV
200	cynomolgus monkey E-selectin UniProtKB G8F370 (leader sequence is underlined)	MIASQFLSAL TLVLLIKESG AWSYNTSTEA MTYDEASAYC QORYTHLVAI QNKEEIEYLN SILSYSPSYWIGIRKVNNV WVWVGTVQKPL TEEAKNWAPG EPNNRQKDED CVEIYIKRDK DVGMWNDERC SKKKLALCYT AACTNTSCSG HGECVETINN YTCKCDPGFS GLECEQIVNC TALESPEHGS LVCSHPLGNF SYSSSCSVSC DRGYLPSSVE TTQCMSSGEW SVP IPACKVV ECDAVTNPAN GFVECFQNP G SFPWNTTCTF DCEEGFELMG AQLQCTSSG NWDNEKPTCK AVTCRAIRQP QNGSVRCSHS PAGEFTFKSS CNFTCEEGFM LQGAQVECT TQGWTTQVP VCEAFQCTAL SNPERGYMNC LPSASGSFRN GSSCEFSCEQ GFVLKGSKRL QCPTGEWDN EKPTCEAVRC DAVHQPQRGL VRCASPIGE FTYKSSCAFS CEEGFELHGS TQLECTSQGQ WTEEVPSQV VKCSSLAVLE KINMSCSGEP VFGTVCNFAC PEGWRLNGSA AMTCGATGHW SGMLPTCEAP TESNTPLVAG LSAAGLSLLT LAPFLLWLRK CFRKAKKFVP ASSCQSLES GSYQKPSYIL
201	cynomolgus monkey E-selectin (amino acid residues 22-610 of UniProtKB sequence G8F370)	WSYNTSTEAMTYDEASAYCQORYTHLVAIQNKEE IEYLNSILSYSPSYWIGIRKVNNVWVWVGTVQKPL LTEEAKNWAPGEPNNRQKDED CVEIYIKRDKDV MWNDERCSKKKLALCYTAACTNTSCSGHGECVET INNYTCKCDPGFSGLECEQIVNCTALESPEHGS VCSHPLGNFSYSSSCSVSCDRGYLPSSVETTQCM SSGEWSVP IPACKVVECDAVTNPANGFVECFQNP GSFPWNTTCTFDCEEGFELMG AQLQCTSSGNWD NEKPTCKAVTCRAIRQPQNGSVRCSHS PAGEFTF KSSCNFTCEEGFMLQGAQVECTTQGWTTQVPV CEAFQCTALSNPERGYMNC LPSASGSFRNGSSCE FSCEQGFVLKGSKRLQCPTGEWDNEKPTCEAVR CDAVHQPQRGLVRCASPIGEFTYKSSCAFSCEE GFELHGSTQLECTSQGQWTEEVPSQVVKCSSLA VLEKINMSCSGEPVFGTVCNFACPEGWRLNGSAA MTCGATGHWSGMLPTCEAPTESNTPLVAGLSAAG LSLLTLAPFLLWLRKCFRKAKKFVPASSCQSLES DGSYQKPSYIL
202	IGHV3-07*01 (DP-54) heavy chain germline	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWM SWVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRF TISRDNKNSLYLQMNSLRAEDTAVYYCAR
203	IGHJ4*01	YFDYWGGQTLVTSS
204	IGKV1-39*01 (DPK-9) light chain germline	DIQMTQSPSSLSASVGRVTITCRASQSISSYLN WYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQSYSTP

205	IGKJ1*01	WTFGQGTKVEIK
		GAAGTGCAGC TTGTGGAATC CGGGGGCGGC TTGGTCCAAC CGGGTGGCAG CCTCCGTCTG TCGTGCGCGG CTTCCGGGCTA TGCCATCCGT TCTGCCTACA TGCACTGGGT TCGCCAGGCG CCTGGGAAGG GCCTGGAATG GGTGGCCAGG ATTGATCCTG CAAACGGAAA TACTATATAT GTGGACTCCG TGACCGGCCG CTTTACAATC AGCGCCGACA ACGCTAAGAA TTCCGCCTAC CTGCAAAATGA ATAGCCTGCG GGCAGAGGAT ACCGCGGTGT ACTATTGTGC CATGGATTTA TATTCCACGT CTGAATATTG GGGCCAAGGA ACCCTGGTAA CGGTGTCGTC GGCCTCGACC AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG GCCCTGGGCT GCCTGGTCAA GGACTIONTTC CCCGAACCGG TGACGGGTGTC GTGGAACCTCA GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT GACAAAACCTC ACACATGCCC ACCGTGCCCC GCACCTGAAG CCGCTGGGGC ACCGTGAGTC TTCCTCTTCC CCCCCAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGAGGA GATGACCAAG AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC GACGGCTCCT TCTTCCTCTA TAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC CTCTCCCTGT CCCCAGGA
206	1444 HC nucleic acid sequence (without C- terminal lysine (K))	

[0151] Table 3. Anti-E-selectin antibodies.

Antibody	HCD R-1	HCD R-2	HCD R-3	LCD R-1	LCD R-2	LCD R-3	VH	VL	JH (FW- H4)	JK (FW- L4)	CL	CH	HC	LC	L Leader	H Leader	FV- H DNA	FV- L DNA
1444 optimized	8	9	10	2	3	4	11	5	12	6	14	15/ 16	7/ 13	1	129	130	136	137
0841 humanized	23	24	10	18	19	20	25	21	12	6	14	16	22	17	129	129	144	145
0978 humanized	23	29	10	18	3	20	30	27	12	6	14	16	28	26	129	129	146	147
0164 chimera	23	24	10	18	19	20	35	32	36	33	14	15	34	31	131	131	148	149
1448 optimized	8	38	10	2	3	4	39	5	12	6	14	16	37	1	129	129	150	137
1284 optimized	8	41	10	2	3	4	42	5	12	6	14	16	40	1	129	129	151	137
1282 optimized	8	44	10	2	3	4	45	5	12	6	14	16	43	1	129	129	152	137
0525 humanized	52	53	54	47	48	49	55	50	12	6	14	16	51	46	129	129	153	154
0039 chimera	52	53	54	47	48	49	60	57	61	58	14	15	59	56	131	131	155	156
0265_0254 humanized	63	64	65	68	69	70	66	71	12	6	14	16	62	67	129	129	157	158
0158 chimera	63	64	65	68	69	70	75	73	36	33	14	15	74	72	131	131	159	160
0929_0548 humanized	77	78	79	82	83	84	80	85	12	6	14	16	76	81	129	129	161	162
0159 chimera	77	78	79	82	83	84	90	87	36	88	14	15	89	86	131	131	163	164
0955_0300 humanized	92	93	94	97	98	99	95	100	12	6	14	16	91	96	129	129	165	166

Antibody	HCD R-1	HCD R-2	HCD R-3	LCD R-1	LCD R-2	LCD R-3	VH	VL	JH (FW _{H4})	JK (FW _{L4})	CL	CH	HC	LC	L Leader	H Leader	FV _H DNA	FV _L DNA
0170 chimera	92	93	94	97	98	99	104	102	36	88	14	15	103	101	131	131	167	168
0564 humanized	111	112	113	106	107	108	114	109	12	6	14	16	110	105	129	129	169	170
0180 chimera	111	112	113	106	107	108	118	116	36	88	14	15	117	115	131	131	171	172
0027 chimera	125	126	127	18	120	121	128	122	12	123	14	15	124	119	131	131	173	174

[0152] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VH domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:11. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VH domain comprising, or
5 consisting of, an amino acid of SEQ ID NO:11.

[0153] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VH domain may comprise an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identical to the amino acid sequence of any one of SEQ ID NOs: 11, 25, 30, 35, 39, 42, 45, 55, 60, 66, 75, 80, 90, 95, 104, 114, 118 and
10 128. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VH domain may comprise or consist of the amino acid sequence of any one of SEQ ID NOs: 11, 25, 30, 35, 39, 42, 45, 55, 60, 66, 75, 80, 90, 95, 104, 114, 118 and 128.

[0154] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence at least 90% identical
15 to the amino acid sequence of SEQ ID NO:5. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising, or consisting of, an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:5.

[0155] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment
20 thereof, may comprise a VL domain may comprise an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identical to the amino acid sequence of any one of SEQ ID NOs: 5, 21, 27, 32, 50, 57, 71, 73, 85, 87, 100, 102, 109, 116 and 122. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain may comprise or consist of the amino acid sequence of any one of SEQ ID NOs: 5,
25 21, 27, 32, 50, 57, 71, 73, 85, 87, 100, 102, 109, 116 and 122.

[0156] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:5 and a VH domain comprising an amino acid sequence of SEQ ID NO:11. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid
30 sequence of SEQ ID NO:5 and a VH domain comprising an amino acid sequence of any one of SEQ ID NO:39, 42 or 45. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:21 and a VH domain comprising an amino acid sequence of SEQ ID NO:25. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of

SEQ ID NO:27 and a VH domain comprising an amino acid sequence of SEQ ID NO:30. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:32 and a VH domain comprising an amino acid sequence of SEQ ID NO:35. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:50 and a VH domain comprising an amino acid sequence of SEQ ID NO:55. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:57 and a VH domain comprising an amino acid sequence of SEQ ID NO:60. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:71 and a VH domain comprising an amino acid sequence of SEQ ID NO:66. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:73 and a VH domain comprising an amino acid sequence of SEQ ID NO:75. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:85 and a VH domain comprising an amino acid sequence of SEQ ID NO:80. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:87 and a VH domain comprising an amino acid sequence of SEQ ID NO:90. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:100 and a VH domain comprising an amino acid sequence of SEQ ID NO:95. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:102 and a VH domain comprising an amino acid sequence of SEQ ID NO:104. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:109 and a VH domain comprising an amino acid sequence of SEQ ID NO:114. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:116 and a VH domain comprising an amino acid sequence of SEQ ID NO:118. An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of SEQ ID NO:122 and a VH domain comprising an amino acid sequence of SEQ ID NO:128.

[0157] An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL domain comprising an amino acid sequence of any one of SEQ ID NOs:5, 21, 27, 32, 50, 57, 71, 73, 85, 87, 100, 102, 109, 116 and 122 and a VH domain comprising an amino acid sequence of any one of SEQ ID NOs:11, 25, 30, 35, 39, 42, 45, 55, 60, 66, 75, 80, 90, 95, 104,

114, 118 and 128.

[0158] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises of a LCDR-1, a LCDR-2, and a LCDR-3 as set forth in the amino acid sequence of at least one of SEQ ID Nos:5, 21, 27, 32, 50, 57, 71, 73, 85, 87, 100, 102, 109, 116 and 122.

[0159] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, further comprises a HCDR-1, a HCDR-2, and a HCDR-3 as set forth in the amino acid sequence of at least one of SEQ ID NOs:11, 25, 30, 35, 39, 42, 45, 55, 60, 66, 75, 80, 90, 95, 104, 114, 118 and 128.

[0160] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a LCDR-1, a LCDR-2, a LCDR-3 as set forth in the amino acid sequence of SEQ ID NO:5, and a HCDR-1, a HCDR-2, and a HCDR-3 as set forth in the amino acid sequence of SEQ ID NO:11.

[0161] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a LCDR-1 comprising an amino acid of SEQ ID NO:2, a LCDR-2 comprising an amino acid sequence of SEQ ID NO:3 and a LCDR-3 comprising an amino acid of SEQ ID NO:4. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a HCDR-1 comprising an amino acid of SEQ ID NO:8, a HCDR-2 comprising an amino acid sequence of SEQ ID NO:9 and a HCDR-3 comprising an amino acid of SEQ ID NO:10. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a HCDR-1 comprising an amino acid sequence of SEQ ID NO:8, a HCDR-2 comprising an amino acid sequence of any one of SEQ ID NO:38, 41 or 44 and a HCDR-3 comprising an amino acid sequence of SEQ ID NO:10.

[0162] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a LCDR-1 comprising an amino acid of any one of SEQ ID NO:2, 18, 47, 68, 82, 97 or 106, a LCDR-2 comprising an amino acid sequence of any one of SEQ ID NO:3, 19, 48, 69, 83, 98, 107 or 120 and a LCDR-3 comprising an amino acid of SEQ ID NO:4, 20, 49, 70, 84, 99, 108 or 121. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a HCDR-1 comprising an amino acid of SEQ ID NO:8, 23, 52, 63, 77, 92, 111 or 125, a HCDR-2 comprising an amino acid sequence of SEQ ID NO:9, 24, 29, 38, 41, 44, 53, 64, 78, 93, 112 or 126 and a HCDR-3 comprising an amino acid of SEQ ID NO:10, 54, 65, 79, 94, 113 or 127.

[0163] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a LCDR-1, a LCDR-2, and a LCDR-3 as set forth in the amino acid

sequence encoded by the insert of the plasmid deposited with the ATCC having the Accession number PTA-126530.

[0164] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a HCDR-1, a HCDR-2, and a HCDR-3 as set forth in the amino acid sequence encoded by the insert of the plasmid deposited with the ATCC having Accession number PTA-126529.

[0165] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a LCDR-1, a LCDR-2, and a LCDR-3 amino acid sequence encoded by the insert of the plasmid deposited with the ATCC having the Accession number PTA-126530, and a HCDR-1, a HCDR-2, and a HCDR-3 amino acid sequence encoded by the insert of the plasmid deposited with the ATCC having Accession number PTA-126529.

[0166] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a light chain variable region comprising the amino acid sequence encoded by the insert of the plasmid deposited with the ATCC having the Accession number PTA-126530.

[0167] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a heavy chain variable region comprising the amino acid sequence encoded by the insert of the plasmid deposited with the ATCC having the Accession number PTA-126529.

[0168] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a light chain comprising the amino acid sequence encoded by the insert of the plasmid deposited with the ATCC having the Accession number PTA-126530.

[0169] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a heavy chain comprising the amino acid sequence encoded by the insert of the plasmid deposited with the ATCC having the Accession number PTA-126529.

[0170] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a LC comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:1. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises, or consist of, an amino acid sequence of SEQ ID NO:1

[0171] A LC may comprise an amino acid sequence at least 90, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identical to the amino acid sequence of any one of SEQ ID NO:1, 17, 26, 31, 46, 56, 67, 72, 81, 86, 96, 101, 105, 115, or 119. In some embodiments, an antibody LC may comprise an amino acid sequence comprising or consisting of any one of SEQ

ID Nos: 1, 17, 26, 31, 46, 56, 67, 72, 81, 86, 96, 101, 105, 115, or 119.

[0172] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a HC comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:7 or 13. In some embodiments, an anti-E-selectin antibody,
5 or antigen-binding fragment thereof, may comprise a HC comprising, or consisting of, an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:7 or 13.

[0173] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a heavy chain comprising an amino acid sequence at least 90, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identical to the amino acid sequence of any
10 one of SEQ ID NOs:7, 13, 22, 28, 34, 37, 40, 43, 51, 59, 62, 74, 76, 89, 91, 103, 110, 117 or 124. In some embodiments, an antibody HC may comprise an amino acid sequence comprising, or consisting of, any one of SEQ ID NOs:7, 13, 22, 28, 34, 37, 40, 43, 51, 59, 62, 74, 76, 89, 91, 103, 110, 117 or 124.

[0174] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment
15 thereof, comprises a LC comprising, or consisting of the amino acid sequence of SEQ ID NO:1 and a HC comprising, or consisting of, the amino acid sequence of SEQ ID NO:7 or SEQ ID NO:13. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, lacks effector function (i.e., is effector null).

[0175] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment
20 thereof, may comprise a heavy chain comprising a VH domain comprising the amino acid sequence of any one of SEQ ID NOs: 11, 25, 30, 35, 39, 42, 45, 55, 60, 66, 75, 80, 90, 95, 104, 114, 118 and 128 (e.g., SEQ ID NO: 11), and further comprising an IgG1 constant domain (e.g., an IgG1 constant domain comprising the amino acid sequence of SEQ ID NO:15 or SEQ ID NO:16).

[0176] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment
25 thereof, comprises a heavy chain comprising a VH domain comprising the amino acid sequence of SEQ ID NO:11 and further comprises an IgG1 constant domain comprising the amino acid sequence of SEQ ID NO:15 or SEQ ID NO:16. In some embodiments, an antibody, or antigen-binding fragment thereof, comprises a VH domain consisting of the amino acid sequence of
30 SEQ ID NO:11 and further comprises an IgG1 constant domain consisting of the amino acid sequence of SEQ ID NO:15 or SEQ ID NO:16. In some embodiments, an anti-E-selectin antibody lacks effector function(s).

[0177] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a light chain constant region chosen from, e.g., a (e.g., human) kappa light chain

constant region (e.g., encoded by the amino acid sequence of SEQ ID NO:14) or a lambda light chain constant region.

[0178] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a light chain comprising a VL domain comprising the amino acid sequence of any one of SEQ ID NOs: 5, 21, 27, 32, 50, 57, 71, 73, 85, 87, 100, 102, 109, 116 and 122 (e.g., SEQ ID NO:5), and further comprises a kappa constant domain comprising the amino acid sequence of SEQ ID NO:14. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, comprises a light chain comprising a VL domain consisting of the amino acid sequence of SEQ ID NO:5 and further comprises a kappa constant domain consisting of the amino acid sequence of SEQ ID NO:14.

[0179] In some embodiments, the constant region of an anti-E-selectin antibody, or antigen-binding fragment thereof, can be altered, e.g., mutated, to modify the properties of the antibody (e.g., to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, and/or complement function).

[0180] In some aspects, an antibody, or antigen-binding fragment, variant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 conservative or non-conservative substitutions, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 additions and/or deletions to the full length heavy chain (e.g., a HC of the amino acid sequence of SEQ ID NO:7 or 13) and/or the full length light chain. In a further aspect, a variant antibody shares at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the full length heavy chain, and wherein said antibody or antigen-binding fragment specifically binds E-selectin. In a further aspect, a variant antibody shares at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the full length light chain (e.g., a LC of the amino acid sequence of SEQ ID NO:1), and wherein said antibody or antigen-binding fragment specifically binds E-selectin.

Germline substitutions

[0181] A wide variety of acceptor human germline sequences are available and the process for "humanizing" a non-human species antibody to use in humans is well-known in the art and also discussed elsewhere herein. Therefore, the skilled artisan would appreciate that the above CDR sequences from a mouse, rat, etc., can be placed in the context of human variable domain amino acid sequences. In doing so, changes to the acceptor human germline sequences are generally made to preserve antibody binding and other desirable characteristics of the original parent (i.e., donor) antibody. Both the CDRs and framework regions (FW) may be engineered

as follows.

[0182] In certain embodiments, a substitution is a human germline substitution in which a (donor) CDR residue is replaced with the corresponding human germline (acceptor) residue, to increase the human amino acid content and potentially reduce immunogenicity of the antibody as described in, e.g., U.S. Patent Application Publication No. 2017/0073395 and Townsend et al., Proc. Nat. Acad. Sci. USA 2015; 112(50):15354-15359, both of which are herein incorporated by reference in their entirety.

[0183] An antibody, or antigen-binding fragment thereof, may comprise a VH framework comprising a human germline VH framework sequence. In some aspects, a VH framework from the following germlines may be used: IGHV1-2*02, IGHV1-3*01, IGHV1-46*01, IGHV1-69*01, IGHV1-69*02, IGHV1-8*01, IGHV3-7*01, IGHV3-13*01, IGHV3-23*01, IGHV3-23*04, IGHV3-30*01, IGHV3-30*18, IGHV5-10-1*01, IGHV5-10-1*04, or IGHV5-51*01 (germline names are based on IMGT germline definition). In some embodiments, an anti-E-selectin antibody, or antigen binding fragment thereof, uses the VH framework from germline IGHV3-7*01 (SEQ ID NO:202). In some embodiments, an anti-E-selectin antibody, or antigen binding fragment thereof, uses the VH framework from germline IGHV3-7*01 (SEQ ID NO:202) for the CDR regions and IGHJ4*01 (SEQ ID NO:203) for the framework region.

[0184] Preferred human germline light chain frameworks are frameworks derived from VK or V λ germlines. In some aspects, a VL framework from the following germlines may be used: IGKV1-12*01, IGKV1-13*02, IGKV1-33*01, IGKV1-39*01, IGKV1-5*01, IGKV3-11*01, IGKV3-15*01, IGKV3-20*01, IGKV3D-20*02, and IGKV4-1*01 (germline names are based on IMGT germline definition). In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, uses the VL framework from germline IGHV1-39*01 (SEQ ID NO:204). In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, uses the VL framework from germline IGHV1-39*01 (SEQ ID NO:204) for the CDR regions and IGKJ1*01 (SEQ ID NO:205) for the framework region.

[0185] Alternatively, or in addition, the framework sequence may be a human germline consensus framework sequence, such as the framework of human V λ 1 consensus sequence, VK1 consensus sequence, VK2 consensus sequence, VK3 consensus sequence, VH3 germline consensus sequence, VH1 germline consensus sequence, VH5 germline consensus sequence, or VH4 germline consensus sequence. Sequences of human germline frameworks are available from various public databases, such as V-base, IMGT, NCBI, or Abysis.

[0186] An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VL framework comprising a human germline VL framework sequence. A VL framework may

comprise one or more amino acid substitutions, additions, or deletions, while still retaining functional and structural similarity with the germline from which it was derived. In some aspects, a VL framework is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a human germline VL framework sequence. In some embodiments, an antibody, or antigen binding fragment thereof, comprises a VL framework comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid substitutions, additions or deletions relative to the human germline VL framework sequence. In some embodiments, the 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions or deletions are only in the framework regions. In some embodiments, the percent identity is based on similarity with VL domain excluding those portions herein defined as CDRs.

[0187] A human germline VL framework may be, for example, the framework of IGKV1-39*01. A human germline VL framework may be, for example, the framework of IGKV1-33*01. A human germline VL framework may be the framework of any one of human consensus sequence including: V λ , V λ 1, V λ 3, VK, VK1, VK2 or VK3.

[0188] In some embodiments, a VL framework is IGK-39*01_IGKJ1*01. Other similar framework regions are also predicted to deliver advantageous antibodies of the invention comprising CDRs of SEQ ID NOs: 2-4, 18-20, 47-49, 68-70, 82-84, 97-99, 106-108, 120 and 121; and CDRs specified by the following VL amino acid sequences: SEQ ID NOs: 5, 21, 27, 32, 50, 57, 71, 73, 85, 87, 100, 102, 109, 116, 122, which may comprise 99%, 97%, 97%, 96%, 80%, 76%, 74% and 66%, identity respectively to the framework region of any one of IGKV1-12*01, IGKV1-13*02, IGKV1-33*01, IGKV1-39*01, IGKV1-5*01, IGKV3-11*01, IGKV3-15*01, IGKV3-20*01, IGKV3D-20*02, and IGKV4-1*01. In some embodiments, the percent identity is based on similarity with VL excluding those portions herein defined as CDRs.

[0189] An anti-E-selectin antibody, or antigen-binding fragment thereof, may comprise a VH framework comprising a human germline VH framework sequence. A VH framework may comprise one or more amino acid substitutions, additions, or deletions, while still retaining functional and structural similarity with the germline from which it was derived. In some aspects, a VH framework is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a human germline VH framework sequence. In some embodiments, an antibody, or antigen binding fragment thereof, comprises a VH framework comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid substitutions, additions or deletions relative to the human germline VH framework sequence. In some embodiments, the 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions or deletions are only in the framework regions. In some embodiments, the percent identity is based

on similarity with VH domain excluding those portions herein defined as CDRs.

[0190] A human germline VH framework may be, for example, the framework of IGHV3-7*01. A human germline VH framework may be, for example, the framework of IGHV1-46*01. A human germline VH framework may be, for example, IGHV1-69*01. A human germline VH framework may be the framework of human VH germline consensus sequence. The human germline VH framework may be the framework of a human germline consensus sequence including: VH3, VH5, VH1 or VH4.

[0191] In some embodiments, a VH framework is IGHV3-7*01. Other similar framework regions are also predicted to deliver advantageous antibodies of the invention comprising CDRs of SEQ ID NOs:8-10, 23, 24, 29, 38, 41, 44, 52-54, 63-65, 77-79, 92-94, 111-113, 125-127, and CDRs specified by any of the following VH amino acid sequences: SEQ ID NOs:11, 25, 30, 35, 39, 42, 45, 55, 60, 66, 75, 80, 90, 95, 104, 114, 118, 128, including IGHV1-2*02, IGHV1-3*01, IGHV1-46*01, IGHV1-69*01, IGHV1-69*02, IGHV1-8*01, IGHV3-7*01, IGHV3-13*01, IGHV3-23*01, IGHV3-23*04, IGHV3-30*01, IGHV3-30*18, IGHV5-10-1*01, IGHV5-10-1*04, or IGHV5-51*01, which may comprise 92, 93, 94, 95, 96, 97, 98, 99% identity respectively to the FW region of DP-54 and one or fewer amino acid differences in common structural features (Kabat Numbering). In some aspects, the percent identity is based on similarity with VH domain excluding those portions herein defined as CDRs.

[0192] In certain embodiments, the antibody, or antigen-binding fragment thereof, described herein comprises (i) a VH domain comprising an amino acid sequence that is at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:11, and/or (ii) a VL domain comprising an amino acid sequence that is at least 50%, at least 60%, at least 66%, at least 70%, at least 75%, at least 76%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5. Any combination of these VL and VH sequences is also encompassed by the invention.

[0193] In certain embodiments, the antibody, or antigen-binding fragment thereof, described herein comprises (i) a HC comprising an amino acid sequence that is at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7 or SEQ ID NO:13; and/or (ii) a LC comprising an amino acid sequence that is at least 50%, at least 60%, at least 70%, at

least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:1. Any combination of these HC and LC sequences is also encompassed by the invention.

- 5 **[0194]** In certain embodiments, the antibody, or antigen-binding fragment thereof, described herein comprises an Fc domain. The Fc domain can be derived from IgA (e.g., IgA₁ or IgA₂), IgG, IgE, or IgG (e.g., IgG₁, IgG₂, IgG₃, or IgG₄). In some embodiments, an anti-E-selectin antibody is and IgG1 antibody.

Biological activity of anti-E-selectin antibodies

- 10 **[0195]** In addition to binding an epitope on E-selectin, an antibody, or antigen-binding fragment thereof, of the disclosure can mediate a biological activity. That is, the disclosure includes an isolated antibody, or antigen-binding fragment thereof, that specifically binds E-selectin and mediates at least one detectable activity selected from the following:
- (i) binds specifically to human E-selectin
 - 15 (ii) binds specifically to cynomolgus monkey E-selectin;
 - (iii) reduces, inhibits and/or neutralizes interaction (e.g., binding) between soluble E-selectin (e.g., human, cynomolgus) and an E-selectin ligand (e.g., sialyl-Lewis A and/or sialyl-Lewis X ligand);
 - (iv) reduces, inhibits and/or neutralizes interaction (e.g., binding) between cell surface
20 expressed E-selectin (e.g., human, cynomolgus) and an E-selectin ligand (e.g., sialyl-Lewis A and/or sialyl-Lewis X ligand);
 - (v) reduces, inhibits and/or neutralizes interaction (e.g., adhesion) between cell surface expressed E-selectin (e.g., human, cynomolgus) and cell surface expressed E-selectin ligands (e.g., sialyl-Lewis A and/or sialyl-Lewis X ligand on,
25 e.g., HL-60 cells);
 - (vi) reduces, inhibits and/or neutralizes interaction (e.g., adhesion) of soluble E-selectin to cells expressing E-selectin ligand (e.g., HL-60);
 - (vii) reduces, inhibits and/or neutralizes adhesion of cells expressing E-selectin ligand (e.g., HL-60) to cells expressing E-selectin under static and physiological flow
30 conditions;
 - (viii) reduces, inhibits and/or neutralizes adhesion of activated human neutrophils to cells expressing E-selectin (e.g., human and cynomolgus) under physiological flow conditions;
 - (ix) binds to at least one amino acid residue selected from: T7, E8, A9, M10, T11, P46,

S47, Y48, N82, N83, Q85, E88, E92, Y94, R97, N105, E107, R108, S110, K111, K112 and K113 of human E-selectin;

- (x) has a viscosity of about 38 +/- 7 cP at a concentration of about 187 mg/mL at 25°C;
- 5 (xi) has a half-life of about 21.5 days (518 hours) when administered SC at a dose of 3 mg/kg;
- (xii) shows suitable formulation properties, including a high degree of thermal stability and minimal aggregation at high concentration; and
- 10 (xiii) may show reproducible expression and purity in large-scale manufacturing conditions.

[0196] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a binding affinity, expressed as K_D for soluble human E-selectin that is less than or equal to 200 nM, for example, less than or equal to 195 nM, 190 nM, 180 nM, 160 nM, 140 nM, 120 nM, 110 nM, 100 nM, 90 nM, 80 nM, 75 nM, 50 nM. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a binding affinity, expressed as K_D for soluble human E-selectin that is less than or equal to 200 nM as measured by SPR. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or an antigen-binding fragment thereof, has a binding affinity, expressed as K_D for soluble human E-selectin that is about 61.8 to about 68.4 +/- 3.18 nM as measured, for example, by SPR.

20 **[0197]** In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a binding affinity, expressed as K_D , for soluble cynomolgus E-selectin that is less than or equal to 200 nM, for example, less than or equal to 195 nM, 190 nM, 180 nM, 160 nM, 140 nM, 120 nM, 110 nM, 100 nM, 90 nM, 80 nM, 75 nM, 50 nM. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or an antigen-binding fragment thereof, has a binding affinity, expressed as K_D , for soluble cynomolgus E-selectin that is about 64.9 +/- 1.13 nM to about 81.5 nM as measured, for example, by SPR.

30 **[0198]** In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a binding affinity, expressed as EC_{50} , for cell-surface expressed human E-selectin that is less than or equal to 50 nM, for example, less than or equal to 48 nM, 45 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.75 nM, 0.5 nM, 0.25 nM or 0.1 nM, as measured, for example, by FACS. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or an antigen-binding fragment thereof, has a binding affinity, expressed as EC_{50} , for cell-surface expressed human E-selectin that is about 0.66 nM, as measured, for example, by FACS.

[0199] In some embodiments, an anti-E-selectin antibody, or an antigen-binding fragment thereof, has a binding affinity, expressed as EC_{50} , for cell-surface expressed cynomolgus E-selectin that is less than or equal to 50 nM, for example, less than or equal to 48 nM, 45 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.75 nM, 0.5 nM, 0.25 nM or 0.1 nM as measured, for example, by FACS. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or an antigen-binding fragment thereof, has a binding affinity, expressed as EC_{50} , for cell-surface expressed cynomolgus E-selectin that is about 0.75 nM, as measured, for example, by FACS.

[0200] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a binding affinity, expressed as EC_{50} , for cell-surface expressed human P-selectin that is greater than or equal to 350 nM, for example, greater than or equal to 400 nM, 450 nM, 500 nM, 550 nM, 600 nM, 650 nM or more, as measured, for example, by FACS.

[0201] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has weak or no binding to soluble rat, mouse or rabbit E-selectin or soluble human L- or P-selectin. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, demonstrates no binding to soluble rat, mouse or rabbit E-selectin or soluble human L- or P-selectin up to 405 nM as measured, for example, by SPR. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, demonstrates weak-non-saturable binding (e.g., >100x lower) to soluble mouse or rat E-selectin up to 133.3 nM as measured, for example, by direct binding ELISA.

[0202] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, binds to soluble human E-selectin with an EC_{50} of less than or equal to 2 nM, for example, less than or equal to 0.010 nM, 0.015 nM, 0.020 nM, 0.025 nM, 0.030 nM, 0.035 nM, 0.040 nM, 0.045 nM, 0.05 nM, 0.055 nM, 0.06 nM, 0.065 nM, 0.070 nM, 0.075 nM, 0.080 nM, 0.085 nM, 0.090 nM, 0.10 nM, 0.12 nM, 0.15 nM, 0.2 nM, 0.5 nM, 0.9 nM, 0.95 nM, 1 nM, 1.5 nM, 1.8 nM or 1.9 nM. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or an antigen-binding fragment thereof, binds to soluble human E-selectin with an EC_{50} of about 0.085 nM to about 0.12 as measured, for example, by direct binding ELISA.

[0203] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to soluble human E-selectin with an EC_{50} of less than or equal to 2 nM, for example, less than or equal to 0.010 nM, 0.015 nM, 0.020 nM, 0.025 nM, 0.030 nM, 0.035 nM, 0.040 nM, 0.045 nM, 0.05 nM, 0.055 nM, 0.06 nM, 0.065 nM, 0.070 nM, 0.075 nM, 0.080 nM, 0.085 nM, 0.090 nM, 0.10 nM, 0.12 nM, 0.15 nM, 0.2 nM, 0.5 nM, 0.9 nM, 0.95 nM, 1 nM, 1.5 nM, 1.8 nM or 1.9 nM, as measure, for example, by a AlphaLisa

homogenous competition assay.

[0204] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, binds to soluble cynomolgus E-selectin with an EC_{50} of less than or equal to 1 nM, for example, less than or equal to 0.010 nM, 0.015 nM, 0.020 nM, 0.025 nM, 0.030 nM, 0.035 nM, 5 0.040 nM, 0.045 nM, 0.05 nM, 0.055 nM, 0.06 nM, 0.065 nM, 0.070 nM, 0.075 nM, 0.080 nM, 0.085 nM, 0.090 nM, 0.10 nM, 0.12 nM, 0.15 nM, 0.2 nM, 0.5 nM, 0.9 nM or 0.95 nM. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or an antigen-binding fragment thereof binds to soluble cynomolgus E-selectin with an EC_{50} of 0.071 nM to 0.093 nM as measured, for example, by direct binding ELISA.

10 **[0205]** In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, binds free soluble human E-selectin in human serum with an IC_{50} of about 1 nM to about 3 nM, and preferably with an IC_{50} of about 1.2 nM.

[0206] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to soluble human E-selectin with an IC_{50} of 15 less than or equal to 100 nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 2 nM or 1 nM as measured, for example, by competition ELISA under static conditions. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to soluble human E-selectin with an IC_{50} of about 2.87 nM to about 3.01 nM as 20 measured, for example, by competition ELISA under static conditions.

[0207] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to soluble cynomolgus E-selectin with an IC_{50} of less than or equal to 100 nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 25 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 2 nM or 1 nM as measured, for example, by competition ELISA under static conditions. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to soluble cynomolgus E-selectin with an IC_{50} of about 2.39 nM to about 2.91 nM as measured, for example, by competition ELISA under static conditions.

[0208] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment 30 thereof, neutralizes binding of a sialyl-Lewis A ligand to cell-surface expressed human E-selectin with an IC_{50} of less than or equal to 50 nM, for example, less than or equal to 48 nM, 45 nM, 40 nM, 20 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM or 1 nM as measured, for example, by competition ELISA under static conditions. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to cell-surface

expressed human E-selectin with an IC_{50} of about 1.88 nM to about 2.89 nM as measured, for example, by competition ELISA under static conditions.

[0209] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to cell-surface expressed cynomolgus E-selectin with an IC_{50} of less than or equal to 100 nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 20 nM, 10 nM, 5 nM, 2 nM or 1 nM as measured, for example, by competition ELISA under static conditions. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, neutralizes binding of a sialyl-Lewis A ligand to cell-surface expressed cynomolgus E-selectin with an IC_{50} of about 1.47 nM to about 2.65 nM as measured, for example, by competition ELISA under static conditions.

[0210] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed human E-selectin with an IC_{50} of less than or equal to 100 nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 20 nM, 10 nM, 5 nM, 2 nM or 1 nM as measured, for example, under static conditions. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed human E-selectin with an IC_{50} of about 3.36 nM to about 4.7 nM as measured, for example, under static conditions.

[0211] In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed cynomolgus E-selectin with an IC_{50} of about 3.84 nM as measured, for example, under static conditions.

[0212] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed human E-selectin with an IC_{50} of less than or equal to 100 nM, for example, less than or equal to 95 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 2 nM or 1 nM as measured, for example, under physiological flow conditions. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed human E-selectin with an IC_{50} of about 4.25 nM to about 4.56 nM as measured, for example, under physiological flow conditions.

[0213] In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed cynomolgus E-selectin with an IC_{50} of about 4.32 nM to about 4.35 nM as measured, for example, under physiological flow conditions.

[0214] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to soluble human E-selectin with an IC_{50} of less than or equal to 300 nM, for example, less than or equal to 290 nM, 280 nM, 270 nM, 260 nM, 250 nM, 150 nM, 100 nM, 90 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 20 nM, 20 nM, 5 nM, 2 nM, or 1 nM as measured, for example, under physiologic flow conditions. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, inhibits adhesion of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to soluble human E-selectin with an IC_{50} of about 13.28 nM to about 15.94 nM as measured, for example, under physiologic flow conditions.

[0215] In some embodiments, anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, inhibits adhesion of activated human neutrophils to cell-surface expressed human, or cell surface expressed cynomolgus, E-selectin with an IC_{50} of about 2.87 nM to about 4.65 nM or 9.45 nM to about 16.33 nM as measured, for example, under physiologic flow conditions. In some embodiments, neutrophils are activated by TNF- α .

[0216] In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, inhibits adhesion of blood cells from SCD patients to soluble human E-selectin with an IC_{50} of about 6.17 nM to about 18.66 nM as measured, for example, under physiologic flow conditions. In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, inhibits adhesion of blood cells from SCD patients to soluble human E-selectin with an IC_{50} of about 12.4 nM as measured, for example, under physiologic flow conditions.

[0217] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, binds to at least one of three epitopes of human E-selectin as determined by, for example a competition assay using, for example an Octet biosensor.

[0218] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, binds to at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or more amino acid residues of human E-selectin selected from the group consisting of T7, E8, A9, M10, T11, P46, S47, Y48, N82, N83, Q85, E88, E92, Y94,

R97, N105, E107, R108, S110, K111, K112, K113 and a combination thereof, optionally, according to the crystal structure.

[0219] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, interacts with amino acid residues T7, E8, A9, M10, T11, P46, S47, Y48, N82, N83, Q85, E88, E92, Y94, R97, N105, E107, R108, S110, K111, K112 and K113 of human E-selectin, optionally, according to the crystal structure.

[0220] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof interacts with at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or more amino acid residues of human E-selectin within 3.8 Å selected from the group consisting of T7, E8, A9, T11, P46, S47, Y48, N82, N83, Q85, E92, Y94, N105, E107, R108, S110, K111, K112 and a combination thereof, optionally, according to the crystal structure.

[0221] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof interacts with amino acid residues T7, E8, A9, T11, P46, S47, Y48, N82, N83, Q85, E92, Y94, N105, E107, R108, S110, K111 and K112 of human E-selectin within 3.8 Å, optionally, according to the crystal structure.

[0222] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof interacts with at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or more amino acid residues of human E-selectin with a buried surface area ($\text{\AA}^2$) of $>5 \text{\AA}^2$ selected from the group consisting of T7, E8, A9, T11, P46, S47, Y48, N82, N83, Q85, E88, E92, Y94, R97, E107, R108, S110, K111, K112, K113 and a combination thereof, optionally, according to the crystal structure.

[0223] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof interacts with amino acid residues T7, E8, A9, T11, P46, S47, Y48, N82, N83, Q85, E88, E92, Y94, R97, E107, R108, S110, K111, K112 and K113 of human E-selectin with a buried surface area ($\text{\AA}^2$) of $>5 \text{\AA}^2$, optionally, according to the crystal structure.

[0224] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof interacts with at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 or more amino acid residues of human E-selectin via a hydrogen bond selected from the group consisting of E8, S47, N82, N83, E88, E92, Y94, N105, E107, R108, S110, K112, and a combination thereof, optionally, according to the crystal structure.

[0225] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof interacts with amino acid residues E8, S47, N82, N83, E88, E92, Y94, N105, E107,

R108, S110 and K112 of human E-selectin via a hydrogen bond, optionally, according to the crystal structure.

[0226] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, interacts with at least one amino acid residue of human E-selectin via a salt bridge selected from the group consisting of K111, K112, and a combination thereof, optionally, according to the crystal structure.

[0227] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, interacts with amino acid residues K111 and K112 of human E-selectin via a salt bridge, optionally, according to the crystal structure.

[0228] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, interacts with at least one amino acid residue of human E-selectin via a water-mediated hydrogen bond selected from the group consisting of R97, K112, and a combination thereof, optionally, according to the crystal structure.

[0229] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, interacts with amino acid residues R97 and K112 of human E-selectin via a water-mediated hydrogen bond, optionally, according to the crystal structure.

[0230] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, interacts with at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, more amino acid residues of human E-selectin which also interacts within 3.8Å of an sLex amino acid residue contact selected from the group consisting of Y48, N82, N83, E92, Y94, R97, N105, E107 and a combination thereof, optionally, according to the crystal structure.

[0231] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, interacts with amino acid residues Y48, N82, N83, E92, Y94, R97, N105 and E107 of human E-selectin which also interact within 3.8Å of an sLex amino acid residue contact, optionally, according to the crystal structure.

[0232] Binding, or interaction of, an anti-E-selectin antibody, or antigen-binding fragment thereof with at least one or more amino acid residues of human E-selectin may be determined according to methods known in the art, including analysis of a crystal structure of the bound molecules as described in the Examples herein.

[0233] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, is at low risk for polyreactivity, as measured by, for example an AC-SINS assay, a DNA binding assay and/or an insulin binding assay. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof has a low immunogenicity risk, e.g., has a T-reg Adjusted score of about -44, -45, -46 or -47.

[0234] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, demonstrated at least one predicted human pharmacokinetic (PK) parameter, when administered IV, chosen from: (i) a systemic clearance of about 0.15 mL/h/kg to about 0.39 mL/h/kg; (ii) an apparent volume of distribution at steady state of 22 mL/kg to 36 mL/kg; (iii) a mean half-life of about 102 hours to about 345 hours. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, administered SC demonstrates a half-life of about 243 to about 518 hours.

[0235] In some embodiments, a mean half-life of an anti-E-selectin antibody, or antigen-binding fragment thereof, is at least about 102 hours (about 4.25 days) following IV administration at a dose of 0.3 mg/kg. In some embodiments, a mean half-life of an anti-E-selectin antibody, or antigen-binding fragment thereof, is at least about 264 hours (about 11 days) following IV administration at a dose of 0.6 mg/kg. In some embodiments, a mean half-life of an anti-E-selectin antibody, or antigen-binding fragment thereof, is at least about 188 hours (about 7.8 days) following IV administration at a dose of 1.0 mg/kg. In some embodiments, a mean half-life of an anti-E-selectin antibody, or antigen-binding fragment thereof, is at least about 345 hours (about 14.4 days) following IV administration at a dose of 10 mg/kg.

[0236] In some embodiments, a mean half-life of an anti-E-selectin antibody, or antigen-binding fragment thereof, is at least about 243 hours (about 10 days) following SC administration at a dose of 1.0 mg/kg. In some embodiments, a mean half-life of an anti-E-selectin antibody, or antigen-binding fragment thereof, is at least about 518 hours (about 21.5 days) following SC administration at a dose of 3.0 mg/kg.

[0237] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a viscosity selected from the group consisting of about 7.97 +/- 1.83 cP at a concentration of about 23 mg/mL, about 12.38 +/- 5.28 cP at a concentration of about 48 mg/mL, about 4.26 +/- 0.6 cP at a concentration of about 90 mg/mL, about 5.58 +/- 0.99 cP at a concentration of about 102 mg/mL, about 8.44 +/- 1.54 cP at a concentration of about 121 mg/mL, about 9.78 +/- 2.32 cP at a concentration of about 140 mg/mL, about 17.47 +/- 3.24 cP at a concentration of about 158 mg/mL and about 37.99 +/- 7.03 cP at a concentration of about 188 mg/mL, when measured at 25°C by, for example, dynamic light scattering (DLS).

[0238] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a viscosity of about 15 cP to 40 cP at a concentration of about 150 mg/mL to about 190 mg/mL when measured at 25°C by, for example DLS.

[0239] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a viscosity of 33.4 cP at 185.7 mg/mL when measured at 25°C by, for example an

Anton Parr method.

Immunogenicity

- [0240]** Immunogenicity is a major barrier to the development and utilization of protein therapeutics, including antibodies and Fc fusion proteins. Several factors can contribute to protein immunogenicity, including but not limited to the protein sequence, the route and frequency of administration, and the patient population. Although immune responses are typically most severe for non-human proteins, such as murine antibodies, even therapeutics with mostly or entirely human sequence content may be immunogenic. Immunogenicity is a complex series of responses to a substance that is perceived as foreign and may include production of neutralizing and non-neutralizing antibodies, formation of immune complexes, complement activation, mast cell activation, inflammation, and anaphylaxis. Unwanted immune responses may reduce the efficacy of antibody and Fc fusion protein therapeutics by directly interfering with antigen recognition, altering interactions with effector molecules, or perturbing the serum half-life or tissue distribution of the therapeutic.
- [0241]** Protein therapeutics can be analyzed to predict the presence of potential immunogenic epitopes using commercially available services such as provided by Epivax, Inc. of Providence, R.I. Potential immunogenic epitopes may also be predicted using methods such as the IEDB Consensus method. In some embodiments, *in silico* algorithms can predict epitopes that bind to Class II MHC molecules. Analysis of a data set of the polypeptide with such algorithms provides predicted epitopes. Predicted epitopes are used to make peptides prepared by standard methods of automated peptide synthesis or recombinant DNA techniques. Scoring information provided from Epivax can provide an indication of how widespread a predicted epitope is recognized in the population. A lower score predicts a lower immunogenic potential.
- [0242]** As used herein, "T-regitopes" are amino acid sequences within the monoclonal antibody framework region that can potentially activate natural regulatory T cells and reduce unwanted immune responses. In some embodiments, an anti-E-selectin antibody, or antigen binding-fragment thereof, comprises 8, 7, 6, 5, 4, 3, 2, 1 or 0 non-germline T-cell epitopes. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, has a low immunogenicity risk, e.g., has a T-reg Adjusted Score of about -45.11, -45.32, -46.16 or -46.26. In some embodiments, anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, has a T-reg Adjusted Score of about -45.11 and 0 non-germline T-cell epitopes.

Nucleic Acids Encoding Anti-E-selectin Antibodies

[0243] The disclosure also provides polynucleotides encoding any of the antibodies of the invention, including antibody portions and modified antibodies described herein. The invention also provides a method of making any of the polynucleotides described herein. Polynucleotides
5 can be made and the proteins expressed by procedures known in the art.

[0244] A sequence of a desired antibody, or antigen-binding fragment thereof, and nucleic acid encoding such antibody, or antigen-binding fragment thereof, can be determined using standard sequencing techniques. A nucleic acid molecule encoding a desired antibody, or antigen-binding fragment thereof, may be inserted into various vectors (such as cloning and
10 expression vectors) for recombinant production and characterization. A nucleic acid molecule encoding the heavy chain, or an antigen-binding fragment of the heavy chain, and a nucleic acid molecule encoding the light chain, or an antigen-binding fragment of the light chain, can be cloned into the same vector, or different vectors.

[0245] In some embodiments, the disclosure provides polynucleotides encoding the amino acid sequences of any of the following anti-E-selectin antibodies and antigen-binding fragments thereof: antibody 1444, 0841, 0978, 0164, 1448, 1284, 1282, 0525, 0039, 0265_0254, 0158, 0929_548, 0159, 0955_0300, 0170, 0564, 0180 and 0027. In one embodiment, the invention provides polynucleotides encoding the amino acid sequence of anti-E-selectin antibody 1444 and antigen-binding fragments thereof.

[0246] In some embodiments, the disclosure provides polynucleotides encoding one or more anti-E-selectin antibody HC polypeptides comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs:7, 13, 22, 28, 34, 37, 40, 43, 51, 59, 62, 74, 76, 89, 91, 103, 110, 117 and 124. In some embodiments, the disclosure provides a polynucleotide encoding an anti-E-selectin antibody HC polypeptide comprising, or consisting of, the amino
20 acid sequence of SEQ ID NO:7 or SEQ ID NO:13.

[0247] In some embodiments, the disclosure provides polynucleotides encoding one or more anti-E-selectin antibody LC polypeptides comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs:1, 17, 26, 31, 46, 56, 67, 72, 81, 86, 96, 101, 105, 115 and 119. In some embodiments, the disclosure provides a polynucleotide encoding an anti-
30 E-selectin antibody LC polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:1.

[0248] In some embodiments, the disclosure provides polynucleotides encoding one or more anti-E-selectin antibody VH domain polypeptides comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs:11, 25, 30, 35, 39, 42, 45, 55, 60, 66, 75,

80, 90, 95, 104, 114, 118 and 128. In some embodiments, the disclosure provides a polynucleotide encoding an anti-E-selectin antibody VH domain polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:11.

[0249] In some embodiments, the disclosure provides polynucleotides encoding one or more anti-E-selectin antibody VL domain polypeptides comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs:5, 21, 27, 32, 50, 57, 7, 73, 85, 87, 100, 102, 109, 116 and 122. In some embodiments, the disclosure provides a polynucleotide encoding an anti-E-selectin antibody VL domain polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:5.

10 **[0250]** The invention provides a polynucleotide comprising the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having Accession No. PTA-126529 encoding the HC domain of antibody 1444. The invention also provides a polynucleotide comprising the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having Accession No. PTA-126530 encoding the LC of antibody 1444. In addition, the invention
15 provides a polypeptide comprising the amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC and having Accession No. PTA-126529, encoding the VH domain of antibody 1444. The invention further provides a polypeptide comprising the amino acid sequence encoded by the insert of the plasmid deposited with the ATCC and having Accession No. PTA-126530 encoding the VL domain of antibody 1444.

20 **[0251]** The invention also provides a polynucleotide comprising the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having Accession No. PTA-126529, encoding the HCDR-1, HCDR-2 and HCDR-3 of antibody 1444 and the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having Accession No. PTA-126530, encoding the LCDR-1, LCDR-2 and LCDR-3 of antibody 1444.

25 **[0252]** The invention also provides a polynucleotide comprising the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having Accession No. PTA-126529, encoding the VH domain of antibody 1444 and the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having Accession No. PTA-126530, encoding the VL domain of antibody 1444.

30 **[0253]** The invention also provides a polynucleotide comprising the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having Accession No. PTA-126529, encoding the heavy chain of antibody 1444 and the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and having Accession No. PTA-126530, encoding the light chain of antibody 1444.

[0254] In some embodiments, the disclosure provides polynucleotides and variants thereof encoding an anti-E-selectin antibody, wherein such variant polynucleotides share at least 70%, at least 75%, at least 80%, at least 85%, at least 87%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% nucleic acid sequence identity to any of the nucleic acid sequences disclosed in Table 2. These amounts are not meant to be limiting and increments between the recited percentages are specifically envisioned as part of the disclosure.

[0255] In one embodiment, the VH and VL domains, or antigen-binding fragment thereof, or full-length HC or LC, are encoded by separate polynucleotides. Alternatively, both VH and VL, or antigen-binding fragment thereof, or HC and LC, are encoded by a single polynucleotide.

[0256] Polynucleotides complementary to any such sequences are also encompassed by the present disclosure. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present disclosure, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

[0257] Polynucleotides may comprise a nucleic acid sequence that encodes an antibody or a fragment thereof or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions such that the binding characteristics of the encoded polypeptide is not diminished relative to a native antibody molecule. The effect on the binding characteristics of the polypeptide encoded by the variant nucleic acid sequence may generally be assessed as described herein. In some embodiments, polynucleotide variants exhibit at least about 70% identity, at least about 80% identity, at least about 90% identity, at least about 95% identity, at least 98% identity or at least 99% identity to a polynucleotide sequence that encodes the original (parent) antibody not comprising any substitution, addition, deletion and/or insertion, or a fragment thereof. These percent identities are not meant to be limiting and increments between the recited percentages are specifically envisioned as part of the disclosure.

[0258] Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described herein. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and

compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, or 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. In some embodiments, a polynucleotide is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% identical to a polynucleotide disclosed herein.

[0259] In some embodiments, an anti-E-selectin antibody VL domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:137, 145, 147, 149, 154, 165, 158, 160, 162, 164, 166, 168, 170, 172 and 174. In some embodiments, an anti-E-selectin antibody VL domain is encoded by a polynucleotide comprising a nucleic acid sequence at least 80%, 85%, 90%, 95%, 98% or 99% identical to a nucleic acid selected from the group consisting of SEQ ID NO:137, 145, 147, 149, 154, 165, 158, 160, 162, 164, 166, 168, 170, 172 and 174.

[0260] In some embodiments, an anti-E-selectin antibody VL domain is encoded by a polynucleotide comprising a nucleic acid sequence at least 90% identical to the nucleic acid sequence of SEQ ID NO:137. In some embodiments, an anti-E-selectin antibody VL domain is encoded by a polynucleotide comprising, or consisting of, a nucleic acid sequence of SEQ ID NO:137.

[0261] In some embodiments, an anti-E-selectin antibody VH domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:136, 144, 146, 148, 150, 151, 152, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171 and 173. In some embodiments, an anti-E-selectin antibody VH domain is encoded by a polynucleotide comprising a nucleic acid sequence at least 80%, 85%, 90%, 95%, 98% or 99% identical to a nucleic acid selected from the group consisting of SEQ ID NO: 136, 144, 146, 148, 150, 151, 152, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171 and 173.

[0262] In some embodiments, an anti-E-selectin antibody VH domain is encoded by a polynucleotide comprising a nucleic acid sequence at least 90% identical to SEQ ID NO:136. In some embodiments, an anti-E-selectin antibody VH domain is encoded by a polynucleotide comprising, or consisting of, a nucleic acid sequence of SEQ ID NO:136.

[0263] In some embodiments, an anti-E-selectin antibody HC is encoded by a polynucleotide comprising a nucleic acid sequence at least 90% identical to SEQ ID NO:206 or 207. In some embodiments, an anti-E-selectin antibody HC is encoded by a polynucleotide comprising, or consisting of, a nucleic acid sequence of SEQ ID NO:206 or 138.

[0264] In some embodiments, an anti-E-selectin antibody LC is encoded by a

polynucleotide comprising a nucleic acid sequence at least 90% identical to SEQ ID NO:139. In some embodiments, an anti-E-selectin antibody LC is encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO:139.

[0265] Polynucleotide variants may also, or alternatively, be substantially homologous to a gene, or a fragment or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding an antibody (or a complementary sequence).

[0266] Suitable "moderately stringent conditions" include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at about 50 °C to 65 °C, 5X SSC (0.75 M NaCl, 0.075 M sodium citrate), overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS.

[0267] As used herein, "highly stringent conditions" or "high stringency conditions" are those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50 °C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42 °C; or (3) employ 50% formamide, 5X SSC, 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X Denhardt's solution, sonicated salmon sperm DNA (50 µg/mL), 0.1% SDS, and 10% dextran sulfate at 42 °C, with washes at 42 °C in 0.2X SSC (sodium chloride/sodium citrate) and 50% formamide at 55 °C, followed by a high-stringency wash consisting of 0.1X SSC containing EDTA at 55 °C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0268] It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode the amino acid sequence of a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. That is, there are 64 different codons to encode 20 natural amino acids, with some amino acids having multiple codons that encode it (e.g., 6 different codons encode leucine). Therefore, a large number of nucleic acid sequences can encode the same protein sequence such that two nucleic acids encoding the same polypeptide amino acid sequence can share very low nucleic acid sequence identity. Therefore, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present disclosure.

[0269] Further, alleles of the genes comprising the polynucleotide sequences provided

herein are within the scope of the present disclosure. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

[0270] The polynucleotides of this disclosure can be obtained using chemical synthesis, recombinant methods, or PCR. Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

[0271] For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as further discussed herein. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook *et al.*, 1989.

[0272] Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis *et al.* eds., Birkauwer Press, Boston, 1994.

[0273] RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook *et al.*, 1989, for example.

[0274] As used herein, the term "vector" means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest (e.g., a nucleic acid encoding a HC, a LC, a VH, a VL and/or a fragment thereof, of an anti-E-selectin antibody) in a host cell. Examples of vectors include, but are not limited to, viral vectors (e.g. AAV), naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

[0275] Suitable cloning and expression vectors can include a variety of components, such

as promoter, enhancer, and other transcriptional regulatory sequences. The vector may also be constructed to allow for subsequent cloning of an antibody variable domain into different vectors. Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector
5 selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1,
10 pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Stratagene, and Invitrogen.

[0276] Expression vectors are further provided. Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the disclosure. It is implied
15 that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal
20 sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

[0277] In some embodiments, a cell (e.g., isolated or within an organism) is transduced with
25 a recombinant AAV (rAAV) comprising a recombinant nucleic acid encoding a heterologous polynucleotide (e.g., a HC, a LC, a VH domain, a VL domain, or an antigen-binding fragment thereof, of an anti-E-selectin antibody) and an AAV capsid. A recombinant nucleic acid may further comprise regulatory elements (e.g., a promoter, an enhancer, an intron, an exon, polyA) for expression of the heterologous polynucleotide within a transduced cell. A recombinant
30 nucleic acid may further comprise viral inverted tandem repeat (ITR) sequences. In some embodiments, an AAV capsid is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or any other wild type or recombinant AAV capsid known in the art. ITR sequences may be AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or any other wild type or recombinant ITR sequences (e.g., AAV2) known in the art. In some

embodiments, a rAAV comprises a recombinant nucleic acid encoding a HC, a LC, a VH domain, a VL domain, or an antigen-binding fragment thereof, of an anti-E-selectin antibody, a promoter, an AAV ITR and a viral capsid. Such rAAV is suitable for expression of an anti-E-selectin antibody, or antigen-binding fragment thereof in a cell to treat or prevent a disease, disorder or condition (e.g., SCD) mediated by E-selectin in a subject (e.g., a patient).

[0278] The vectors containing the polynucleotides of interest and/or the polynucleotides themselves, can be introduced into a host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride or polyethylenimine (PEI), rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[0279] In some embodiments, a vector comprises a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 137, 145, 147, 149, 154, 165, 158, 160, 162, 164, 166, 168, 170, 172 and 174. In some embodiments, a vector comprises a polynucleotide comprising a nucleic acid sequence at least 70%, 80%, 85%, 90%, 95%, 98% or 99% identical to the nucleic acid sequence of SEQ ID NO:137. In some embodiments, a vector comprises a polynucleotide comprising, or consisting of, a nucleic acid sequence of SEQ ID NO:137.

[0280] In some embodiments, a vector comprises polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 136, 144, 146, 148, 150, 151, 152, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171 and 173. In some embodiments, a vector comprises a polynucleotide comprising a nucleic acid sequence at least 70% 80%, 85%, 90%, 95%, 98% or 99% identical to SEQ ID NO:136. In some embodiments, a vector comprises a polynucleotide comprising, or consisting of, a nucleic acid sequence of SEQ ID NO:136.

[0281] In some embodiments, a vector comprises a polynucleotide comprising i) a nucleic acid sequence of SEQ ID NO:136; ii) a nucleic acid of SEQ ID NO:137; or iii) both.

[0282] In some embodiments, a vector comprises a polynucleotide comprising a nucleic acid sequence at least 70% 80%, 85%, 90%, 95%, 98% or 99% identical to SEQ ID NO:206 or 138. In some embodiments, a vector comprises a polynucleotide comprising, or consisting of, a nucleic acid sequence of SEQ ID NO:206 or 138.

[0283] In some embodiments, a vector comprises a polynucleotide comprising a nucleic acid sequence at least 70% 80%, 85%, 90%, 95%, 98% or 99% identical to SEQ ID NO:139. In some embodiments, a vector comprises a polynucleotide comprising, or consisting of, a nucleic

acid sequence of SEQ ID NO:139.

[0284] In some embodiments, a vector comprises a polynucleotide comprising i) a nucleic acid sequence of SEQ ID NO:206 or 138; ii) a nucleic acid of SEQ ID NO:139; or iii) both.

[0285] As used herein, the terms "host cell," "host cell line," and "host cell culture" are used interchangeably and mean an individual cell or cell culture that can be or has been a recipient for a polynucleotide and/or vector(s) for incorporation of polynucleotide inserts. Host cells include "transformants," "transformed cells," and "transduced cells," which include the primary transformed or transduced cell and progeny derived therefrom without regard to the number of passages. Host cell progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected and/or transformed *in vivo* with a polynucleotide of this invention (e.g., a polynucleotide encoding an amino acid sequence of an anti-E-selectin antibody) or a vector comprising the same.

[0286] Host cells may be prokaryotic cells or eukaryotic cells. Exemplary eukaryotic cells include mammalian cells, such as primate or non-primate animal cells; fungal cells, such as yeast; plant cells; and insect cells.

[0287] An antibody, or antigen-binding fragment thereof, may be made recombinantly using a suitable host cell. A nucleic acid encoding an anti-E-selectin antibody, or antigen-binding fragment thereof, of the present disclosure can be cloned into an expression vector, which can then be introduced into a host cell, where the cell does not otherwise produce an immunoglobulin protein, to obtain the synthesis of an antibody in the recombinant host cell. Any host cell susceptible to cell culture, and to expression of protein or polypeptides, may be utilized in accordance with the present invention. In certain embodiments, the host cell is mammalian. Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). Nonlimiting exemplary mammalian cells include, but are not limited to, NS0 cells, HEK 293 and Chinese hamster ovary (CHO) cells, and their derivatives, such as 293-6E and CHO DG44 cells, CHO DXB11, and Potelligent® CHOK1SV cells (BioWa/Lonza, Allendale, NJ). Mammalian host cells also include, but are not limited to, human cervical carcinoma cells (HeLa, ATCC CCL 2), baby hamster kidney (BHK, ATCC CCL 10) cells, monkey kidney cells (COS), and human hepatocellular carcinoma cells (e.g., Hep G2). Other non-limiting examples of mammalian cells that may be used in accordance with the present invention include human retinoblasts (PER.C6®; CruCell, Leiden, The Netherlands); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line 293 (HEK 293)

or 293 cells subcloned for growth in suspension culture (Graham et al., J. Gen Virol. 1997; 36:59); mouse sertoli cells (TM4, Mather, Biol. Reprod. 1980; 23:243-251); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1 587); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather et al., Annals N.Y. Acad. Sci. 1982; 383:44-68); MRC 5 cells; FS4 cells; a human hepatoma line (Hep G2); and numerous myeloma cell lines, including, but not limited to, BALB/c mouse myeloma line (NS0/1, ECACC No: 85110503), NS0 cells and Sp2/0 cells.

[0288] Additionally, any number of commercially and non-commercially available cell lines that express polypeptides or proteins may be utilized in accordance with the present invention. One skilled in the art will appreciate that different cell lines might have different nutrition requirements and/or might require different culture conditions for optimal growth and polypeptide or protein expression, and will be able to modify conditions as needed.

Uses

Methods of Treatment

[0289] In some embodiments, the disclosure provides for therapeutic methods for reducing or inhibiting E-selectin activity using an anti-E-selectin antibody or antigen-binding fragment thereof, wherein the therapeutic method comprises administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of E-selectin activity (e.g., SCD).

[0290] Methods of treatment using anti-E-selectin antibodies, or antigen-binding fragments thereof, include, but are not limited to, methods of treating and/or preventing diseases, disorders and conditions associated with, or mediated by, E-selectin expression and/or E-selectin binding to a ligand (e.g., sLex A and/or X determinants), including, but not limited to, SCD, vaso-occlusive crisis, pain, organ infarction, ischemia, stroke, end organ dysfunction, acute chest and vascular obstruction. An anti-E-selectin antibody, or antigen-binding fragment thereof of the present disclosure, may also be used to treat and/or prevent other diseases, disorders and conditions such as, skin diseases (e.g., psoriasis), inflammatory diseases (e.g., rheumatoid arthritis) and complications of diabetes.

[0291] The present disclosure provides novel therapeutic antibodies that specifically bind to E-selectin and are capable of neutralizing E-selectin functional activity. These antibodies may

be used advantageously to prevent, or reduce occurrence of, a VOC when utilized as a prophylactic treatment for SCD. In some embodiments, occurrence of a VOC in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is reduced by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% as compared to occurrence of a VOC in a subject, or group of subjects, (e.g., patient(s) with SCD) who are not treated with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, occurrence of a VOC in subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is reduced by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% as compared to occurrence of a VOC in the same subject prior to treatment with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, occurrence of a VOC in subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is not significantly different from occurrence of VOC in a subject, or population of subjects, who do not have SCD. In some embodiments, occurrence of a VOC (e.g., number of VOC events) is measured over a period of time. In some embodiments, occurrence of a VOC (e.g., number of VOC events) is measured over days, weeks, months or years.

[0292] In some embodiments, an antibody, or antibody-binding fragment thereof, of the disclosure may reduce an annualized rate of sickle cell-related pain crisis. Sickle cell-related pain crises, as referred to herein, are acute episodes of pain, with no medically determined cause other than a vaso-occlusive event, that may result in a medical facility visit and/or may result in treatment with oral or parenteral narcotic agents or with a parenteral nonsteroidal anti-inflammatory drug. In some embodiments, acute chest syndrome, hepatic sequestration, splenic sequestration, and priapism are considered sickle cell-related pain crises.

[0293] In some embodiments, an annualized rate of sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% lower than an annualized rate of sickle cell-related pain crisis in a subject, or group of subjects, (e.g., patient(s) with SCD) who are not treated with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, an annualized rate of sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% lower than an annualized rate of sickle cell-related pain crisis in the same subject prior to treatment with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, an annualized rate of sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated

with an anti-E-selectin antibody, or antigen-binding fragment thereof, is not significantly different from an annualized rate of sickle cell-related pain crisis in a subject, or population of subjects, who do not have SCD.

[0294] In some embodiments, a median time to a first sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% longer than a median time to a first sickle cell-related pain crisis in a subject, or group of subjects, (e.g., patient(s) with SCD) who are not treated with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, a median time to a first sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% longer than a median time to a first sickle cell-related pain crisis in the same subject prior to treatment with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, a median time to a first sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is not significantly different from a median time to first sickle cell-related pain crisis in a subject, or population of subjects, who do not have SCD.

[0295] In some embodiments, a median time to a second sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% longer than a median time to a second sickle cell-related pain crisis in a subject, or group of subjects, (e.g., patient(s) with SCD) who are not treated with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, a median time to a second sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% longer than a median time to a second sickle cell-related pain crisis in the same subject prior to treatment with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, a median time to a second sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is not significantly different from a median time to second sickle cell-related pain crisis in a subject, or population of subjects, who do not have SCD.

[0296] In some embodiments, a median rate of uncomplicated sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%

lower than a median rate of uncomplicated sickle cell-related pain crisis in a subject, or group of subjects, (e.g., patient(s) with SCD) who are not treated with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, an uncomplicated crisis was a crisis other than acute chest syndrome, hepatic sequestration, splenic sequestration and/or priapism.

- 5 In some embodiments, a median rate of uncomplicated sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% longer than a median rate of uncomplicated sickle cell-related pain crisis in the same subject prior to treatment with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some
- 10 embodiments, a median rate of uncomplicated sickle cell-related pain crisis in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is not significantly different from a median rate of uncomplicated sickle cell-related pain crisis in a subject, or population of subjects, who do not have SCD.

- [0297]** Antibodies of the disclosure may also be used advantageously to treat an acute VOC
- 15 in a patient with SCD by decreasing the duration (e.g., reduction in the time to resolve a VOC), severity and/or intensity of a VOC. In some embodiments, duration, severity and/or intensity of a VOC in a subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is reduced by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% as compared to duration, severity and/or intensity of a VOC in a subject, or group
- 20 of subjects, (e.g., patient(s) with SCD) who are not treated with an anti-E-selectin antibody, or antigen-binding fragment thereof. In some embodiments, duration, severity and/or intensity of a VOC in subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody is reduced by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% as compared to duration, severity and/or intensity of a VOC in the same subject prior to treatment with an anti-E-selectin
- 25 antibody, or antigen-binding fragment thereof. In some embodiments, duration, severity and/or intensity of a VOC in subject (e.g., a patient with SCD) treated with an anti-E-selectin antibody, or antigen-binding fragment thereof, is not significantly different from duration, severity and/or intensity of a VOC in a subject, or population of subjects, who do not have SCD.

- [0298]** An anti-E-selectin antibody, or antigen-binding fragment thereof, may also be used,
- 30 to treat, prevent and/or ameliorate at least one sign and/or symptom of SCD, for example, those affecting the cardiothoracic system (e.g., chronic restrictive lung disease, left ventricular diastolic disease, pulmonary hypertension, acute chest syndrome, dysrhythmias, sudden death, vaso-occlusive crisis), the nervous system (e.g., hemorrhagic stroke, venous sinus thrombosis, silent cerebral infarction of the brain, chronic pain, acute ischemic stroke of the brain,

proliferative retinopathy, orbital infarction, cognitive impairment) the reticuloendothelial system (e.g., splenic sequestration, functional hyposplenism, anemia, hemolysis), the musculoskeletal system (e.g., avascular necrosis, skin ulcerations), the urogenital system (e.g., papillary necrosis, proteinuria, renal failure, hematuria, nocturnal enuresis, priapism) and the
5 gastrointestinal system (e.g., cholelithiasis, cholangiopathy, hepatopathy, mesenteric vaso-occlusion).

[0299] In some embodiments, the present disclosure includes an isolated antibody, or antigen-binding fragment thereof, that specifically binds E-selectin and modulates at least one detectable E-selectin biological activity such that the antibody: (a) decreases leukocyte
10 tethering to endothelial cells; (b) decreases activation of stable adhesion to endothelial cells; (c) reduces slow rolling of leukocytes to arrest; (d) reduces efficient trans-endothelial migration of leukocytes; (e) decreases affinity and avidity of CD18 integrins; (f) reduces trafficking of leukocytes to sites of acute inflammation; (g) decreases cytosolic calcium; (h) decreases tyrosine phosphorylation that activates p38 MAP kinase and Syk kinase; (i) reduces recruitment
15 of platelets and leukocytes from the blood to the vascular endothelium; and/or (j) does not create a pro-inflammatory environment.

[0300] Methods of treating using an anti-E-selectin antibody, or antigen-binding fragment thereof, of the present disclosure includes prophylactic and/or therapeutic treatments. If a treatment is administered prior to clinical manifestation of a condition, the treatment is
20 considered prophylactic. For example, administration of an anti-E-selectin antibody, or antigen-binding fragment thereof, may be used to prevent VOC (e.g., reduce the frequency of VOC events) when utilized as a prophylactic treatment (e.g., one or more doses, over a period of time) for SCD. Therapeutic treatment includes, e.g., ameliorating or reducing the severity of a disease, or shortening the length of the disease. For example, administration of an anti-E-
25 selectin antibody, or antigen-binding fragment thereof, may be used to treat an acute VOC in patients with SCD by decreasing the duration, intensity and/or severity of the VOC.

[0301] In some embodiments, a subject to be treated may be mammal, and in particular a human patient, for example, a patient with SCD. A subject may be in need of treatment because, as a result of one or more mutations in the coding sequence of the HBB gene, the β -
30 globin protein is expressed inappropriately, e.g., has an incorrect amino acid sequence. Administration of an anti-E-selectin antibody, or antigen-binding fragment thereof, may be used to treat and/or prevent at least one sign and/or symptom of SCD (e.g., VOC), a variant of SCD or SC disease. Administration of an anti-E-selectin antibody, or antigen-binding fragment thereof, may be used to treat and/or prevent at least one sign and/or symptom of disease in a

subject who has a HBSS, HBSC, HBSE, HBS/ β° thal, HBS/ β^{+} thal or HBS-variant genotype.

[0302] The disclosure further encompasses an anti-E-selectin antibody, or antigen binding fragment thereof, or pharmaceutical composition, as defined herein for use in the defined methods of treatment and/or prevention. In embodiments that refer to a method of treatment and/or prevention as described herein, such embodiments also include further embodiments concerning an anti-E-selectin antibody, or antigen binding fragment thereof, or pharmaceutical composition, for use in that treatment and/or prevention, or alternatively use in the manufacture of a medicament for treatment and/or prevention of a symptom of SCD.

Combination therapies

[0303] An antibody, or antigen-binding fragment thereof of the present disclosure, may be administered in combination with one or more additional therapeutically active compounds or treatment modalities which are effective in treating and/or preventing at least one sign and/or symptom of SCD. The disclosure encompasses a method of treating and/or preventing at least one sign and/or symptom of SCD comprising administering to a patient in need thereof an amount of an anti-E-selectin antibody, or antigen-binding fragment thereof, in combination with an amount of a therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and/or symptom of SCD. In some embodiments, treating a symptom of SCD includes decreasing the duration and intensity of an acute VOC. In some embodiments, preventing a symptom of SCD includes reducing the frequency of VOC events.

[0304] The invention also encompasses a method of treating and/or preventing at least one sign and/or symptom of SCD comprising administering to a patient in need thereof an amount of an anti-E-selectin antibody, or antigen-binding fragment thereof and an amount of a therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and/or symptom of SCD, wherein the amounts together are effective in treating and/or preventing at least one sign and/or symptom of SCD.

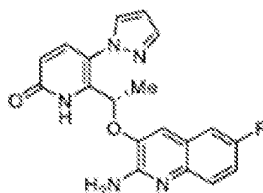
[0305] In another embodiment, the invention is related to a method of treating and/or preventing at least one sign and/or symptom of SCD comprising administering to a patient in need thereof an amount of an anti-E-selectin antibody, or antigen-binding fragment thereof, and an amount of a therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and/or symptom of SCD, wherein the amounts together achieve synergistic effects in the treatment and/or prevention of at least one sign and/or symptom of SCD, that is, the combination is "synergistic," (i.e., the combination provides an effect greater than a merely additive effect of two or more individual therapies). Such

synergistic combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0306] Additional therapeutically active compounds useful for the treatment and/or prevention of at least one sign and/or symptom of SCD include, for example, penicillin prophylaxis to prevent pneumococcal infection, hydroxyurea (e.g., DROXIA, HYDREA), L-glutamine (e.g., ENDARI), crizanlizumab (ADAKVEO), voxelotor (OXBRYTA), apixaban (ELIQUIS), rivaroxaban (XARELTO), a non-steroidal anti-inflammatory drug, an analgesic generally, an opioid analgesic, IW-1701, riociguat (ADEMPAS), ticagrelor (BRILINTA), memantine (NAMENDA) and a combination thereof.

[0307] Additional therapeutically active compounds useful for the treatment and/or prevention of at least one sign and/or symptom of SCD include compounds such as an anti-P-selectin antibody (e.g., crizanlizumab (ADAKVEO)), a compound that modulates HbS, a compound that modulates oxygen affinity of HbS (e.g., voxelotor (OXBRYTA)), a compound that targets HbS polymerization by modulating generation of 2,3-disphosphoglyceric acid, a compound that targets HbS polymerization by inducing expression of fetal hemoglobin (HbF) (e.g., hydroxyurea), a compound that targets dysfunctional cellular adhesion, vascular dysfunction and/or inflammation (e.g., a phosphodiesterase-9 inhibitor), a compound that increases levels of nitric oxide in the blood (e.g., soluble guanylate cyclase stimulators, e.g., IW-1701, riociguat (ADEMPAS)), intravenous IG, a compound that targets hypercoagulability (e.g., riociguat (ADEMPAS), apixaban (ELIQUIS), rivaroxaban (XARELTO)), a compound that blocks NMDA receptor binding (e.g., memantine (NAMENDA)).

[0308] An additional therapeutically active compound useful for the treatment and/or prevention of at least one sign and/or symptom of SCD (e.g., a VOC) is a compound that modulates HbS so as to maintain it in its R state (i.e., oxygenated state), such as those described in WO 2020/109994, and incorporated herein by reference. In some embodiments, a compound that modulates HbS so as to maintain it in its R state includes a 2-aminoquinoline compound. In some embodiments, a compound that modulates HbS so as to maintain it in its R state is 6-((1S)-1-[(2-amino-6-fluoroquinolin-3-yl)oxy]ethyl)-5-(1H-pyrazol-1-yl)pyridin-2-ol, optionally prepared in solid form as its amide tautomer (S)-6-(1-((2-amino-6-fluoroquinolin-3-yl)oxy)ethyl)-5-(1H-pyrazol-1-yl)pyridin-2(1H)-one,



[0309] A therapeutically active treatment modality useful for treatment and/or prevention of at least one sign and/or symptom of SCD includes supplemental oxygen, blood transfusion, optionally with iron chelation, bone marrow transplant, gene therapy (e.g., LentiGlobin®), a gene editing therapy by CRISPR (e.g., CTX001), a zinc finger technique and a combination thereof.

[0310] The present disclosure encompasses a pharmaceutical composition comprising an anti-E-selectin antibody, or antigen-binding fragment thereof, a therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and/or symptom of SCD, and a pharmaceutically acceptable carrier for use in the treatment and/or prevention of at least one sign and/or symptom of SCD (e.g., VOC). The disclosure

encompasses a pharmaceutical composition comprising a synergistic, therapeutically effective amount of an anti-E-selectin antibody, a synergistic, therapeutically effective amount of a therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and/or symptom of SCD, and a pharmaceutically acceptable carrier for use in the treatment and/or prevention of at least one sign and/or symptom of SCD (e.g., VOC). The composition can further comprise an additional therapeutic agent, such as, but not limited to at least one other therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and/or symptom of SCD (e.g., VOC).

[0311] One skilled in the art would understand, based on the disclosure provided therein, that the method of treating and/or preventing at least one sign and/or symptom of SCD encompasses administering a synergistic, therapeutically effective amount of an anti-E-selectin antibody and a synergistic, therapeutically effective amount of a therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and/or symptom of SCD, to a patient either previously treated with, or currently receiving, at least one additional therapeutic agent to treat and/or prevent at least one sign and/or symptom of SCD (e.g., VOC).

[0312] Such additional therapeutic agent encompasses an agent that is standard of care to treat and/or prevent at least one sign and/or symptom of SCD. That is, the combination therapy of the invention may be added to the therapeutic regimen of a SCD patient already receiving a different therapy including, but not limited to, L-glutamine, hydroxyurea, a blood transfusion

(optionally with iron chelation) and any other therapy known in the art.

[0313] Those skilled in the art will be able to determine, according to known methods, the appropriate amount, dose or dosage of each compound, as used in the combination of the present disclosure, to administer to a patient with SCD, taking into account factors such as age, weight, general health, the compound administered, the route of administration, the nature and advancement of the treatment of SCD, and the presence of other medications.

[0314] A prophylactic or therapeutic agent of the combination therapies, including an anti-E-selectin antibody, or antigen-binding fragment thereof, can be administered to a subject in the same pharmaceutical composition (e.g., the therapies are co-formulated). Alternatively, a prophylactic or therapeutic agent of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions (e.g., the therapies are co-administered). Prophylactic or therapeutic agent of the combination therapies can be administered according to the same dosing regimen (e.g., both therapies are administered daily) or according to different dosing regimens (e.g., one therapy is administered daily, the other therapy is administered weekly). Prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

[0315] A prophylactic or therapeutic agent of the combination therapies, including an anti-E-selectin antibody, or an antigen-binding fragment thereof, and 6-((1S)-1-[(2-amino-6-fluoroquinolin-3-yl)oxy]ethyl)-5-(1H-pyrazol-1-yl)pyridin-2-ol, optionally prepared in solid form as its amide tautomer (S)-6-(1-((2-amino-6-fluoroquinolin-3-yl)oxy)ethyl)-5-(1H-pyrazol-1-yl)pyridin-2(1H)-one, can be administered concurrently to a subject in separate pharmaceutical compositions (e.g., the therapies are co-administered). Such prophylactic or therapeutic agent of the combination therapies can be administered according to the same dosing regimen (e.g., both therapies are administered daily) or according to different dosing regimens (e.g., one therapy is administered daily, the other therapy is administered weekly). Furthermore, such prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

[0316] Prophylactic or therapeutic agents including an anti-E-selectin antibody, or an antigen-binding fragment thereof, and 6-((1S)-1-[(2-amino-6-fluoroquinolin-3-yl)oxy]ethyl)-5-(1H-pyrazol-1-yl)pyridin-2-ol, optionally prepared in solid form as its amide tautomer (S)-6-(1-((2-amino-6-fluoroquinolin-3-yl)oxy)ethyl)-5-(1H-pyrazol-1-yl)pyridin-2(1H)-one, may be administered in combination to reduce the duration (e.g., reduction in the time to resolve a VOC), severity and/or intensity of a VOC, to reduce an annualized rate of sickle cell-related pain crisis, to increase the median time to a first sickle cell-related pain crisis, to increase the median

time to a second sickle cell-related pain crisis, and/or to reduce the median rate of uncomplicated sickle cell-related pain crisis in a subject.

[0317] The disclosure further encompasses a prophylactic or therapeutic agent of the combination therapies, including an anti-E-selectin antibody, or antigen-binding fragment thereof, as defined herein for use in the defined methods of treatment and/or prevention. In 5
embodiments that refer to a method of treatment and/or prevention as described herein, such embodiments also include further embodiments concerning a combination therapy, including an anti-E-selectin antibody, or antigen binding fragment thereof, or pharmaceutical composition, for use in that treatment and/or prevention, or alternatively for the manufacture of a medicament 10
for use in that treatment and/or prevention of at least one sign and/or symptom of SCD.

[0318] The disclosure provides protocols for the administration of pharmaceutical composition comprising E-selectin antibodies, or antigen-binding fragments thereof, of the disclosure alone or in combination with other therapies to a subject in need thereof. The therapies (e.g., prophylactic or therapeutic agents) of the combination therapies of the present 15
disclosure can be administered concomitantly or sequentially to a subject. The therapy (e.g., prophylactic or therapeutic agents) of the combination therapies of the present disclosure can also be cyclically administered. Cycling therapy involves the administration of a first therapy (e.g., a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (e.g., a second prophylactic or therapeutic agent) for a period of time and 20
repeating this sequential administration, i.e., the cycle, in order to reduce the development of resistance to one of the therapies (e.g., agents) to avoid or reduce the side effects of one of the therapies (e.g., agents), and/or to improve, the efficacy of the therapies.

[0319] The therapies (e.g., prophylactic or therapeutic agents) of the combination therapies of the disclosure can be administered to a subject concurrently. The term "concurrently" is not 25
limited to the administration of therapies (e.g., prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a pharmaceutical composition comprising E-selectin antibodies, or antigen-binding fragments thereof, of the disclosure are administered to a subject in a sequence and within a time interval such that the antibodies of the disclosure or conjugates thereof can act together with the other therapy(ies) to provide an increased benefit than if they 30
were administered otherwise. For example, each therapy may be administered to a subject at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapy can be administered to a subject separately, in any appropriate form and by any suitable route. In various embodiments,

the therapies (e.g., prophylactic or therapeutic agents) are administered to a subject less than 15 minutes, less than 30 minutes, less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, 24 hours apart, 48 hours apart, 72 hours apart, or 1 week apart. In other embodiments, two or more therapies (e.g., prophylactic or therapeutic agents) are administered to a within the same patient visit.

- 10 **[0320]** Prophylactic or therapeutic agents of a combination therapy can be administered to a subject in the same pharmaceutical composition. Alternatively, a prophylactic or therapeutic agent of a combination therapy can be administered concurrently to a subject in separate pharmaceutical compositions. Prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

15 **Diagnostic Uses**

- [0321]** The anti-E-selectin antibodies, antibody compositions, and methods of the present invention have *in vitro* and *in vivo* utilities including immunoassays and use for the diagnosis and assessment of treatment of E-selectin mediated disorders. The methods are particularly suitable for diagnosing, assessing, and treating human patients having a disorder associated with the existence of E-selectin. This disorder associated with the existence of E-selectin includes, but is not limited to, SCD, skin diseases (e.g., psoriasis), inflammatory diseases (e.g., rheumatoid arthritis) and complications of diabetes.

- 20 **[0322]** The invention provides a method for detecting the presence of E-selectin in a sample, the method comprising contacting a sample suspected of comprising E-selectin with an antibody specific for E-selectin and detecting the presence of E-selectin bound with the antibody thereby detecting E-selectin in the sample. Methods for detecting E-selectin bound with the antibody are well-known in the art including, but not limited to, an assay where E-selectin is bound to a solid support and a sample is added thereto allowing the antibody to bind E-selectin in the sample. A second E-selectin antibody that is either the same or different from the
- 25 antibody bound to the solid support is added and can be detected by either direct labeling (i.e., the second antibody is conjugated to a detectable label) or by adding a third antibody, e.g., from another species which reacts with the constant domain of the second antibody and which comprises a detectable label. Thus, the assay can be used to detect the presence or absence of E-selectin in a sample.
- 30

[0323] The invention also provides a method for determining the concentration of E-selectin in a sample, said method comprising providing a labeled competitor comprising E-selectin coupled to a detectable label; providing an antibody, or antigen binding fragment thereof, that specifically binds E-selectin; combining the sample, the antibody, and the labeled competitor, wherein the E-selectin in the sample competes with the labeled competitor for binding to the antibody; and determining the concentration of E-selectin in said sample by measuring the amount of labeled competitor not bound to antibody by detection of the label. The amount of labeled competitor bound to the antibody in the absence of the sample is compared with the amount of labeled competitor bound to the antibody when the sample is added. The amount of decrease of bound labeled-competitor in the presence of the sample is an indicator of the amount of non-labeled E-selectin present in the sample such that the assay can be used to assess the presence and level of E-selectin in a sample. In some embodiments, E-selectin is soluble E-selectin. In some embodiments, E-selectin is membrane bound E-selectin.

[0324] In one embodiment, the invention provides a method for assessing the effectiveness of a treatment for a disease or disorder associated with an increased level of E-selectin in a subject, the method comprising administering a treatment to the subject and comparing the level of E-selectin in a sample obtained from the subject prior to the treatment with the level of E-selectin in an otherwise identical sample obtained from the subject after the treatment, wherein the level of E-selectin in a sample is assessed using a E-selectin specific antibody, and further wherein a lower, level of E-selectin in the sample collected from the subject after the treatment compared with the level of E-selectin in a sample collected from the subject prior to treatment is an indication of the effectiveness of the course of treatment.

[0325] The term "labeled," with regard to the E-selectin specific antibody or labeled competitor, includes direct labeling by coupling (i.e., physically linking) a detectable substance to the antibody or labeled competitor, as well as indirect labeling of the antibody or labeled competitor by coupling it with another reagent that is directly labeled. An example of indirect labeling includes detection of a primary antibody using a fluorescent-labeled secondary antibody. *In vitro* techniques for detection of a polypeptides of the invention include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitation, and immunofluorescence.

[0326] The term "sample" is intended to include tissues, cells, and biological fluids (e.g., blood, CSF, urine, etc.) isolated from a subject, as well as tissues, cells, and fluids present within a subject.

[0327] The antibodies, labeled competitors, and potential therapeutic compounds described

herein are also suitable for use with any of a number of other homogeneous and heterogeneous immunoassays with a range of detection systems.

Compositions

5 **[0328]** An anti-E-selectin antibody, or antigen-binding fragment thereof, of the disclosure may be formulated as a pharmaceutical composition. The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, excipient, and/or stabilizer (Remington: The Science and practice of Pharmacy 21st Ed., 2005, Lippincott Williams and Wilkins, Ed. K. E. Hoover), in the form of lyophilized formulation or aqueous solution. As used herein, the term
10 “pharmaceutically acceptable carrier” or “pharmaceutically acceptable excipient” includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject’s immune system.

[0329] Examples include, but are not limited to, any of the standard pharmaceutical carriers such as phosphate buffered saline (PBS), water, normal saline (0.9%), emulsions (e.g., oil/water
15 emulsions) and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are PBS and normal saline.

[0330] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations, and may comprise buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as
20 octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as
25 glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, polysorbate (e.g., polysorbate 80 (PS80), polysorbate 60 (PS60),
30 polysorbate 20 (PS20)), or polyethylene glycol (PEG). Pharmaceutically acceptable excipients are further described herein.

[0331] Pharmaceutical compounds of the disclosure may include one or more pharmaceutically acceptable salts. Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as

hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanolic acids, hydroxy alkanolic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[0332] A pharmaceutical composition of the disclosure also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[0333] Examples of suitable aqueous and non-aqueous carriers that may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0334] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0335] Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and

suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be suitable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0336] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration.

[0337] Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0338] A pharmaceutical composition of the present disclosure may be prepared, packaged, or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1%-1.0% (w/w) solution or suspension of the active ingredient in an aqueous or oily liquid carrier. Such drops may further comprise buffering agents, salts, or one or more other of the additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form or in a liposomal preparation.

[0339] As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the disclosure are known in the art and described, for example in Remington's Pharmaceutical Sciences, Genaro, ed., Mack Publishing Co., Easton, PA (1985), which is incorporated herein by reference.

- [0340]** In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof is formulated in a vial containing 100 mg of an anti-E-selectin antibody (e.g., antibody 1444), or an antigen binding fragment thereof, in 1 mL of an aqueous buffered solution. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof is formulated in a vial containing 150 mg of an anti-E-selectin antibody (e.g., antibody 1444), or an antigen binding fragment thereof, in 1 mL of an aqueous buffered solution. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof is formulated in a vial containing 15 mg, 40 mg, 100 mg, 300 mg, or 600 mg of an anti-E-selectin antibody (e.g., antibody 1444), or an antigen binding fragment thereof, in 1 mL of an aqueous buffered solution, optionally for subcutaneous administration. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof is formulated in a vial containing 500 mg of an anti-E-selectin antibody (e.g., antibody 1444), or an antigen binding fragment thereof, in 1 mL of an aqueous buffered solution, optionally for intravenous administration.
- [0341]** In some embodiments, a pharmaceutical composition comprises about 25 mg/mL, 50 mg/mL, 75 mg/mL, 100 mg/mL, 125 mg/mL, 150 mg/mL antibody, or antigen binding fragment thereof. In some embodiments, a pharmaceutical composition comprises about 100 mg/mL antibody, or antigen binding fragment thereof. In some embodiments, a pharmaceutical composition suitable for SC and/or IV administration comprises about 100 mg/mL anti-E-selectin antibody (e.g., antibody 1444), or antigen binding fragment thereof.
- [0342]** In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, is administered in an intravenous or subcutaneous formulation as a sterile aqueous solution comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:7 or SEQ ID NO:13. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, is administered in an intravenous or subcutaneous formulation as a sterile aqueous solution comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:1. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, is administered in an intravenous or subcutaneous formulation as a sterile aqueous solution comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:7 or SEQ ID NO:13 and polypeptide comprising the amino acid sequence of SEQ ID NO:1.
- [0343]** In some embodiments, an anti-E-selectin antibody (e.g., antibody 1444), or antigen-binding fragment thereof, is administered as an intravenous or subcutaneous formulation that is a sterile aqueous solution containing about 5 mg/mL, about 10 mg/mL, about 15 mg/mL, about 20 mg/mL of antibody, about 25 mg/mL, about 50 mg/mL, about 75 mg/mL, about 100 mg/mL, about 125 mg/mL, or about 150 mg/mL of antibody or antigen-binding fragment thereof. In some

embodiments, an intravenous or subcutaneous formulation is a sterile aqueous solution comprising sodium acetate, polysorbate 80, and sodium chloride at a pH ranging from about 5 to 6. In some embodiments, an intravenous formulation is a sterile aqueous solution containing 5 or 10 mg/mL of antibody, with 20 mM sodium acetate, 0.2 mg/mL polysorbate 80, and 140 mM sodium chloride at pH 5.5. Further, a solution comprising an antibody, or antigen-binding fragment thereof, can comprise, among many other compounds, glutamic acid, histidine, mannitol, sucrose, trehalose, glycine, poly(ethylene) glycol, EDTA, methionine, polysorbate 80 and any combination thereof, and many other compounds known in the relevant art.

[0344] In one embodiment, a pharmaceutical composition of the present disclosure comprises the following components: 50 mg/mL or 100 mg/mL of an anti-E-selectin antibody (e.g., antibody 1444) or antigen-binding fragment of the present disclosure, 20 mM histidine, 8.5% sucrose, and 0.02% polysorbate 80, 0.005% EDTA at pH 5.8. In one embodiment, a pharmaceutical composition of the present invention comprises the following components: 100 mg/mL anti-E-selectin antibody (e.g., antibody 1444) or antigen-binding fragment of the present disclosure, 10 mM histidine, 5% sucrose, and 0.01% polysorbate 80 at pH 5.8.

[0345] In some embodiments, a pharmaceutical composition comprises an anti-E-selectin antibody, or antigen-binding fragment thereof (e.g., antibody 1444) at a concentration of 100 mg/mL, 1.12 mg/mL L-histidine, 2.67 mg/mL L-histidine hydrochloride monohydrate, 85 mg/mL sucrose, 0.05 mg/mL edetate disodium dihydrate and 0.2 mg/mL polysorbate 80 at pH 5.8 in a nominal fill volume of 1.0 mL. Such pharmaceutical composition is suitable for SC or IV administration.

[0346] Such pharmaceutical compositions may be provided as a liquid formulation or as a lyophilized powder. When a powder is reconstituted at full volume, the composition retains the same formulation. Alternatively, a powder may be reconstituted at half volume, in which case the composition comprises the same components but at twice the concentration.

[0347] With regard to a therapeutic agent, where the agent is, e.g., a small molecule to be used in a combination therapy, it can be present in a pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

[0348] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

[0349] In some embodiments, a composition of the disclosure is a pyrogen-free formulation which is substantially free of endotoxins and/or related pyrogenic substances. Endotoxins include toxins that are confined inside a microorganism and are released when the microorganisms are broken down or die. Pyrogenic substances also include fever-inducing, thermostable substances (glycoproteins) from the outer membrane of bacteria and other microorganisms. These substances can cause fever, hypotension and shock if administered to humans. Due to the potential harmful effects, it is advantageous to remove even low amounts of endotoxins from intravenously administered pharmaceutical drug solutions. The Food and Drug Administration ("FDA") has set an upper limit of 5 endotoxin units (EU) per dose per kilogram body weight in a single one-hour period for intravenous drug applications (The United States Pharmacopeial Convention, Pharmacopeial Forum 26 (1):223 (2000)). When therapeutic proteins are administered in amounts of several hundred or thousand milligrams per kilogram body weight it is advantageous to remove even trace amounts of endotoxin. In one embodiment, endotoxin and pyrogen levels in the composition are less than 10 EU/mg, or less than 5 EU/mg, or less than 1 EU/mg, or less than 0.1 EU/mg, or less than 0.01 EU/mg, or less than 0.001 EU/mg. In another embodiment, endotoxin and pyrogen levels in the composition are less than about 10 EU/mg, or less than about 5 EU/mg, or less than about 1 EU/mg, or less than about 0.1 EU/mg, or less than about 0.01 EU/mg, or less than about 0.001 EU/mg.

20 **Dosing and Administration**

[0350] To prepare a pharmaceutical or sterile composition including an anti-E-selectin antibody, or antigen-binding fragment thereof, of the disclosure, the antibody is mixed with a pharmaceutically acceptable carrier or excipient (see above). Formulations of therapeutic and diagnostic agents can be prepared by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, e.g., lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, e.g., Hardman, et al. (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y.; Gennaro (2000) Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N. Y.; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie (2000) Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y.).

[0351] Selecting an administration regimen for a therapeutic depends on several factors,

- including the serum or tissue turnover rate of the entity, the level of a symptom, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. In certain embodiments, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available (see, e.g., Wawrzynczak, 1996, Antibody Therapy, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.), 1991, Monoclonal Antibodies, Cytokines and Arthritis, Marcel Dekker, New York, N.Y.; Bach (ed.), 1993, Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases, Marcel Dekker, New York, N. Y.; Baert, et al., New Engl. J. Med. 2003; 348:601-608; Milgrom, et al., New Engl. J. Med. 1999; 341:1966-1973; Slamon, et al., New Engl. J. Med. 2001; 344:783-792; Beniaminovitz, et al., New Engl. J. Med. 2000; 342:613-619; Ghosh, et al., New Engl. J. Med. 2003; 348:24-32; Lipsky, et al., New Engl. J. Med. 2000; 343:1594-1602).
- 15 **[0352]** Determination of the appropriate dose is made by a clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. A specific dose protocol may be one involving the maximal dose or dose frequency that avoids significant undesirable side effects.
- 20 **[0353]** "Reducing, decreasing, neutralizing or inhibiting interaction with E-selectin" as the terms are used herein, means to reduce, decrease, neutralize or inhibit interaction of E-selectin with a ligand as compared to the level of interaction prior to any therapeutic intervention. As used herein, "free E-selectin," means E-selectin that is not bound or otherwise in a complex with another molecule (e.g., a free or cell surface expressed E-selectin ligand).
- 25 **[0354]** The level of E-selectin includes the level of free E-selectin in a subject where the level is assessed using the methods disclosed herein or any other method for assessing the level of free E-selectin known in the art.
- [0355]** In one embodiment, a level of free E-selectin is reduced as compared to the level of E-selectin in a subject prior to administration of an anti-E-selectin antibody of the disclosure. In one embodiment, a level of free E-selectin is reduced compared to a standard level of free E-selectin that is associated with or indicates that a subject is afflicted with a disease, disorder or condition associated with or mediated by free E-selectin.
- 30 **[0356]** In some embodiments, the disclosure encompasses reducing the level of free E-

selectin to a level where there is a decrease, or complete lack of, detectable deleterious effect(s) mediated by or associated with free E-selectin. In some embodiments, an effective dose of an anti-E-selectin antibody is administered to neutralize E-selectin functional activity, such as (a) leukocyte tethering to endothelial cells; (b) activation of stable adhesion to endothelial cells; (c) slow rolling of leukocytes to arrest; (d) trans-endothelial migration of leukocytes; (e) affinity and avidity of CD18 integrins; (f) trafficking of leukocytes to sites of acute inflammation; (g) increase in cytosolic calcium; (h) increase in tyrosine phosphorylation that activates p38 MAP kinase and Syk kinase; (i) recruitment of platelets and leukocytes from the blood to the vascular endothelium; and/or (j) does not create a pro-inflammatory environment.

10 **[0357]** As used herein, an “effective dosage,” “effective dose,” “effective amount,” or “therapeutically effective amount” of a drug, compound, or pharmaceutical composition is an amount sufficient to effect any one or more beneficial or desired results. For prophylactic use, beneficial or desired results include eliminating or reducing the risk, reducing the frequency of occurrence (e.g., over a period of time, e.g., weekly, monthly, yearly), lessening the severity, 15 and/or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include detectable clinical results such as reducing, or decreasing the rate of, VOC or reducing one or more symptoms resulting from expression of E-selectin, decreasing the dose of other 20 medications required to treat the disease, enhancing the effect of another medication, and/or delaying the progression of the disease of patients. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an 25 effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective dosage” may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

30 **[0358]** In some embodiments, an effective dose of an anti-E-selectin antibody is administered to a patient with SCD, skin diseases (e.g., psoriasis), inflammatory diseases (e.g., rheumatoid arthritis) and complications of diabetes.

[0359] In some embodiments, an effective dose on an anti-E-selectin antibody (e.g., antibody 1444) is administered to treat and/or prevent diseases, disorders and conditions

associated with, or mediated by, E-selectin expression and/or E-selectin binding to a ligand (e.g., sLex A and/or X determinants), including, but not limited to, SCD, vaso-occlusive crisis, pain, organ infarction, ischemia, stroke, end organ dysfunction, acute chest and vascular obstruction.

- 5 **[0360]** In some embodiments, an effective dose on an anti-E-selectin antibody (e.g., antibody 1444) is administered to prevent, or reduce the occurrence of, a VOC when utilized as a prophylactic treatment for SCD. In some embodiments, prophylactic administration of an anti-E-selectin antibody (e.g., antibody 1444) is administered one time. In some embodiments, prophylactic administration of an anti-E-selectin antibody (e.g., antibody 1444) is administered
- 10 on an on-going basis (e.g., one or more than one dose, over a period of time).

[0361] In some embodiments, an effective dose on an anti-E-selectin antibody (e.g., antibody 1444) is administered to treat acute VOC in patients with SCD by decreasing the duration, intensity and/or severity of the VOC.

- [0362]** In some embodiments, an effective dose on an anti-E-selectin antibody (e.g., antibody 1444) is administered to treat, prevent and/or ameliorate at least one sign and/or symptom of SCD, for example, those affecting the cardiothoracic system (e.g., chronic restrictive lung disease, left ventricular diastolic disease, pulmonary hypertension, acute chest syndrome, dysrhythmias, sudden death, vaso-occlusive crisis), the nervous system (e.g., hemorrhagic stroke, venous sinus thrombosis, silent cerebral infarction of the brain, chronic pain, acute ischemic stroke of the brain, proliferative retinopathy, orbital infarction, cognitive impairment) the reticuloendothelial system (e.g., splenic sequestration, functional hyposplenism, anemia, hemolysis), the musculoskeletal system (e.g., avascular necrosis, skin ulcerations), the urogenital system (e.g., papillary necrosis, proteinuria, renal failure, hematuria, nocturnal enuresis, priapism) and the gastrointestinal system (e.g., cholelithiasis, cholangiopathy,
- 20 hepatopathy, mesenteric vaso-occlusion).
- 25

- [0363]** In some embodiments, the method or use comprises administering a dose of about 1 mg to about 800 mg. In some embodiments, the method or use comprises administering a dose of about 1 mg to 800 mg as an initial fixed dose. In some embodiments, the method or use comprises administering a dose of about 1 mg to about 2 mg, about 2 mg to about 5 mg, about
- 30 5 mg to about 10 mg, about 10 mg to about 20 mg, about 20 mg to about 30 mg, about 30 mg to about 40 mg, about 40 mg to about 50 mg, about 50 mg to about 60 mg, about 60 mg to about 70 mg, about 70 mg to about 80 mg, about 80 mg to about 90 mg, about 90 mg to about 100 mg, about 100 mg to about 150 mg, about 150 mg to about 200 mg, about 200 mg to about 300 mg, about 300 mg to about 400 mg, about 400 mg to about 500 mg, about 500 mg to about 600

mg, about 600 mg to about 700 mg or about 700 mg to about 800 mg, optionally as an initial fixed dose. In some embodiments, the method or use comprises administering a dose of about 15 mg, 40 mg, 100 mg, 150 mg, 300 mg, 500 mg or 600 mg of an anti-E-selectin antibody, or antigen binding fragment thereof, or a pharmaceutical composition thereof, of the invention. In
5 some embodiments, the dose is an initial fixed dose.

[0364] In some embodiments, the method or use comprises administering a dose of about 150 mg of an anti-E-selectin antibody, or antigen binding fragment thereof, or a pharmaceutical composition thereof, of the invention. In some embodiments, the method or use comprises administering a dose of about 150 mg of an anti-E-selectin antibody, or antigen binding
10 fragment thereof, or a pharmaceutical composition thereof, of the invention on a weekly basis. In some embodiments, the method or use comprises administering a dose of about 150 mg of an anti-E-selectin antibody (e.g., antibody 1444), or antigen binding fragment thereof, or a pharmaceutical composition thereof, of the invention, subcutaneously, on a weekly basis.

[0365] In some embodiments, the method or use comprises administering dose of about
15 0.01 mg/kg to about 30 mg/kg of an antibody, or antigen binding fragment thereof, or a pharmaceutical composition of the invention, optionally as an initial dose. The initial dose may be followed by one or more subsequent doses. In some embodiments, one or more subsequent dose may be administered at least any of weekly, every other week, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every
20 nine weeks, every ten weeks, every eleven weeks, or every twelve weeks.

[0366] An initial dose may be followed by one or more subsequent doses. In some embodiments, a subsequent dose is the same dose, a lower dose or a higher dose of anti-E-selectin antibody as compared to the initial dose. In some embodiments, one or more subsequent dose may be administered at least any of weekly, every other week, every three
25 weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every nine weeks, every ten weeks, every eleven weeks, or every twelve weeks. A specific dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects.

[0367] A pharmaceutical composition of the present disclosure may also be administered
30 via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Selected routes of administration for antibodies of the disclosure include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. Parenteral

administration may represent modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, a composition of the disclosure can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

[0368] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof of the disclosure is administered intravenously. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof of the disclosure is administered subcutaneously.

[0369] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof of the disclosure is administered intravenously or subcutaneously on a weekly basis. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof of the disclosure is administered at a unit dose of about 15 mg, about 40 mg, about 100 mg, about 150 mg, about 300 mg, or about 600 mg subcutaneously on a weekly basis. In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof of the disclosure is administered at a unit dose of about 150 mg or about 500 mg intravenously on a weekly basis.

[0370] In some embodiments, a subject is administered an anti-E-selectin antibody, or antigen-binding fragments thereof, of the disclosure on a weekly basis and a therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and/or symptom of a disease (e.g., SCD) on a daily, weekly, biweekly, monthly, or on an as needed basis. The disclosure provides protocols for the administration of pharmaceutical composition comprising anti-E-selectin antibodies, or antigen-binding fragments thereof, of the disclosure alone or in combination with other therapies to a subject in need thereof. The therapies (e.g., prophylactic or therapeutic agents) of the combination therapies of the present disclosure can be administered concomitantly or sequentially to a subject. The therapy (e.g., prophylactic or therapeutic agents) of the combination therapies of the present disclosure can also be cyclically administered.

[0371] In some embodiments, an anti-E-selectin antibody, or antigen-binding fragment thereof, or a pharmaceutical composition comprising the same, is administered about twice a week, once a week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, twice a month, once a month, once

every two months, once every three months, once every four months, once every five months, once every six months, once every seven months, once every eight months, once every nine months, once every ten months, once every eleven months or once every twelve months.

[0372] In some embodiments, part of a dose is administered by an intravenous bolus and the rest of the dose is administered by infusion of the antibody formulation. For example, a 0.01 mg/kg intravenous injection of the anti-E-selectin antibody, or antigen-binding fragment thereof, may be given as a bolus, and the rest of the antibody dose may be administered by intravenous injection. A predetermined dose of the anti-E-selectin antibody, or antigen-binding fragment thereof, may be administered, for example, over a period of an hour and a half to two hours to five hours.

[0373] Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. In addition, pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entirety.

Kits

[0374] The disclosure also provides a kit comprising any or all of the anti-E-selectin antibodies or antigen-binding fragments thereof described herein. A kit of the disclosure includes one or more containers comprising an anti-E-selectin antibody, or antigen-binding fragment thereof, described herein and instructions for use in accordance with any of the methods of the disclosure described herein. Generally, these instructions comprise a description of administration of an antibody for the above described therapeutic treatments. In some embodiments, a kit is provided for producing a single-dose administration unit. In certain embodiments, a kit can contain both a first container having a dried protein and a second container having an aqueous formulation. In certain embodiments, a kit containing an applicator, e.g., single and multi-chambered pre-filled syringe or device (e.g., liquid syringes and lyosyringes), is included.

[0375] In some embodiments, a kit contains an applicator comprising an anti-E-selectin antibody, or antigen-binding fragment thereof, wherein the applicator is designed, or acceptable for self-administration, by a patient (e.g., a patient with SCD). In some embodiments, self-administration is by subcutaneous administration.

[0376] Instructions relating to use of an anti-E-selectin antibody, or antigen-binding fragment thereof, generally include information as to dosage, dosing schedule, and route of administration (e.g., SC or IV) for the intended treatment. A container may be a unit dose, a bulk package (e.g., a multi-dose package) or a sub-unit dose. Instructions supplied in a kit of the disclosure are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

[0377] A kit of this disclosure is in suitable packaging. Suitable packaging includes, but is not limited to, a vial, bottle, jar, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example a container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). A container may also have a sterile access port (for example a container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-E-selectin antibody of the disclosure. A container may further comprise an additional therapeutic agent.

[0378] In one embodiment, the invention includes a kit further comprising a second therapeutically active compound, or treatment modality, which is effective in treating or preventing symptoms of SCD, wherein the amount of the anti-E-selectin antibody and the second compound or modality together achieve synergistic effects in the treatment or prevention of symptoms of SCD, that is, the combination is "synergistic," (i.e., the combination provides an effect greater than a merely additive effect of two or more individual therapies). Such a kit comprising synergistic combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0379] In some embodiments, a kit may further comprise at least one additional therapeutically active compound useful for the treatment or prevention of a sign and/or symptom of SCD including, for example, penicillin, hydroxyurea (e.g., DROXIA, HYDREA), L-glutamine (e.g., ENDARI), crizanlizumab (ADAKVEO), voxelotor (OXBRYTA), apixaban (ELIQUIS), rivaroxaban (XARELTO), a non-steroidal anti-inflammatory drug, an analgesic generally, an opioid analgesic, IW-1701, riociguat (ADEMPAS), ticagrelor (BRILINTA) or memantine (NAMENDA).

[0380] A kit may optionally provide additional components such as a buffer and interpretive

information. Normally, a kit comprises a container and a label or package insert(s) on, or associated with, the container.

[0381] The disclosure also provides a diagnostic kit comprising any or all of the antibodies, or antigen-binding fragments thereof, described herein. A diagnostic kit is useful for, for example, detecting the presence of E-selectin in a sample. In some embodiments, a diagnostic kit can be used to identify an individual with a latent disease, disorder or condition that may put them at risk of developing an E-selectin-mediated disease, disorder or condition. In some embodiments, a diagnostic kit can be used to detect the presence and/or level of E-selectin in an individual suspected of having an E-selectin mediated disease, disorder or condition.

[0382] Diagnostic kits of the disclosure include one or more containers comprising an anti-E-selectin antibody, or antigen-binding fragment thereof, described herein and instructions for use in accordance with any of the methods of the disclosure described herein. Generally, the instructions comprise a description of use of an E-selectin antibody, or antigen-binding fragment thereof, to detect the presence of E-selectin in individuals at risk for, or suspected of having, an E-selectin mediated disease, disorder or condition, such as a VOC in a patient suspected or known to be afflicted with SCD and or any other condition associated with or mediated by an abnormal form of β -globin including: HbS, HbC, Hb β^+ -thalassemia, Hb β^0 -thalassemia or other Hb variant. In some embodiments, an exemplary diagnostic kit can be configured to contain reagents such as, for example, an E-selectin antibody, or antigen-binding fragment thereof, a negative control sample, a positive control sample, and directions for using the kit.

Biological Deposits

[0383] The heavy and light chains of anti-E-selectin antibody 1444 were deposited under terms in accordance with the Budapest Treaty with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209, USA, on December 6, 2019.

Antibody	Description	ATCC Accession No.
1444	Ab 1444-HC (heavy chain of antibody 1444)	PTA-126529
1444	Ab 1444-LC (light chain of antibody 1444)	PTA-126530

[0384] The deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Pfizer Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the

deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. Section 122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. Section 1.14 with particular reference to 886 OG 638).

5 [0385] The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention
10 of the rights granted under the authority of any government in accordance with its patent laws.

General Techniques

[0386] It is to be understood that this invention is not limited to specific synthetic methods of making that may of course vary. Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly
15 understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art.

20 [0387] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook
25 (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of
30 Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd. ed., Cold Spring Harbor Laboratory

Press, Cold Spring Harbor, NY (2001); Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, NY (2002); Harlow and Lane Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1998); Coligan et al., Short Protocols in Protein Science, John Wiley & Sons, NY (2003); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

[0388] Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, biochemistry, immunology, molecular biology, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

EQUIVALENTS

[0389] The foregoing description and following Examples detail certain specific embodiments of the disclosure and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the disclosure may be practiced in many ways and the disclosure should be construed in accordance with the appended claims and any equivalents thereof.

[0390] Although the disclosed teachings have been described with reference to various applications, methods, kits, and compositions, it will be appreciated that various changes and modifications can be made without departing from the teachings herein and the claimed disclosure below. The following examples are provided to better illustrate the disclosed teachings and are not intended to limit the scope of the teachings presented herein. While the present teachings have been described in terms of these exemplary embodiments, the skilled artisan will readily understand that numerous variations and modifications of these exemplary embodiments are possible without undue experimentation. All such variations and modifications are within the scope of the current teachings.

[0391] All references cited herein, including patents, patent applications, papers, text books,

and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

5

EXAMPLES

[0392] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

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Example 1: Isolation of rat monoclonal antibodies that bind to human E-selectin.

[0393] Sprague Dawley Rats were immunized with soluble human E-selectin extracellular domain (Uniprot P16581 residues 22-556; SEQ ID NO:133) using a Rappid IP Immunization method. Hybridoma supernatants were screened for binding to soluble human (ID NO:133) and mouse (SEQ ID NO:135) E-selectin extracellular domain and truncated human E-selectin (UniProtKB P16581 residues 22-178; SEQ ID NO:197) and truncated mouse E-selectin (UniProtKB Q00690 residues 22-178; SEQ ID NO:199) E-selectin using a direct binding HRP-ELISA. Of the set of binders, 6 antibodies (0039, 0158, 0159, 0164, 0170 and 0180) were selected that bound to soluble and truncated human E-selectin and one antibody (0027) was selected that bound to soluble and truncated mouse E-selectin. These seven antibodies were chosen for molecular cloning and subsequence analysis.

20

Example 2: Cloning of rat anti-E-selectin antibody heavy and light chain variable regions.

[0394] Heavy chain (HC) and light chain (LC) variable regions of the anti-E-selectin antibodies that bound to human E-selectin (antibodies 0039, 0158, 0159, 0164, 0170 and 0180) or mouse E-selectin (antibody 0027) were cloned using the SMART® cDNA synthesis system (Clontech Laboratories Inc. of Mountain View, California) followed by PCR amplification. The cDNA was synthesized from 1 µg total RNA isolated from approximately 500,000 hybridoma cells (for each clone 0027, 0039, 0158, 0159, 0164, 0170 and 0180), using the RNEasy kit (Qiagen) and the SMART® IIA oligo (Clontech Laboratories Inc.) with Superscript™ III reverse transcriptase (Invitrogen). The cDNA was then amplified by PCR using a primer that anneals to the SMART® IIA oligo sequence and rat constant region-specific primer (rat Kappa for the light chain and rat IgG1 for the heavy chain) with Q5® High-Fidelity 2X Master Mix (New England

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Biolabs Inc.). Heavy and light chain PCR products were subcloned into the pCR4-TOPO vector (Invitrogen) and the nucleic acid sequence was determined. This method is advantageous in that no prior knowledge of the DNA sequence is required. In addition, the resultant DNA sequence is not altered by use of degenerate PCR primers.

- 5 **[0395]** The variable heavy regions (VH for antibody 0039 (amino acid sequence of SEQ ID NO:60), VH for antibody 0158 (amino acid sequence of SEQ ID NO:75), VH for antibody 0159 (amino acid sequence of SEQ ID NO:90), VH for antibody 0164 (amino acid sequence of SEQ ID NO:35), VH for antibody 0170 (amino acid sequence of SEQ ID NO:104), VH for antibody 0180 (amino acid sequence of SEQ ID NO:118), and VH for antibody 0027 (amino acid
10 sequence of SEQ ID NO:128)) were cloned into the pTT5 mammalian expression vector containing the human IgG1 constant region that was mutated to abolish effector function (Leu234Ala, Leu235Ala and Gly237Ala, EU numbering; US Patent No. 5,624,821) (amino acid sequence of SEQ ID NO:15), producing chimeric heavy chains. Variable light regions (VL for antibody 0039 (amino acid sequence of SEQ ID NO:57), VL for antibody 0158 (amino acid
15 sequence of SEQ ID NO:73), VL for antibody 0159 (amino acid sequence of SEQ ID NO:87), VL for antibody 0164 (amino acid sequence of SEQ ID NO:32), VL for antibody 0170 (amino acid sequence of SEQ ID NO:102), VL for antibody 0180 (amino acid sequence of SEQ ID NO:116) and VL for antibody 0027 (amino acid sequence of SEQ ID NO:122)) were cloned into the pTT5 mammalian expression vectors containing the constant region of human kappa (amino acid
20 sequence of SEQ ID NO:14) to produce chimeric light chains.

Example 3: Binding and neutralization of anti-E-selectin antibodies.

- [0396]** The binding of anti-E-selectin antibodies to human (amino acid sequence of SEQ ID NO:133 plus a 10 His purification tail) and mouse (amino acid sequence of SEQ ID NO:135 plus
25 a 10 His purification tail) E-selectin extracellular domains was measured using a recombinant antigen binding ELISA with HPR detection. 384-well Maxisorp plates were used to capture the human and mouse His-tagged recombinant E-selectin polypeptides. The polypeptides were incubated with chimeric antibodies of varying concentrations and detected with HRP-labeled anti-human IgG1 antibodies. Antibodies 0039, 0158, 0159, 0164, 0170 and 0180 bound to
30 human E-selectin with EC₅₀s of 0.02 nM, 0.03 nM, 0.02 nM, 0.02 nM, 0.01 nM and 0.01 nM, respectively. None of these antibodies bound to mouse E-selectin, with EC₅₀ of >133 nM. Antibody 0027 bound to mouse E-selectin with an EC₅₀ of 0.02 nM, but did not bind to human E-selectin, with an EC₅₀ of >133 nM.

[0397] The neutralization ability of anti-E-selectin antibodies was measured in an AlphaLISA

homogenous competition assay. Here the antibodies competed against the interaction of human E-selectin extracellular domain (amino acid sequence of SEQ ID NO:133 plus 10 His purification tail), mouse E-selectin extracellular domain (amino acid sequence of SEQ ID NO:135 plus 10 His purification tail), or cynomolgus E-selectin extracellular domain (amino acid sequence of SEQ ID NO:175 plus 10 His purification tail) to a sialyl-Lewis A ligand. For this experiment 1 μ L of an antibody was added to a well of a 384-well plate followed by 1 μ L of sialyl Lewis A-PAA-biotin to a final concentration of 1.75 nM. This was incubated at room temperature for 15 minutes, followed by addition of 1.4 μ L of human, mouse or cynomolgus monkey E-selectin added to a final concentration of 7 nM. This was again incubated at room temperature for 15 minutes. To this, 1.4 μ L AlphaLISA beads was added (Ni-donor/SA-acceptor) for a final assay concentration of 10 μ g/mL. This was incubated in the dark at room temperature for at least 10 hours. The plates were then read using the AlphaLISA protocol (Envision).

[0398] The EC_{50} of neutralization of human, mouse and cynomolgus monkey E-selectin was measured for chimeric anti-E-selectin antibodies 0027, 0158, 0159, 0164, 0170 and 0180. Antibodies 0039, 0158, 0159, 0164, 0170 and 0180 had EC_{50} values to human E-selectin of <1 nM, while antibody 0027 did not neutralize human E-selectin and had an EC_{50} of >133 nM. Antibodies 0158, 0159, 0164, 0170 and 0180 had EC_{50} values to cynomolgus E-selectin of <1 nM, antibody 0039 had an EC_{50} to cynomolgus E-selectin of >63 nM and antibody 0027 didn't neutralize cynomolgus E-selectin and had an EC_{50} of >133 nM. Antibody 0027 had an EC_{50} value of 1 nM to mouse E-selectin while antibodies 0039, 0158, 0159, 0164, 0170 and 0180 did not neutralize mouse E-selectin and had an EC_{50} of >133 nM (Table 4).

[0399] **Table 4.** Binding and neutralization of anti-E-selectin chimeric antibodies to human, mouse and cynomolgus monkey E-selectin using an HRP binding ELISA and AlphaLISA neutralization assay.

antibody	HC SEQ ID NO	LC SEQ ID NO	HRP binding EC_{50} (nM)		alphaLISA neutralization EC_{50} (nM)		
			human E- selectin	mouse E- selectin	human E- selectin	mouse E- selectin	cynomolgus monkey E-selectin
0027	124	119	>133	0.02	>133	1	>133 nM
0039	59	56	0.02	>133	<1	>133 nM	>63
0158	74	72	0.03	>133	<1	>133 nM	<1
0159	89	86	0.02	>133	<1	>133 nM	<1

0164	34	31	0.02	>133	<1	>133 nM	<1
0170	103	101	0.01	>133	<1	>133 nM	<1
0180	117	115	0.01	>133	<1	>133 nM	<1

Example 4: Kinetic evaluation of anti-E-selectin using surface plasmon resonance (SPR).

[0400] Human (amino acid sequence of SEQ ID NO:133), mouse (amino acid sequence of SEQ ID NO:135), and cynomolgus monkey (amino acid sequence of SEQ ID NO:175) E-selectin extracellular domain proteins were prepared. Each protein contained a C-terminal 10 residue histidine tag utilized for purification. An anti-human Fc sensor chip was prepared by amine coupling of anti-human IgG antibody (catalog number BR-1008-39, GE Healthcare) to all four flow cells of a carboxymethylated dextran coated sensor chip (CM5) (catalog number BR100530, GE Healthcare) according to the manufacturer's protocols. The flow cells were activated by injecting a 1:1 mixture of 400 mM 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 100 mM N-hydroxysuccinimide (NHS) for 7 minutes at a flow rate of 10 μ L/minute. Anti-human IgG antibody was diluted to 25 μ g/mL in 10 mM sodium acetate pH 5.0 and injected over all flow cells for 7 minutes at 10 μ L/minute. All flow cells were blocked with 1M Ethanolamine-HCl (ETH) for 7 minutes at 10 μ L/minute. Final immobilization level of the capture antibody was approximately 10,000 resonance units (RU). The running buffer for immobilization and kinetics was HBS-EP+ (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4, 150 mM sodium chloride, 3 mM ethylenediaminetetraacetic acid (EDTA), 0.05% (v/v) Tween-20).

[0401] To characterize binding of antibodies to human, cynomolgus monkey, and mouse E-selectin, an antibody was diluted to 0.5 μ g/mL in HBS-EP+ and captured by the anti-human IgG immobilized on flow cells 2, 3 and 4 for 30 seconds to 1 minute at a flow rate of 10 μ L/minute to achieve a capture level of 70 to 300 RU. Flow cell 1 was used as a reference surface. After antibody capture, the flow rate was increased to 50 μ L/minute and buffer or E-selectin proteins ranging in concentration from 15 nM to 405 nM in HBS-EP+ were injected over all flow cells for a 1.0 minute association period and then allowed to dissociate for 6 to 15 minutes. HBS-EP+ buffer cycles collected for each captured antibody were used for double-referencing (Myszka DG. J. Mol. Recognition 1999; 12(5):279-84). At the end of each cycle, the entire anti-IgG surface was regenerated by a 30 second pulse of 3 M $MgCl_2$ and a 30 second pulse of ionic buffer (1.83 M $MgCl_2$, 0.92 M Urea, 1.83 M Guanidine HCl, pH 7.4) (Andersson K. Anal. Chem. 1999; 71(13): 2475-81). Kinetic assays were conducted at 25°C at a collection rate of 10 Hertz

(Hz) on a BIAcore™ T200 instrument (GE Healthcare). Rate constants and affinities were determined by fitting the data to a 1:1 binding model in BIAcore™ T200 Evaluation software version 3.0 (GE Healthcare).

[0402] Binding affinities were determined for human and cynomolgus monkey E-selectin for antibodies 0039, 0158, 0159, 0164, 0170 and 0180, and for mouse E-selectin antibody 0027 by first binding the antibody to immobilized anti-human IgG. After antibody capture, dilutions of the E-selectin proteins were flowed over and the association rate constant (k_a), disassociation rate constant (k_d), $t_{1/2}$, and K_D values were determined for binding to human, cynomolgus and mouse E-selectin (Tables 5-7). In this SPR analysis the affinity (K_D) of antibodies 0039, 0158, 0159, 0164, 0170 and 0180 to human E-selectin was 68.0 nM, 21.6 nM, 324 nM, 54.4 nM, 628 nM and 2940 nM, respectively. The affinity K_D of chimeric antibodies 0039, 0159, 0164, 0170 and 0180 to cynomolgus monkey E-selectin was 45.8 nM, 243.5 nM, 45.4 nM, 492 nM, and 3145 nM, respectively. Antibody 0158 did not show binding to cynomolgus monkey E-selectin. The affinity K_D of chimeric antibody 0027 to mouse E-selectin was 1.2 nM.

[0403] **Table 5.** Affinity of anti-E-selectin antibodies to human E-selectin measured by surface plasmon resonance.

antibody	HC SEQ ID NO	LC SEQ ID NO	analyte			
			human E-selectin			
			k_a	k_d	$t_{1/2}$	K_D
			(1/Ms)	(1/s)	(s)	(nM)
0039	59	56	6.42E+04	4.21E-03	173.64	68.0
0158	74	72	8.10E+04	1.75E-03	397.16	21.6
0159	89	86	1.15E+05	3.71E-02	18.71	324
0164	34	31	2.07E+05	1.13E-02	61.61	54.4
0170	103	101	8.74E+04	5.49E-02	12.64	628.5
0180	117	115	2.62E+05	6.66E-01	1.05	2940

[0404] **Table 6.** Affinity of anti-E-selectin antibodies to cynomolgus monkey E-selectin measured by surface plasmon resonance.

antibody	HC SEQ ID NO	LC SEQ ID NO	analyte			
			cynomolgus monkey E-selectin			
			k_a	k_d	$t_{1/2}$	K_D
			(1/Ms)	(1/s)	(s)	(nM)

0039	59	56	8.24E+04	3.77E-03	184.07	45.8
0158	74	72	ND	ND		weak
0159	89	86	1.54E+05	3.74E-02	18.53	243.5
0164	34	31	2.65E+05	1.20E-02	57.75	45.4
0170	103	101	1.15E+05	5.64E-02	12.30	492
0180	117	115	2.45E+05	7.49E-01	0.93	3145

ND: not detected

[0405] **Table 7.** Affinity of anti-mouse E-selectin antibody to mouse E-selectin measured by surface plasmon resonance.

antibody	HC SEQ ID NO	LC SEQ ID NO	analyte			
			mouse E-selectin			
			ka	kd	t _{1/2}	K _D
			(1/Ms)	(1/s)	(s)	(nM)
0027	124	119	1.92E+05	2.31E-04	3010.95	1.2

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Example 5: Neutralization of ligand binding of anti-E-selectin antibodies in a static binding assay.

[0406] The ability of anti-E-selectin antibodies to neutralize ligand binding was assessed in a static neutralization assay using a competition enzyme-linked immunosorbent assay (ELISA) to evaluate the neutralization of the sialyl Lewis antigen to Chinese hamster ovary cells (CHO) engineered to express E-selectin.

[0407] Briefly, CHO cells expressing human E-selectin were grown in culture medium (MEM (Minimum Essential Medium) Alpha Medium, supplemented with 10% dialyzed fetal bovine serum, 100 nM methotrexate and 100 Units/mL penicillin and 100 µg/mL streptomycin) and were seeded at 12,500 cells per well into a 96 well tissue culture plate and incubated at 37°C, 5% CO₂ for 48 hours to form a confluent monolayer. Plates were subsequently washed twice with 200 µL of calcium magnesium free phosphate buffered saline (PBS). Anti-E-selectin antibodies 0039, 0158, 0159, 0164, 0170 and 0180 or an IgG1 isotype control were added to the assay in the presence of a small synthetic conjugate of biotinylated, sialyl Lewis A polyacrylamide (Carbosynth LLC; OS45446) with a streptavidin/horseradish peroxidase (100 µL total) and incubated at 37°C, 5% CO₂ for 2 hours. Plates were then washed twice with 200 µL of calcium magnesium free PBS. Following the incubation, the cells were washed twice with 200 µL of wash buffer containing 2 mM of calcium chloride (50 mM TRIS [trisaminomethane], 150

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mM sodium chloride; 0.05% polysorbate 20, pH 7.4; 2 mM of calcium chloride). Signal was developed by adding 100 μ L of 1-Step Ultra TMB substrate (Thermo Scientific, 34028) and incubating for 30 minutes at room temperature. Reactions were stopped using 2 M of sulfuric acid and absorbance read at 450 nm (Spectramax M5e). Data were analyzed and graphed using GraphPad Prism software (Version 8.0.2). Estimated half maximal inhibitory concentration (IC₅₀) values derived from the analysis (Table 8).

[0408] The tested antibodies dose-dependently neutralized ligand binding to cell surface expressed human E-selectin. The estimated IC₅₀ values in the neutralization assay for chimeric antibodies 0039, 0164, 0158, 0159, 0170 and 0180 were 3.76 nM, 2.77 nM, 2.87 nM, 3.24 nM, 2.3 nM and 3.83 nM, respectively. Antibody 0027 did not neutralize binding of the sialyl Lewis A polyacrylamide ligand binding to cell surface expressed human E-selectin. A human IgG control antibody did not demonstrate an effect in these assays.

Example 6: Inhibition of cellular binding in a static adhesion assay of anti-E-selectin antibodies.

[0409] The ability of chimeric anti-E-selectin antibodies 0039, 0164, 0158, 0159, 0170 and 0180 to inhibit adhesion of cells expressing an E-selectin ligand, under a static condition, to CHO cells expressing human E-selectin was evaluated.

[0410] CHO cells expressing human E-selectin were cultured in Alpha medium (MEM [Minimum Essential Medium] Alpha Medium, Corning)/10 % dialyzed fetal bovine serum (GIBCO)/100 Units/mL penicillin and 100 μ g/mL streptomycin (Gibco)/100 nM methotrexate and were maintained at 37°C with 5% CO₂ and 95% humidity. HL-60 cells (a human promyelocytic cell line which expresses the E selectin ligand, PSGL-1 and other sialyl Lewis ligands) were maintained at 37° C with 5% CO₂ and 95% humidity in RPMI/Glutamine growth medium (Gibco)/10% fetal bovine serum (Gibco)/ 100 Units/mL penicillin and 100 μ g/mL streptomycin (Gibco). CHO cells expressing human E-selectin were seeded in 96-well flat bottom black plates at a density of 11,500 cells/well and incubated to form a confluent monolayer for 48 hours at 37°C, 5% CO₂ and 95% air in a humidified incubator.

[0411] For the static adhesion neutralization assay, HL-60 cells at log phase were washed three times in PBS and resuspended in 500 μ L of PBS and an equal volume of 2 μ M carboxyfluorescein diacetate succinimidyl ester fluorescent dye [(CFSE) (ThermoFisher)]. The cells were incubated for 10 minutes at room temperature in the dark. The labeling was stopped with the addition of 4 mLs of cold HL-60 growth medium on ice for 5 minutes. The labeled HL-60 cells were washed three times in Hanks Balanced Salt Solution (HBSS)/1% Bovine Serum

Albumin (BSA)/0.1% sodium azide, resuspended at a density of 400,000 cells/mL for use in the static adhesion assays.

[0412] Anti-E-selectin chimeric antibodies 0039, 0164, 0158, 0159, 0154, 0170 and 0180, negative control anti-P-selectin antibodies and a control human IgG were serially
5 (concentrations ranging from 1333 nM to 1.83 nM) diluted in HBSS/1%BSA/0.1% sodium azide. A no antibody control was prepared for each antibody. The E-selectin CHO cell monolayer was gently washed three times with HBSS/1%BSA/0.1% sodium azide and 100 μ L of the cellular suspension was added to the wells and incubated for one hour on ice. Fifty μ L (20,000) CFSE-labeled HL-60 cells were added per well without removing the antibody. The labeled HL-60
10 cells were incubated with the CHO cell monolayer for 1 hour at 37°C at 5% CO₂ and 95% air in a humidified incubator. The plate was washed three times with HBSS/1%BSA/0.1% sodium azide, a 100 μ L of HBSS/1% Triton-X100 was added and incubated for 10 minutes in the dark. The fluorescence of the bound HL-60 cells was measured at excitation/emission wavelengths of 494/521 nanometer using Spectramax i3X (Molecular Devices) to determine the number of
15 adhered HL-60 cells. Background binding was subtracted using the signal from the no cell control wells and the % adhesion was determined using the human IgG control as 100%. The estimated EC₅₀ values were determined in the GraphPad Prism suite.

[0413] Under static adhesion conditions, antibodies 0158 and 0164 inhibited HL-60 cell adhesion to CHO cells expressing human E-selectin with >50% inhibition at 16.5 nM. Antibody
20 0159 inhibited HL-60 cell adhesion to CHO cells expressing human E-selectin with >50% inhibition at 49.4 nM. Antibodies 0170 and 0180 inhibited HL-60 adhesion to CHO cells expressing human E-selectin with >50% inhibition at 444 nM. Antibody 0039 at a concentration up to 1333 nM did not inhibit HL-60 cell adhesion to CHO cells expressing human E-selectin. Negative control antibodies did not inhibit the adhesion of HL-60 cells (Table 8).

Example 7: Cell surface binding (FACS) of the anti-E-selectin antibodies.

[0414] Antibody binding was evaluated using Fluorescence Activated Cell Sorting (FACS) to cells engineered to express E-selectin or P-selectin. Species cross-reactivity to cynomolgus monkey was also evaluated.

30 [0415] CHO cells expressing human E-selectin or P-selectin were cultured in Alpha medium (MEM [Minimum Essential Medium]) Alpha Medium, Corning) supplemented with 10 % dialyzed fetal bovine serum (GIBCO), 100 Units/mL penicillin and 100 μ g/mL streptomycin (Gibco). For the CHO cells expressing human E-selectin, the growth media was also supplemented with 100 nM methotrexate. CHO cells expressing cynomolgus monkey E-selectin were cultured in R1

- medium (RI Dulbecco's Modified Eagle's Medium/Ham's F12 modification) supplemented with 10% heat inactivated fetal bovine serum (GIBCO), 100 Units/mL penicillin and 100 µg/mL streptomycin and 125 µg/mL Zeocin (GIBCO). Cells were maintained in growth medium at 37°C, 5% CO₂ and 95% air in a humidified incubator. Methotrexate or Zeocin were removed
- 5 from the medium prior to culture for experiments. CHO-E selectin cells or P-selectin cells were detached from T-150 flasks using citric saline (135 mM KCl, 15 mM sodium citrate) and immediately re-calcified with the addition of CaCl₂ to the detached cells to a final concentration of 1 mM. Growth media was immediately added to the detached cells, then centrifuged at 300 x g for 5 minutes at room temperature to pellet the cells. Cells were plated into 96-well V-bottom
- 10 plates in cold CHO buffer consisting of phosphate buffered saline (PBS), pH 7.4 containing bovine serum albumin (BSA; Sigma-Aldrich) at final concentration of 1%, calcium chloride at final concentration of 1 mM, and sodium azide at a final concentration of 0.1%, for 5 minutes on wet ice. After incubation, the cells were centrifuged at 300 x g for 2 minutes at 4°C to remove the buffer.
- 15 **[0416]** Test antibody solutions were prepared with serial dilutions (ranging from 1333.3 nM to 0.0022 nM) using CHO buffer as diluent and 100 µL was added and incubated in the designated volume of serially-diluted antibody and incubated for 45 minutes on ice. After incubation, antibodies were removed and the cells were washed three times by centrifugation at 300 x g for 1 minute at 4°C and resuspended in cold CHO buffer. The washed cells were
- 20 incubated for 30 minutes on wet ice with 100 µL of a 1:1000 dilution of APC-conjugated AffiniPure F(ab')₂ Fragment Goat Anti-human IgG, Fcy (Jackson ImmunoResearch). The cells were washed three or four times prior to resuspension in CHO buffer for flow cytometric analysis. A Becton Dickinson LSRFortessa instrument using 640 nanometer laser line was used to acquire cell fluorescence data. Geometric mean fluorescence of cells was analyzed by
- 25 FlowJo software package (FlowJo, LLC) and plotted against antibody concentration. The estimated EC₅₀ values were calculated using nonlinear regression analysis in the Prism suite (GraphPad, version 8) (Table 8).
- [0417]** Chimeric antibodies 0039, 0164, 0158, 0159, 0154, 0170 and 0180 exhibited binding to cell surface expressed human E-selectin on CHO cells with estimated EC₅₀ values ranging
- 30 from 1.99 nM to 4.99 nM (Table 8). Antibodies 0039, 0164, 0158, 0159, 0170 and 0180 also bound to CHO cells expressing cynomolgus monkey E-selectin with estimated EC₅₀ values ranging from 3.37 to 13.98. Antibodies 0164, 0158, 0159, 0154, 0170 and 0180 did not bind to CHO-expressed human P-selectin, indicating selectivity for E-selectin. Antibody 0039 exhibited minimal binding to CHO-expressed human P-selectin with an estimated EC₅₀ value > 354 nM.

[0418] Table 8. Cell binding and neutralization of anti-E-selectin antibodies.

antibody	static neutralization		cell surface antibody binding by FACS		
	sialyl Lewis ligand adhesion CHO-human E-selectin cells IC ₅₀ (nM)	HL-60 Cell adhesion CHO-Human E-selectin cells IC ₅₀ (nM)	binding to CHO-human E-selectin cells EC ₅₀ (nM)	binding to CHO-cynomolgus monkey E-selectin cells EC ₅₀ (nM)	binding to CHO-human P-selectin cells EC ₅₀ (nM)
0039	3.76	ND	4.99	4.07	> 354 nM
0164	2.77	> 50 % at 16.5 nM	4.43	1.73	ND
0158	2.87	> 50 % at 16.5 nM	3.75	13.98	ND
0159	3.24	> 50 % at 49.4 nM	2.44	3.37	ND
0170	2.30	> 50 % at 444 nM	1.99	3.76	ND
0180	3.83	> 50 % at 444 nM	4.31	3.66	ND
ND = Not Detected					

Example 8: *Ex vivo* neutralization of anti-E-selectin antibodies using a physiological flow assay

- 5 **[0419]** *Ex vivo* cellular adhesion was analyzed under physiological flow conditions using a BioFlux™ system (Fluxion, San Francisco, CA, USA) using BioFlux™ plates/chambers (Fluxion Biosciences, 48-well low shear, 0-20 dynes/cm²; 910-0004). Adhesion assays were performed using recombinant purified soluble E- or P-selectin proteins and human HL-60 cells (ATCC CCL-240). HL-60 cells express the E selectin ligand, PSGL-1 and other sialyl Lewis ligands.
- 10 HL-60 cells were maintained at 37°C with 5% CO₂ and 95% humidity in RPMI/Glutamine growth medium (Gibco) with 10% fetal bovine serum (Gibco); 1X penicillin/streptomycin (Gibco). The cells were maintained at 37°C prior to perfusion and BioFlux™ assays were performed at room temperature. First, to mimic a surface of an activated endothelial cell, the flow chambers were coated with equal amounts of both human E- and P-selectin soluble recombinant proteins.
- 15 BioFlux™ flow microchannels were coated with 10 µg/mL each of soluble recombinant human

P-selectin (R & D Systems, ADP3) and E-selectin (R & D Systems, ADP1) prepared in PBS, perfused at 1 dyne/cm² for 5 minutes, incubated at room temperature for 1 hour and washed with PBS for 3-5 minutes at 1 dyne/cm². The E-selectin chimeric antibodies, 0039, 0158, 0159, 0164, 0170 and 0180, were diluted in PBS and the plates were treated with the test articles (concentrations from 1.6 to 1000 nM), or the vehicle control for 1 hour at 1 dyne/cm². The HL-60 cells were centrifuged at 1000 rpm for 5 minutes, stained with Calcein AM (Calcein; Life Technologies™; C3099) for 30 minutes, washed twice with phosphate buffered saline (PBS; Gibco) and resuspended to a concentration of $\sim 3 \times 10^6$ cells/mL in PBS. Diluted antibody stocks were also added to the cellular suspension prior to perfusion in the flow chambers. An aliquot of 100 μ L of HL-60 cells was perfused in each channel for 10 minutes at physiological shear flow of 1 dyne/cm². PBS was perfused for 2-3 minutes at 1 dyne/cm² to remove the non-adherent cells. Adherent cells were visualized and counted using a Nikon Eclipse Ti-S (NIS-Elements BR 3.2 64-bit) inverted-stage phase-contrast fluorescent microscope with 40X magnification. Cellular counts were normalized to phosphate buffer vehicle control. Each experiment has two wells per conditions. Data was graphed and analyzed using GraphPad Prism (8.0.2). Estimated half-maximal inhibitory concentration (IC₅₀) values were derived from the analysis.

[0420] In these studies, which were designed to mimic blood flow through the vasculature *ex vivo*, E-selectin antibodies 0039, 0164, 0158, 0159, 0170 and 0180 neutralized the adhesion of HL-60 cells to soluble recombinant E-selectin protein with estimated IC₅₀ values of 264.7 nM, 1.7 nM, 17.9 nM, 21 nM, 74.2 nM, and 224.7 nM, respectively (Table 9).

[0421] **Table 9.** Neutralization of HL-60 binding to soluble human E-selectin protein under physiological flow.

antibody	neutralization of HL-60 cell binding under physiological flow to soluble human E-selectin protein
	IC ₅₀ (nM)
0039	264.7
0164	1.7
0158	17.9
0159	21
0170	74.2
0180	224.7

Example 9: Epitope grouping of anti-E-selectin antibodies

[0422] The six neutralizing chimeric antibodies (0039, 0158, 0159, 0164, 0170 and 0180) were grouped into epitope bins based on a competition assay using an Octet biosensor. Anti-human FC (AHC) coated tips were transferred to a well containing 20µg/mL of one of the seven antibodies for 600 seconds to allow the first antibody to bind to the anti-human FC. The tips were transferred to a second well with 300 nM human E-selectin extracellular domain (SEQ ID NO:133 plus a 10 His purification tail) and allowed to associate for 100 seconds. Finally, the tips were transferred to a third well containing a second antibody for 100 seconds. Antibodies were scored as non-competing if the second antibody showed an increase in biosensor signal above that produced by the first antibody, and they were scored as competing if the second antibody did not produce an additional increase in signal (Table 10).

[0423] Based on these competition data, antibodies 0159, 0164, 0170 and 0180 defined one epitope group, 0158 defined a second epitope group and 0039 defined a third epitope group. The first group and the second group partially overlap, the second and third groups partially overlap, and the first and third group are non-overlapping. In Table 10 a "+" indicates that the second antibody (indicated by the column heading) bound in the presence of the first antibody (indicated by row headings). A "-" indicates that no increase in signal was observed upon addition of the second antibody.

[0424] **Table 10.** Epitope grouping of anti-IL-E-selectin antibodies by Octet biosensor

1 st Ab (rows)	0039	0158	0159	0164	0170	0180
2 nd Ab (column)						
0039	-	-	+	+	+	+
0158	-	-	-	-	-	-
0159	+	-	-	-	-	-
0164	+	-	-	-	-	-
0170	+	-	-	-	-	-
0180	+	-	-	-	-	-

Example 10: Crystal structure and epitope

[0425] The Fab fragment of the anti-E-selectin chimeric antibody 0164 was obtained by cleaving the full-length IgG with papain and removing the Fc using a Protein A resin. The Fab

was mixed with truncated human E-selectin (described in Example 1) at a 1:1 molar ratio and the resulting complex was purified by size exclusion chromatography (SEC). Crystals of the complex formed in 200 mM calcium acetate pH 7.5, 20% PEG 3350. Data was collected to 2.68 Å at beamline 17-ID at the Advanced Photon Source. The structure was solved by molecular replacement and refined to R/Rfree of 0.243/0.252 (**FIG. 1**). The crystal structure has a single copy of E-selectin bound to a single 0164 Fab fragment in the asymmetric unit in space group P3121.

[0426] Based upon the crystal structure, the residues of E-selectin that interacted with antibody 0164 were determined based upon: i) residues that were within 3.8 Å, ii) normalized surface area (Å²) buried due to interactions between pairs of 0164 antibody residues and E-selectin residues of >5Å², iii) formation of a salt bridge, iv) formation of a hydrogen bond or v) formation of a water-mediated hydrogen bond. The list of residues, whose numbering is based upon the mature human E-selectin sequence (SEQ ID NO:132) includes T7, E8, A9, M10, T11, P46, S47, Y48, N82, N83, Q85, E88, E92, Y94, R97, N105, E107, R108, S110, K111, K112 and K113 (Table 11).

[0427] The interaction of E-selectin with its natural ligand Sialyl-Lewis X (sLeX) by x-ray crystallography is shown in the PDB structure (1g1t) (see www.rcsb.org/structure/1g1t). In this structure, the E-selectin residues that are within 3.8 Å of sLeX and are similar to the interaction with antibody 0164 are Y48, N82, N83, E92, Y94, R97, N105, and E107 based on the numbering of SEQ ID NO:132. Additional residues that are also within 3.8 angstroms of sLeX are E80, E98 and D106 (based on the numbering of SEQ ID NO:132). Given the significant overlap of the epitopes of sLeX and antibody 0164, it is likely that the neutralization activity demonstrated by this antibody is a result of directly blocking the interaction of sLeX and other similar ligands like Sialyl-Lewis A (sLeA) with E-selectin.

[0428] **Table 11.** Interaction of E-selectin with anti-E-selectin antibody 0164 or sLeX.

E-selectin position	amino acid	Interaction with Antibody 0164					Interaction with sLeX
		within 3.8 Å	buried surface area (Å ²)	hydrogen bond	salt bridge	H ₂ O mediated interaction	within 3.8 Å
7	T	X	13.3				
8	E	X	94.5	1			

9	A	X	39.2				
10	M		5				
11	T	X	7.3				
46	P	X	56.2				
47	S	X	36.1	1			
48	Y	X	13.1				X
82	N	X	29.5	1			X
83	N	X	29.4	1			X
85	Q	X	35.7				
88	E		9.9	1			
92	E	X	6.9	1			X
94	Y	X	9.3	1			X
97	R		29.7			X	X
105	N	X	1.4	1			X
107	E	X	30.6	1			X
108	R	X	72.6	2			
110	S	X	29.3	1			
111	K	X	63.9		X		
112	K	X	112.6	1	X	X	
113	K		15.1				
80	E						X
98	E						X
106	D						X

Example 11: Humanization of rat anti-E-selectin antibodies

- [0429]** Humanized versions of neutralizing rat chimeric antibodies 0039, 0158, 0159, 0164, 0170 and 0180 were generated by complementarity determining region (CDR) grafting (referred to hereafter as "CDR-grafted"). For antibodies 0039, 0158, 0164 and 0180, heavy chain CDRs were grafted onto a human DP-54 framework region (VH3 sub-group). Humanized antibody 0039 contained rat amino acid residues including methionine at position 48, glycine at position 49, and valine at position 78. Humanized antibody 0158 contained rat amino acid residues including valine at position 24, methionine at position 48, glycine at position 49, threonine at position 73, and valine at position 78. Humanized antibody 0164

contained rat amino acid residues including alanine at position 71, alanine at position 78 and methionine at position 94. Humanized antibody 0180 contained rat amino acid residues including isoleucine at position 48, glycine at position 49, leucine at position 69, serine at position 71, threonine at position 73, alanine at position 78 and isoleucine at position 94.

5 **[0430]** For antibody 0159, heavy chain CDRs were grafted onto DP-7 framework region (VH1 sub-group). Humanized antibody 0159 contained rat amino acid residues including valine at position 24, leucine at position 69, alanine at position 78 and methionine at position 94.

10 **[0431]** For antibody 0170, heavy chain CDRs were grafted onto DP-10 framework region (VH1 sub-group). Humanized antibody 0170 contained rat amino acid residues including valine at position 24, isoleucine at position 69, threonine at position 73, and valine at position 94.

[0432] All 6 humanized antibodies were grafted with the JH4 J-segment (amino acid sequence of SEQ ID NO:6).

15 **[0433]** For antibodies 0039, 0158, 0164, 0170 and 0180, light chain CDRs were grafted onto human DPK9 framework region (VKI sub-group). Humanized antibody 0039 contained rat amino acid residue cysteine at L87. Humanized antibody 0158, contained rat amino acid residue methionine at position 4. Humanized antibodies 0164 and 180 contained rat amino acid residue phenylalanine at position 87. Humanized antibody 0170 contained rat amino acid residues including methionine at position 48 and phenylalanine at position 87.

20 **[0434]** For antibody 0159, light chain CDRs were grafted onto human DPK1 framework region (VKI sub-group).

25 **[0435]** The humanized V_H regions were joined to the effector-function mutated human IgG1 constant region (amino acid sequence of SEQ ID NO:16) and then sub-cloned into a proprietary expression vector to generate the CDR-grafted heavy chains SEQ ID NO:51 (0039 CDR graft; heavy chain of antibody 0525), SEQ ID NO:62 (0158 CDR graft; heavy chain of antibody 265_254), SEQ ID NO:76 (0159 CDR graft; heavy chain of antibody 0929_0548), SEQ ID NO:22 (0164 CDR graft; heavy chain of antibody 0841), SEQ ID NO:91 (0170 CDR graft; heavy chain of antibody 0955_0300) and SEQ ID NO:110 (0180 CDR graft; heavy chain of antibody 0564).

30 **[0436]** The humanized V_L regions were fused to the human kappa constant region (amino acid sequence of SEQ ID NO:14) and then sub-cloned into a proprietary expression vector to create the CDR-grafted light chains SEQ ID NO:46 (0039 CDR graft; light chain of antibody 0525), SEQ ID NO:67 (0158 CDR graft; light chain of antibody 0265_0254), SEQ ID NO:81

(0159 CDR graft; light chain of antibody 0929_0548), SEQ ID NO:17 (0164 CDR graft; light chain of antibody 0841), SEQ ID NO:96 (0170 CDR graft; light chain of antibody 0955_0300) and SEQ ID NO:105 (0180 CDR graft; light chain of antibody 0564).

[0437] Table 12. Anti-E-selectin antibodies.

Chimeric antibody	Humanized antibody	Optimized Antibody
0164	0841	
	0978	1444 1448 1282 1284
0039	0525	
0158	0265_0254	
0159	0929_0548	
0170	0955_0300	
0180	0564	
0027		

5

Example 12: Kinetic evaluation of humanized anti-E-selectin using surface plasmon resonance.

[0438] Binding affinity was determined for human and cynomolgus monkey E-selectin for the humanized antibody 0841 (HC SEQ ID NO:22, LC SEQ ID NO:17) using SPR in a method similar to the method described in Example 4. This was done by first binding the antibody to immobilized anti-human IgG. After antibody capture, dilutions of the E-selectin proteins were flowed over and the association rate constant (k_a), disassociation rate constant (k_d), $t_{1/2}$, and K_D values were determined for binding to human and cynomolgus E-selectin (Table 13). In this SPR analysis, the affinity of humanized anti-E-selectin antibody 0841 to human E-selectin and cynomolgus monkey E-selectin was 92.85 nM and 138.5 nM, respectively.

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15

[0439] Table 13. Affinity of anti-E-selectin antibodies to human and cynomolgus monkey E-selectin measured by surface plasmon resonance.

antibody	analyte							
	human E-selectin				cynomolgus monkey E-selectin			
	K_a	k_d	$t_{1/2}$	K_D	k_a	k_d	$t_{1/2}$	K_D
	(1/Ms)	(1/s)	(s)	(nM)	(1/Ms)	(1/s)	(s)	(nM)

0841	2.55E+05	2.37E-02	29.31	92.85	1.82E+05	2.52E-02	27.56	138.5
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Example 13: Cell surface binding (FACS) of humanized anti-E-selectin antibody (0841)

[0440] Antibody binding was evaluated using flow cytometry (FACS) of cells engineered to express E-selectin. Species cross-reactivity to cynomolgus monkey was also evaluated.

- 5 **[0441]** CHO cells expressing human E-selectin were cultured in Alpha medium (MEM [Minimum Essential Medium] Alpha Medium, Corning) supplemented with 10 % dialyzed fetal bovine serum (GIBCO) and 100 Units/mL penicillin and 100 µg/mL streptomycin in the culture medium (Gibco). For the CHO cells expressing human E-selectin, the growth media was also supplemented with 100 nM methotrexate. CHO cells expressing cynomolgus monkey E-selectin
- 10 were cultured in R1 medium (RI Dulbecco's Modified Eagle's Medium/Ham's F12 modification) supplemented with 10% heat inactivated fetal bovine serum (GIBCO), 100 Units/mL penicillin and 100 µg/mL streptomycin and 125 µg/mL Zeocin (GIBCO). Cells were maintained in growth medium at 37°C, 5% CO₂ and 95% air in a humidified incubator. Methotrexate or Zeocin were removed from the medium prior to culture for experiments. CHO-E selectin cells were detached
- 15 from T-150 flasks using citric saline (135 mM KCl, 15 mM sodium citrate) and immediately re-calcified with the addition of CaCl₂ to the detached cells to a final concentration of 1 mM. Growth media was immediately added to the detached cells, then centrifuged at 300 x g for 5 minutes at room temperature to pellet the cells. Cells were plated into 96-well round-bottom plates in cold CHO buffer consisting of phosphate buffered saline (PBS), pH 7.4 containing bovine serum
- 20 albumin (BSA; Sigma-Aldrich) at final concentration of 1%, calcium chloride at final concentration of 1 mM, and sodium azide at a final concentration of 0.1%, for 5 minutes on wet ice. After incubation, the cells were centrifuged at 300 x g for 2 minutes at 4°C to remove the buffer.

- [0442]** Test antibody solutions were prepared with serial dilutions (ranging from 1333.3 nM
- 25 to 0.0022 nM) using CHO buffer as diluent, 100 µL was added in the designated volume of serially-diluted antibody and incubated for 45 minutes on ice. After incubation, the antibodies were removed and the cells were washed three times by centrifugation at 300 x g for 1 minute at 4°C and resuspended in cold CHO buffer. The washed cells were resuspended and incubated for 30 minutes on wet ice with 100 µL of 1:1000 dilution of APC-conjugated AffiniPure
- 30 F(ab')₂ Fragment Goat Anti-human IgG, Fcγ (Jackson ImmunoResearch). The cells were washed three or four times prior to resuspension in CHO buffer for flow cytometric analysis. A Becton Dickinson LSRFortessa instrument using 640 nanometer laser line was used to acquire

cell fluorescence data. Geometric mean fluorescence of cells was analyzed by FlowJo software package (FlowJo, LLC) and plotted against antibody concentration. The estimated EC₅₀ values were calculated using nonlinear regression analysis in the GraphPad Prism suite.

[0443] Binding of humanized antibody 0841 to human and cynomolgus monkey E-selectin expressed on the surface of CHO cells was similar with estimated EC₅₀ values of 1.62 and 1.54 nM, respectively (Table 14). The estimated EC₅₀ values for antibody 0164 in the same assay were similar with estimated EC₅₀ values of 1.39 and 1.73 nM for CHO expressed human and cynomolgus monkey E-selectin, respectively (Table 14).

10 **Example 14: Humanized anti-E-selectin antibody neutralization of ligand binding to CHO cells expressing human or cynomolgus monkey E-selectin**

[0444] In a static ligand cellular adhesion assay, horse radish peroxidase (HRP) conjugated E-selectin ligand and E-selectin antibodies compete for binding to human or cynomolgus E-selectin expressed on Chinese hamster ovary (CHO) cells. The ability of anti-E-selectin antibodies to neutralize the ligand binding was assessed in a static neutralization assay using a competition enzyme-linked immunosorbent assay (ELISA) to evaluate the neutralization of the sialyl Lewis antigen to Chinese hamster ovary cells (CHO) engineered to express human or cynomolgus monkey E-selectin.

[0445] CHO cells expressing human E-selectin were grown in culture medium (MEM (Minimum Essential Medium) Alpha Medium, supplemented with 10% dialyzed fetal bovine serum, 100 nM Methotrexate and 100 Units/mL penicillin and 100 µg/mL streptomycin) and CHO cells expressing cynomolgus monkey E-selectin were cultured in R1 medium (RI Dulbecco's Modified Eagle's Medium/Ham's F12 modification) supplemented with 10% heat inactivated fetal bovine serum (GIBCO), 100 Units/mL penicillin and 100 µg/mL streptomycin and 125 µg/mL Zeocin (GIBCO). CHO cells expressing E-selectin were seeded at 12,500 cells per well into a 96 well tissue culture plate and incubated at 37°C, 5% CO₂ for 48 hours to form a confluent monolayer. Plates were subsequently washed twice with 200 µL of calcium magnesium free PBS. Anti-E-selectin antibodies, or an IgG1 isotype control, were added to the assay in the presence of a small synthetic conjugate of biotinylated, sialyl Lewis A polyacrylamide (Carbosynth LLC, OS45446) or sialyl Lewis X polyacrylamide (Glycotect, 01-045) with a streptavidin/horseradish peroxidase (100 µL total) and incubated at 37°C, 5% CO₂ for 2 hours. Plates were then washed twice with 200 µL of calcium magnesium free PBS. Following the incubation, the cells were washed twice with 200 µL of wash buffer containing 2 mM calcium chloride (50 mM TRIS [trisaminomethane], 150 mM sodium chloride; 0.05%

polysorbate 20, pH 7.4; 2 mM of calcium chloride). Signal was developed by adding 100 μ L of 1-Step Ultra TMB substrate (Thermo Scientific, 34028) and incubating for 30 minutes at room temperature. Reactions were stopped using 2 M of sulfuric acid and absorbance was read at 450 nm (Spectramax M5e). Data was analyzed and graphed using GraphPad Prism software (Version 8.0.2). Estimated half maximal inhibitory concentration (IC_{50}) values were derived from the analysis.

[0446] Neutralization of sialyl Lewis ligand binding by humanized antibody 0841 to human and cynomolgus monkey E-selectin expressed on the surface of CHO cells was similar with estimated IC_{50} values of 3.81 and 1.74 nM, respectively (Table 14). The estimated IC_{50} values for 0164 in the same assay were similar with estimated IC_{50} values of 5.17 and 2.00 nM for CHO expressed human and cynomolgus monkey E-selectin, respectively.

Example 15: Humanized anti-E-selectin antibody neutralization of cellular adhesion under physiological flow

[0447] *Ex vivo* cellular rolling/adhesion studies were conducted to evaluate the potency and the neutralizing activity of antibodies 0841 and 0164 to inhibit the adhesion of HL-60 cells to CHO-human E-selectin cells using a physiological flow system.

[0448] CHO-human E-selectin cells were maintained in Alpha medium/10% dialyzed fetal bovine serum (GIBCO)/ penicillin/streptomycin (1X) and 100 nM methotrexate at 37°C with 5% CO₂ and 95% humidity. The BioFlux™ plates were coated with 50 μ g/mL of fibronectin in PBS at a flow rate of 1 dyne/cm² and incubated for 1 hour at room temperature. Chinese Hamster Ovary cells expressing human E-selectin (human CHO-E-selectin cells) were prepared for assay by treatment with a 1:1 ratio of 0.05% Trypsin-Ethylenediaminetetraacetic acid (EDTA), and StemPro Accutase (Gibco; A1110501) for 5 minutes at 37°C, neutralized with an equal volume of growth medium, collected, centrifuged at 1000 rpm for 5 minutes and re-suspended to a final concentration of $\sim 30 \times 10^6$ cells/mL in growth medium. The plates were perfused twice with CHO-human E-selectin growth medium at 1 dyne/cm² for 2-3 minutes at room temperature. All medium was removed and 30 μ L of CHO- human E-selectin cells (at $\sim 30 \times 10^6$ cells/mL) were perfused for 25 seconds at 3-4 dyne/cm². The plates were removed from the instrument and were incubated at 37°C in a 5% CO₂ incubator for 1 hour. Medium was removed and 500 μ L of fresh growth medium was added and the cells were incubated at 37°C in a 5% CO₂ incubator overnight. Following the incubation, the CHO-E monolayers were washed (3-4 dynes/cm²) on the BioFlux instrument with fresh growth medium. Antibody 0841 (serial dilutions), isotype control antibody, and IgG1 control antibody (200 nM), in PBS and the vehicle

control were perfused at 1 dyne/cm² for 1 hour.

[0449] HL-60 cells were maintained at 37°C with 5% CO₂ and 95% humidity in RPMI/Glutamine growth medium (Gibco) with 10% fetal bovine serum (Gibco); and 1X penicillin/streptomycin (Gibco). The cells were maintained at 37°C prior to perfusion. The HL-60 cells were centrifuged at 1000 rpm for 5 minutes, stained with Calcein AM (Life Technologies™; C3099) for 30 minutes, washed twice with phosphate buffered saline (PBS; Gibco) and resuspended to a concentration of ~3 x 10⁶ cells/mL in PBS. Diluted antibody stocks were also added to the cellular suspension prior to perfusion in the flow chambers. An aliquot of 100 µL of HL-60 cells, plus inhibitor, were perfused in each channel for 10 minutes at physiological shear flow of 1 dyne/cm². PBS was perfused for 2-3 minutes at 1 dyne/cm² to remove the non-adherent cells and the plates were imaged and analyzed. Assays were performed at room temperature.

[0450] Antibodies 0841 and 0164 inhibited the neutralization of HL-60 cells to CHO cells expressing human E-selectin under physiological flow conditions with estimated IC₅₀ values of 4.17 and 3.73 nM, respectively (Table 14).

[0451] **Table 14.** Binding and neutralization by antibody 0841 and 0164.

	static neutralization		neutralization under physiological flow	cell surface antibody binding by FACS	
antibody	sialyl Lewis ligand adhesion CHO-human E-selectin cells IC ₅₀ (nM)	sialyl Lewis ligand adhesion CHO-cynomolgus monkey E-selectin cells IC ₅₀ (nM)	HL-60 cell adhesion to CHO-human E-selectin cells IC ₅₀ (nM)	binding to CHO-human E-selectin cells EC ₅₀ (nM)	binding to CHO-cynomolgus monkey E-selectin cells EC ₅₀ (nM)
0841	3.81	1.74	4.17	1.62	1.54
0164	5.17	2.00	3.73	1.39	1.73

Example 16: Biophysical characterization of humanized anti-E-selectin

Thermal stability

[0452] Differential scanning calorimetry was used to determine the stability of humanized

antibody 0841 (comprising HC amino acid sequence of SEQ ID NO:22 and LC amino acid sequence of SEQ ID NO:17). For this analysis, samples at 0.3 mg/mL were dispensed into a sample tray of a MicroCal VP-Capillary DSC with an Autosampler (Malvern Instruments, Inc.), equilibrated for 5 minutes at 10° C and then scanned up to 110°C at a rate of 100°C per hour. A filtering period of 16 seconds was selected. Raw data was baseline corrected and the protein concentration was normalized. Origin Software 7.0 (OriginLab Corporation, Northampton, MA) was used to fit the data to an MN2-State Model with an appropriate number of transitions. Table 15 below shows the melting temperatures (T_{m1} – T_{m4} and FAB) of the molecule in Tris/Suc buffer (20mM Tris, 8.5% sucrose, at pH 7.5) His/Suc buffer (20mM histidine, 8.5% sucrose, 0.005% EDTA, at pH 5.8) and Glu/Tre buffer (20mM glutamic acid, 8.5% trehalose, at pH 4.5). This molecule showed good stability, with the first transition in the CH2 domain (T_{m1}) of greater than 65°C in Tris/Suc and Glu/Tre buffers and just under 65°C in the Glu/Tre buffer.

[0453] Table 15. Thermal transitions of humanized antibody 0841.

formulation	$T_{m1}(^{\circ}\text{C})$	$T_{m2}(^{\circ}\text{C})$	$T_{m3}(^{\circ}\text{C})$	$T_{m4}(^{\circ}\text{C})$	$T_{m\text{ Fab}}(^{\circ}\text{C})$
Tris/Suc	75.04 $\pm$ 0.24	77.99 $\pm$ 0.04	85.31 $\pm$ 0.03	---	77.8
His/Suc	68.65 $\pm$ 0.05	77.48 $\pm$ 0.24	79.44 $\pm$ 0.08	84.65 $\pm$ 0.09	78.9
Glu/Tre	64.78 $\pm$ 0.13	77.44 $\pm$ 0.21	79.71 $\pm$ 0.10	---	79.0

15 **Forced Degradation**

[0454] Humanized antibody 0841 was dialyzed extensively into three buffers: Tris (20 mM Tris at pH 7.5), His (20 mM histidine at pH 5.8) and Glu (20 mM glutamic acid at pH 4.5) for a 4 week forced degradation study at 40°C. After dialysis, sample concentrations were adjusted to 5 mg/mL. At T0, T2 and T4 weeks, samples were taken for analytical size exclusion chromatography (aSEC), imaged capillary electrophoresis (iCE) and bioassay testing.

[0455] The aggregation state of the heated samples was determined by aSEC. Samples were injected onto a YMC-Pack Diol-200 column (300 x 8.0 mm, pore size 200 Å) connected to the Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA) with 20 mM sodium phosphate, 400 mM arginine at pH 7.2 as the mobile phase. An isocratic program at a flow rate of 0.75 mL/minute for 20 minutes was applied to the aSEC column for sample elution and the data was analyzed using the Agilent OpenLAB Data Analysis software to integrate and quantify peak area of aggregate, protein of interest and low molecular weight species. All samples showed minimal change in % HMMS (high molecular mass species) and % LMMS (low molecular mass species), i.e., the percentage was less than 5% (Table 16).

[0456] Table 16. aSEC of a T4-week forced degradation sample of humanized 0841.

	antibody 0841 (HC SEQ ID NO: 22; LC ID SEQ NO: 17)		
formulation	concentration (mg/mL)	% HMMS	% LMMS
Tris pH 7.5	5	3.05	0.48
His pH 5.8	5.2	2.75	0.39
Glu pH 4.5	5.3	2.73	0.84

[0457] iCE was used to detect the charge-based heterogeneity of the forced degradation samples. iCE separates the protein charge species under high voltage and detection at absorbance 280 nm. Carrier ampholytes produce a pH gradient and proteins migrate until their net charge is zero. Electropherograms were analyzed to determine pI values and peak areas for acidic, main, and basic species. Protein Simple iCE3 instrument with PrinCE Autosampler was used to analyze samples. Proteins were diluted to 2 mg/mL in water. Sample diluent contained 0.01 mg/mL pI marker 4.65, 0.01 mg/mL pI marker 9.5, 4.0% Pharmalyte pH 3-10, 0.25% methyl cellulose, and 2.0 M urea. Samples contained 15 µL protein at 2 mg/mL and 85 µL sample diluent. Samples were focused for 1 minute at 1500 Volts and then 6 minutes at 3000 Volts. Empower software was used for data analysis.

[0458] For samples with a high percentage of acidic/basic species in iCE analysis, additional mass spectroscopy analysis was required to identify sequence liabilities on CDR regions. Analysis of samples in the histidine and glutamic acid buffers showed typical increases in acidic and basic species. However, there was a larger increase in basic species than expected for a stable antibody in the Tris buffer, suggesting a possible chemical modification (Table 17). Samples in all three buffers were further analyzed using mass spectroscopy three-part analysis and peptide mapping.

[0459] Table 17. iCE analysis of forced degradation samples of humanized antibody 0841 (HC SEQ ID NO:22 and LC SEQ ID NO:17).

antibody 0841	40° C	pI	% acidic species	% main species	% basic species
Tris, pH 7.5	T0wks	8.8	23.6	72.1	4.3
	T2wks	8.8	37.4	51.9	10.7
	T4wks	8.8	45.3	41.4	13.3

His, pH 5.8	T0wks	8.9	24.3	71.5	4.2
	T2wks	8.9	32.9	60.8	6.3
	T4wks	8.9	40.9	51.7	7.4
Glu, pH 4.5	T0wks	8.8	24.2	71.3	4.4
	T2wks	8.8	35.1	56.0	8.9
	T4wks	8.8	42.0	47.3	10.8

Mass spectroscopy analysis

[0460] For 3-part mass spectroscopy analysis antibodies were digested using FabALACTICA. 50 µg of antibody mAb was added to 100-150 mM NaPO₄ pH 7.0 for a volume of 25 µL. 1.5 µLs of FabALACTICA enzyme (40 U/µL) was added and incubated overnight (16 - 18 hours) at 37° C with gentle shaking. Following this, 50 µL of 8M Guan-HCl, 12.5 µL 1 M DTT and 12.5 µL 150 mM sodium phosphate buffer pH 7.0 was added. This was incubated for 1 hour at 37° C with gentle shaking. 7-8 µg (about 15 µL) were used for 3-part Liquid Chromatography Mass Spectrometry (LC-MS) analysis.

[0461] LC-MS analysis was performed using a Bruker maXis II mass spectrometer (Bruker, Billerica, MA) coupled to a Waters H-Class (Waters, Milford, MA) UPLC. The FabALACTICA digested and reduced samples were separated over a Waters BEH C4 (1.7Å, 2.1 x 150 mm) column maintained at 65° C with a flow rate of 300 µL/min. Mobile phase A was water with 5% acetonitrile and 0.05% TFA, mobile phase B was 50:50 acetonitrile:2-Propanol and 0.05%TFA. The mass spectrometer was run in positive MS only mode and the data were acquired with oTOF control software. The TOF-MS signal was deconvoluted using Maximum Entropy in Compass DataAnalysis v 4.2 (Bruker). Analysis of the forced degradation 0841 samples in glutamic acid, histidine and Tris buffer at time four weeks (T4W) showed similar profiles as compared to samples at time 0 (T0) (**FIG. 2**). PyroE-Fd (Fd fragment from the IgG heavy chain with pyroglutamic acid (PyroE) at the N-termini) showed a slight increase in T4W samples particularly in the Tris buffer (~3.5%). In addition, about 5% Fd D/P clip (Fd fragment with cleaved aspartic acid/proline (D/P) site) was detected in the T0 and T4W samples.

Peptide Mapping by LC-MS

[0462] To further investigate sample stability, peptide mapping LC-MS was utilized. For peptide mapping, denaturation and reduction of antibody 0841 was carried out in 5 M Guan:HCl NaAc, pH 5.0, 10 mM TCEP at 37°C for 1 hour. The reaction mixture was diluted 10 times with 100 mM MES, pH 6.0, 0.5 mM TCEP buffer. Next, LysC/Trypsin mix was added at a 1:10 ratio followed by a 37°C overnight digestion. The reaction was quenched with 0.5% TFA/H₂O (final

concentration). This digestion mixture was then injected onto Thermo Scientific Orbitrap Fusion Lumos for LC-MS analysis

[0463] LC-MS peptide mapping analysis was performed using an Orbitrap Fusion Lumos mass spectrometer (Thermo Scientific, Waltham, MA) coupled to a Waters H-Class (Waters, Milford, MA) UPLC. The digested samples were separated over a Waters BEH C18 (300Å, 1.7µm 2.1 x 150 mM) column maintained at 60°C with a flow rate of 200 µL/min. Mobile phase A was 0.05% TFA in water and mobile phase B was 0.05% TFA in acetonitrile. Peptides were eluted from the column using a gradient: 0.5% to 35% B in 135 minutes. The mass spectrometer was run in positive MS only mode scanning from 300 to 1600 m/z and data was acquired with Xcalibur software. The data analysis and database search were performed using both Xcalibur software (Thermo Fisher Scientific, Waltham MA) and Peaks AB (Bioinformatics Solutions Inc, Toronto, Canada).

[0464] High fidelity peptide mapping analysis of T4W samples for Tris, histidine and glutamic acid buffer identified trace amounts of deamidation (< 1%) in a HC CDR2 peptide with the sequence IDPAN*GNTIYAEK (NG deamidation site is underlined; SEQ ID NO:176). NG is a common deamidation site, but in this case was mostly stable. Potential deamidation (in the form of succinimide-N) was detected in a LC CDR1 peptide with the sequence TSQNIN*RYLNWYQQKPGK (NR deamidation site is underlined; SEQ ID N:177), the deamidation increased to about 5.1% in the Tris T4W sample (MS2 spectrum indicated the N(30)R site was the most likely deamidation site, however, N28 and N34 could also potentially have trace amounts of deamidation) (Table 18).

[0465] **Table 18.** LC/MS peptide mapping of forced degradation samples of humanized antibody 0841.

HC CDR2 peptide	% regular peptide	% deamidation	% succinimide peptide	total deamidated peptide
T0	99.74%	0.07%	0.15%	0.26%
Glu T4W	99.62%	0.18%	0.16%	0.38%
His T4W	99.59%	0.16%	0.17%	0.41%
Tris T4W	99.36%	0.30%	0.15%	0.65%
LC CDR1 peptide	% regular peptide	% deamidation	% succinimide peptide	total deamidated peptide

T0	99.12%	0.00%	0.88%	88.00%
Glut T4W	98.08%	0.00%	1.48%	1.48%
His T4W	95.20%	0.08%	3.95%	4.03%
Tris T4W	93.46%	0.27%	4.85%	5.12%

Competition binding ELISA

[0466] To additionally assess stability of the forced degradation samples, the activity of the stressed samples was evaluated in a competition binding ELISA. Here the stability of the T0, T2W and T4W samples were compared for their ability to compete with biotinylated chimeric antibody 0164 (comprising HC SEQ ID NO:34 and LC SEQ ID NO:31) for binding to human E-selectin. The human E-selectin was stored in PBS with Ca/Mg at 500 µg/mL and was diluted with PBS with Ca/Mg to a concentration of 1 µg/mL. This was added to a 384 well MaxiSorp plate, sealed and incubated at 4°C with gentle shaking overnight. The next day the reagents in each well were removed, followed by addition of 50 µL of blocking buffer (1% BSA in PBS with Ca/Mg) to each well. The wells were sealed and incubated at room temperature for about 1 hour with gentle shaking. At a starting concentration of 0.2 mg/mL, antibody 0841 was serially diluted in steps of 1/3 dilutions with PBS with Ca/Mg for a total of 12 concentrations.

[0467] Biotinylated antibody 0164 was diluted to 0.04 µg/mL in 2% BSA/PBS with Ca/Mg. Equal volumes of diluted antibody 0841 were mixed with biotinylated antibody 0164. Next, the wash plate was blocked with 1% BSA with PBST (0.05% Tween) on a TITERTEK for 3 cycles using 80 µL for each wash. The plate was tapped on a paper towel to drain. Twenty-five µL of the mixture of antibody 0841 and Biotin-labeled antibody 0164 was added to each well of the 384-well plate, in duplicate wells and incubated at room temperature with gentle shaking for 2 – 4 hours. The wash step was repeated with 3 cycles of 2 washes. Then, dilute Streptavidin-HRP (~ 1/7500 diluted in blocking buffer) was added to each well and the plate was incubated at room temperature with gentle shaking ~ 40 minutes to 1 hour. The wash step was again repeated with 3 cycles of 2 washes, tapped dry and followed by the addition of 25 µL of TMB substrate (one component HRP microwell substrate). This was followed by the addition of 25 µL of 0.18 M sulfuric acid to stop the reaction when the color reached saturation (determined by observing the color development). The OD450 measurement of the plate was then read on the Envision plate reader, and the data was analyzed with GraphPad, Prism7.

[0468] The competition binding results are shown in **FIG. 3** and no difference was seen between the T0, T2W and T4W samples in any of the three buffers. These data demonstrate that antibody 0841 retained its ability to bind to E-selectin following storage under high-

temperature conditions (i.e., 40°C).

High concentration stability

[0469] Humanized antibody 0841 was dialyzed extensively into three buffers, Tris/Suc (20 mM Tris, 8.5% sucrose, at pH 7.5), His/Suc (20 mM histidine, 8.5% sucrose, 0.005% EDTA, at pH 5.8) and Glu/Tre (20 mM glutamic acid, 8.5% trehalose, at pH 4.5). After dialysis, the samples were concentrated to 150 mg/mL using a spin filter concentrator and stored at 4°C and 25°C. At T0, T1, T2, T4 and T6 weeks, samples were taken for real time aSEC analysis.

[0470] The aggregation state of the high concentration samples was determined by aSEC as described above for the forced degradation analysis. Samples stored at 4°C and 25°C were stable at high concentration at T6 weeks (T6W) with little increase in percentage of HMMS (<5%) under all three buffer conditions (Table 19).

[0471] **Table 19.** aSEC analysis of high concentration samples of humanized antibody 0841 (HC SEQ ID NO:22, LC SEQ ID NO:17) at T0 and T6 weeks.

	antibody 0841			
formulation	concentration (mg/mL)	% HMMS T0	% HMMS T6W 4°C	% HMMS T6W 25°C
Tris/Suc, pH 7.5	149.6	3.3	3.99	4.66
His/Suc, pH 5.8	157.1	3.2	3.34	3.56
Glu/Tre, pH 4.5	150.5	3.1	3.24	3.37

[0472] At T0, T3 (T3W) and T6 weeks, samples were analyzed by iCE and capillary gel electrophoresis (CGE). iCE was used to detect charge-based heterogeneity of the high concentration samples using a method similar to the method used in the forced degradation study. Minimal changes in the % acidic species and the % basic species were observed over 6 weeks of storage at 25°C in His/Suc and Glu/Tre buffers with larger changes associated with the Tris/Suc buffer in the acidic and basic species (Table 20).

[0473] **Table 20.** iCE analysis of high concentration samples of humanized antibody 0841 (HC SEQ ID NO:22, LC SEQ ID NO:17).

antibody 0841	25°C sampling time	pI	% acidic species	% main species	% basic species
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Tris/Suc, pH 7.5	T0wks	9.0	25.8	69.9	4.3
	T3wks	9.0	32.3	60.7	6.9
	T6wks	9.0	37.6	53.1	9.3
His/Suc, pH 5.8	T0wks	9.0	25.9	69.4	4.7
	T3wks	9.0	27.6	67.2	5.1
	T6wks	9.0	28.7	65.6	5.7
Glu/Tre, pH 4.5	T0wks	9.0	25.6	69.6	4.8
	T3wks	9.0	28.0	65.8	6.2
	T6wks	9.0	30.1	63.1	6.9

[0474] CGE was performed to determine the fragmentation of the high concentration samples of antibody 0841. Samples were analyzed using LabChip Touch HT Protein Express Assay from Perkin Elmer. The assay was set up according to manufacturer instruction and samples were prepared using a modified method. Briefly, 5 μ L of sample at 1 mg/mL was mixed with 35 μ L of sample buffer containing either iodoacetamide for non-reducing (nrCGE), or dithiothreitol for reducing CGE, and incubated at 70° C for 10 minutes. After heating, 70 μ L of de-ionized water was added to each sample prior to analysis on the Touch system. The size and level of fragmentation for each sample was quantified using the LabChip GX software.

[0475] Quantification of the % fragmentation and % HMMS demonstrated that antibody 0841, at a high concentration, was stable in all three buffers (Table 21).

[0476] **Table 21.** CGE analysis of high concentration samples of humanized antibody 0841(HC SEQ ID NO:22, LC SEQ ID NO:17).

% fragmentation (25°C)				
	nrCGE	Tris/Suc pH 7.5	His/Suc pH 5.8	Glu/Tre pH 4.5
antibody 0841	T0wks	0.49	0.87	0.51
	T3wks	0.75	0.3	0.75
	T6wks	1.1	0.31	1.69
% HMMS (25°C)				
	nrCGE	Tris/Suc pH 7.5	His/Suc pH 5.8	Glu/Tre pH 4.5
antibody 0841	T0wks	0.03	0.03	0
	T3wks	0.31	0	0

	T6wks	0	0.38	0
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Example 17: In Silico immunogenicity risk and t-cell epitope prediction of humanized anti-E-selectin.

[0477] Immunogenicity risk of the humanized anti-E-selectin antibody 0841 was predicted using *in silico* tools for prediction of MHCII peptide binding. These tools were used: (1) for epitope identification of potential MHCII binding for each individual peptide in the sequence, (2) for epitope classification, to assess the risk of potential MHCII peptide binders and (3) for overall sequence score, to predict the overall risk of the entire sequence having MHCII binding associated immunogenicity risk. The methods are described below.

10 Epitope Identification

[0478] Sequences were analyzed using two protocols (described below) to identify epitopes. Any sequence flagged by the rules described herein for either protocol was considered an epitope. These methods examine sequences primarily at the level of amino acid 9-mers.

Protocol 1: ISPRI/EpiMatrix

15 [0479] Sequences were submitted for EpiMatrix analysis in the ISPRI software package (ISPRI v 1.8.0, EpiVax Inc., Providence, RI (2017); Schafer JRA et al. (1998) Vaccine 16(19): 1880-84). The raw results provided ranking of likelihood of binding of each 9-mer amino acid fragment against 8 different HLA types. Thus, there were 8 predictions ("observations") for each 9-mer. The 9-mers were generated starting at each individual linear numbering position of the sequence (thus, it was possible for the same 9-mer to occur more than once in the same sequence). If any 4 observations indicated that the 9-mer was in the top 5% of binders (meaning it was predicted to be in the top 5% of binders for at least 4 HLA types), the 9-mer was considered a predicted epitope ("epitope"). Alternatively, if any 1 of the 8 predictions indicated that the 9-mer was in the top 1% of binders, the 9-mer was also considered a predicted epitope.

Protocol 2: IEDB Consensus Method

30 [0480] Sequences were submitted for analysis using the MHC-II binding Consensus method (Wang P. et al. (2010) BMC Bioinformatics 11: 568; Wang P. et al. (2008) PLoS Comput. Biol. 4(4),e1000048) in IEDB (Vita R. et al., Nucleic Acids Res. 2015; 28 (43): D405-12; IEDB MHC-II Binding Predictions, www.iedb.org). The output of the software arranged results by 15-mer. A consensus score and percentile ranking were provided for each combination of 15-mer and HLA type. Individual scores from which each 15-mer's consensus was derived were rankings of certain 9-mers found in the 15-mer: each method used for the consensus reported a percentile

rank for a 9-mer within the 15-mer. The consensus taken as the value for the overall 15-mer was the prediction for the 9-mer having the median score. A 9-mer was classified as an epitope if (a) it was chosen as the consensus representative for the 15-mer AND (b) had a percentile ranking in the top 10% of binders for the HLA type being considered, AND if criteria (a) and (b) occurred for three or more distinct HLA types for the same 9-mer (i.e., three observations). The HLA types considered were DRB1*01, 1*03, 1*04, 1*07, 1*08, 1*11, 1*13, and 1*15, which were the same HLA types in a standard ISPRI/EpiMatrix report. Thus, although the primary output of the method was a ranking of 15-mers, we reinterpreted the data to obtain a list of predicted 9-mer epitopes, for ease of comparison with Protocol 1.

10 ***Epitope Classification***

[0481] Each epitope was classified as a germline or non-germline epitope. For antibodies, we further classified each epitope based on its location within the antibody (CDR or non-CDR). We filtered sequences of human V domains obtained from IMGT (www.imgt.org) to remove germlines annotated as pseudogenes or open reading frames (ORFs). Any predicted 9-mer epitopes found in the remaining sequences were considered a germline epitope. Epitopes found in the J or C regions (including IgG1, IgG2, IgG3, and IgG4), or the junctions between these regions, were also classified as germline epitopes. Otherwise, an epitope was classified as a non-germline epitope. Variable domain residues were numbered based on the numbering system of Kabat (Kabat EA, et al. (1991) US Department of Health and Human Services, NIH Publication No. 91-3242). After numbering, CDRs were defined to include the following residues: CDR-H1 (H26-H35 including insertions such as H35A, up to but not including H36), CDR-H2 (H50-H65 inclusive), CDR-H3 (H95-H102 inclusive) CDR-L1 (L24-L34 inclusive), CDR-L2 (L50-L56 inclusive), CDR-L3 (L89-L97, inclusive). A predicted 9-mer epitope was a CDR epitope if any one of its amino acids was part of a CDR region. Note that our chosen start position (H26) for CDR-H1 differs from some other publications using Kabat annotation.

25 ***Overall Sequence Score (T-reg Adjusted Score)***

[0482] For an individual chain, or for a pairing of an antibody VH and VL domain, an overall score was calculated by summing over each of the constituent 9-mers as follows. All individual combinations of 9-mer and HLA type ("observations") were examined, regardless of whether the 9-mer was an epitope. If a particular observation indicated that the peptide is in the top 5% of binders for the given HLA type, the EpiMatrix Z-score for this observation was added to a running total associated with the entire protein sequence. The total number of observations examined was also recorded. The only exception was that all observations of 9-mers identified by ISPRI as "T-regitopes" were assumed to have EpiMatrix scores of zero. In the running total,

a baseline score of $0.05 * 2.2248$ was subtracted from each observation (including T-regitopes). The final score was computed as follows:

[0483] T-reg Adjusted Score = (Running total) * 1000 / (Number of observations)

[0484] Lower scores indicated lower predicted immunogenic potential. Note that the score only included predictions from ISPRI/EpiMatrix, and did not include information from IEDB. Therefore, any strong HLA binders predicted by IEDB but not ISPRI did not contribute to the score. In theory, sequences may contain many IEDB-predicted HLA binders and still have a favorable T-reg Adjusted Score if EpiMatrix does not also predict the same sequences to be likely binders.

[0485] These tools were used to predict the number of non-germline T-cell epitopes and the overall sequence score in the humanized antibody 0841 (comprising VH amino acid sequence of SEQ ID NO:25 and VL amino acid sequence of SEQ ID NO: 21). Using the two protocols, 8 non-germline epitopes were identified in the VH and VL sequences (H27: YNIRSSYMH (SEQ ID NO:178), H63: FKIRFTISA (SEQ ID NO:179), H65: IRFTISADN (SEQ ID NO:180), L29: INRYLNWYQ (SEQ ID NO:181), L46: LLIYNANSL (SEQ ID NO:182), L47: LIYNANSLQ (SEQ ID NO:183), L48: IYNANSLQT (SEQ ID NO:184), and L49: YNANSLQTG (SEQ ID NO:185) (**FIG. 4**). Additionally, the overall sequence score was -30.34. Reduction in the number of epitopes and the overall sequence score is predicted to decrease the overall immunogenicity risk of the sequence.

Example 18: Optimization of humanized anti-E-selectin antibodies.

Structure-Based Rational Mutagenesis of antibody 0841

[0486] Anti-E-selectin antibody 0841 contained several predicted T-cell epitopes that may increase the risk for immunogenicity when administered to a subject. These included 8 non-germline epitopes contained in the VH and VL sequences (H27: YNIRSSYMH, H63: FKIRFTISA, H65: IRFTISADN, L29: INRYLNWYQ, L46: LLIYNANSL, L47: LIYNANSLQ, L48: IYNANSLQT, and L49: YNANSLQTG). In addition, peptide mapping of the antibody following heat forced degradation identified an NR deamidation liability at the L30 position. In addition to the NR deamidation liability at L30, other potential liability sites were identified including a NG site at heavy chain position H54, a NS site at position L52 and a NS site at position L92. Structure based optimization methods were utilized to remove the predicted T-cell epitopes and sequence liability sites.

[0487] Initially though, a further humanized antibody, 0978 (comprising a HC amino acid sequence of SEQ ID NO:28 and a LC amino acid sequence of SEQ ID NO:26), was generated

that germlines the CDR-L2 to the DPK9 CDR-L2 (AASSLQS (SEQ ID NO:3) and the end of the CDR-H2 to the DP-54 sequence of (H59-H65 YVDSVKG (SEQ ID NO:186)). Antibody 0978 was shown to maintain binding similar to antibody 0841 using the competition binding assay described in Example 16. Germlining these two stretches removed 5 of the predicted T-cell epitopes (H63: FKIRFTISA (SEQ ID NO:179), L46: LLIYNANSL (SEQ ID NO:182), L47: LIYNANSLQ (SEQ ID NO:183), L48: IYNANSLQT (SEQ ID NO:184), and L49: YNANSLQTG (SEQ ID NO:185) but introduced a new predicted T-cell epitope (H63: VKGRFTISA (SEQ ID NO:187)). Additionally, mutation of the L2 removed the potential NS liability site at position L52, but mutation of the end of H2 added a DS site at position H61 that was another potential liability site. Structure based optimization was performed on antibody 0978 using an x-ray crystal structure of antibody 0164 bound to c-type lectin domain of E-selectin (residues 22-178) to remove the remaining 3 predicted T-cell epitopes and the sequence liabilities.

[0488] To determine suitable positions for mutagenesis, computational prediction of mutations for both the changes in affinity and changes in stability were made using Discovery Studio 4.5 (Dassault Systems Biovia Corp.) and FoldX. Tolerated mutations are those that are not predicted to have a $\Delta\Delta G > 1$ kcal/mol for both the Discovery Studio and FoldX methods. From these predictions, a set of residues that would be predicted to have a minimal impact on binding and stability, while removing the predicted T-cell epitopes and sequence liabilities, were identified and shown in Table 22.

[0489] **Table 22.** List of mutations predicted to remove sequence liabilities motifs or predicted T-cell epitopes identified through structure based rational design.

Feature	WT	Mutant
L30 deamidation site motif NR	L30 NR	L30 ER
L92 deamidation site motif NS	L92 NS	L92 NA
H61 isomerization site motif DS	H61 DS	H61 ES
H27 predicted T-cell epitope	H27: YNIRSSYM (SEQ ID NO:178)	H27: YAIRSAYMH (SEQ ID NO:188)
H63 predicted T-cell epitope	H63: VKGRFTISA (SEQ ID NO:187)	H63: VEGRFTISA (SEQ ID NO:189)
	H63: VKGRFTISA (SEQ ID NO:187)	H63: VTGRFTISA (SEQ ID NO:190)
	H63: VKGRFTISA (SEQ ID NO:187)	H63: VKERFTISA (SEQ ID NO:191)

L29 predicted T-cell epitope	L29: INRYLNWYQ (SEQ ID NO:181)	L29: IERYLNWYQ (SEQ ID NO:192)
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- [0490]** Using this set of mutations, 4 optimized constructs were generated which removed the 3 sequence liabilities and the 3 T-cell epitopes identified in Table 22. These constructs are antibody 1282 (comprising HC amino acid sequence of SEQ ID NO:43 and LC amino acid sequence of SEQ ID NO:1) , antibody 1284 (comprising HC amino acid sequence of SEQ ID NO:40 and LC amino acid sequence of SEQ ID NO:1), antibody 1444 (comprising HC amino acid sequence of SEQ ID NO:13 and LC amino acid sequence of SEQ ID NO:1) and antibody 1448 (comprising HC amino acid sequence of SEQ ID NO:37 and LC amino acid sequence of SEQ ID NO:1). Mutations relative to antibody 0978 are shown in Table 23. The binding affinity of these mutants were confirmed to be similar to antibody 0978 using the competition binding assay described in Example 16.

[0491] **Table 23.** List of new sequences optimized to remove t-cell epitopes and sequence liabilities.

antibody	HC mutants	LC mutants
1282	K64E	N30E, S93A
1284	D61E, K64E	N30E, S93A
1444	K64T	N30E, S93A
1448	G64E	N30E, S93A

- Example 19: Neutralization of optimized anti-E-selectin antibodies. (0164, 0841, 1282, 1284, 1444, 1448)**

Neutralization of ligand binding to CHO expressing cell surface E-selectin

- [0492]** In a static ligand cellular adhesion assay, horse radish peroxidase (HRP) conjugated E-selectin ligand (sialyl Lewis antigen) and E-selectin antibodies compete for binding to human or cynomolgus E-selectin expressed on Chinese hamster ovary (CHO) cells. The ability of anti-E-selectin antibodies to neutralize ligand binding was assessed in a static neutralization assay using a competition enzyme-linked immunosorbent assay (ELISA) to evaluate the neutralization of the sialyl Lewis antigen to Chinese hamster ovary cells (CHO) engineered to express human or cynomolgus monkey E-selectin.

- [0493]** CHO cells expressing human E- selectin were grown in culture medium (MEM (Minimum Essential Medium) Alpha Medium, supplemented with 10% (volume/volume) dialyzed fetal bovine serum, 100 nM methotrexate and 100 Units/mL penicillin and 100 µg/mL

streptomycin). CHO cells expressing cynomolgus monkey E-selectin were cultured in R1 medium (RI Dulbecco's Modified Eagle's Medium/Ham's F12 modification) supplemented with 10% heat inactivated fetal bovine serum (GIBCO), 100 Units/mL penicillin and 100 µg/mL streptomycin and 125 µg/mL Zeocin (GIBCO).

- 5 **[0494]** CHO cells expressing E-selectin were seeded at 12,500 cells per well into a 96 well tissue culture plate and incubated at 37° C, 5% CO₂ for 48 hours to form a confluent monolayer. Plates were subsequently washed twice with 200 µL of calcium magnesium free PBS. Anti-E-selectin antibodies or an IgG1 isotype control (IgG control) were added to the assay in the presence of a small synthetic conjugate of biotinylated, sialyl Lewis A polyacrylamide
- 10 (Carbosynth LLC, OS45446) or sialyl Lewis X polyacrylamide (Glycotect, 01-045) with streptavidin/horseradish peroxidase (100 µL total) and incubated at 37°C, 5% CO₂ for 2 hours. Plates were then washed twice with 200 µL of calcium and magnesium free PBS. Following the incubation, the cells were washed twice with 200 µL of wash buffer containing 2 mM of calcium chloride (50 mM TRIS [trisaminomethane], 150 mM sodium chloride; 0.05% polysorbate 20, pH
- 15 7.4; 2 mM calcium chloride). Signal was developed by adding 100 µL of 1-Step Ultra TMB substrate (Thermo Scientific, 34028) and incubating for 30 minutes at room temperature. Reactions were stopped using 2 M of sulfuric acid and absorbance was read at 450 nm (Spectramax M5e). Data were analyzed and graphed using GraphPad Prism software (Version 8.0.2). Estimated half maximal inhibitory concentration (IC₅₀) values derived from the analysis
- 20 ranged from 1.38 to 2.89 nM for human E-selectin and from 1.85 to 2.65 nM for cynomolgus monkey E-selectin (**FIG. 5** and **FIG. 6**) (Table 24).

[0495] **Table 24.** Neutralization of ligand binding to CHO expressing cell surface E-selectin

antibody	static neutralization of sialyl Lewis ligand adhesion	
	CHO-human E-selectin cells IC ₅₀ (nM)	CHO-cynomolgus monkey E-selectin cells IC ₅₀ (nM)
0164	2.69	2.25
0841	2.56	NT
1282	1.38	1.29
1284	2.77	1.91
1444	2.89	2.65
1448	2.78	1.85

NT = Not determined in this assay

[0496] In further static neutralization assays, using methods similar to those described above, antibody 1444 neutralized ligand binding to both human or cynomolgus E-selectin protein expressed by CHO cells with an estimated IC_{50} value of 1.88 nM – 2.33 nM and 1.47 nM – 1.49 nM, respectively.

Neutralization of Ligand Binding to Soluble Recombinant Proteins

[0497] Recombinant histidine-tagged E-selectin; cynomolgus monkey E-selectin (Sino Biologicals; 190169-C08H) and human E-selectin (Sino Biologicals; 13025-H08H) stock solutions were prepared to a final concentration of 3 μ g/mL in phosphate buffered saline (PBS) without calcium and magnesium. One hundred μ L per well of the diluted recombinant E-selectin protein was added into a Ni-NTA HisSorb 96 well plate (Qiagen, 35061) and plates were incubated on an orbital shaker at room temperature for 2 hours. Sialyl Lewis A polyacrylamide biotin, a synthetic E-selectin ligand (GlycoTech corporation 01-044), was conjugated to streptavidin HRP (horse radish peroxidase) (Ultra Streptavidin HRP, Thermo Scientific N504) and diluted in assay diluent containing 2 mM calcium chloride (BD OptEIA buffer; BD Biosciences, 555213).

[0498] Two-fold concentrated stock solutions of antibodies (IgG1 control, 0160, 1282, 1284, 1444 and 1448) were also made in assay diluent containing 2 mM calcium chloride. Following the incubation, the plates were washed 5 times with 200 μ L of wash buffer containing 2 mM of calcium chloride (50 mM TRIS [trisaminomethane]; 150 mM sodium chloride, 0.05% polysorbate 20, pH 7.4; 2 mM of calcium chloride). Following this, 100 μ L of a mixture of antibody (IgG1 control, 0160, 1282, 1284, 1444 and 1448) and HRP conjugated Sialyl Lewis A were added in duplicate and the plates were incubated on an orbital shaker at room temperature for 2 hours. The final antibody concentration ranged from 200 nM to 0.09 nM and the final concentration of HRP conjugated Sialyl Lewis A was 5 μ g/mL. Plates were washed as mentioned before, and 100 μ L per well of 1-Step Ultra TMB substrate (Thermo Scientific, 34028) was added and incubated for 5 minutes at room temperature. Reactions were stopped with 100 μ L of 2M sulfuric acid and absorbance read at 450 nm (Spectramax M5e). Data were analyzed and graphed using GraphPad Prism software (Version 8.0.2). Estimated half-maximal inhibitory concentration (IC_{50}) values derived from the analysis (Table 25).

[0499] The estimated half maximal inhibitory concentration (IC_{50}) values derived from the analysis and ranged 2.96 nM to 3.59 nM for antibody neutralization of ligand binding to soluble recombinant human E-selectin and 2.74 nM to 3.29 nM for antibody neutralization of ligand

binding to soluble recombinant cynomolgus monkey E-selectin.

[0500] Table 25. Neutralization of ligand binding to soluble recombinant E-selectin

antibody	static neutralization of sialyl Lewis ligand adhesion	
	recombinant human E-selectin - IC ₅₀ (nM)	recombinant cynomolgus monkey E-selectin - IC ₅₀ (nM)
0164	3.39	3.29
1282	3.39	3.23
1284	3.59	3.39
1444	3.01	2.91
1448	2.96	2.74

[0501] In further static neutralization assays, using methods similar to those described above, antibody 1444 neutralized ligand binding to both human or cynomolgus E-selectin protein with an estimated IC₅₀ value of 2.87 nM – 2.91 nM and 2.39 nM – 2.45 nM, respectively.

Example 20: Kinetic evaluation of optimized anti-E-selectin using surface plasmon resonance.

[0502] Binding affinities were determined for human and cynomolgus monkey E-selectin for the optimized antibodies 1282, 1284, 1444, and 1448 using SPR in a method similar to Example 4. This was done by first binding the antibody to immobilized anti-human IgG. After antibody capture, dilutions of the E-selectin proteins were flowed over and the association rate constant (ka), disassociation rate constant (kd), t_{1/2}, and K_D values were determined for binding to human and cynomolgus (Table 26). In this SPR analysis, the affinity of 1282, 1284, 1444 and 1448 to human E-selectin was 70.3 nM, 65.2 nM, 61.8 nM, and 60.5 nM, respectively. The affinity of 1282, 1284, 1444 and 1448 to cynomolgus monkey E-selectin was 78.3 nM, 76.5 nM, 81.5 nM, and 67.8 nM, respectively.

[0503] Table 26. Affinity of anti-E-selectin antibodies to human and cynomolgus monkey E-selectin measured by surface plasmon resonance.

antibody (HC SEQ ID NO.; LC	analyte							
	human E-selectin				cynomolgus monkey E-selectin			
	ka	Kd	t _{1/2}	K _D	ka	kd	t _{1/2}	K _D
	(1/Ms)	(1/s)	(s)	(nM)	(1/Ms)	(1/s)	(s)	(nM)

SEQ ID NO.)								
1282 (43;1)	2.01E+05	1.41E-02	49.3	70.3	2.27E+05	1.76E-02	39.4	78.3
1284 (40;1)	2.22E+05	1.45E-02	48	65.2	2.36E+05	1.80E-02	38.5	76.5
1444 (13;1)	2.64E+05	1.63E-02	42.7	61.8	2.11E+05	1.71E-02	40.5	81.5
1448 (37;1)	2.10E+05	1.26E-02	55	60.5	2.22E+05	1.50E-02	46.2	67.8

Example 21: Cell surface binding (FACS) and neutralization of optimized anti-E-selectin antibodies (0164, 0841, 1282, 1284, 1444 and 1448).

Cell Surface Binding by FACS

- 5 [0504] The binding of antibodies 0841, 0164, 1282, 1284, 1444 and 1448 to membrane-bound E-selectin was analyzed by FACS. CHO cells expressing human E-selectin were cultured in Alpha medium (MEM [Minimum Essential Medium] Alpha Medium, 50-012-PC, Corning;) supplemented with dialyzed fetal bovine serum (GIBCO,) at a final concentration of 10% and penicillin/streptomycin (1X) and supplemented with methotrexate. Briefly, the thawed cells were
- 10 initially grown in the presence of 100 nM methotrexate. For subsequent cultures, the methotrexate was omitted from the growth media.

- [0505] Endothelial cells were thawed from frozen stocks and initially plated in T-25 flasks (Corning, 353108) re-coated with sterile 0.1% gelatin solution (Sigma, ES-006-B). Human umbilical vein endothelial cells (HUVEC) were grown in Endothelial Cell Growth Medium
- 15 (Sigma Aldrich, 211-500) while cynomolgus monkey lung microvascular endothelial cells (CLMEC) were grown in Complete Monkey Endothelial Cell Medium (Cell Biologics, MK1168). Cells were split upon reaching 90% confluency and plated into three T-175 flasks precoated with 0.1% gelatin.

- [0506] CHO cell lines expressing human E-selectin were detached from confluent flasks
- 20 using enzyme-free Cell Dissociation Buffer (GIBCO, 13151014). Growth media was immediately added to the detached cells, then centrifuged at 300 x g for 5 minutes at room temperature to pellet the cells. The cells were then resuspended in cold CHO buffer consisting of phosphate buffered saline (PBS), pH 7.4 containing bovine serum albumin (BSA; Sigma-Aldrich, A3059) at final concentration of 1%, calcium chloride at final concentration of 1 mM, and sodium azide at a

final concentration of 0.1%.

[0507] For primary cells, HUVEC or CLMEC, cultures at 90% confluency were serum-starved by replacing the growth media with Endothelial Cell Basal Medium (Sigma-Aldrich, 210-500). Cells were incubated at 37°C, 5% CO₂ and 95% air in a humidified incubator for 2 hours.

5 Immediately after serum starvation, Endothelial Cell Basal Medium containing 10 ng/mL of human tissue necrosis factor-alpha (TNF- α ; GIBCO,) was added to the cells to induce E-selectin expression. Following 4 hours of TNF- α stimulation, the cells were detached using citric saline (135 mM potassium chloride, 15 mM sodium citrate) and immediately recalcified by addition of calcium chloride to 1 mM final concentration. The cells were centrifuged at 300 x g at 4° C and
10 fixed by resuspension in PBS containing 4% paraformaldehyde (PFA; Electron Microscopy Sciences, 15710) in wet ice for 15 minutes. The PFA was removed by centrifugation and the cells resuspended in CHO buffer overnight.

[0508] Binding studies with CHO cells were performed in duplicate using two rows of a 96-well round bottom plate, whereas, binding studies with primary endothelial cells were performed
15 in a single row of a 96-well V-bottom plate. The plates were centrifuged at 300 x g for 2 minutes at 4°C and the cells were resuspended and blocked by incubation in the CHO buffer for 10 minutes on wet ice. After incubation, the cells were centrifuged at 300 x g for 2 minutes at 4° C to remove the buffer. The cell pellet in each well was then resuspended and incubated in the designated volume of serially-diluted antibody (human IgG1 control, 0841, 1282, 1284, 1444
20 and 1448) and allowed to incubate for 30 minutes on wet ice. The test antibody solutions were prepared by three-fold dilution series (1333 nM to 0.0226 nM) using CHO buffer as diluent. After incubation with the antibody, the cells were washed three times by centrifugation at 300 x g for 1 minute at 4° C and resuspended in cold CHO buffer. The washed cells were resuspended and incubated with 100 μ L of 1:1000 dilution of AlexaFluor647-conjugated goat anti-human F(ab')₂
25 Fragment (Fc γ fragment specific antibody, [Jackson ImmunoResearch, 109-606-170] for 45 minutes (CHO cells) or 30 minutes (endothelial cells) on wet ice. The cells were washed three or four times prior to resuspension in CHO buffer for flow cytometric analysis. A Becton Dickinson LSRFortessa instrument using 640 nanometer laser line was used to acquire cell fluorescence data. Geometric mean fluorescence of cells was analyzed by FlowJo software
30 package (FlowJo, LLC) and plotted against antibody concentration. The estimated EC₅₀ values were calculated using nonlinear regression analysis in the Prism suite (GraphPad, version 8).

[0509] Antibodies 0841, 1282, 1284, 1444 and 1448 bound to CHO cells engineered to express human E-selectin with similar estimated EC₅₀ values in the sub to low nM range (Table 27). The control human IgG did not exhibit any binding to the CHO cells expressing human E-

selectin.

- [0510]** Antibodies 0164, 1282, 1284, 1444 and 1448 bound to cell surface human and cynomolgus monkey E-selectin on HUVECs and CLMECs that were treated for 4 hours with TNF- α to induce expression of E-selectin. Compared to the IgG1 isotype control, in which no binding was detected, binding to endogenously expressed E-selectin on the cell surface of TNF- α activated HUVEC or CLMEC was observed. A high fluorescence background was observed and EC₅₀ values were not calculated (Table 27).

[0511] **Table 27.** Cell surface antibody binding by FACS

antibody	binding to CHO-human E- selectin cells EC ₅₀ (nM)	binding to activated HUVEC cells	binding to activated CLMEC cells
0164	NT	binding	binding
0841	0.71	NT	NT
1282	0.85	binding	binding
1284	0.64	binding	binding
1444	0.66	binding	binding
1448	0.75	binding	binding

10 Example 22: Static neutralization of cellular adhesion by optimized anti-E-selectin antibodies

- [0512]** E-selectin binds to sialylated Lewis modified ligands expressed on the surfaces of blood cells. In this study, the human promyelocytic cell line (HL-60) was used as a cellular source for E-selectin ligands. The neutralizing activity of antibodies 0164, 1282, 1284, 1444 and 1448 on the adhesion of HL-60 cells to cell surface E-selectin (on CHO cells) was evaluated in a static neutralization adhesion assay.

- [0513]** CHO cells expressing human E-selectin, were grown in fresh growth media supplemented with 100 nM Methotrexate. After this initial passage of the cells, the methotrexate was omitted from the growth media for subsequent cultures. CHO cells expressing cynomolgus monkey E-selectin were cultured in R1 medium. After this initial passage of cells, the Zeocin was omitted from the growth media for subsequent cultures.

[0514] HL-60 cells at log phase were labeled using carboxyfluorescein diacetate succinimidyl ester fluorescent dye (CFSE) labeling kit (ThermoFisher, 65-0850-84). The labeled HL-60 cells were resuspended in CHO buffer (PBS, pH 7.4/1% bovine serum albumin (BSA)/1

mM calcium chloride (CaCl_2)/0.1% sodium azide (NaN_3)) at density of 400,000 cells/mL for use in the static adhesion assays.

- [0515]** The ability of antibodies 0164, 1282, 1284, 1444 and 1448 to inhibit cellular adhesion of HL-60 cells to E-selectin expressing CHO cells under static condition was evaluated. Briefly, CHO cells were seeded on two rows of a 96-well (technical replicates) flat bottom black plates at a density of 100,000 cells/well and were allowed to form a confluent monolayer (typically after 24 hours) at 37° C, 5% CO_2 and 95% air in a humidified incubator. The cell monolayer was gently washed using CHO buffer then designated wells were incubated with antibody solution (0164, 1282, 1284, 1444 and 1448) in a three-fold dilution series (ranging from 200 $\mu\text{g/mL}$ or 1333 nM to 0.003 $\mu\text{g/mL}$ or 0.022 nM). An appropriate volume of the CFSE-labeled HL-60 cell suspension (400,000 cells/mL) was immediately added to the wells so that the final antibody concentration was maintained (ranging from 200 $\mu\text{g/mL}$ or 1333 nM to 0.003 $\mu\text{g/mL}$ or 0.022 nM). The labeled HL-60 cells were incubated with the CHO cell monolayer for 45 minutes at 37°C at 5% CO_2 and 95% air in a humidified incubator. After incubation, the plate was washed three times with CHO buffer and the fluorescence of the bound HL-60 cells was measured at excitation/emission wavelengths of 494/521 nanometer using Spectramax i3X (Molecular Devices). Fluorescence units were plotted as a function of the antibody concentration and IC_{50} was estimated using nonlinear regression analysis using GraphPad Prism software (Version 8.0.2).
- [0516]** Estimated IC_{50} values for the antibodies were similar and ranged from 3.4 nM to 6.2 nM (**FIG. 7**) (Table 28).

[0517] **Table 28.** Static neutralization of HL-60 cell adhesion.

static neutralization of HL-60 cell adhesion	
antibody	CHO-human E-selectin cells - IC_{50} (nM)
0164	4.4
1282	3.4
1284	4.4
1444	4.7
1448	6.2

- [0518]** In a further static neutralization assay, using methods similar to those described above, antibody 1444 neutralized HL-60 adhesion to both cell surface human and cynomolgus monkey E-selectin with estimated IC_{50} values of 3.36 nM and 3.84 nM, respectively (Table 38).

Example 23: Endogenous human E-selectin binding of optimized anti-E-selectin antibodies.

- [0519]** Analysis of E-selectin binding inhibition in human plasma by optimized anti-E-selectin antibodies 1282, 1284, 1444 and 1448 was performed using an IP-LC/MS/MS method. This method quantified free soluble human E-selectin in human serum or free soluble monkey E-selectin in cynomolgus monkey serum. The method used 50 μ L of serum in 250 μ L of PBS which was incubated overnight at 4° C (in cold room, shaking at 500 rpm) with 1.0 μ g of biotinylated anti-E-selectin antibody 0164. After incubation, 30 μ L of Invitrogen T1 streptavidin magnetic beads were added to each sample followed by incubation for 40 minutes at room temperature (shaking at 1000rpm). Using a Hamilton Microlabstar, the magnetic beads were then washed two times with PBS/0.05% Tween-20 followed by one wash with PBS. Soluble E-selectin was then eluted from the beads with 145 μ L of 30 mM hydrochloric acid (HCl) and neutralized with 32 μ L of 1 M Tris HCl, pH 8. Subsequently 50 fmol of stable isotope labeled (SIL) internal standard was added, followed by the addition of dithiothreitol (DTT, 10 μ L x 50 mM). After incubating 35 minutes at 60°C the samples were allowed to cool to room temperature prior to the addition of iodoacetamide (IAA, 10 μ L x 100 mM). Samples were then placed in the dark for 30 minutes to which 1 μ g of Promega LysC/trypsin (10 μ L x 100 μ g/mL) was added and then digested overnight at 37°C.
- [0520]** After overnight incubation, 50 μ L of prepared sample was injected onto the nano ESILC-MS/MS. Quantification occurred by monitoring tryptic peptides for soluble E-selectin (CSSSLAVLEK (SEQ ID NO:193)). This quantification showed that antibodies 1282, 1284, 1444 and 1448 had similar inhibition of human E-selectin with IC₅₀s of 2.4 nM, 1.1 nM, 1.2 nM and 1.8 nM, respectively, and IC₉₀s of 4.9 nM, 4.8 nM, 6.5 nM and 8.6 nM, respectively.

Example 24: Biophysical characterization of humanized anti-E-selectin.***Thermal stability***

- [0521]** Differential scanning calorimetry was used to determine the stability of the four optimized anti-E-selectin antibodies 1282, 1284, 1444 and 1448. The DSC method similar to the method described in Example 16 was used. Table 29 below shows the melting temperatures (T_{m1} – T_{m3} and FAB) of these molecules in Tris/Sucrose ("Tris/Suc") buffer (20 mM Tris, 8.5% sucrose, at pH 7.5), Histidine/Sucrose ("His/Suc") buffer (20 mM histidine, 8.5% sucrose, 0.005% EDTA, at pH 5.8) and Glutamic Acid/Trehalose ("Glu/Tre") buffer (20 mM glutamic acid, 8.5% trehalose, at pH 4.5). All four molecules demonstrated good stability, with the first transition in the CH2 domain (T_{m1}) of greater than 65 °C in the Tris/Suc, His/Suc and

Glu/Tre buffers. These molecules showed a slight increase in stability in the His/Suc and Glu/Tre buffers, and a slight decrease in stability in the Tris/Suc buffer.

[0522] Table 29. Thermal stability of optimized anti-E-selectin antibodies determined by differential scanning calorimetry.

antibody	formulation	Tm1(°C)	Tm2(°C)	Tm3(°C)	apparent Tm of Fab(°C)
1282	Tris/Suc, pH 7.5	71.41 ± 0.01	77.23 ± 0.04	85.86 ± 0.02	71.7
	His/Suc/EDTA, pH 5.8	71.70 ± 0.02	77.64 ± 0.05	84.42 ± 0.01	72.0
	Glu/Tre, pH 4.5	65.99 ± 0.02	74.81 ± 0.01	82.99 ± 0.02	74.9
1284	Tris/Suc, pH 7.5	71.56 ± 0.01	78.12 ± 0.02	85.52 ± 0.02	71.7
	His/Suc/EDTA, pH 5.8	71.52 ± 0.06	76.09 ± 0.16	84.23 ± 0.02	73.0
	Glu/Tre, pH 4.5	65.95 ± 0.02	75.42 ± 0.01	83.07 ± 0.02	75.5
1444	Tris/Suc, pH 7.5	71.25 ± 0.01	77.78 ± 0.02	85.47 ± 0.02	71.4
	His/Suc/EDTA, pH 5.8	71.69 ± 0.01	78.17 ± 0.02	84.34 ± 0.01	71.8
	Glu/Tre, pH 4.5	65.79 ± 0.01	74.28 ± 0.01	82.88 ± 0.02	74.3
1448	Tris/Suc, pH 7.5	72.06 ± 0.01	77.70 ± 0.02	85.47 ± 0.01	72.2
	His/Suc/EDTA, pH 5.8	73.10 ± 0.10	76.24 ± 1.54	84.34 ± 0.02	73.5
	Glu/Tre, pH 4.5	65.65 ± 0.01	74.62 ± 0.03	76.58 ± 0.02	75.2

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Forced Degradation

[0523] Optimized anti-E-selectin antibodies 1282, 1284, 1444 and 1448 were prepared for the 4 week forced degradation study at 40°C by extensive dialysis into three buffers: Tris (20 mM Tris at pH 7.5), His (20 mM histidine at pH 5.8) and Glu (20 mM glutamic acid at pH 4.5).

10 After dialysis, sample concentrations are adjusted to 5 mg/mL. At T0, T2 and T4 weeks, samples were aliquoted for aSEC, iCE and bioassay testing.

[0524] The aggregation state of the forced degradation samples was determined by aSEC as described in Example 16. All samples showed minimal change, less than 5% LMMS and less than 1% HMMS species at T4 weeks (Table 30).

15 **[0525] Table 30.** aSEC of forced degradation samples of optimized anti-E-selectin antibodies.

Week 4, 40°C	antibody 1282		
	% HMMS	% main	% LMMS
Tris (pH 7.5)	0.38	97.18	2.43

His (pH 5.8)	0.33	97.44	2.23
Glu (pH 4.5)	0.47	95.91	3.62
antibody 1284			
Week 4, 40 °C	% HMMS	% main	% LMMS
Tris (pH 7.5)	0.62	96.38	3.00
His (pH 5.8)	0.34	97.51	2.15
Glu (pH 4.5)	0.51	95.65	3.84
antibody 1444			
Week 4, 40 °C	% HMMS	% main	% LMMS
Tris (pH 7.5)	0.56	95.55	3.89
His (pH 5.8)	0.31	97.70	2.00
Glu (pH 4.5)	0.59	95.63	3.78
antibody 1448			
Week 4, 40 °C	% HMMS	% main	% LMMS
Tris (pH 7.5)	0.68	94.96	4.36
His (pH 5.8)	0.33	97.56	2.11
Glu (pH 4.5)	0.61	95.68	3.72

[0526] Imaged capillary isoelectric focusing (iCE) was used to detect the charge-based heterogeneity of the forced degradation samples. This was done using a method similar to the method used in Example 16. For samples with a high % acidic/basic species in iCE analysis, additional mass spectroscopy analysis was required to identify sequence liabilities in CDR regions. Analysis of the samples in the Tris, His and Glu buffers showed typical increases in the acidic and basic species (Table 31). Antibody 1444 was further analyzed using Mass spectroscopy three-part analysis and peptide mapping for comparison to humanized 0841.

[0527] Table 31. iCE Analysis of forced degradation samples of optimized anti-E-selectin antibodies.

antibody 1282	40 °C	iCE pI	% acidic species	% main	% basic species
Tris, pH 7.5	T0W	7.66	19.7	75.4	5.0
	T2W	7.66	38.8	48.4	12.8
	T4W	7.66	45.6	38.5	15.9
His, pH 5.8	T0W	7.74	18.2	77.7	4.1

	T2W	7.73	25.9	65.2	8.9
	T4W	7.74	33.7	55.9	10.4
Glu, pH 4.5	T0W	7.67	20.2	74.5	5.3
	T2W	7.67	31.5	55.5	13.0
	T4W	7.66	40.6	42.8	16.6
antibody 1284	40°C	iCE pl	% acidic species	% main	% basic species
Tris, pH 7.5	T0W	7.66	22.3	73.4	4.4
	T2W	7.66	36.3	51.7	12.0
	T4W	7.66	49.2	36.0	14.8
His, pH 5.8	T0W	7.74	19.5	76.8	3.7
	T2W	7.75	27.5	65.6	6.9
	T4W	7.74	35.6	55.7	8.8
Glu, pH 4.5	T0W	7.67	21.1	74.3	4.6
	T2W	7.67	32.1	56.6	11.4
	T4W	7.67	41.9	43.8	14.2
antibody 1444	40°C	iCE pl	% acidic species	% main	% basic species
Tris, pH 7.5	T0W	8.02	25.1	71.3	3.6
	T2W	8.02	36.2	52.3	11.5
	T4W	8.02	51.4	34.2	14.4
His, pH 5.8	T0W	8.08	25.7	70.8	3.6
	T2W	8.08	33.2	59.7	7.1
	T4W	8.09	44.6	47.1	8.4
Glu, pH 4.5	T0W	8.03	20.7	75.8	3.6
	T2W	8.03	36.3	52.4	11.3
	T4W	8.03	44.3	40.9	14.8
antibody 1448	40°C	iCE pl	% acidic species	% main	% basic species
Tris, pH 7.5	T0W	8.01	24.5	71.8	3.7
	T2W	8.01	39.1	50.4	10.5
	T4W	8.01	54.0	33.0	13.0
His, pH 5.8	T0W	8.07	25.6	70.7	3.8

Glu, pH 4.5	T2W	8.07	34.8	57.2	8.0
	T4W	8.08	40.0	51.0	9.0
	T0W	8.02	22.3	73.8	3.9
	T2W	8.02	31.6	54.5	13.9
	T4W	8.02	39.9	42.3	17.8

Mass spectroscopy analysis

[0528] 3-part mass spectroscopy analysis was performed on optimized antibody 1444. Analysis of the forced degradation of antibody 1444 at T4W in Glu, His and Tris buffer showed similar profiles as compared to T0. There were low levels of scFc (single chain Fc) oxidation and D/P Clip observed, low levels of oxi_Fd (oxidized Fd) detected, trace Fd+ HexNAc (glycated Fd) detected, trace amount Fd D/P clip (Fd fragment with cleaved D/P site) detected, and low level PyroE Fd (Fd fragment with pyroglutamic acid at the N-termini) observed in stressed samples (**FIG. 8**).

[0529] Peptide mapping was carried out for antibody 1444 using a method similar to the method used in Example 16. High fidelity peptide mapping analysis of T0 and T4 week forced degradation samples in Tris, His or Glu buffer indicated that only trace level isomerization and deamidation was detected in anti-E-selectin antibody 1444 HC CDR 2 peptide IDPANGNTIYVDSVTGR (SEQ ID NO:194), (Table 32). All samples had > 96% regular peptide with less than 1% found as a deamidated peptide and less than 2% found as peptide containing isomerization (IsoD). This optimized variant no longer contains a chemically labile motif at light chain position 30 which was mutated to Glu. For LC CDR1 peptide TSQNIERYLNWYQQKPGK (SEQ ID NO:195), no modification was detected.

[0530] **Table 32.** LC/MS peptide mapping of forced degradation samples of HC CDR2 peptide from optimized anti-E-selectin antibody 1444.

HC CDR2 peptide	% regular peptide	% deamidation peptide	% IsoD peptide
T0	99.00%	0.07%	0.32%
Glu T4W	98.30%	0.18%	0.56%
His T4W	96.60%	0.16%	0.65%
Tris T4W	97.80%	0.30%	1.20%

[0531] To additionally assess the stability of the forced degradation samples, the activity of the stressed samples (antibodies 1282, 1284, 1444, 1448) was evaluated in a competition

binding ELISA. Here the T0, T2W and T4W samples were compared for their ability to compete with biotinylated chimeric antibody 0164 for binding to human E-selectin. This method was similar to the method described in Example 16. The competition binding results are shown in **FIG. 9A-9D** and no difference was seen between the T0, T2W and T4W samples, indicating that the ability of the antibodies to bind to E-selectin was stable following high-temperature incubation.

High Concentration Stability

[0532] Optimized anti-E-selectin antibodies 1282, 1284, 1444 and 1448 were dialyzed extensively into three buffers, Tris/Suc (20 mM Tris, 8.5% sucrose, at pH 7.5), His/Suc (20 mM histidine, 8.5% sucrose, 0.005% EDTA, at pH 5.8) and Glu/Tre (20 mM glutamic acid, 8.5% trehalose, at pH 4.5). After dialysis, the samples were concentrated to 150 mg/mL using spin filter concentrator and store at 4°C and 25°C. At time zero (T0), one week (T1W), two weeks (T2W), four weeks (T4W), six weeks (T6W) and seven weeks (T7W), samples were aliquoted for real time aSEC analysis, with iCE and CGE analysis at T0, T3 and T7 weeks performed at the end of study.

[0533] The aggregation state of the high concentration samples was determined by aSEC as described in Example 16. Samples at 4°C and 25°C showed good high concentration stability at all time points with little increase in HMMS (<5% of the total) in all three buffer conditions (**FIG. 10A-10D**).

[0534] iCE was used to detect the charge-based heterogeneity of the high concentration samples using a method similar to the method described in Example 16. Minimal change in the % acidic and the % basic species was seen in antibodies stored at 25°C in Tris/Suc, His/Suc and Glu/Tre buffers over the 7 week study (Table 33).

[0535] **Table 33.** iCE analysis of 25°C high concentration samples of optimized anti-E-selectin antibodies.

antibody 1282			
buffer/time	% acidic species	% main	% basic species
Tris/Suc T0	17.7	77.5	4.8
Tris/Suc T3W	25.1	67.0	7.9
Tris/Suc T7W	35.1	54.1	10.8
His/Suc T0	13.2	82.7	4.1
His/Suc T3W	15.4	79.1	5.5

His/Suc T7W	18.3	75.6	6.1
Glu/Tre T0	16.9	77.3	5.8
Glu/Tre T3W	18.1	74.6	7.3
Glu/Tre T7W	21.1	69.5	9.5
antibody 1284			
buffer/time	% acidic species	% main	% basic species
Tris/Suc T0	22.4	73.2	4.4
Tris/Suc T3W	29.6	63.3	7.1
Tris/Suc T7W	37.2	53.4	9.4
His/Suc T0	16.5	79.8	3.7
His/Suc T3W	19.3	76.1	4.6
His/Suc T7W	22.4	71.9	5.7
Glu/Tre T0	18.6	77.0	4.5
Glu/Tre T3W	20.0	73.7	6.2
Glu/Tre T7W	22.7	69.9	7.4
antibody 1444			
buffer/time	% acidic species	% main	% basic species
Tris/Suc T0	24.0	72.4	3.6
Tris/Suc T3W	31.9	61.0	7.1
Tris/Suc T7W	39.2	51.1	9.8
His/Suc T0	24.7	71.1	4.3
His/Suc T3W	24.4	70.9	4.7
His/Suc T7W	29.5	64.1	6.5
Glu/Tre T0	24.0	72.4	3.6
Glu/Tre T3W	26.7	67.0	6.4
Glu/Tre T7W	29.7	63.0	7.3
antibody1448			
buffer/time	% acidic species	% main	% basic species
Tris/Suc T0	30.4	65.3	4.4
Tris/Suc T3W	35.1	58.6	6.3

Tris/Suc T7W	43.3	48.0	8.7
His/Suc T0	28.1	67.5	4.4
His/Suc T3W	30.7	63.5	5.8
His/Suc T7W	32.4	61.2	6.3
Glu/Tre T0	27.9	68.4	3.8
Glu/Tre T3W	29.2	63.9	6.9
Glu/Tre T7W	32.8	57.9	9.2

- [0536]** CGE analysis was conducted to determine the fragmentation of the high concentration samples of optimized antibodies 1282, 1284, 1444 and 1448. The analysis was done using a method similar to the method described in Example 16. The quantification of the % fragmentation and % HMMS demonstrated good high concentration stability for antibodies 1282, 1284, 1444 and 1448 in all three buffers at 25°C (Table 34).

[0537] **Table 34.** CGE analysis of 25°C high concentration samples of optimized anti-E-selectin antibodies.

antibody 1282 buffer/time	sum of % HLC	% non HLC	% non HLC	
			% fragment (LMMS)	% other (HMMS)
Tris/Suc, T0	99.89	0.11	0.07	0.04
Tris/Suc, T3W	99.88	0.12	0.05	0.07
Tris/Suc, T7W	99.86	0.14	0.07	0.07
His/Suc, T0	99.92	0.08	0.04	0.04
His/Suc, T3W	99.95	0.05	0.04	0.01
His/Suc, T7W	99.90	0.10	0.07	0.03
Glu/Tre, T0	99.96	0.04	0.00	0.04
Glu/Tre, T3W	99.84	0.16	0.11	0.05
Glu/Tre, T7W	99.77	0.23	0.20	0.03

antibody 1284 buffer/time	sum of % HLC	% non HLC	% non HLC	
			% fragment (LMMS)	% other (HMMS)
Tris/Suc, T0	99.99	0.01	0.00	0.01
Tris/Suc, T3W	99.79	0.21	0.10	0.11

Tris/Suc, T7W	99.79	0.21	0.11	0.10
His/Suc, T0	99.92	0.08	0.05	0.03
His/Suc, T3W	99.90	0.10	0.05	0.05
His/Suc, T7W	99.75	0.25	0.19	0.06
Glu/Tre, T0	99.86	0.14	0.09	0.05
Glu/Tre, T3W	99.84	0.16	0.11	0.05
Glu/Tre, T7W	99.76	0.24	0.19	0.05
% non HLC				
antibody 1444 buffer/time	sum of % HLC	% non HLC	% fragment (LMMS)	% other (HMMS)
Tris/Suc, T0	99.68	0.32	0.19	0.13
Tris/Suc, T3W	99.79	0.21	0.07	0.14
Tris/Suc, T7W	99.03	0.97	0.66	0.31
His/Suc, T0	99.70	0.30	0.20	0.10
His/Suc, T3W	99.85	0.15	0.05	0.10
His/Suc, T7W	99.43	0.57	0.42	0.15
Glu/Tre, T0	99.76	0.24	0.12	0.12
Glu/Tre, T3W	99.57	0.43	0.32	0.11
Glu/Tre, T7W	99.31	0.69	0.59	0.10
% non HLC				
antibody 1448 buffer/time	sum of % HLC	% non HLC	% fragment (LMMS)	% other (HMMS)
Tris/Suc, T0	99.74	0.26	0.14	0.12
Tris/Suc, T3W	99.52	0.48	0.20	0.28
Tris/Suc, T7W	99.28	0.72	0.35	0.37
His/Suc, T0	99.62	0.38	0.27	0.11
His/Suc, T3W	99.29	0.71	0.56	0.15
His/Suc, T7W	99.25	0.75	0.57	0.18
Glu/Tre, T0	99.59	0.41	0.27	0.14
Glu/Tre, T3W	99.18	0.82	0.68	0.14
Glu/Tre, T7W	99.23	0.77	0.65	0.12

Viscosity

[0538] Viscosity of the optimized antibodies 1282, 1284, 1444 and 1448 were measured using the following DLS (dynamic light-scattering) bead-based method. Proteins in PBS were extensively dialyzed against 20 mM histidine, 85 mg/mL sucrose, 0.05 mg/mL EDTA, pH 6.0 using membrane cassette devices 10K MWCO (Thermo Scientific). Proteins were harvested from dialysis and filtered using a 0.2 µM syringe filter. Proteins were concentrated using Vivaspin centrifugal concentrators 10K MWCO (GE Healthcare). Sample aliquots (12 µL) were removed from the concentrator retentate as the protein volume was reduced and the protein concentration increased. 300 nm beads (Nanosphere, Thermo Scientific) were added to the protein samples and buffer blank. The beads were diluted 1:10 in 20 mM histidine, 85 mg/mL sucrose, 0.05 mg/mL EDTA, pH 6.0 and 0.75 µL diluted beads were spiked into the protein sample. The protein/bead and buffer/bead samples were mixed by gently vortexing. Eight µL of sample was transferred to 1536 well plate (SensioPlate, glass bottom, Greiner Bio-One) for analysis by DLS. The plate was sealed with optically clear tape and centrifuged at 2000 RPM for 2 minutes to remove bubbles.

[0539] The DLS measurements were made using a DynaPro Plate Reader (Wyatt Technology, Santa Barbara, Calif.). Samples were incubated at 25°C and measured with 15 consecutive 25 second acquisitions. Radius of the bead was averaged for data acquisitions that had acceptable decay curves. The viscosity was calculated based on the Stokes-Einstein equation. Sample viscosity was calculated as the measured apparent radius divided by the nominal bead radius times 0.893 centipoise (cP), the viscosity of water at 25°C.

[0540] High concentration formulation is a desirable property for antibodies, allowing higher and less frequent dosing. Optimized antibodies all showed good viscosity profiles with their viscosity not reaching 20 cP until approximately 160 mg/mL. To confirm this measurement, an additional viscosity measurement at a single concentration for each optimized antibody was done using the Anton Paar method described below.

[0541] Protein samples were concentrated to a target of 170-180 mg/mL using 50 kDa molecular weight cut-off Amicon centrifugal filter units (EMD Millipore, Billerica, MA). Protein concentrations were determined by 280 nm analysis on the SoloVPE Variable Pathlength System (C Technologies, Inc, Bridgewater, NJ). Viscosity measurements were performed using the CP25-1 cone and plate on the MCR-302 rheometer (Anton Paar USA Inc., Ashland, VA) at a constant rotational speed of 150 rpm at 25°C. A total of 10 measurements of 10 seconds each were collected per sample and the data was analyzed using the Rheoplus (Anton Paar USA Inc.) V 3.62 software. The viscosity of 1282 was measured to be 36.29 cP at 179.5 mg/mL.

The viscosity of 1284 was measured to be 27.38 cP at 171.9 mg/mL. The viscosity of 1444 was measured to be 33.43 cP at 185.7 mg/mL. The viscosity of 1448 was measured to be 33.35 cP at 186.8 mg/mL. All of these measurements fit well to the curve fit of the DLS viscosity measurements, reinforcing their accuracy (**FIG. 11**).

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Example 25: *In silico* immunogenicity risk and T-cell epitope prediction of optimized anti-E-selectin.

[0542] The immunogenicity risk of optimized anti-E-selectin antibodies 1282, 1284, 1444 and 1448 was predicted using *in silico* tools to predict MHCII peptide binding. The method used was similar to the method described in Example 17. The optimized antibodies showed improved overall sequence score decreasing from -30.34 for humanized antibody 0841 to scores of -46.26 for antibody 1282, -46.16 for antibody 1284, -45.11 for antibody 1444 and -45.32 for antibody 1448. Additionally, 8 predicted non-germline T-cell epitopes were removed in the optimized antibodies (Table 35).

[0543] **Table 35.** *In silico* immunogenicity analysis of optimized anti-E-selectin antibodies.

antibody	VH Seq ID NO	VL Seq ID NO	overall sequence score	epitopes			
				all	non- germline	CDR	non-germline CDR
0841	25	21	-30.34	15	8	9	8
1282	45	5	-46.26	10	0	4	0
1284	42	5	-46.16	10	0	4	0
1444	11	5	-45.11	10	0	4	0
1448	39	5	-45.32	10	0	4	0

Example 26: Self-interaction and polyreactivity of optimized anti-E-selectin antibodies.

[0544] The optimized antibodies 1444 (comprising HC amino acid sequence of SEQ ID NO:13 and LC amino acid sequence of SEQ ID NO:1), 1282 (comprising HC amino acid sequence of SEQ ID NO:43 and LC amino acid sequence of SEQ ID NO:1), 1284 (comprising HC amino acid sequence of SEQ ID NO:40 and LC amino acid sequence of SEQ ID NO:1) and 1448 (comprising HC amino acid sequence of SEQ ID NO:37 and LC amino acid sequence of SEQ ID NO:1) were tested for binding to DNA and insulin, and in addition were tested for self-interaction in an AC-SINS assay (affinity-capture self-interaction nanoparticle spectroscopy; Liu et al. (2014) mAbs 6:483-92). In the AC-SINS assay, mAbs captured on gold nanospheres induce a shift in the absorbance maximum if they bind to one another and thereby cause the

25

beads to cluster. High scores in this assay have been suggested to correlate with low or poor solubility and nonspecific membrane interactions (Liu et al., mAbs 2014; 6:483-92). Antibodies 1282, 1284, 1444 and 1448 had very low AC-SINS scores and DNA/Insulin scores, comparable to those of the negative control (Table 36). These data indicate that these antibodies are at low risk for polyreactivity, similar to that of the negative control.

[0545] Table 36. Non-specific binding and self-interaction of E-selectin antibodies.

antibody	AC-SINS (wavelength of maximum absorbance relative to blank, nm)	DNA binding normalized to blank	insulin binding normalized to blank
1282	1	2	1
1284	1	2	2
1444	1	3	2
1448	1	2	2
Polyreactivity negative control	1	2	4
Polyreactivity positive control (MJ4-2 v1.1/P33)	22	34	36

Example 27: Cross-reactivity evaluation of optimized anti-E-selectin using surface plasmon resonance.

[0546] Binding affinities were determined for human and cynomolgus monkey E-selectin for the optimized antibody 1444 (comprising HC amino acid sequence of SEQ ID NO:13 and LC amino acid sequence of SEQ ID NO:1) using SPR as described in Example 20. Using a similar method with a high analyte concentration of 405 nM, we also evaluated the binding affinity of this antibody to homologs of rat E-selectin, mouse E-selectin, rabbit E-selectin, human L-selectin and human P-selectin using a method similar to the method described in Example 5. At 25°C no binding of antibody 1444 to rat E-selectin, mouse E-selectin, rabbit E-selectin, human L-selectin and human P-selectin was observed up to 405 nM (**FIG. 12**). Antibody 1444 bound to human E-selectin with a K_D of 68.35 +/- 3.18 nM and bound to cynomolgus monkey E-selectin with a K_D of 64.9 +/- 1.13 nM.

Example 28: Plate based direct binding ELISA analysis of antibodies 1282, 1284, 1444

and 1448.

- [0547]** Direct binding ELISAs were used to characterize the binding of antibodies 0841, 1282, 1284, 1444 and 1448 to various immobilized soluble recombinant selectins. Recombinant histidine tagged human P-, E- or L-selectin (Sino Biologicals), mouse E-selectin (Sino Biologicals), rat E-selectin (Sino Biologicals) and Cynomolgus monkey E-selectin (Sino Biologicals) stock solutions were prepared to a final concentration of 1 µg/mL in phosphate buffered saline without calcium and magnesium. Controls included an L-selectin directed antibody (BioLegend; 304811) or P-selectin directed antibody or a negative control IgG. One hundred µL per well of the diluted recombinant selectin proteins were added into a Ni-NTA HisSorb 96 well plate (Qiagen; 35061). Plates were incubated on an orbital shaker at room temperature for 2 hours. Following the incubation, the plates were washed 3 times with 200 µL of wash buffer (50 mM TRIS [trisaminomethane]; 150 mM sodium chloride; 0.05% polysorbate 20, pH 7.4). To decrease nonspecific binding, plates were treated with 200 µL of blocking buffer (BD OptEIA buffer; 555213; BD Biosciences), incubated on an orbital shaker for 1 hour at room temperature and washed as described above. Antibodies (IgG1 control antibody, anti-E-selectin antibodies, anti-P-selectin antibody or anti L-selectin antibody) were added to the reaction at 100 µL per well in duplicate with a four-fold dilution series (ranging from 133.33 nM to 0.004 nM made in BD OptEIA buffer), incubated for 1 hour on an orbital shaker and washed as described above. One hundred µL specific detection antibodies (described below) were added and incubated for 1 hour:
- IgG1 control, 0841, 1282, 1284, 1444, 1448, anti-mouse-E-selectin and anti P-selectin antibodies were detected using 1 to 25000 or 1 to 50000 dilution of Peroxidase-conjugated Goat anti-human IgG Fc gamma (Jackson ImmunoResearch Lab; 109-035-008);
 - Anti L-selectin and anti-rat-E-selectin antibodies were detected using 1 to 25000 dilution of Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (H+L); (Jackson ImmunoResearch Lab;).

- [0548]** Plates were washed and 100 µL per well of 1-Step Ultra TMB substrate (Thermo Scientific,) was added and incubated for 5 minutes at room temperature. Reactions were stopped with 100 µL of 2 M sulfuric acid and absorbance read at 450 nm (Spectramax M5e). Data was analyzed and graphed using GraphPad Prism software (8.0.2). Estimated half-maximal concentration (EC₅₀) values derived from the analysis.

[0549] Dose-dependent binding to immobilized human L-selectin or human P-selectin was observed with L-, or P-selectin directed antibodies, with estimated EC₅₀ values of 0.362 nM and

0.081 nM, respectively. In contrast, no cross reactivity of 0841, 1282, 1284, 1444 or 1448 to soluble human L- or P-selectin protein was observed (**FIG. 13**). These studies demonstrate that the antibodies (e.g., 1444) bind to E-selectin selectively with no observed binding to human L- or P-selectin.

- 5 **[0550]** Dose-dependent binding to mouse E-selectin and rat E-selectin was observed with control rat or mouse anti-E-selectin antibodies with low to sub-nanomolar binding (0.153 nM and 0.0139 nM, respectively). In contrast, weak binding of 0841, 1282, 1284, 1444 or 1448 to mouse and rat E-selectin was observed, and the binding curves were right shifted as compared to the positive control antibodies. The binding curves indicated weaker, non-saturable or
- 10 variable binding compared to control rat or mouse E-selectin directed antibodies (Table 37).

[0551] **Table 37.** Antibody cross reactivity to soluble recombinant E- selectin proteins.

antibody	human E-selectin EC ₅₀ (nM)	cynomolgus monkey E-selectin EC ₅₀ (nM)	mouse E-selectin EC ₅₀ (nM)	rat E-selectin EC ₅₀ (nM)
0841	0.17	0.081	Weak, non-saturable	Weak, variable
1282	NT	NT	Weak, non-saturable	Weak, variable
1284	0.10	0.082	Weak, non-saturable	Weak, variable
1444	0.12	0.093	Weak, non-saturable	Weak, variable
1448	NT	NT	Weak, non-saturable	Weak, variable
NT = Not determined				

- [0552]** In a further direct binding ELISA, using methods similar to those described above, antibody 1444 bound to immobilized soluble recombinant human E-selectin with an EC₅₀ of
- 15 0.085 and to immobilized soluble recombinant cynomolgus monkey E-selectin with an EC₅₀ of 0.071 to 0.075 nM (Table 38).

Example 29: Pharmacokinetics of optimized antibody 1444 in monkey following

intravenous or subcutaneous administration.

[0553] A pharmacokinetics study of optimized antibody 1444 was conducted in cynomolgus monkeys using intravenous and subcutaneous administration. The quantification of serum antibody 1444 (comprising HC amino acid sequence of SEQ ID NO:7 (which includes a terminal lysine) and LC amino acid sequence of SEQ ID NO:1) concentrations were determined using an immunoassay on the Meso-Scale Discovery® (MSD) assay platform. In the assay, to quantify anti-E-selectin antibody 1444, biotinylated goat anti-human immunoglobulin G (IgG) was bound onto a blocked streptavidin-coated small spot plate. Anti-E-selectin antibody 1444 was captured by the ligand, recombinant human soluble E-selectin. Bound anti-E-selectin antibody 1444 was detected with a ruthenylated goat anti-human IgG. Plates were read on the MSD SECTOR Imager 6000, and final detection was conducted by using the ruthenium labeled goat anti-human IgG antibody and Tripropylamine (TPA) to produce an electrochemiluminescent signal within the MSD instrument that is representative of the amount of bound anti-E-selectin antibody 1444. Sample concentrations were determined by interpolation from a standard curve that is fit using a 5-parameter logistic equation (weighting formula for standard curve is $1/y^2$). The range of quantitation in 100% serum was 40.0 ng/mL to 2560 ng/mL.

[0554] Following intravenous (IV) administration at 0.3, 0.6, 1, 3 or 10 mg/kg to two animals each, antibody 1444 exhibited a mean systemic clearance calculated from time 0 to the last measurable concentration ($CL_{0-t_{last}}$) of 0.39, 0.31, 0.15, 0.18 and 0.18 mL/h/kg, respectively, and an apparent volume of distribution at steady state ($V_{ss(0-t_{last})}$) of 25, 22, 27, 35 and 36 mL/kg, respectively. Antibody 1444 displayed dose dependent pharmacokinetics, the clearance decreased as the dose increased indicating a target-mediated drug disposition. The mean half-life ($t_{1/2}$) for the IV administration at 0.3, 0.6, 1, 3 or 10 mg/kg was 102, 264, 188, 287 and 345 hours respectively.

[0555] After subcutaneous (SC) administration at 1 or 3 mg/kg to two animals each, antibody 1444 had a mean bioavailability (%F) of 50% following SC administration at 1 mg/kg when comparing to the IV administration of the antibody at 1 mg/kg. Following SC administration of antibody 1444 at 3 mg/kg, antibody 1444 had a mean %F of 65% when compared to IV administration of antibody 1444 at 3 mg/kg. The mean half-life ($t_{1/2}$) for SC administration of antibody 1444 at 1 and 3 mg/kg was 243 and 518 hours, respectively.

[0556] A ligand binding assay was validated to detect the presence of anti-drug antibodies (ADA) in cynomolgus monkey serum on the Meso Scale Discovery platform. In this method, positive controls at 2 different concentrations, consisting of a mouse monoclonal anti-antibody 1444 antibody spiked into cynomolgus monkey serum, and a negative control, consisting of

pooled normal cynomolgus monkey serum, were included on each plate to monitor assay performance. The overall incidence of ADA induction to antibody 1444 was 0% (0/18 animals) across all dose groups.

5 **Example 30: *Ex vivo* neutralization of cellular adhesion by optimized antibody 1444 in physiological flow assays**

[0557] *Ex vivo* cellular adhesion experiments were performed inside microfluidic channels coated with recombinant proteins or cell monolayers to evaluate the potency and the neutralization activity of antibody 1444 to inhibit adhesion of cells that express E-selectin
10 ligands. Cellular adhesion was analyzed under physiological flow conditions using a BioFlux™ (S/N-008-0180-08) system (Fluxion, San Francisco, CA, USA). Adhesion assays were performed using recombinant purified soluble E- or P-selectin proteins or Chinese Hamster Ovary (CHO) cells engineered to express E-selectin (human or cynomolgus monkey) on the cell surface. Human HL-60 cells, which express the E-selectin ligand, PSGL-1 and other sialyl
15 Lewis X ligands, purified human neutrophils, which express E-selectin ligands, or whole blood obtained from patients with sickle cell disease were used to test adhesion to recombinant E- and P-selectin proteins and CHO cells that expressed E-selectin.

[0558] BioFlux™ plates/chambers (Fluxion Biosciences, 48-well low shear, 0-20 dynes/cm²; 910-0004) were used in these studies. CHO cells expressing human E-selectin (human CHO-E, Pfizer) or cynomolgus monkey E-selectin (cynomolgus monkey CHO-E, Pfizer) and HL-60
20 cells were maintained at 37°C with 5% CO₂ and 95% humidity in their respective growth medium. In studies using HL-60 cells, or purified human neutrophils, the cells were centrifuged at 1000 rpm for 5 minutes, stained with Calcein AM (Calcein; Life Technologies™; C3099) for 30 minutes, washed twice with phosphate buffered saline (PBS) with calcium and magnesium
25 (Gibco; Catalog number 14040-133) and resuspended to a concentration of 3 x 10⁶ cells/mL in PBS prior to perfusion in the flow chambers. The cells were maintained at 37°C prior to perfusion and BioFlux™ assays were performed at room temperature. Adherent cells were visualized and counted using a Nikon Eclipse Ti-S (NIS-Elements BR 3.2 64-bit) inverted-stage phase-contrast fluorescent microscope with 40X magnification. Cellular counts were normalized
30 to phosphate buffer vehicle control. Each experiment had two wells per condition. Data were graphed and analyzed using GraphPad Prism (8.0.2). Estimated half-maximal inhibitory concentration (IC₅₀) values derived from the analysis were rounded to two significant digits from the decimal point. The estimated IC₅₀ values were calculated by normalizing data to vehicle control (=100%, with no inhibition).

Neutralization of Cellular Adhesion to Recombinant E-selectin

[0559] First, to mimic the surface of an activated endothelial cell, the flow chambers were coated with equal amounts of both human E- and P-selectin soluble recombinant proteins. BioFlux™ flow microchannels were coated with 20 µg/mL each of soluble recombinant human P-selectin and E-selectin (Sino Biologicals) prepared in PBS, perfused at 1 dyne/cm² for 5 minutes, incubated at room temperature for 1 hour and washed with 0.1% bovine serum albumin (BSA)/PBS for 3-5 minutes at 1 dyne/cm². The plates were treated with test articles including antibody 1444 (concentrations from 0.78 to 200 nM), isotype negative control antibody (200 nM), positive control anti-E selectin antibody (200 nM) in PBS and the vehicle control for 1 hour at 1 dyne/cm². An aliquot of 100 µL of HL-60 cells were perfused in each channel for 10 minutes at physiological shear flow of 1 dyne/cm². PBS was perfused for 2-3 minutes at 1 dyne/cm² to remove the non-adherent cells and analyzed as described.

[0560] Antibody 1444 (0.78 to 200 nM), isotype negative control antibody, or vehicle were perfused in the chambers to inhibit E-selectin binding. In these studies, HL-60 cells, which express the E-selectin ligand, PSGL-1 and other sialyl Lewis X ligands, were flowed at 1 dyne/cm. Antibody 1444 reduced HL-60 cellular adhesion to purified soluble human E-selectin protein, with an estimated IC₅₀ value of 15.26 nM. The isotype negative control had no effect in the assay. The positive control anti-E selectin antibody reduced HL-60 adhesion by about 43%.

[0561] In further flow adhesion neutralization assays, using methods similar to those described above, antibody 1444 neutralized HL-60 adhesion to recombinant human E-selectin protein with an estimated IC₅₀ value of 13.28 nM – 15.94 nM (Table 38).

Neutralization of Cellular Adhesion to Chinese Hamster Ovary Cells Expressing E-selectin

[0562] The BioFlux™ plates were coated with 100 µg/mL of fibronectin (Advanced BioMatrix, Catalog number 5050-1MG) in PBS at a flow rate of 1 dyne/cm² and incubated for 1 hour at room temperature. Chinese Hamster Ovary cells expressing E-selectin (human or cynomolgus monkey CHO-E) cells were prepared for assay by treatment with a 1:1 ratio of 0.05% Trypsin- Ethylenediaminetetraacetic acid (EDTA), (Gibco, 25300054) and StemPro Accutase (Gibco; A1110501) for 5 minutes at 37°C, neutralized with an equal volume of growth medium, collected, centrifuged at 1000 rpm for 5 minutes and re-suspended to a final concentration of 60 x 10⁶ cells/mL in their respective growth medium. The plates were perfused twice with 250 µL of CHO growth medium at 1 dyne/cm² for 2-3 minutes at room temperature, all medium was removed and 10-20 µL of CHO-E cells (at 60 x 10⁶ cells/mL) were perfused for 15 seconds at 2-3 dyne/cm². The plates were removed from the instrument and were incubated

at 37°C in a 5% CO₂ incubator for 1 hour. Medium was removed and 500 µL of fresh growth medium was added and the cells were incubated at 37°C in a 5% CO₂ incubator overnight. Following the incubation, the CHO-E monolayers were washed (3-4 dynes/cm²) on the BioFlux instrument with fresh growth medium. The plates were treated with test articles including
5 antibody 1444 (concentrations from 0.78 to 200 nM), isotype negative control antibody (200 nM), positive control commercial anti-E selectin antibody (200 nM) in PBS and vehicle control by perfusion at 1 dyne/cm² for 1 hour. An aliquot of 100 µL of HL-60 cells was perfused in each channel for 10 minutes at physiological shear flow of 1 dyne/cm². PBS was perfused for 2-3 minutes at 1 dyne/cm² to remove the non-adherent cells and the plates were imaged and
10 analyzed as described.

[0563] E-selectin is expressed on the endothelial cell surface. CHO cells that express E-selectin (human or cynomolgus monkey) were attached to the surface of the flow chambers to form a cellular monolayer. Antibody 1444 was perfused in the chambers (0.78 to 200 nM) and HL-60 cells were perfused under physiological flow at 1 dyne/cm². Negative controls included
15 isotype negative control antibody and vehicle. Anti-E-selectin antibody 1444, neutralized cellular adhesion of HL-60 cells to human CHO-E-selectin cells with an estimated IC₅₀ value of 4.41 nM. In addition, the estimated IC₅₀ value for the neutralization of HL-60 to cells expressing cynomolgus monkey E-selectin was 4.34 nM. The isotype negative control antibody did not neutralize HL-60 binding to CHO cells expressing either human or cynomolgus monkey E-
20 selectin. The positive control antibody testing at 200 nM reduced HL-60 adhesion to cells expressing human E-selectin by about 44%

[0564] In further flow adhesion neutralization assays, using methods similar to those described above, antibody 1444 neutralized HL-60 adhesion to human or cynomolgus monkey E-selectin expressing CHO cells with an estimated IC₅₀ value of 4.25 nM – 4.56 nM and 4.32
25 nM – 4.35 nM, respectively (Table 38).

Neutralization of Purified Neutrophils Adhesion Chinese Hamster Ovary Cells Expressing E-selectin

[0565] Human and cynomolgus monkey CHO cell monolayers were prepared on the Bioflux plates as described. Whole blood was obtained from four healthy volunteer donors (Pfizer) and
30 3 mL of each were pooled and neutrophils were purified using Miltenyi Kit (MACSxpress whole blood neutrophil isolation kit, human, 130-104-434) according to the manufacturer's protocol.

[0566] Test articles including antibody 1444 (concentrations from 0.78 to 200 nM), isotype negative control antibody (200 nM), positive control anti-E selectin antibody (200 nM) in PBS and the vehicle control were perfused at 1 dyne/cm² for 1 hour. The purified neutrophils were

stained with Calcein and treated with tumor necrosis factor-alpha (TNF- α) (Biotang Inc, 50-751-5681) for 10 minutes at 37° C, prior to assay. The neutrophils were collected, 300 x g for 10 minutes at room temperature and resuspended in PBS (with calcium and magnesium). The neutrophils were perfused at 1 dyne/cm² for 10 minutes. PBS was perfused for 2-3 minutes at 1 dyne/cm² to remove the non-adherent cells and the plates were imaged and analyzed.

[0567] Neutrophils express E-selectin ligands and E-selectin mediates neutrophil adherence to the endothelium. Anti-E-selectin antibody neutralization of human neutrophils to CHO monolayers that express E-selectin (human or cynomolgus monkey) under *ex vivo* physiological flow was assessed. Antibody 1444, isotype negative control antibody or vehicle were perfused into the chambers. Human neutrophils were purified from 4 healthy donors, pooled and TNF- α activated (10 ng/mL) neutrophils were evaluated in the assay. Antibody 1444 neutralized the adhesion of activated human neutrophils to human CHO-E-selectin cells with estimated IC₅₀ values of 2.87 nM. The estimated IC₅₀ for neutralization of adhesion of activated human neutrophils to cynomolgus monkey CHO-E-selectin cells was 16.33 nM.

[0568] In a further flow adhesion neutralization assay, using methods similar to those described above, antibody 1444 neutralized adhesion of activated human neutrophils to human or cynomolgus monkey E-selectin expressing CHO cells with an estimated IC₅₀ value of 4.65 nM and 9.45 nM, respectively (Table 38).

Neutralization of SCD Blood Cell Adhesion

[0569] A pro-inflammatory response in SCD is promoted by alterations in the sickled red blood cell and the activated endothelial cell (Manwani D and Frenette PS Blood 2013; 122(24):3892-98). Red blood cell sickling, hemolysis, and endothelial cell dysfunction lead to inflammation and cellular activation and contribute to SCD pathogenesis. Whole blood from 2 donors with SCD, were labeled with Calcein and Hoechst to visualize rolling and adhesion. Briefly, the blood was assayed 24 hours after arrival and diluted to a hematocrit of 15% with HBSS (Hank's Balanced Salt Solution) buffer containing 1 mM calcium chloride, 1 mM magnesium chloride, 10 mM N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid, 0.25% BSA, 0.1% glucose, pH 7.4. Blood cells were stained with Calcein and Hoechst (Thermo Scientific, catalog number 33342) dye (1:1000) for 30 minutes at 37°C prior to assay. The flow chambers were coated with 20 μ g/mL each of soluble recombinant human E- and P-selectin protein (Sino Biologicals) prepared in PBS at 1 dyne/cm² for 5 minutes, incubated at room temperature for 1-2 hours and washed with 0.1% BSA/PBS for 3-5 minutes at 1 dyne/cm².

[0570] Blood from both donors was evaluated for adhesion to recombinant human E- and P-selectin proteins. Test articles including antibody 1444, anti-P-selectin antibody control in PBS

(0.78-200 nM), isotype negative control antibody (200 nM), control anti-E-selectin antibody (200 nM), or vehicle were flowed at 1 dyne/cm² for 1 hour. Diluted SCD blood was flowed for 10 minutes at 0.6 dyne/cm². PBS was perfused for 2-3 minutes at 1 dyne/cm² to remove the non-adherent cells and the plates were imaged and analyzed. The estimated IC₅₀ values were calculated as described above. The estimated IC₂₀, IC₈₀ and IC₉₀ were calculated based on the formula: (IC₅₀* effect)/(100-effect). The calculation is based on an E_{max} Tmodel

$$[E=(E_{max}+C)/(C+EC_{50})]$$
.

[0571] The isotype negative control antibody did not neutralize binding. The positive control antibody, tested at 200 mM, reduced cell binding adhesion between 10-35%. Antibody 1444 neutralized the binding of human SCD cells to recombinant E-selectin with an estimated IC₅₀ value of 6.17 nM and 18.66 nM, with a mean of 12.4 nM (**FIG. 14**). The IC₂₀, IC₈₀, and IC₉₀ values were estimated to be 3.1 nM, 49.6 nM, and 111.6 nM, respectively. Greater neutralization of cellular adhesion was observed with E-selectin inhibition compared to P-selectin inhibition under similar physiological flow conditions using soluble E-and P-selectin protein coated chambers.

[0572] **Table 38.** Summary of key pharmacologic properties of antibody 1444.

assay	pharmacodynamic activity
<i>In vitro</i> assays	
SPR binding to soluble recombinant protein	
human E-selectin	K _D = 68.4 +/- 3.18 nM
cynomolgus monkey E-selectin	K _D = 64.9 +/- 1.13 nM
mouse E-selectin	no binding (up to 405 nM)
rat E-selectin	no binding
rabbit E-selectin	no binding
Direct binding ELISA binding to soluble recombinant protein	
human E-selectin	EC ₅₀ = 0.085 nM – 0.12 nM
cynomolgus E-selectin	EC ₅₀ = 0.071 nM – 0.093 nM
human P-selectin	no binding
human L-selectin	no binding
mouse E-selectin	weak non-saturable binding (up to 133.3 nM)
rat E-selectin	weak non-saturable binding (up to 133.3 nM)
FACS binding to cell surface expressed E-selectin	
CHO cells	

human E-selectin	EC ₅₀ = 0.66 nM – 2.33 nM
cynomolgus monkey E-selectin	EC ₅₀ = 0.75 nM – 1.37 nM
Primary cells – TNFα activated	
Human – HUVECS	binding
Cynomolgus monkey – CLMEC	binding
Selectivity	
CHO human P-selectin cells	no binding
CHO DUKX parental	no binding
Functional assay	
Neutralization of ligand binding (sialyl Lewis A)	
CHO cells	
human E-selectin	IC ₅₀ = 1.88 nM – 2.89 nM
cynomolgus monkey E-selectin	IC ₅₀ = 1.47 nM – 2.65 nM
Soluble recombinant protein	
human E-selectin	IC ₅₀ = 2.87 nM – 3.01 nM
cynomolgus monkey E-selectin	IC ₅₀ = 2.39 nM – 2.91 nM
Static adhesion neutralization of HL-60 cells (endogenous ligand)	
CHO cells	
human E-selectin	IC ₅₀ = 3.36 nM – 4.7 nM
cynomolgus monkey E-selectin	IC ₅₀ = 3.84 nM
Flow adhesion neutralization of HL-60 cells (endogenous ligand)	
Soluble recombinant protein	
human E-selectin	IC ₅₀ = 13.28 nM – 15.94 nM
CHO cells	
human E-selectin	IC ₅₀ = 4.25 nM – 4.56 nM
cynomolgus monkey E-selectin	IC ₅₀ = 4.32 nM – 4.35 nM
Neutralization of Human Neutrophils (endogenous ligand)	
CHO cells	
human E- selectin	IC ₅₀ = 2.87 nM – 4.65 nM
cynomolgus E-selectin	IC ₅₀ = 9.45 nM – 16.33 nM
Neutralization of SCD patient cells	
Soluble human recombinant protein	IC ₅₀ = 12.4 nM
	IC ₂₀ = 3.2 nM

	IC ₈₀ = 49.6 nM
	IC ₉₀ = 111.6 nM

Example 31: Cell surface binding (FACS) and neutralization of optimized anti-E-selectin antibody 1444.

[0573] E-selectin is expressed on the endothelial cell surface and under conditions of endothelial cell dysfunction, such as an inflammatory response, circulating blood cells adhere to the vascular endothelium through ligand-adhesion molecule interactions. The ability of antibody 1444 to bind cell surface expressed E-selectin was assessed. In these studies, CHO cell lines expressing membrane-bound human or cynomolgus E-selectin were used to characterize binding of antibody 1444 to cell surface expressed E-selectin.

[0574] CHO cells that express E-selectin (human or cynomolgus monkey) or human P-selectin on the cell surface and control non-transfected CHO cells (CHO-DUKX) were evaluated by FACS analysis using antibody 1444, isotype control IgG1 and two control P-selectin antibodies. Antibody 1444 bound to human E-selectin with estimated EC₅₀ of 0.66 to 2.33 nM (based on 6 experiments). Antibody 1444 bound to cynomolgus E-selectin with an estimated EC₅₀ of 0.75 to 1.37 nM (based on 6 experiments). Antibody 1444 did not bind to control CHO-DUKX indicating absence of detectable cell surface binding. Furthermore, antibody 1444 did not bind cell-surface P-selectin. Dose dependent binding of the control anti-P-selectin antibody was observed to the CHO-P-selectin cells indicating expression of P-selectin. No binding was observed with the isotype control IgG1 (Table 38).

Example 32: Tolerability and toxicokinetic study in cynomolgus monkeys

[0575] The cynomolgus monkey was the only pharmacologically relevant nonclinical species based on in vitro pharmacology studies designed to assess the ability of antibody 1444 to inhibit E-selectin binding and/or biological activity. The binding affinity to human and cynomolgus monkey E-selectin were similar across assays evaluated, in contrast to the absence of potent binding to E-selectin from mouse, rabbit, or rat. In in vitro functional assays, antibody 1444 dose-dependently neutralized cellular adhesion of human promyelocytic cells to soluble recombinant human E-selectin protein and cellularly expressed recombinant human or cynomolgus monkey E-selectin. In addition, antibody 1444 neutralized the adhesion of purified neutrophils to both human and cynomolgus monkey cellular E-selectin with estimated 50% inhibitive concentration (IC₅₀) values in the low nM range. An SC dose of 30 mg/kg was associated with an overall maximum observed concentration (C_{max}) of 353 µg/mL and area

under the concentration-time curve from time 0 to 336 hours (AUC_{336}) of 92,500 $\mu\text{g}\cdot\text{h}/\text{mL}$; while the IV dose of 100 mg/kg was associated with overall C_{max} of 693 $\mu\text{g}/\text{mL}$ and AUC_{336} of 108,000 $\mu\text{g}\cdot\text{h}/\text{mL}$. Antibody 1444 was administered to male and female cynomolgus monkeys at doses up to 200 mg/kg/week IV once weekly for 1 month and as well tolerated. *In vitro* human complement component 1q (C1q) and fragment crystallizable gamma Fc receptor (Fc γ R) binding assays and a tissue cross reactivity evaluation did not yield safety concerns

Example 33: Pharmacokinetic and Product Metabolism in Animals

[0576] Single-dose PK and repeat-dose TK were evaluated in cynomolgus monkeys following IV and/or SC dosing of antibody 1444. After single IV dosing in cynomolgus monkeys, dose dependent clearance (CL) was observed and was consistent with saturable target mediated drug dispositions (TMDD). After single IV dosing at a dose range from 0.3 to 10 mg/kg, antibody 1444 exhibited a mean CL and mean V_{SS} from approximately 0.39 to 0.15 mL/h/kg and 22 to 36 mL/kg, respectively. After single SC doses of antibody 1444 at 1 mg/kg or 3 mg/kg, T_{max} was observed at 72 and 96 hours, respectively and SC bioavailability was >50%. The estimated $T_{1/2}$ of antibody 1444 at 3 mg/kg IV was approximately 12 days.

[0577] In a 1-month GLP repeat-dose toxicity study, there were no apparent sex-related differences in systemic exposure (as assessed by the maximum observed concentration [C_{max}] an area under the concentration-time curve [AUC]) following repeat SC and IV dosing. Systemic exposure increased with increasing dose (SC) and was higher after repeat SC and IV dosing. Antibody 1444 was administered to male and female cynomolgus monkeys at doses of 15 or 50 mg/kg/week SC or 200 mg/kg/week IV once weekly for 1 month (5 total doses). After once weekly repeat dosing at 15 and 50 mg/kg/week, mean T_{max} was between 72 and 112 hours post dose on Days 1 and 22. Systemic exposure was higher after repeat dosing with mean accumulation ratios (AUC_{168} , Day22/Day1) of 5.5, 3.2 and 1.9 for 15 (SC), 50 (SC) and 200(IV) mg/kg/week, respectively.

[0578] The predicted efficacious dose in humans is a single SC administration of antibody 1444 at 150 mg. The predicted minimal effective concentration and exposure at 150 mg SC dosing are 14 $\mu\text{g}/\text{mL}$ for average concentration (C_{av} [1 to 168 hours]) and 2400 $\mu\text{g}\cdot\text{h}/\text{mL}$ for AUC_{168} , respectively.

Example 34: E-selectin Levels, Aggregates and ICAM-1 Expression in Animals

[0579] Soluble E-selectin was characterized in serum from cynomolgus monkeys following doses of antibody 1444 ranging from 0.3 to 10 mg/kg by liquid chromatography-mass-

spectrometry (LC-MS). Antibody 1444 showed effective binding of E-selectin in a concentration-dependent manner, and a >90% inhibitory effect on soluble E-selectin was observed at all dose groups indicating *in vivo* E-selectin binding activity of antibody 1444.

- [0580]** Following dosing with antibody 1444, blood was collected from cynomolgus monkeys and analyzed using fluorescent activated cell sorting (FACS) to detect cellular aggregates and adhesion markers. The monocyte-platelet aggregate and neutrophil-platelet aggregates were examined. In this FACS analysis, CD14 was used to differentiate monocytes and neutrophils using size scan, and CD41 was used to detect monocyte-platelet aggregates and neutrophil-platelet aggregates. Antibody 1444 showed effects on decrease in monocyte-platelet and neutrophil-platelet aggregates in cynomolgus monkeys after treatment.

[0581] Leukocyte Intercellular Adhesion Molecule 1 (ICAM-1) expression was examined in leukocytes from cynomolgus monkeys after antibody 1444 dosing using FACS analysis. A decrease in ICAM-1 was observed following dosing.

CLAIMS

We claim:

1. An isolated antibody, or antigen binding fragment thereof, that specifically binds to
5 human E-selectin, comprising at least one of the following:
 - (a) a light chain complementarity determining region 1 (LCDR-1) comprising the amino acid sequence of SEQ ID NO:2, a LCDR-2 comprising the amino acid sequence of SEQ ID NO:3, a LCDR-3 comprising the amino acid sequence of SEQ ID NO:4, a
10 heavy chain complementarity determining region 1 (HCDR-1) comprising the amino acid sequence of SEQ ID NO:8, a HCDR-2 comprising the amino acid sequence of SEQ ID NO:9, and a HCDR-3 comprising the amino acid sequence of SEQ ID NO:10;
 - (b) a LCDR-1 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:2, 18, 47, 68, 82, 97 and 106;
 - (c) a LCDR-2 amino acid sequence selected from the group consisting of the sequence of
15 SEQ ID NO:3, 19, 48, 69, 83, 98, 107 and 120;
 - (d) a LCDR-3 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:4, 20, 49, 70, 84, 99, 108 and 121;
 - (e) a heavy chain complementarity determining region 1 (HCDR-1) amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:8, 23, 52, 63, 77,
20 92, 111 and 125;
 - (f) a HCDR-2 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:9, 24, 29, 38, 41, 44, 53, 64, 78, 93, 112 and 126;
 - (g) a HCDR-3 amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:10, 54, 65, 79, 94, 113 and 127;
 - 25 (h) the HCDR-1, HCDR-2, and HCDR-3 amino acid sequences as set forth in the amino acid sequence of SEQ ID NO:11, and the LCDR-1, LCDR-2, and LCDR-3 amino acid sequences as set forth in the amino acid sequence of SEQ ID NO:5;
 - (i) the LCDR-1, LCDR-2, and LCDR-3 amino acid sequences as set forth in the amino acid sequence encoded by the insert of the plasmid deposited at the ATCC and having
30 ATCC Accession No. PTA-126530, and HCDR-1, HCDR-2, and HCDR-3 amino acid sequences as set forth in the amino acid sequence encoded by the insert of the plasmid deposited at the ATCC and having ATCC Accession No. PTA-126529;
 - (j) a heavy chain variable domain (VH) comprising an amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:11, 25, 30, 35, 39, 42, 45, 55, 60,

- 66, 75, 80, 90, 95, 104, 114, 118 and 128, and a light chain variable domain (VL) comprising an amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:5, 21, 27, 32, 50, 57, 71, 73, 85, 87, 100, 102, 109, 116 and 122;
- 5 (k) a VH comprising an amino acid sequence at least 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO:11, and a light chain variable domain (VL) comprising an amino acid sequence at least 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO:5;
- (l) a VH comprising the amino acid sequence of SEQ ID NO:11 and a VL comprising the amino acid sequence of SEQ ID NO:5;
- 10 (m) a VH comprising the amino acid sequence as set forth in the amino acid sequence encoded by the insert of the plasmid deposited at the ATCC and having ATCC Accession No. PTA-126529 and a VL comprising the amino acid sequence as set forth in the amino acid sequence encoded by the insert of the plasmid deposited at the ATCC and having ATCC Accession No. PTA-126530;
- 15 (n) a heavy chain (HC) comprising an amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:7, 13, 22, 28, 34, 37, 40, 43, 51, 59, 62, 74, 76, 89, 91, 103, 110, 117 and 124, and a light chain (LC) comprising an amino acid sequence selected from the group consisting of the sequence of SEQ ID NO:1, 17, 26, 31, 46, 56, 67, 72, 81, 86, 96, 101, 105, 115 and 119;
- 20 (o) a HC comprising an amino acid sequence at least 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO:7 or SEQ ID NO:13, and a LC comprising an amino acid sequence at least 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO:1;
- (p) a HC comprising the amino acid sequence of SEQ ID NO:7 or SEQ ID NO:13, and a LC comprising the amino acid sequence of SEQ ID NO:1; and
- 25 (q) a HC comprising the amino acid sequence as set forth in the amino acid sequence encoded by the insert of the plasmid deposited at the ATCC and having ATCC Accession No. PTA-126529 and a LC comprising the amino acid sequence as set forth in the amino acid sequence encoded by the insert of the plasmid deposited at the ATCC and having ATCC Accession No. PTA-126530.
- 30
2. An isolated antibody that specifically binds to human E-selectin, comprising:
an amino acid sequence comprising a LCDR-1, a LCDR-2 and a LCDR-3 as specified by the amino acid sequence of SEQ ID NO:5; and

an amino acid sequence comprising a HCDR-1, a HCDR-2 and a HCDR-3 as specified by the amino acid sequence of SEQ ID NO:11.

3. The isolated antibody of claim 2, comprising an antibody heavy chain constant region (CH) comprising the amino acid sequence of SEQ ID NO: 15 or 16, and an antibody light chain constant region (CL) comprising the amino acid sequence of SEQ ID NO:14.
4. An isolated antibody that specifically binds to human E-selectin, comprising a LC consisting of the amino acid sequence of SEQ ID NO:1, and a HC consisting of the amino acid sequence of SEQ ID NO:7 or 13.
5. An isolated antibody that specifically binds human E-selectin, comprising at least one of the following:
 - a) an antibody VL region comprising the LCDR1, LCDR2 and LCDR3 of the VL comprising the amino acid sequence of SEQ ID NO:5;
an antibody VH region comprising the HCDR1, HCDR2 and HCDR3 of the VH comprising the amino acid sequence of SEQ ID NO:11;
an antibody CL region comprising the amino acid sequence of SEQ ID NO:14; and
an antibody heavy chain (CH) constant region comprising the amino acid sequence of SEQ ID NO:15 or 16;
 - b) an antibody VL region comprising an amino acid sequence at least 95% identical to SEQ ID NO:5; and an antibody VH region comprising an amino acid sequence at least 95% identical to SEQ ID NO:11; and
 - c) an antibody LC region comprising an amino acid sequence at least 95% identical to SEQ ID NO:1, and an antibody HC comprising an amino acid sequence at least 95% identical to SEQ ID NO:7 or 13.
6. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to human E-selectin, comprising an antibody HC comprising an amino acid sequence encoded by the insert deposited with the ATCC and having the Accession Number PTA-126529, and an antibody LC comprising an amino acid sequence encoded by the insert deposited with the ATCC and having the Accession Number PTA-126530.
7. The isolated antibody, or antigen binding fragment thereof, of any one of claims 1-6,

comprising a human Fc domain selected from the group consisting of an Fc domain of IgA₁, IgA₂, IgD, IgE, IgM, IgG₁, IgG₂, IgG₃, or IgG₄; and/or a light chain constant region chosen from kappa or lambda.

- 5 8. The isolated antibody or antigen-binding fragment thereof, of any one of claims 1-7, wherein the antibody heavy chain isotype is IgG1 and/or wherein the light chain constant region is a kappa light chain.
9. The isolated antibody or antigen-binding fragment thereof, of any of claims 1-8, wherein
10 the antibody is a humanized antibody, a human antibody, a murine antibody, chimeric antibody, or a camelid antibody.
10. The isolated antibody, or antigen binding fragment thereof, of any one of claims 1-9, wherein the antibody, or antigen binding fragment thereof, binds human or cynomolgus monkey
15 E-selectin with a K_D about or less than a value selected from the group consisting of 200 nM, 175 nM, 150 nM, 125 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 900pM, 800pM, 700pM, 600pM and 500pM.
11. The isolated antibody, or antigen binding fragment thereof, wherein the antibody or
20 antigen-binding fragment thereof, binding human or cynomolgus monkey E-selectin with a K_D of about 200 nM to about 2 nM.
12. An isolated antibody, or antigen-binding fragment thereof, that specifically binds E-selectin and demonstrates at least one detectable characteristic selected from the following:
25 (i) binds to human E-selectin with a K_D of 200 nM or less, as measured, optionally, by SPR;
(ii) binds to cynomolgus monkey E-selectin with a K_D of 200 nM or less, as measured, optionally, by SPR;
(iii) binds with an EC_{50} of 50 nM or less, as measured, optionally, by FAC, to cell-
30 surface expressed E-selectin;
(iv) binds with an EC_{50} of 350 nM or greater, as measured, optionally, by FACS, to cell-surface expressed P-selectin;
(v) does not detectably bind to soluble rat, mouse or rabbit E-selectin or soluble human L- or P-selectin up to 405 nM as measured, optionally, by SPR;

- (vi) binds with an EC_{50} of 2 nM or less, as measured, optionally, by ELISA, to soluble human E-selectin;
- (vii) neutralizes binding with an EC_{50} of 2 nM or less as measured, optionally, by AlphaLisa competition assay, of a sialyl-Lewis A ligand to soluble human E-selectin;
- (viii) binds with an IC_{50} of about 1 nM to about 3 nM to free soluble human E-selectin in human serum;
- (ix) neutralizes binding with an IC_{50} of 100 nM or less, as measured, optionally, by competition ELISA under static conditions, of a sialyl-Lewis A ligand to soluble human E-selectin;
- (x) neutralizes binding with an IC_{50} of 50 nM or less, as measured, optionally, by competition ELISA under static conditions, of a sialyl-Lewis A ligand to cell-surface expressed human E-selectin;
- (xi) inhibits adhesion with an IC_{50} of 100 nM or less, as measured, optionally, under static conditions, of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed human E-selectin;
- (xii) inhibits adhesion with an IC_{50} of 100 nM or less, as measured, optionally, under physiological flow conditions, of cells expressing an E-selectin ligand (e.g., E selectin ligand, PSGL-1 and other sialyl Lewis ligands) to cell-surface expressed human E-selectin;
- (xiii) inhibits adhesion with an IC_{50} of about 6.17 nM to about 18.66 nM as measured, optionally, under physiologic flow conditions, of blood cells from SCD patients to soluble human E-selectin;
- (xiv) binds to at least one amino acid residue of human E-selectin selected from: T7, E8, A9, M10, T11, P46, S47, Y48, N82, N83, Q85, E88, E92, Y94, R97, N105, E107, R108, S110, K111, K112 and K113;
- (xv) has a mean half-life of about 14.4 days (345 hours) following IV administration at a dose of 10 mg/kg;
- (xvi) has a mean half-life of about 21.5 days (518 hours) following SC administration at a dose of about 3 mg/kg;
- (xvii) has a viscosity of 33.4 cP at 185.7 mg/mL when measured at 25°C by, optionally an Anton Parr method;
- (xviii) exhibits commercially suitable formulation properties, including a high degree of

thermal stability and minimal aggregation at high concentration; and
(xix) exhibits reproducible expression and purity under large-scale manufacturing conditions.

13. An isolated antibody or antigen-binding fragment thereof that competes for binding to
5 human E-selectin with at least one antibody or antigen binding-fragment thereof of any one of
claims 1-12.

14. An isolated nucleic acid molecule encoding the antibody, or antigen-binding fragment
thereof, of any one of claims 1-13.

10

15. An isolated nucleic acid molecule comprising at least one of the following:
a) the nucleic acid sequence of SEQ ID NO:136, the nucleic acid sequence of SEQ ID
NO:137, or both;
b) a nucleic acid sequence selected from the group consisting of the sequence of SEQ
15 ID NO:136, 144, 146, 148, 150, 151, 152, 153, 155, 157, 159, 161, 163, 165, 167,
169, 171 and 173 or a nucleic acid sequence selected from the group consisting of
the sequence of SEQ ID NO:137, 145, 147, 149, 154, 156, 158, 160, 162, 164, 166,
168, 170, 172 and 174, or both;
c) a nucleic acid sequence of SEQ ID NO:206 or 138, the nucleic acid sequence of
20 SEQ ID NO:139, or both; and
d) the nucleic acid sequence of the insert of the plasmid deposited with the ATCC and
having the Accession Number PTA-126529, the nucleic acid sequence of the insert
of the plasmid deposited with the ATCC and having the Accession Number PTA-
126530, or both.

25

16. A vector comprising the nucleic acid of claim 14 or 15.

17. A host cell comprising the vector of claim 16.

30 18. The host cell of claim 17, wherein said host cell is a mammalian cell selected from the
group consisting of a CHO cell, a COS cell, a HEK-293 cell, an NS0 cell, a PER.C6® cell, or an
Sp2.0 cell.

19. A method of making an antibody, or antigen binding fragment thereof, comprising

culturing the host cell of claim 17 or 18 under a condition wherein said antibody, or antigen binding fragment thereof, is expressed by said host cell.

20. A pharmaceutical composition comprising an antibody, or antigen binding fragment thereof, of any one of claims 1-13, and a pharmaceutically acceptable carrier or excipient.

21. The pharmaceutical composition of claim 20, comprising i) an antibody or antigen-binding fragment thereof comprising an antibody heavy chain encoded by the amino acid sequence of SEQ ID NO:7 and an antibody light chain encoded by the amino acid sequence of SEQ ID NO:1, ii) an antibody or antigen-binding fragment thereof comprising an antibody heavy chain encoded by the amino acid sequence of SEQ ID NO:13 and an antibody light chain encoded by the amino acid sequence of SEQ ID NO:1, or iii) both.

22. A method of treating a medical condition, disease or disorder mediated by or associated with expression of E-selectin and/or binding of E-selectin to a ligand, in a subject in need thereof, wherein the method comprises administering a therapeutically effective amount of an antibody, or antigen binding fragment thereof, of any one of claims 1-13 that specifically binds to E-selectin.

23. The method of claim 22, wherein the medical condition, disease or disorder is SCD, a skin disease (e.g., psoriasis), inflammatory disease (e.g., rheumatoid arthritis) and/or complications of diabetes.

24. A method of treating SCD, in a subject in need thereof, wherein the method comprises administering a therapeutically effective amount of an antibody, or antigen binding fragment thereof, of any one of claims 1-13 that specifically binds to E-selectin and, treating, preventing and/or ameliorating at least one sign and/or symptom of SCD selected from the group consisting of those affecting the cardiothoracic system (e.g., chronic restrictive lung disease, left ventricular diastolic disease, pulmonary hypertension, acute chest syndrome, dysrhythmias, sudden death, vaso-occlusive crisis (VOC)), the nervous system (e.g., hemorrhagic stroke, venous sinus thrombosis, silent cerebral infarction of the brain, chronic pain, acute ischemic stroke of the brain, proliferative retinopathy, orbital infarction, cognitive impairment) the reticuloendothelial system (e.g., splenic sequestration, functional hyposplenism, anemia, hemolysis), the musculoskeletal system (e.g., avascular necrosis, skin ulcerations), the

urogenital system (e.g., papillary necrosis, proteinuria, renal failure, hematuria, nocturnal enuresis, priapism) and the gastrointestinal system (e.g., cholelithiasis, cholangiopathy, hepatopathy, mesenteric vaso-occlusion).

- 5 25. The method of treating SCD of claim 24, wherein treatment of VOC includes i) treatment of acute VOC by decreasing the duration and/or intensity of the VOC, ii) prevention, or reduction in the occurrence, of VOC or iii) both.

- 10 26. A method of decreasing an E-selectin biological activity in a subject in need thereof, wherein the method comprises administering a therapeutically effective amount of an antibody, or antigen binding fragment thereof, of any one of claims 1-13 that specifically binds to E-selectin.

- 15 27. The method of claim 26, wherein the antibody, or antigen binding fragment thereof decreases at least one detectable E-selectin biological activity wherein the decreased activity is selected from the group consisting of (a) leukocyte tethering to endothelial cells; (b) activation of stable adhesion to endothelial cells; (c) slow rolling of leukocytes to arrest; (d) efficient trans-endothelial migration of leukocytes; (e) affinity and avidity of E-selectin to CD18 integrins; (f) trafficking of leukocytes to sites of acute inflammation; (g) cytosolic calcium; (h) tyrosine phosphorylation that activates p38 MAP kinase and Syk kinase; (i) recruitment of platelets and leukocytes from the blood to the vascular endothelium; and (j) creation of a pro-inflammatory environment.

- 25 28. The method of any one of claims 22-27, comprising administration of a therapeutically effective amount of at least one additional therapeutically active compound or treatment modality which additional compound and modality is effective in treating and/or preventing at least one sign and/or symptom of SCD.

- 30 29. The method of claim 28, wherein the at least one additional therapeutically active compound is selected from the group consisting of penicillin prophylaxis, hydroxyurea (DROXIA, HYDREA), L-glutamine (ENDARI), crizanlizumab (ADAKVEO), voxelotor (OXBRYTA), apixaban (ELIQUIS), rivaroxaban (XARELTO), a non-steroidal anti-inflammatory drug, an analgesic (e.g., an opioid analgesic), IW-1701, riociguat (ADEMPAS), ticagrelor (BRILINTA), memantine (NAMENDA), and a combination thereof.

30. The method of claim 28, wherein the at least one additional therapeutically active compound is selected from the group consisting of an anti-P-selectin antibody, a compound that modulates HbS so as to maintain it in its R state (i.e., oxygenated), a compound that modulates oxygen affinity of HbS, a compound that targets HbS polymerization by modulating generation of 2,3-disphosphoglyceric acid, a compound that targets HbS polymerization by inducing expression of fetal hemoglobin (HbF), a compound that targets dysfunctional cellular adhesion, vascular dysfunction and/or inflammation, a compound that increases levels of nitric oxide (NO) in the blood as compared with the level of NO before administration of the compound, intravenous IG, a compound that targets hypercoagulability, a compound that blocks NMDA receptor binding and a combination thereof.

31. The method of claim 30, wherein the compound that modulates HbS so as to maintain it in its R state (i.e., oxygenated) is selected from the group consisting of 6-((1S)-1-((2-amino-6-fluoroquinolin-3-yl)oxy)ethyl)-5-(1H-pyrazol-1-yl)pyridin-2-ol, (S)-6-(1-((2-amino-6-fluoroquinolin-3-yl)oxy)ethyl)-5-(1H-pyrazol-1-yl)pyridin-2(1H)-one and a combination thereof.

32. The method of claim 28, wherein the at least one additional therapeutically active treatment modality is selected from the group consisting of supplemental oxygen, blood transfusion, optionally with iron chelation, bone marrow transplant, gene therapy, a gene editing therapy by CRISPR or a zinc finger technique and a combination thereof.

33. The method of any one of claims 28-32, wherein the administration of the at least one additional therapeutically active compound or treatment modality, which is effective in treating and/or preventing at least one sign and/or symptom of SCD, is simultaneous, sequential or separate from the administration of the therapeutically effective amount of the anti-E-selectin antibody, or antigen-binding fragment thereof.

34. A kit for the treatment of SCD, comprising a therapeutically effective amount of an anti-E-selectin antibody of any one of claims 1-13.

35. The kit of claim 34, further comprising a synergistic therapeutically effective amount of an at least one additional therapeutically active compound or treatment modality which is effective in treating and/or preventing at least one sign and/or symptom of SCD.

36. An antibody, or antigen-binding fragment thereof, of any one of claims 1-13, or the pharmaceutical composition of claims 20 or 21 for use in treating and/or preventing a disease, disorder or condition associated with, or mediated by, E-selectin expression and/or E-selectin binding to a ligand.
37. The antibody, or antigen-binding fragment thereof, or the pharmaceutical composition for use of claim 36, wherein the disease, disorder or condition is SCD.
38. Use of an antibody, or antigen-binding fragment thereof, of any one of claims 1-13 in the manufacture of a medicament for treating and/or preventing a disease, disorder or condition associated with, or mediated by, E-selectin expression and/or E-selectin binding to a ligand.
39. The use of claim 38, wherein the disease, disorder or condition is SCD.
40. The antibody or antigen-binding fragment thereof according to any of claims 1-13, or the pharmaceutical composition of claims 20 or 21 for use in reducing E-selectin activity in a subject in need thereof, for treatment of SCD.