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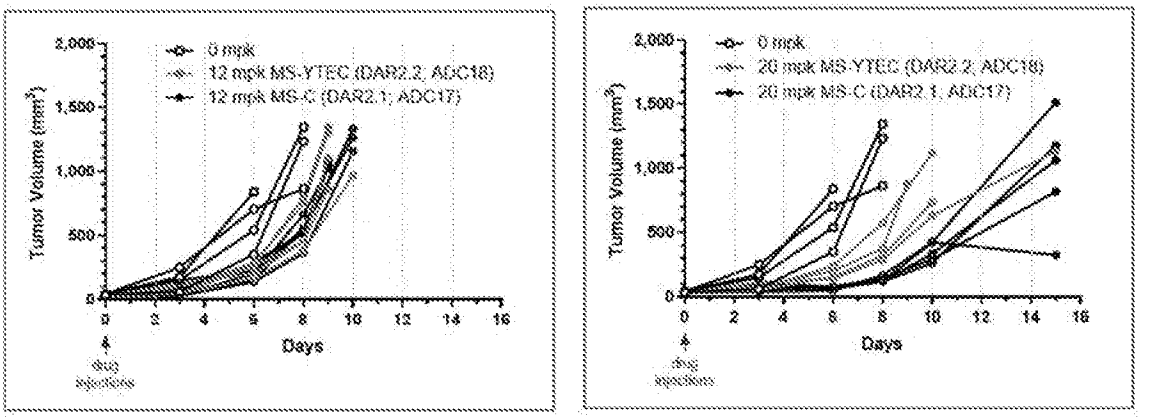
(54) Titre : CONJUGUES D'ANTICORPS-MEDICAMENT COMPRENANT DES ANTICORPS ANTI-TM4SF1 ET LEURS METHODES D'UTILISATION

(54) Title: ANTIBODY-DRUG CONJUGATES COMPRISING ANTI-TM4SF1 ANTIBODIES AND METHODS OF USING THE SAME

FIG. 11



(57) Abrégé/Abstract:
Antibody-drug conjugates (ADCs) are described, comprising anti-TM4SF1 antibodies, and antigen-binding fragments thereof. Methods of use of said ADCs are also described.

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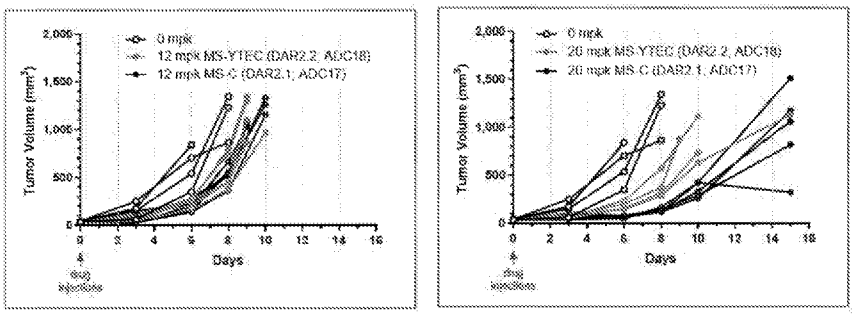
(54) Title: ANTIBODY-DRUG CONJUGATES COMPRISING ANTI-TM4SF1 ANTIBODIES AND METHODS OF USING THE SAME

FIG. 11



(57) Abstract: Antibody-drug conjugates (ADCs) are described, comprising anti-TM4SF1 antibodies, and antigen-binding fragments thereof. Methods of use of said ADCs are also described.

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ANTIBODY-DRUG CONJUGATES COMPRISING ANTI-TM4SF1 ANTIBODIES AND METHODS OF USING THE SAME

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/811,411 filed February 27, 2019, and U.S. Provisional Application No. 62/967, 377 filed January 29, 2020, each incorporated by reference herein in its entirety.

BACKGROUND

[0002] There remains a need in the art for cancer therapeutics, and in particular therapeutics with improved therapeutic margins that can regress primary tumors as well as invasive tumor cells and metastases.

[0003] Cancer therapies designed to destroy tumor blood vessels have in the past failed in clinical trials due to toxicity. Examples include the vascular disrupting agents such as Combretastatin (CA4P). *See, e.g.*, Grisham *et al.* Clinical trial experience with CA4P anticancer therapy: focus on efficacy, cardiovascular adverse events, and hypertension management. Gynecol Oncol Res Pract. 2018; 5:1.. CA4P reduced overall survival from 16.2 to 13.6 months in the Phase II FALCON study, and seven patients have experienced heart attacks while being treated with CA4P. *Id.* As coronary heart disease and stroke are leading causes of death, any vascular targeted toxic therapy may lead to a risk of lethal toxicity.

[0004] TM4SF1 is an endothelial marker with a functional role in angiogenesis. *See, e.g.*, Shih *et al.* The L6 protein TM4SF1 is critical for endothelial cell function and tumor angiogenesis. Cancer Res. 2009; 69(8):3272-7. Although antibody-drug conjugates targeting TM4SF1 have been considered previously, *see, e.g.*, Visintin *et al.* Novel Anti-TM4SF1 Antibody–Drug Conjugates with Activity against Tumor Cells and Tumor Vasculature, Mol Cancer Ther 2015 (14) (8) 1868-1876, in order to enable anti-TM4SF1 ADCs to fulfill their promise as therapies for solid tumors, TM4SF1 targeted ADCs with reduced toxicity to normal vessels, especially arteries, are needed.

INCORPORATION BY REFERENCE

[0005] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

SUMMARY OF THE INVENTION

[0006] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat. In some embodiments, said IgG Fc region comprises said mutation at position N297. In some embodiments, said mutation at position N297 comprises N297C. In some embodiments, said IgG Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

[0007] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat. In some embodiments, said IgG Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, T356, M428, and N434; as numbered by the EU index as set forth in Kabat. In some embodiments, said IgG Fc region comprises said mutation at position N297. In some embodiments, said mutation at position N297 comprises N297C.

[0008] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising a cysteine residue at position N297, as numbered by the EU index as set forth in Kabat. In some embodiments, said IgG Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat.

[0009] In some embodiments, said IgG Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region

comprising a human IgG1 Fc region comprising a cysteine residue at position N297 and a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat. In some embodiments, said IgG Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

[0010] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising a cysteine residue at position N297, as numbered by the EU index as set forth in Kabat, wherein said antibody-drug conjugate comprises a drug to antibody ratio (DAR) of greater than or equal to about 1. In some embodiments, said IgG Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat. In some embodiments, said IgG Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

[0011] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising a cysteine residue at position N297 and an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, wherein numbering is according to the EU index as set forth in Kabat. In some embodiments, said IgG Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat. In some embodiments, said one or more amino acid residues after position K447 are independently selected from the group consisting of: a lysine, a proline, an arginine, or any combinations thereof.

[0012] In some embodiments, said one or more amino acid residues after position K447 are independently selected from the group consisting of: said lysine and said proline. In some embodiments, said IgG Fc region comprises said mutation at position E233. In some embodiments, said mutation at position E233 comprises E233P. In some embodiments, said IgG Fc region comprises said mutation at position L234. In some embodiments, said mutation at

position L234 comprises L234A. In some embodiments, said IgG Fc region comprises said mutation at position L235. In some embodiments, said mutation at position L235 comprises L235A. In some embodiments, said IgG Fc region comprises said mutation at position G237. In some embodiments, said mutation at position G237 comprises G237A. In some embodiments, said IgG Fc region comprises said mutation at position M252. In some embodiments, said mutation at position M252 comprises M252Y. In some embodiments, said IgG Fc region comprises said mutation at position S254. In some embodiments, said mutation at position S254 comprises S254T. In some embodiments, said IgG Fc region comprises said mutation at position T256. In some embodiments, said mutation at position T256 comprises T256E. In some embodiments, said IgG Fc region comprises said mutation at position M428. In some embodiments, said mutation at position M428 comprises M428L. In some embodiments, said IgG Fc region comprises said mutation at position N434. In some embodiments, said mutation at position N434 comprises N434S or N434A. In some embodiments, said IgG Fc region comprises said mutation at position T250. In some embodiments, said mutation at position T250 comprises T250Q. In some embodiments, said IgG Fc region comprises said mutation at position D265. In some embodiments, said mutation at position D265 comprises D265A. In some embodiments, said IgG Fc region comprises said mutation at position K322. In some embodiments, said mutation at position K322 comprises K322A. In some embodiments, said IgG Fc region comprises said mutation at position P331. In some embodiments, said mutation at position P331 comprises P331G. In some embodiments, said IgG Fc region comprises T250Q and M428L. In some embodiments, said IgG Fc region comprises M428L. In some embodiments, said IgG Fc region comprises M428L and N434S.

[0013] In some embodiments, said IgG Fc region comprises N434A. In some embodiments, said IgG Fc region comprises L234A, L235A, and G237A. In some embodiments, said IgG Fc region comprises L234A, L235A, G237A, and P331G. In some embodiments, said IgG Fc region comprises L234A, L235A, G237A, N297C, and P331G. In some embodiments, said IgG Fc region comprises L234A, L235A, G237A, K322A, and P331G. In some embodiments, said IgG Fc region comprises E233P, L234A, L235A, G237A, and P331G. In some embodiments, said IgG Fc region comprises E233P, L234A, L235A, G237A, and N297C. In some embodiments, said IgG Fc region comprises E233P, L234A, L235A, G237A, and N297C. In some embodiments, said IgG Fc region comprises L234A, L235A, G237A, N297C, K322A, and P331G. In some embodiments, said IgG Fc region comprises E233P, L234A, L235A, G237A, D265A, N297C, K322A, and P331G. In some embodiments, said IgG Fc region comprises E233P, L234A, L235A, G237A, D265A, N297C, K322A, and P331G. In some embodiments, said IgG Fc region comprises E233P and D265A. In some embodiments, said IgG Fc region

comprises M252Y, S254T, and T256E. In some embodiments, said IgG Fc region comprises M252Y, S254T, T256E, and N297C. In some embodiments, said IgG Fc region comprises K322A and P331G, and wherein said IgG Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447. In some embodiments, said IgG Fc region comprises an amino acid sequence selected from the group consisting of SEQ ID Nos. 87-88, 135-145, and 151-153. In some embodiments, said IgG Fc region exhibits reduced or ablated binding with C1q. In some embodiments, said IgG Fc region exhibits reduced or ablated binding to an Fc receptor. In some embodiments, said anti-TM4SF1 antibody exhibits reduced or ablated ADCC or CDC effector function.

[0014] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297, as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region comprises said mutation at position N297. In some embodiments, said mutation at position N297 comprises N297C. In some embodiments, said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

[0015] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297, as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region comprises said mutation at position N297. In some embodiments, said mutation at position N297 comprises N297C.

[0016] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a cysteine residue at position N297, as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, and H435, , as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

[0017] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a cysteine residue at position N297 and a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, and H435, , as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

[0018] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a cysteine residue at position N297, as numbered by the EU index as set forth in Kabat, wherein said antibody-drug conjugate comprises a drug to antibody ratio (DAR) of greater than or equal to 1. In some embodiments, said human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, and H435, as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said

extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

[0019] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a cysteine residue at position N297 and an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, wherein numbering is according to the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, and H435, as numbered by the EU index as set forth in Kabat. In some embodiments, said one or more amino acid residues after position K447 is independently selected from the group consisting of: a lysine, a proline, an arginine, or any combinations thereof. In some embodiments, said one or more amino acid residues after position K447 is independently selected from the group consisting of: said lysine and said proline. In some embodiments, said human IgG4 Fc region comprises said mutation at position S228. In some embodiments, said mutation at position S228 comprises S228P. In some embodiments, said human IgG4 Fc region comprises said mutation at position F234. In some embodiments, said mutation at position F234 comprises F234A. In some embodiments, said human IgG4 Fc region comprises said mutation at position L235. In some embodiments, said mutation at position L235 comprises L235E. In some embodiments, said human IgG4 Fc region comprises S228P and L235E. In some embodiments, said human IgG4 Fc region comprises S228P, L235E, and N297C. In some embodiments, said human IgG4 Fc region comprises S228P, F234A, L235E, and N297C. In some embodiments, said human IgG4 Fc region comprises S228P, L235E, and N297C, and wherein said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447. In some embodiments, said human IgG4 Fc region comprises M428L and N434S. In some embodiments, said human IgG4 Fc region comprises mutations at L235 and F234. In some embodiments, said human IgG4 Fc region comprises mutations at positions L328, A330, and T299. In some embodiments, said human IgG4 Fc region comprises S228P, F234A, L235A, G237A, and P238S. In some embodiments, said human IgG4 Fc region comprises F243A and V264A. In some embodiments, said human IgG4 Fc region comprises S228P and L235A. In some embodiments, said human IgG4 Fc region comprises M252Y and M428L; D259I and V308F; or N434S. In some

embodiments, said human IgG4 Fc region comprises T307Q and N434S; M428L and V308F; Q311V and N434S; H433K and N434F; E258F and V427T; or T256D, Q311V, and A378V. In some embodiments, said human IgG4 Fc region comprises one or more of the following properties: (i) reduced or ablated binding with C1q; (ii) reduced or ablated binding to an Fc receptor; and (iii) reduced or ablated ADCC or CDC effector function. In some embodiments, said anti-TM4SF1 antibody or an antigen binding fragment thereof comprising said human IgG4 Fc region comprises an amino acid sequence selected from the group consisting of SEQ ID Nos. 146-150, and 154-155.

[0020] In some embodiments, said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises:

(a) a heavy chain comprising a CDR3 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 8, 20, 32, 44, 56, 68, 80, 96, 118, 119, 120, or 121; a CDR2 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 7, 19, 31, 43, 55, 67, 79, 95, 116, or 117; and a CDR1 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 6, 18, 30, 42, 54, 66, 78, 94, or 115; and

(b) a light chain comprising a CDR3 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 14, 26, 38, 50, 62, 74, 86, 110, or 129; a CDR2 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 13, 25, 37, 49, 61, 73, 85, 109, or 128; and a CDR1 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 12, 24, 36, 48, 60, 72, or 84, 107, 108, 124, 125, 126, or 127.

[0021] In some embodiments, said heavy chain comprises an amino acid sequence that has at least 75% identity to SEQ ID NO: 3, 15, 27, 39, 51, 63, 75, 90, 92, 112, 114, 130, or 132, and a light chain comprises an amino acid sequence that has at least 75% identity to SEQ ID NO: 9, 21, 33, 45, 57, 69, 81, 97, 99, 101, 122, 131, or 133. In some embodiments, said heavy chain comprises a sequence as set forth in SEQ ID NO: 3, 15, 27, 39, 51, 63, 75, 90, 92, 112, 114, 130, or 132, and wherein said light chain variable domain comprises a sequence as set forth in SEQ ID NO: 9, 21, 33, 45, 57, 69, 81, 97, 99, 101, 122, 131, or 133. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 6; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in

SEQ ID NO: 14, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 13, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 12. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 20, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 19, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 18; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 26, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 25, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 24. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 32, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 31, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 30; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 38, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 37, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 36.

[0022] In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 44, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 43, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 42; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 50, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 49, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 48. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 56, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 55, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 54; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 62, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 61, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 60. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 68, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 67, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 66; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 74, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 73, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 72. In some embodiments, said heavy chain comprises a CDR3 domain

comprising the amino acid sequence se set forth in SEQ ID NO: 80, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 79, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 78; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 86, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 85, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 84. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 111 or SEQ ID NO: 110, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 107 or SEQ ID NO: 108.

[0023] In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 110, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 107. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 110, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 108. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 111, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 107. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95,

and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 111, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 108. In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 118, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 116, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 115; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 129, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 128, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 124.

[0024] In some embodiments, said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 118, SEQ ID NO: 119, SEQ IN NO: 120, or SEQ ID NO: 121, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 116 or SEQ ID NO: 117, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 115; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 129, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 128, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, or SEQ ID NO: 127. In some embodiments, said antigen-binding fragment comprises an Fab, an Fab', an $F(ab')_2$, an Fv, or an scFv.

[0025] One embodiment provides an anti-TM4SF1 binding protein comprising a modified human IgG1 Fc region, wherein said modified human IgG1 Fc region comprises one or more amino acid substitutions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434, as numbered by the EU index as set forth in Kabat, wherein said anti-TM4SF1 binding protein demonstrates improved vascular safety compared to an otherwise identical binding protein that does not comprise an amino acid substitution selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434. In some embodiments said modified human IgG1 Fc region comprises a mutation at one or more positions selected from the group consisting of T250, M252, S254, T256, M428, and N434 as numbered by the EU index as set forth in Kabat. In some embodiments said modified human IgG1 Fc region comprises a mutation selected from the group consisting of T250Q, M252Y, S254T, T256E, M428L, and N434S, as numbered by the EU index as set forth in Kabat. In some embodiments, said modified human IgG1 Fc region comprises mutations T250Q and M428L. In some embodiments, said modified human

IgG1 Fc region comprises mutations M252Y, S254T, and T256E. In some embodiments, said modified human IgG1 Fc region comprises mutations M428L and N434S.

[0026] One embodiment provides an anti-TM4SF1 binding protein comprising a modified human IgG4 Fc region, wherein said modified human IgG4 Fc region comprises one or more amino acid substitutions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297, as numbered by the EU index as set forth in Kabat, wherein said anti-TM4SF1 binding protein demonstrates improved vascular safety compared to an otherwise identical binding protein that does not comprise an amino acid substitutions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297. In some embodiments, said modified human IgG4 Fc region comprises a mutation at one or more positions selected from the group consisting of T250, M428, and N434 as numbered by the EU index as set forth in Kabat. In some embodiments, said modified human IgG4 Fc region comprises a mutation selected from the group consisting of T250Q, M428L, and N434S as numbered by the EU index as set forth in Kabat. In some embodiments, said modified human IgG4 Fc region comprises mutations T250Q and M428L. In some embodiments, said modified human IgG4 Fc region comprises M428L and N434S. In some embodiments, said binding protein exhibits increased affinity to FcRn as compared to a control anti-TM4SF1 binding protein comprising a wild type IgG1 Fc or IgG4 Fc. In some embodiments, said anti-TM4SF1 binding protein comprises an anti-TM4SF1 antibody or an antigen binding fragment thereof. In some embodiments, said anti-TM4SF1 antibody or an antigen binding fragment thereof is conjugated to a therapeutic molecule, wherein said therapeutic molecule comprises at least one of: a small molecule, a degrader, a nucleic acid molecule, a CRISPR-Cas9 gene editing system, and a lipid nanoparticle, or any combinations thereof.

[0027] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or said antigen binding fragment thereof comprises a human IgG1 Fc region comprising a mutation at one or more positions selected from the group consisting of T250, M252, S254, T256, M428, and N434 as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG1 Fc region comprises a mutation selected from the group consisting of T250Q, M252Y, S254T, T256E, M428L, and N434S, as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG1 Fc region comprises mutations at

positions T250 and M428. In some embodiments, said human IgG1 Fc region comprises mutations T250Q and M428L. In some embodiments, said human IgG1 Fc region comprises mutations at positions M252, S254, and T256. In some embodiments, said human IgG1 Fc region comprises mutations M252Y, S254T, and T256E. In some embodiments, said human IgG1 Fc region comprises mutations at positions M428 and N434. In some embodiments, said human IgG1 Fc region comprises mutations M428L and N434S. In some embodiments, said human IgG1 Fc region further comprises a mutation at position N297. In some embodiments, said mutation at position N297 is N297C. In some embodiments, said human IgG1 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG1 Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, D265, N297, K322, and P331; as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG1 Fc region comprises a mutation selected from the group consisting of E233P, L234A, L235A, G237A, D265A, N297C, K322A, and P331G.

[0028] In some embodiments, said human IgG1 Fc region comprises 2, 3, 4, 5, 6, or 7 mutations selected from the group consisting of E233P, L234A, L235A, G237A, D265A, N297C, K322A, and P331G. In some embodiments, said human IgG1 Fc region comprises mutations L234A, L235A, and G237A. In some embodiments, said human IgG1 Fc region comprises mutations L234A, L235A, G237A, and P331G. In some embodiments, said human IgG1 Fc region comprises mutations L234A, L235A, G237A, K322A, and P331G. In some embodiments, said human IgG1 Fc region comprises mutations L234A, L235A, G237A, E233P, and P331G. In some embodiments, said human IgG1 Fc region comprises mutations L234A, L235A, G237A, and N297C. In some embodiments, said human IgG1 Fc region comprises mutations L234A, L235A, G237A, N297C, and P331G. In some embodiments, said human IgG1 Fc region comprises mutations L234A, L235A, G237A, N297C, K322A, and P331G. In some embodiments, said human IgG1 Fc region comprises mutations L234A, L235A, G237A, N297C, E233P, and P331G. In some embodiments, said human IgG1 Fc region comprises mutations L234A, L235A, G237A, D265A, N297C, K322A, and P331G.

[0029] One embodiment provides an antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or said antigen binding fragment thereof comprises a human IgG4 Fc region comprising a mutation at one or more positions selected from the group consisting of T250, M428, and N434 as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region comprises a mutation selected from the group

consisting of T250Q, M428L, and N434S as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region comprises mutations at positions T250 and M428. In some embodiments, said human IgG4 Fc region comprises mutations T250Q and M428L. In some embodiments, said human IgG4 Fc region comprises mutations at positions M428 and N434. In some embodiments, said human IgG4 Fc region comprises mutations M428L and N434S. In some embodiments, said human IgG4 Fc region further comprises a mutation at position N297. In some embodiments, said mutation at position N297 is N297C. In some embodiments, said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, and L235 as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region comprises a mutation selected from the group consisting of S228P, F234A, L235E, and N297C as numbered by the EU index as set forth in Kabat. In some embodiments, said human IgG4 Fc region comprises 2, 3,or 4, mutations selected from the group consisting of S228P, F234A, L235E, and N297C. In some embodiments, said IgG4 Fc region comprises a mutation at position S228. In some embodiments, said mutation at position S228 is S228P. In some embodiments, said IgG4 Fc region comprises mutations at positions S228 and L235. In some embodiments, said IgG4 Fc region comprises mutations S228P and L235E. In some embodiments, said IgG4 Fc region comprises mutations at positions S228, L235, and N297. In some embodiments, said IgG4 Fc region comprises mutations S228P, L235E, and N297C. In some embodiments, said antibody drug conjugate exhibits increased affinity to FcRn as compared to a control antibody drug conjugate comprising a wild type IgG1 Fc or IgG4 Fc.

[0030] In some embodiments, said therapeutic molecule comprises at least one of: a small molecule, a degrader, a nucleic acid molecule, a CRISPR-Cas9 gene editing system, and a lipid nanoparticle, or any combinations thereof. In some embodiments, said therapeutic molecule comprises at least one of: a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder, a DHFR inhibitor, a nucleic acid, a CRISPR enzyme, or any combinations

thereof. In some embodiments, said degrader comprises an agent that induces protein degradation. In some embodiments, said agent that induces protein degradation comprises a hydrophobic tag, a proteolysis inducing chimera, an HSP90 inhibitor, a selective estrogen receptor degrader (SERD), and a selective androgen receptor degrader (SARD), or any combinations thereof. In some embodiments, said lipid nanoparticle encapsulates one or more therapeutic molecules. In some embodiments, said nucleic acid molecule comprises an RNA molecule or a DNA molecule. In some embodiments, said RNA molecule comprises an siRNA, an antisense-RNA, an miRNA, an antisense miRNA, an antagomir (anti-miRNA), an shRNA, or an mRNA. In some embodiments, said anti-TM4SF1 antibody or an antigen binding fragment thereof and said therapeutic molecule are conjugated by a linker in a single or a multistep protocol. In some embodiments, said linker comprises a cleavable linker, a non-cleavable linker, a hydrophilic linker, a pro-charged linker, or a dicarboxylic acid based linker. In some embodiments, said cleavable linker comprises a cleavable covalent or non-covalent linker. In some embodiments, said linker comprises a non-cleavable covalent or non-covalent linker. In some embodiments, said cleavable linker comprises an acid-labile linker, a protease-sensitive linker, a photo-labile linker, or a disulfide-containing linker. In some embodiments, said linker comprises a cysteine linker or a non-cysteine linker. In some embodiments, said non-cysteine linker comprises a lysine linker. In some embodiments, said linker comprises a MC (6-maleimidocaproyl), a MCC (a maleimidomethyl cyclohexane-1-carboxylate), a MP (maleimidopropanoyl), a val-cit (valine-citrulline), a val-ala (valine-alanine), an ala-phe (alanine-phenylalanine), a PAB (p-aminobenzyloxycarbonyl), a SPP (N-Succinimidyl 4-(2-pyridylthio) pentanoate), 2,5-dioxopyrrolidin-1-yl 4-(pyridin-2-ylthio)hexanoate, 2,5-dioxopyrrolidin-1-yl 5-methyl-4-(pyridin-2-ylthio)hexanoate, 2,5-dioxopyrrolidin-1-yl 5-methyl-4-(pyridin-2-ylthio)heptanoate, 2,5-dioxopyrrolidin-1-yl 5-ethyl-4-(pyridin-2-ylthio)heptanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclopentyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclohexyl-4-(pyridin-2-ylthio)butanoate, a SMCC (N-Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 carboxylate), or a SIAB (N-Succinimidyl (4-iodo-acetyl)aminobenzoate). In some embodiments, said linker is derived from a cross-linking reagent, wherein the cross-linking reagent comprises N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), 2,5-dioxopyrrolidin-1-yl 3-cyclopropyl-3-(pyridin-2-yldisulfaneyl)propanoate, 2,5-dioxopyrrolidin-1-yl 3-cyclobutyl-3-(pyridin-2-yldisulfaneyl)propanoate, N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-yldisulfaneyl)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-yldisulfaneyl)butanoate, N-succinimidyl 4-(2-

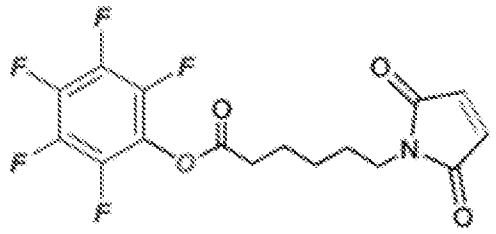
pyridyldithio)butanoate (SPDB), 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-yldisulfaneyl)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-yldisulfaneyl)butanoate, N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC), or 2,5-dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazaheptadecan-1-oate (CX1-1).

[0031] One embodiment provides a method of treating or preventing a disease or disorder in a subject, wherein the disease or disorder is characterized by abnormal endothelial cell (EC)- cell interaction, said method comprising administering to the subject an antibody-drug conjugate according to this disclosure. In some embodiments, the EC-cell interaction comprises one or more of EC-mesenchymal stem cell, EC-fibroblast, EC-smooth muscle cell, EC-tumor cell, EC-leukocyte, EC-adipose cell, and EC-neuronal cell interactions. In some embodiments, the disease or disorder comprises an inflammatory disease or a cancer. One embodiment provides a method of treating or preventing inflammation in a subject, said method comprising administering to the subject an antibody-drug conjugate according to this disclosure. One embodiment provides a method of treating or preventing metastasis in a subject, said method comprising administering to the subject an antibody-drug conjugate according to this disclosure, wherein the subject is in partial or complete remission from a cancer. One embodiment provides a method of treating a subject having a cancer which is associated with a high risk of metastasis, said method comprising administering an antibody-drug conjugate according to this disclosure, to the subject having the cancer which is associated with the high risk of metastasis. One embodiment provides a method of treating or preventing metastasis in a subject having a cancer, said method comprising administering an antibody-drug conjugate according to this disclosure, to the subject having the cancer. In some embodiments, the subject is undergoing a treatment which may induce metastasis. In some embodiments, the treatment comprises surgery, radiation treatment and chemotherapy. In some embodiments, the subject is a human. In some embodiments, the cancer is a carcinoma or a sarcoma. In some embodiments, the carcinoma comprises breast cancer, lung cancer, colon cancer, prostate cancer, pancreatic cancer, liver cancer, gastric cancer, renal cancer, bladder cancer, uterine cancer, cervical cancer, ovarian cancer. In some embodiments, the sarcoma comprises an angiosarcoma, an osteosarcoma, or a soft tissue sarcoma. In some embodiments, the cancer is a glioblastoma. One embodiment provides a method of treating or preventing lymphatic or hematogenous metastasis in a human subject comprising administering to the human subject an antibody-drug conjugate according to

this disclosure. In some embodiments, the antibody drug conjugate exhibits longer serum half-life after administration as compared to a control antibody drug conjugate comprising a wild type IgG1 Fc or IgG4 Fc.

[0032] One embodiment provides a pharmaceutical composition comprising (i) an antibody-drug conjugate according to this disclosure and (ii) a pharmaceutically acceptable carrier. One embodiment provides a pharmaceutical composition comprising the binding protein according to this disclosure.

[0033] One embodiment provides an antibody drug conjugate comprising an anti-TM4SF1 antibody or an antigen binding fragment thereof comprising a modified IgG Fc region comprising one or more mutations selected from the group consisting of: (i) S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297; or (ii) E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434, conjugated to a therapeutic molecule via a linker, wherein said linker is derived from a compound of Formula: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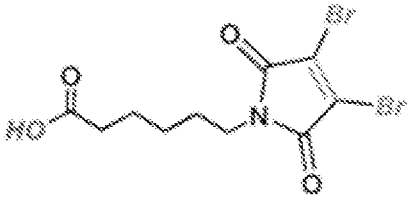
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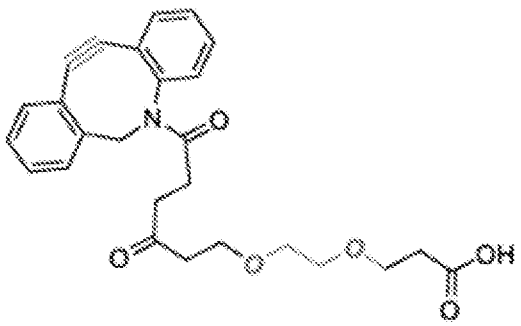
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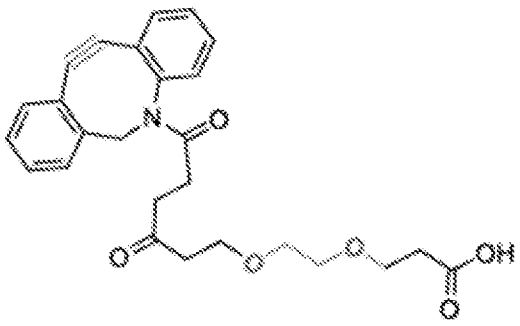
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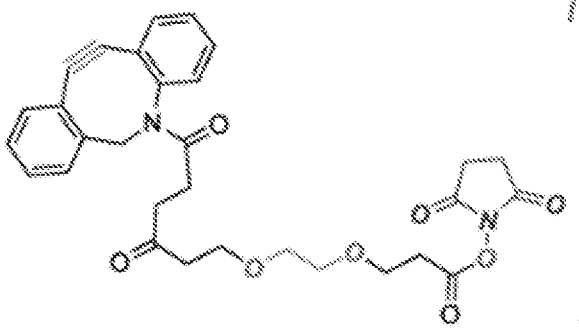
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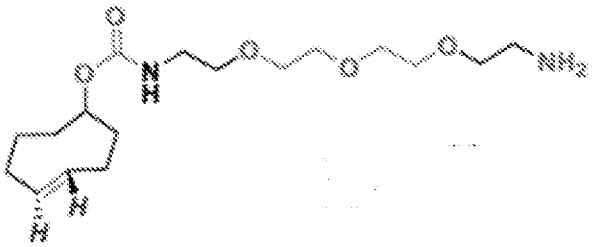
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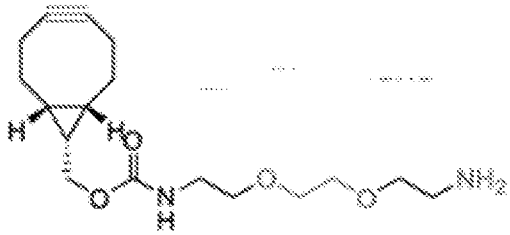
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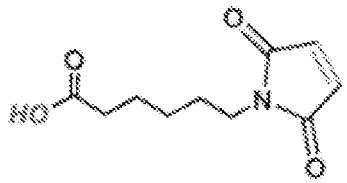
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DESCRIPTION OF THE DRAWINGS

[0034] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0035] FIG. 1 illustrates an exemplary antibody drug conjugate (ADC), using bromoacetamide conjugation.

[0036] FIG. 2 illustrates an exemplary ADC, using maleimide conjugation.

[0037] FIG. 3 illustrates the results of a study assessing the affinity of exemplary anti-TM4SF1 antibodies, in various endothelial cells.

[0038] FIG. 4 illustrates *in vivo* tissue distribution (large intestine, small intestine, stomach) of exemplary anti-TM4SF1 antibodies (murine surrogate, MS) containing various Fc mutations.

[0039] FIG. 5 illustrates *in vivo* tissue distribution (female reproductive tract, skin adjacent to a tumor, and tumor under the skin) of exemplary anti-TM4SF1 antibodies (murine surrogate, MS) containing various Fc mutations.

[0040] FIG. 6 illustrates hydrophobicity of exemplary anti-TM4SF1 antibodies (murine surrogate, MS; and anti-human AGX-A07), assessed by hydrophobic interaction chromatography (HIC).

[0041] FIG. 7 provides a spectrum showing drug to antibody (DAR) ratio of an exemplary anti-TM4SF1 antibody (murine surrogate, MS).

[0042] FIG. 8 provides the results of a study assessing *in vivo* tolerance of exemplary ADCs (maleimide conjugation) containing anti-TM4SF1 antibody (murine surrogate, MS), in mice, following administration at varying doses (40 mg/kg- left panel; 50 mg/kg- middle panel; and 60 mg/kg- right panel). The top half of the figure shows survival percentage and the bottom half shows body weight change, following administration of the ADC.

[0043] FIG. 9 provides the results of a study assessing *in vivo* tolerance of exemplary ADCs (bromoacetamide conjugation) containing anti-TM4SF1 antibodies (murine surrogate, MS), in mice, following administration at 60 mg/kg.

[0044] FIG. 10 provides the results of a pharmacokinetic study using ADCs containing exemplary anti-human TM4SF1 antibodies (AGX-A07) or murine surrogate (MS) TM4SF1 antibodies. The AGX-A07 containing ADCs were tested in cynomolgus monkeys and the MS containing ADCs were tested in mice.

[0045] FIG. 11 provides the results of an *in vivo* study assessing the efficacy of exemplary ADCs containing anti-TM4SF1 antibodies (murine surrogate, MS) containing various Fc region mutations, administered at two doses (12 mg/kg and 20 mg/kg), in regression of tumor growth in mice.

[0046] FIG. 12 provides the results of an *in vivo* study assessing the efficacy of exemplary ADCs containing anti-TM4SF1 antibodies (murine surrogate, MS) containing various Fc region mutations, administered at 24 mg/kg, in regressing of tumor growth in mice.

[0047] FIG. 13 provides the results of an *in vivo* study assessing the efficacy of ADCs containing anti-TM4SF1 antibodies (murine surrogate, MS; anti-human TM4SF1 antibodies (AGX-A07); or combinations of both) containing various Fc region mutations, administered at varying doses (3 mg/kg and 12 mg/kg), in regression of tumor growth in a xenograft model.

DETAILED DESCRIPTION OF THE INVENTION

[0048] Transmembrane-4 L six family member-1 (TM4SF1) is a small membrane glycoprotein with tetraspanin topology that is highly expressed on many human epithelial tumor cells and in endothelial cells, especially endothelial cells in angiogenic vessels.

[0049] Provided herein in one embodiment, is an antibody-drug conjugate (ADC) for a vascular-targeted therapy that, *e.g.*, can regress primary tumors by killing the endothelial cells of tumor blood vessels. This therapy may include various attractive features. Notably, (1) angiogenesis is a hallmark of cancer and a therapy that destroys angiogenic vessels can be a universal treatment for solid tumors; (2) the vascular endothelium is an unmutated host system and might be unable to evolve resistance to therapy. Thus, a vascular-targeted therapy may be able to overcome a common problem with tumor cell targeted therapies, wherein a target tissue evolves and becomes resistant to therapy; and (3) the vascular endothelium of tumors is directly exposed to intravenously (IV)-infused drugs and therefore can be accessible to drugs that cannot reach tumor cells. The inaccessibility of tumor cells can be a major problem in cancers such as pancreatic cancer which have a dense fibrotic stroma which limits access of drugs to tumor cells. A vascular targeted therapy, using an ADC that comprises an anti-TM4SF1 antibody, can advantageously reach the vascular endothelium of tumors.

[0050] In one embodiment, the disclosure provides antibody-drug conjugates (ADCs) comprising TM4SF1 binding proteins, such as anti-TM4SF1 antibodies, and antigen-binding fragments thereof. The disclosure includes, in some examples, methods of using the ADCs for treating or preventing cancer. The disclosure includes, in some embodiments, ADCs in which the drug payload conjugated to the antibody is comprised of a small molecule, RNA, DNA, degrader, protein, or combinations thereof.

I. Definitions

[0051] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting.

[0052] 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. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. 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, 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.

[0053] That the present disclosure may be more readily understood, select terms are defined below. The terms "transmembrane-4 L six family member-1" or "TM4SF1", as used herein refer to a polypeptide of the transmembrane 4 superfamily/tetraspanin family, which is highly expressed on tumor vasculature endothelial cells (ECs), tumor cells (TCs), ECs of developing retinal vasculature, and angiogenic blood vessels. TM4SF1 has two extracellular loops (ECL1 and ECL2) that are separated by four transmembrane domains (M1, M2, M3, and M4), the N- and C-termini, and the intracellular loop (ICL). ECL2 contains two N-glycosylation sites. The amino acid sequence of human TM4SF1 (hTM4SF1) is described in SEQ ID NO: 90 (see also NCBI Ref Seq No. NP_055035.1).

[0054] The term "antibody", as used herein, means any antigen-binding molecule comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.,* TM4SF1). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.,* IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region comprises one domain (CL1). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the disclosure, the FRs of the anti-TMS4F1 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0055] The term "intact antibody" refers to an antibody comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. In one embodiment, the anti-TM4SF1 antibody is an intact antibody. In one embodiment, the intact

antibody is an intact human IgG1, IgG2 or IgG4 isotype. In certain embodiments, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, is a human IgG1, IgG2, or IgG4 isotype.

[0056] The terms "antigen-binding portion" of an antibody, "antigen-binding fragment," or "antibody-fragment," of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from intact antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, e.g., phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0057] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide.

[0058] The term "variable region" or "variable domain" of an antibody, or fragment thereof, as used herein refers to the portions of the light and heavy chains of antibody molecules that include amino acid sequences of complementarity determining regions (CDRs; *i.e.*, CDR-1, CDR-2, and CDR-3), and framework regions (FRs). VH refers to the variable domain of the heavy chain. VL refers to the variable domain of the light chain. According to the methods used in this disclosure, the amino acid positions assigned to CDRs and FRs may be defined according to Kabat (Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md., 1987 and 1991)). Amino acid numbering of antibodies or antigen binding fragments is also according to that of Kabat.

[0059] The term "complementarity determining regions" or "CDRs" as used herein refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat *et al.*, Sequences of Proteins of

Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987) and Chothia *et al.*, Nature 342:877-883 (1989)) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J Mol Biol 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with 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. The methods used herein may utilize CDRs defined according to any of these systems, although preferred embodiments use Kabat or Chothia defined CDRs.

[0060] The term "framework regions" (hereinafter FR) as used herein refers to those variable domain residues other than the CDR residues. Each variable domain typically has four FRs identified as FR1, FR2, FR3 and FR4. Common structural features among the variable regions of antibodies, or functional fragments thereof, are well known in the art. The DNA sequence encoding a particular antibody can generally be found following well known methods such as those described in Kabat, *et al.* 1987 Sequence of Proteins of Immunological Interest, U.S. Department of Health and Human Services, Bethesda MD, which is incorporated herein as a reference. In addition, a general method for cloning functional variable regions from antibodies can be found in Chaudhary, V.K., *et al.*, 1990 Proc. Natl. Acad. Sci. USA 87:1066, which is incorporated herein as a reference.

[0061] The term "Fc region" herein is used to define a C-terminal region of an antibody heavy chain, including, for example, native sequence Fc regions, recombinant Fc regions, and variant Fc regions. Although the boundaries of the Fc region of an antibody heavy chain might vary, the human IgG heavy chain Fc region is often defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system as in Kabat *et al.*) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition

of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Further, a composition of intact antibodies in this disclosure may comprise antibody populations with extension of residues after the C-terminal lysine, K447.

[0062] The term "humanized antibody" as used herein refers to an antibody or a variant, derivative, analog or fragment thereof, which immunospecifically binds to an antigen of interest (*e.g.*, human TM4SF1), and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins that contain minimal sequences derived from non-human immunoglobulin. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin consensus sequence. Methods of antibody humanization are known in the art. See, *e.g.*, Riechmann *et al.*, 1988, Nature 332:323-7; U.S. Patent Nos: 5,530,101; 5,585,089; 5,693,761; 5,693,762; and 6,180,370 to Queen *et al.*; EP239400; PCT publication WO 91/09967; U.S. Patent No. 5,225,539; EP592106; EP519596; Padlan, 1991, Mol. Immunol., 28:489-498; Studnicka *et al.*, 1994, Prot. Eng. 7:805-814; Roguska *et al.*, 1994, Proc. Natl. Acad. Sci. 91:969-973; and U.S. Patent No. 5,565,332, all of which are hereby incorporated by reference in their entireties.

[0063] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible mutations, *e.g.*, naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. In contrast to polyclonal antibody preparations, which typically include different

antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal-antibody preparation is directed against a single epitope on an antigen.

[0064] The term "chimeric antibody" as used herein refers to antibodies (immunoglobulins) that have a portion of the heavy and/or light chain identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison *et al.*, Proc. Natl. Acad. Sci. USA 81 :6851-6855 (1984)).

[0065] The term "epitope" as used herein refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[0066] The terms "payload," "drug payload," "therapeutic molecule," therapeutic payload", "therapeutic agents," "therapeutic moieties," as used interchangeably herein, refers to a chemical or biological moiety that is conjugated to an anti-TMSF1 antibody or antigen binding fragment (*e.g.*, an anti-TM4SF1 antibody or antigen binding fragment disclosed herein), and can include any therapeutic or diagnostic agent, for example, but not limited to, small molecules, both for cancer and for non-cancer angiogenic indications; a V-ATPase inhibitor; a pro-apoptotic agent; a Bcl2 inhibitor; an MCL1 inhibitor; a HSP90 inhibitor; an IAP inhibitor; an mTor inhibitor; a microtubule stabilizer; a microtubule destabilizer; an auristatin; a dolastatin; a maytansinoid; a MetAP (methionine aminopeptidase); an inhibitor of nuclear export of proteins CRM1; a DPPIV inhibitor; proteasome inhibitors; inhibitors of phosphoryl transfer reactions in mitochondria; a protein synthesis inhibitor; a kinase inhibitor (such as, a CDK2 inhibitor, a CDK9 inhibitor); a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder, a DHFR inhibitor, a nucleic acid, a CRISPR enzyme; degraders (such as agents that induce protein degradation, (*e.g.*, HSP90 inhibitor, selective

estrogen receptor degraders (SERDs), selective androgen receptor degraders (SARDs); hydrophobic tags that can be used to recruit chaperones to a protein of interest, *e.g.*, Adamantane, Arg-Boc3; E3 ligase recruiting ligands, *e.g.*, Nutlin-3a (MDM2 ligand), Bestatin (cIAP ligand), VHL ligand, Pomalidomide (CRBN ligand); proteolysis-targeting chimeras (PROTACs) that may utilitize different D3 ligases to target a protein of interest for degradation)) (*see, e.g.*, Lai AC, Crews CM. Induced protein degradation: an emerging drug discovery paradigm. Nat Rev Drug Discov. 2016;16(2):101-114); antisense oligonucleotides; RNAi agents (such as siRNA), CRISPR-Cas9 gene editing systems; RNA molecules; DNA *e.g.*, plasmids; an anti-cancer agent, an anti-inflammatory agent, an anti-infective agent (*e.g.*, anti-fungal, antibacterial, anti-parasitic, anti-viral), an anesthetic agent; RNA polymerase II inhibitor; a DNA intercalating agent, a DNA cross-linking agent; an anti-tubulin agent; a cytotoxic drug, a tumor vaccine, an antibody, a peptide, pepti-bodies, a chemotherapeutic agent, a cytotoxic agent; a cytostatic agent; an immunological modifiers, an interferon, an interleukin, an immuno stimulatory growth hormone, a cytokine, a vitamin, a mineral, an aromatase inhibitor, a Histone Deacetylase (HDAC), an HDAC inhibitor; a lipid nanoparticle to encapsulate one or more therapeutic molecules.

[0067] The term "drug-to-antibody ratio" or "DAR"can refer to the number of drugs (also referred to herein as therapeutic molecules, therapeutic agents, or therapeutic moieties), attached to an anti-TM4SF1 antibody or antigen binding fragments thereof, of the ADCs disclosed herein. The DAR of an ADC typically ranges from 1 to 12, although higher loads, *e.g.*, 16, are also possible depending on the number of linkage sites on an antibody or the use of multivalent linkages in which multiple drug payloads are attached to one linkage site. The term DAR may be used in reference to the number of drug molecules loaded onto an individual antibody, or, alternatively, may be used in reference to the average or mean DAR of a group of ADCs to reflect average drug loading. Compositions, batches, and/or formulations of a plurality of ADCs may be characterized by an average DAR. DAR and average DAR can be determined by various conventional means such as UV spectroscopy, mass spectroscopy, ELISA assay, radiometric methods, hydrophobic interaction chromatography (HIC), electrophoresis and HPLC.

[0068] The term "binding affinity" generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.*, a binding protein such as an antibody) and its binding partner (*e.g.*, an antigen). The affinity of a binding molecule X (*e.g.,* anti-TM4SF1 antibody) for its binding partner Y (*e.g.,* human TM4SF1) can generally be represented by the dissociation constant (K_D). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding

affinity are known in the art, any of which can be used for purposes of the present disclosure. Specific illustrative embodiments include the following. In one embodiment, the "K_D" or "K_D value" may be measured by assays known in the art, for example by a binding assay. The K_D may be measured in a RIA, for example, performed with the Fab version of an antibody of interest and its antigen (Chen et al., 1999, J. Mol Biol 293:865-81). The K_D may also be measured by using FACS or surface plasmon resonance assays by BIACORE, using, for example, a BIACORE 2000 or a BIACORE 3000, or by biolayer interferometry using, for example, the OCTET QK384 system. In certain embodiments, the K_D of an anti-TM4SF1 antibody is determined using a standard flow cytometry assay with HUVEC cells. An "on-rate" or "rate of association" or "association rate" or "k_{on}" and an "off-rate" or "rate of dissociation" or "dissociation rate" or "k_{off}" may also be determined with the same surface plasmon resonance or biolayer interferometry techniques described above using, for example, a BIACORE 2000 or a BIACORE 3000, or the OCTET QK384 system.

[0069] The term "k_{on}", as used herein, is intended to refer to the on rate constant for association of an antibody to the antigen to form the antibody/antigen complex, as is known in the art.

[0070] The term "k_{off}", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex, as is known in the art.

[0071] The term "inhibition" or "inhibit," when used herein, refers to partial (such as, 1%, 2%, 5%, 10%, 20%, 25%, 50%, 75%, 90%, 95%, 99%) or complete (i.e., 100%) inhibition.

[0072] The term "cancer" as used herein, refers to or describes the physiological condition in mammals that is typically characterized by unregulated cell growth.

[0073] The term "cancer which is associated with a high risk of metastasis", as used herein, refers to a cancer that is associated with at least one factor known to increase the risk that a subject having the cancer will develop metastatic cancer. Examples of factors associated with increased risk for metastasis include, but are not limited to, the number of cancerous lymph nodes a subject has at the initial diagnosis of cancer, the size of the tumor, histological grading, and the stage of the cancer at initial diagnosis.

[0074] The term "hematogenous metastasis" as used herein refers to the ability of cancer cells to penetrate the walls of blood vessels, after which they are able to circulate through the bloodstream (circulating tumor cells) to other sites and tissues in the body.

[0075] The term "lymphatic metastasis" as used herein refers to the ability of cancer cells to penetrate lymph vessels and drain into blood vessels.

[0076] In the context of the disclosure, the term "treating" or "treatment", as used herein, means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. By the term "treating

cancer" as used herein is meant the inhibition of the growth and/or proliferation of cancer cells. In one embodiment, the compositions and methods described herein are used to treat metastasis in a subject having metastatic cancer.

[0077] The term "preventing cancer" or "prevention of cancer" refers to delaying, inhibiting, or preventing the onset of a cancer in a mammal in which the onset of oncogenesis or tumorigenesis is not evidenced but a predisposition for cancer is identified whether determined by genetic screening, for example, or otherwise. The term also encompasses treating a mammal having premalignant conditions to stop the progression of, or cause regression of, the premalignant conditions towards malignancy. Examples of premalignant conditions include hyperplasia, dysplasia, and metaplasia. In some embodiments, preventing cancer is used in reference to a subject who is in remission from cancer.

[0078] A variety of cancers, including malignant or benign and/or primary or secondary, may be treated or prevented with a method according to the disclosure. Examples of such cancers are known to those skilled in the art and listed in standard textbooks such as the Merck Manual of Diagnosis and Therapy (published by Merck).

[0079] The term "subject" as used herein, refers to a mammal (*e.g.*, a human).

[0080] The term "administering" as used herein refers to a method of giving a dosage of an antibody or fragment thereof, or a composition (*e.g.*, a pharmaceutical composition) to a subject. The method of administration can vary depending on various factors (*e.g.*, the binding protein or the pharmaceutical composition being administered and the severity of the condition, disease, or disorder being treated).

[0081] The term "effective amount" as used herein refers to the amount of an antibody or pharmaceutical composition provided herein which is sufficient to result in the desired outcome.

[0082] The terms "about" and "approximately" mean within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, within 1%, or less of a given value or range.

[0083] The term "identity," or "homology" as used interchangeable herein, may be to calculations of "identity," "homology," or "percent homology" between two or more nucleotide or amino acid sequences that can be determined by aligning the sequences for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first sequence). The nucleotides at corresponding positions may then be compared, and the percent identity between the two sequences may be a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100). For example, a position in the first sequence may be occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent homology

between the two sequences may be 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. In some embodiments, the length of a sequence aligned for comparison purposes may be at least about: 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 95%, of the length of the reference sequence. A BLAST® search may determine homology between two sequences. The two sequences can be genes, nucleotides sequences, protein sequences, peptide sequences, amino acid sequences, or fragments thereof. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A non-limiting example of such a mathematical algorithm may be described in Karlin, S. and Altschul, S., Proc. Natl. Acad. Sci. USA, 90- 5873-5877 (1993). Such an algorithm may be incorporated into the NBLAST and XBLAST programs (version 2.0), as described in Altschul, S. et al., Nucleic Acids Res., 25:3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, any relevant parameters of the respective programs (e.g., NBLAST) can be used. For example, parameters for sequence comparison can be set at score= 100, word length= 12, or can be varied (e.g., W=5 or W=20). Other examples include the algorithm of Myers and Miller, CABIOS (1989), ADVANCE, ADAM, BLAT, and FASTA. In another embodiment, the percent identity between two amino acid sequences can be accomplished using, for example, the GAP program in the GCG software package (Accelrys, Cambridge, UK).

[0084] The term "manufacturability," as used herein, refers to the stability of a particular protein during recombinant expression and purification of that protein. Manufacturability is believed to be due to the intrinsic properties of the molecule under conditions of expression and purification. Examples of improved manufacturability characteristics include uniform glycosylation of a protein, increased cell titer, growth and protein expression during recombinant production of the protein, improved purification properties, less propensity of aggregation or non-aggregation, and improved stability, including, but not limited to, thermal stability and stability at low pH. In some embodiments are provided TM4SF1 binding proteins that demonstrate the manufacturability, along with retention of *in vitro* and *in vivo* activity, compared with other TM4SF1 antibodies. In some embodiments, humanization of a parent TM4SF1 binding protein, by making amino acid substitutions in the CDR or framework regions, can confer additional manufacturability benefits.

[0085] In some embodiments are provided TM4SF1 binding proteins that demonstrate improved developability characteristics, including, but not limited to improved purification yield, for example, after protein A purification or size exclusion chromatography, improved homogeneity after purification, improved thermal stability. In some cases, the improvement is with respect to

an anti-TM4SF1 antibody produced by a hybridoma mouse cell line 8G4-5-13-13F (PTA-120523), as determined by HLA molecule binding.

[0086] In some examples, binding affinity is determined by Scatchard analysis, which comprises generating a Scatchard plot, which is a plot of the ratio of concentrations of bound ligand to unbound ligand versus the bound ligand concentration.

[0087] The term "vascular toxicity" refers to any effect of an anti-TM4SF1 antibody-therapeutic molecule conjugate (also referred to herein as anti-TM4SF1 ADC or TM4SF1 targeted ADC) which leads to vascular injury either directly due to the antibody or the therapeutic molecule effects on antigen-bearing cells or indirectly through activation of the immune system and resulting inflammation. Such vascular injury may include, but is not limited to, damage or inflammation affecting vascular endothelial cells or underlying smooth muscle cells or pericytes or the basement membrane of any blood vessel, including the endocardium (lining of the heart). Such vascular injury may affect arteries, including major arteries such as the aorta, elastic arteries (such as the aorta), muscuar arteries of varying sizes, such as coronary artery, pulmonary artery, carotid artery, arterioles, capillaries, arteries of the brain or retina; venues, veins; or it may affect angiogenic vessels including vessels serving hair follicles, the digestive tract, and bone marrow. Such vascular injury may include microvascular dysfunction or damage in the heart, lung, kidney, retina, brain, skin, liver, digestive tract, bone marrow, endocrine glands, testes or ovaries, endometrium, and other target organs and may include renal, retinal or cerebrovascular circulation dysfunction.

[0088] The term "antibody-dependent cell-mediated cytotoxicity (ADCC)" as used herein refers to the killing of an antibody-coated target cell by a cytotoxic effector cell through a nonphagocytic process, characterized by the release of the content of cytotoxic granules or by the expression of cell death- inducing molecules. ADCC is triggered through interaction of target-bound antibodies (belonging to IgG or IgA or IgE classes) with certain Fc receptors (FcRs), glycoproteins present on the effector cell surface that bind the Fc region of immunoglobulins (Ig). Effector cells that mediate ADCC include natural killer (NK) cells, monocytes, macrophages, neutrophils, eosinophils and dendritic cells. ADCC is a rapid effector mechanism whose efficacy is dependent on a number of parameters (density and stability of the antigen on the surface of the target cell; antibody affinity and FcR-binding affinity). PBMC-based ADCC assays and natural kill cell-based ADCC assays can be used to detect ADCC. The readout in these assays is endpoint-driven (target cell lysis).

[0089] The term "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 (*See, e.g.*, Gazzano-Santoro et al., 1996, J. Immunol. Methods 202:163) may be performed. Polypeptide variants with altered Fc region amino acid sequences (polypeptides with a variant Fc region) and increased or decreased C1q binding capability have been described (see, e.g., U.S. Pat. No. 6,194,551; WO 1999/51642; Idusogie et al., 2000, J. Immunol. 164: 4178-84). Antibodies (or fragments) with little or no CDC activity may be selected for use.

[0090] The term "effector function" as used herein refers to a function contributed by an Fc effector domain(s) of an IgG (*e.g.*, the Fc region of an immunoglobulin). Such function can be effected by, for example, binding of an Fc effector domain(s) to an Fc receptor on an immune cell with phagocytic or lytic activity or by binding of an Fc effector domain(s) to components of the complement system. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis (ADCP); down regulation of cell surface receptors (*e.g.* B cell receptor); and B cell activation.

[0091] The terms "reduce" or "ablate" as used herein refers to the ability to cause an overall decrease preferably of 20% or greater, more preferably of 50% or greater, and most preferably of 75%, 85%, 90%, 95%, or greater. Reduce or ablate can refer to binding affinity of two molecules, for example the binding of immunoglobulins to C1q or to Fc receptors; or can refer to the symptoms of the disorder (*e.g.*, cancer) being treated, such as the presence or size of metastases or the size of the primary tumor.

[0092] The term "reduced ADCC/CDC function," as used herein refers to a reduction of a specific effector function, *e.g.* ADCC and/or CDC, in comparison to a control (for example an antibody with a Fc region not including the mutation(s)), by at least about 5%, at least about 10%, at least about 15%, 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% at least, at least about 90% or more.

[0093] For all amino acid positions discussed in the present disclosure, in the context of antibodies or antigen binding fragments thereof, numbering is according to the EU index. The "EU index" or "EU index as in Kabat et al." or "EU numbering scheme" refers to the numbering of the EU antibody (*See* Edelman *et al.*, 1969; Kabat *et al.*, 1991).

II. Antibody-drug conjugates containing anti-TM4SF1 antibodies or antigen binding fragments thereof, with modified Fc regions and/or CDR regions

[0094] One embodiment of the disclosure provides antibody-drug conjugates (ADCs) comprising an anti-TM4SF1 antibody or an antigen binding fragment thereof linked to a therapeutic molecule, wherein the anti-TM4SF1 antibody or antigen binding fragment thereof comprises a modified Fc region, such as a modified IgG region (*e.g.*, IgG1, IgG2, IgG3, IgG4) comprising one or more mutations. In some cases, said one or more mutations in the Fc region leads to improvements in a drug comprising such a modified Fc region, in areas of improvement such as: 1) reduction of effector functions, 2) half-life modulation, 3) stability, and 4) downstream processes. In some cases, the modified Fc region can comprise one or more mutations that will reduce or ablate interactions between the antibodies and the immune system. Key interactions may include interactions of the antibody Fc with Fcγ receptors on white blood cells and platelets, and with C1q of the complement system leading to complement dependent cytotoxicity.

[0095] The present disclosure provides, in some cases, an ADC comprising an anti-TM4SF1 antibody or an antigen binding fragment thereof that includes immune ablating mutations, for example, in the Fc region which in such cases is a modified Fc region, for example, a modified IgG Fc region. In some embodiments, the modified Fc region comprises a modification at position N297. In some embodiments, the modified Fc region comprises a modified IgG Fc region (*e.g.*, a modified IgG1, IgG2, IgG3, or IgG4 Fc region) comprising one or more mutations at positions E233, L234 or F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, N297, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, and H435, or any combinations thereof. In some embodiments, the Fc region comprises an extension of residues at its C-terminus, such that positive charge is maintained at the C-terminus (*e.g.*, in some cases, if the anti-TM4SF1 antibody or antigen binding fragment comprises two heavy chains then at least one heavy chain comprises an extension of residues at the C-terminus). Such extension of residues can comprises addition of one or more amino acids, such as, arginine, lysine, proline, or any combinations thereof. In some examples, the extended C-terminus of the Fc regions leads to reduced CDC function of the anti-TM4SF1 antibody or antigen binding fragment thereof, and that of an ADC comprising the anti-TM4SF1 antibody or antigen binding fragment thereof. Such an effect is seen, in some cases, by addition of KP residues after K447 of Fc in IgG1 or IgG4, alone or in combination with other mutations (*e.g.*, K322A, P331G-IgG1).

[0096] In some embodiments, an anti-TM4SF1 antibody or an antigen binding fragment thereof can comprise an antibody with reduced effector function, including substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (*See, e.g.*, U.S. Patent No. 6,737,056). In some cases, such mutations in the Fc region may comprise substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, for example, substitution of residues 265 and

297 to alanine (DANA mutations, *i.e.*, D265A and N297A) (*See, e.g.*, US Pat. No. 7,332,581). In some cases, mutations in the Fc region may comprises substitutions at one or more amino acid positions E233, L234, L235, G237, D265, N297, K322, and P331. In some cases, mutations in the Fc region may comprises at least one of E233P, L234A, L235A, G237A, D265A, N297A, K322A, and P331G, or any combinations thereof. For instance, the mutations in the Fc region can comprise L234A/L235A/G237A (IgG1), or F234A/L235E (IgG4), and an anti-TM4SF1 antibody or antigen binding fragment comprising such mutations may exhibit altered FcgRI interactions.

[0097] In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include an Fc variant comprising the following mutations: an amino acid substitution at position M428 and N434 (M428L, N434S) (*See, e.g.*, US 9803023). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include an Fc variant comprising the following mutations: an amino acid substitution at position T250 and M428 (T250Q, M428L) (*See, e.g.*, US 9803023).

[0098] In some embodiments, the TM4SF1 antibody or antigen binding fragment thereof may comprise mutations D265A and N297A. In some cases, the proline at position 329 (P329) of a wild-type human Fc region may be substituted with glycine or arginine or an amino acid residue large enough to destroy the proline sandwich within the Fc/Fcy receptor interface, that is formed between the P329 of the Fc and tryptophan residues W87 and WHO of FcgRIII (*See, e.g.*, Sondermann *et al.*, Nature 406, 267-273 (20 July 2000)). In a further embodiment, the mutations in the Fc region may comprise one or more amino acid substitutions such as S228P (IgG4), E233P, L234A, L235A, L235E, N297A, N297D, or P331S and in still in other embodiments: L234A and L235A of the human IgGl Fc region or S228P and F234A, L235A, or L235E of the human IgG4 Fc region.

[0099] In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include a modified Fc region which is an Fc variant of a wild-type human IgG Fc region wherein P329 of the human IgG Fc region substituted with glycine and wherein the Fc variant comprises at least two further amino acid substitutions at L234A and L235A of the human IgGl Fc region or S228P and L235E of the human IgG4 Fc region, and wherein the residues are numbered according to the EU numbering (*See, e.g.*, US 8969526). The polypeptide comprising the P329G, L234A and L235A substitutions may exhibit a reduced affinity to the human FcyRIIIA and FcyRIIA, for down-modulation of ADCC to at least 20% of the ADCC induced by the polypeptide comprising the wildtype human IgG Fc region, and/or for down-modulation of ADCP (*See, e.g.*, US 8969526).

[0100] In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include an Fc variant comprising triple mutations: an amino acid substitution at position P329, a L234A and a L235A mutation (P329 / LALA) (*See, e.g.*, US 8969526).

[0101] Certain anti-TM4SF1 antibodies or antigen binding fragments of this disclosure, in some embodiments, can comprise mutations that exhibit improved or diminished binding to FcRs. (*See, e.g.*, US 6737056; WO 2004/056312, and Shields *et al.*, J. Biol. Chem. 9(2): 6591-6604 (2001).)

[0102] In some instances, an anti-TM4SF1 antibody or antigen binding fragment may include an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region. Alterations may be made in the Fc region that result in altered (*i.e.*, either improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US 6194551, WO 99/51642, and Idusogie et al. (2000) J. Immunol. 164: 4178- 4184.

[0103] Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn). FcRn, named after its function for the transfer of maternal IgGs to the fetus, also serves to prevent antibodies from being degraded in lysosomes, by capturing them in endosomes and returning them to circulation. (*See, e.g.*, Guyer et al., J. Immunol. 117:587 (1976) and Kim *et al.*, J. Immunol. 24:249 (1994)), are described in US2005/0014934. Without being bound by any particular theory, it is contemplated that antibodies with improved binding to FcRn detach from TM4SF1 and bind to FcRn, which then recycles the ADC back to circulation, thus reducing vascular toxicity. In some embodiments herein are provided anti-TM4SF1 antibodies or antigen binding fragments that comprise an Fc region with one or more substitutions that enhance FcRn recycling. In some embodiments herein are provided anti-TM4SF1 antibodies or antigen binding fragments thereof that comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn, such as, substitutions at one or more of positions: 238, 250, 252, 254, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 428, 424, 434, and 435, *e.g.*, substitution of Fc region residue 434 (US 7371826) according to EU numbering. *See also* Duncan & Winter, Nature 322:738-40 (1988); US 5648260; US 5624821; US2005/0014934 and WO 94/29351 concerning other examples of Fc region variants, the entirety of which are incorporated herein by reference.

[0104] In some embodiments, provided herein are anti-TM4SF1 antibodies or antigen binding fragments thereof that have pH dependent FcRn binding affinities. Without being bound by any particular theory, it is contemplated that ADC antibodies or antigen binding fragments thereof with pH dependent FcRn binding affinity detach from FcRn at pH >7, and bind to FcRn at pH 6. Accordingly, FcRn in acidic pH subcellular organelles, e.g. endosomes, binds such antibodies

and carries the antibodies back to the cell membrane, and release the antibodies into plasma at pH >7, recycling the antibody and avoiding lysosomal release of ADC payloads.

[0105] In certain embodiments, herein are provided anti-TM4SF1 antibodies or antigen binding fragments thereof that comprise an Fc region with one or more substitutions therein which modulate FcRn recycling. In some embodiments herein are provided anti-TM4SF1 antibodies or antigen binding fragments thereof that comprise one or more substitutions that enhance FcRn binding at acidic pH, e.g., pH 6, and does not affect FcRn binding at neutral or basic pH, e.g. pH 7. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may comprise substitutions at one or more of positions 250, 252, 254, 256, 428, and 434 according to EU numbering. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include an Fc variant comprising one or more of substitutions T250Q, M252Y, S254T, T256E, M428L, and N434S. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include an IgG1 Fc variant comprising substitutions T250Q and M428L (the "QL mutant"). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include an IgG4 Fc variant comprising substitutions T250Q and M428L (the "QL mutant").In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include an IgG1 Fc variant comprising substitutions M252Y, S254T, and T256E (the "YTE mutant"). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include an IgG1 Fc variant comprising substitutions M428L and N434S (the "LS mutant"). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof may include an IgG4 Fc variant comprising substitutions M428L and N434S (the "LS mutant"). Effects of amino acid substitutions in the Fc region that modulate FcRn recycling are described in, e.g. Hamblett et al., Mol. Pharm. 13(7): 2387-96 (2016); Dall'Acqua et al., J. Biol. Chem. 281(33): 23514-24 (2006), Hinton et al., J. Biol. Chem. 279(8): 6213-6 (2003), Hinton et al., J. Immunol., 176(1): 346-56 (2006), US20080181887, US 7361740, and EP2235059, the entirety of which are incorporated herein by reference.

[0106] In certain embodiments, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising one or more substitutions selected from the group consisting of T250Q, M252Y, S254T, T256E, M428L, and N434S. In some embodiments, an anti-TM4SF1 antibody, or antigen binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising one or more substitutions selected from the group consisting of T250Q, M252Y, S254T, T256E, M428L, and N434S. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof is an IgG1 isotype and comprises an Fc region comprising substitutions T250Q and M428L. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof is an IgG1 isotype and comprises an Fc variant

comprising substitutions M252Y, S254T, and T256E. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof is an IgG4 isotype and comprises an Fc variant comprising substitutions M252Y, S254T, and T256E. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof is an IgG1 isotype and comprises an Fc variant comprising substitutions M428L and N434S. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof is an IgG4 isotype and comprises an Fc variant comprising substitutions M428L and N434S.

[0107] In certain embodiments, the ADCs disclosed herein exhibit reduced vascular toxicity, reduced lysosomal toxicity, improved efficacy, and/or improved therapeutic margin. In some embodiments, the ADCs disclosed herein comprise anti-TM4SF1 antibodies or antigen binding fragments thereof comprising mutated Fc regions that have increased FcRn binding affinity and increased serum half life. In certain embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprising mutated Fc regions have serum half life of at least 10 days, at least 15 days, at least 20 days, at least 25 days, at least 30 days, at least 35 days, at least 40 days, at least 50 days, at least 60 days, at least 70 days, at least 80 days, at least 90 days, at least 100 days or more. In some embodiments,

[0108] In certain embodiments, the ADCs of this disclosure exhibit reduced vascular toxicity, improved therapeutic margin, or both. In certain embodiments the ADCs of this disclosure comprise anti-TM4SF1 antibodies or antigen binding fragments thereof comprising mutated Fc regions that have reduced or ablated affinity for an Fc ligand responsible for facilitating effector function compared to an antibody having the same amino acid sequence as the antibody of the disclosure but not comprising the addition, substitution, or deletion of at least one amino acid residue to the Fc region (also referred to herein as an “unmodified antibody”).

[0109] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof comprises an Fc region comprising at least two mutations that reduce or ablate ADCC and/or CDC effector function of the antibody, or antigen-binding fragment thereof. In further embodiments, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, comprises an Fc region comprising at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten or more mutations that reduce or ablate ADCC and/or CDC effector function of the antibody, or antigen-binding fragment thereof.

[0110] In certain embodiments, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising one or more mutations selected from the group consisting of E233P, L234V, L234A, L235A, G236Delta (deletion), G237A, V263L, N297A, N297D, N297G, N297Q, K322A, A327G, P329A, P329G, P329R, A330S, P331A, P331G, and P331S.

[0111] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising an L234A/L235A mutation, with or without a G237A mutation. In one embodiment, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising L234A, L235A, and G237A mutations.

[0112] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising an A327G/A330S/P331S mutation.

[0113] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising an E233P/L234V/L235A/delta G236 (deletion) mutation, which provides reduced binding to FcγRI (also referred to herein as FcgRI), FcγRIIA (also referred to herein as FcgRIIA), FcγRIIIA (also referred to herein as FcgRIIIAI) and reduced ADCC and CDC effector function, as described, for example, in An Z *et al.* Mabs 2009 Nov-Ec; 1(6):572-9, incorporated by reference in its entirety herein.

[0114] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising an N297x mutation, where x = A, D, G, Q.

[0115] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising an A327G/A330S/P331S mutation.

[0116] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising a mutation in one or more of K322A, P329A, and P331A, which provides reduced binding to C1q, as described, for example, in Canfield &Morrison. J Exp Med (1991) 173(6):1483–91.10.1084, incorporated by reference in its entirety herein.

[0117] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising a V263L mutation, which provides enhanced binding to FcγRIIB (also referred to herein as FcgRIIB) and enhanced ADCC, as described in, for example, Hezareh *et al.* J Virol. 2001 Dec;75(24):12161-8, incorporated by reference in its entirety herein.

[0118] In other embodiments, an anti-TM4SF1 antibody or antigen-binding fragment thereof is an IgG1 isotype and comprises an Fc region comprising a L234A/L235A, G237A or L235E mutation.

[0119] In other embodiments, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG1 isotype and comprises an Fc region comprising a L234F, L235E or P331S mutation.

[0120] In certain embodiments, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG2 isotype and comprises an Fc region comprising a one or more mutations selected from the group consisting of V234A, G237A, P238S, H268A or H268Q, V309L, A330S and P331S.

[0121] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG2 isotype and comprises an Fc region comprising an A330S/P331S mutation.

[0122] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG2 isotype and comprises an Fc region comprising an A330S/P331S, V234A/G237A /P238S/H268A/V309L/A330S/P331S or H268Q/V309L/A330S/P331S mutation.

[0123] In other embodiments, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising a one or more mutations selected from the group consisting of S228P, E233P, F234A, F234V, L235E, L235A, G236Delta (deletion), N297A, N297D, N297G, N297Q, P329G, P329R.

[0124] In certain embodiments, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising an S228P mutation, which provides reduced Fab-arm exchange and reduced aggregation, as described for example in Chappel *et al.* Proc Natl Acad Sci U S A (1991) 88(20):9036–40, incorporated by reference in its entirety herein.

[0125] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising an S228P/L235E mutation.

[0126] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising an S228P/E233P/F234V/L235A/delta G236 (deletion) mutation.

[0127] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising an N297x mutation, where x = A, D, G, Q.

[0128] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising an S228P/F234A/L235A mutation.

[0129] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising a L235E mutation, which provides reduced binding to FcγRI, FcγRIIA, FcγRIIIA and reduced ADCC and CDC effector activity, as described in, for example, Saxena *et al.* Front Immunol. 2016 Dec 12; 7:580.

[0130] In other embodiments, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising a S228P/F234A/L235A or E233P/L235A/G236Delta mutation.

[0131] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising at least a S228P mutation. *See, e.g.*, Angal *et al.* (Mol Immunol. 1993 Jan;30(1):105-8) describe an analysis of the hinge sequences of human IgG4 heavy chains to determine that the presence of serine at residue 241 (according to EU numbering system, and now corresponding to residue 228 in Kabat numbering,) as the cause

of heterogeneity of the inter-heavy chain disulphide bridges in the hinge region in a proportion of secreted human IgG4. Silva *et al.* (J Biol Chem. 2015 Feb 27;290(9):5462-9) describe the S228P mutation in human IgG4 that prevents in vivo and in vitro IgG4 Fab-arm exchange.

[0132] In other embodiments, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 isotype and comprises an Fc region comprising a L235E or S228P mutation.

[0133] In other embodiments, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 or IgG1 isotype and comprises an Fc region comprising a N297A, N297D or N297G mutation.

[0134] In other embodiments, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, is an IgG4 or IgG1 isotype and comprises an Fc region comprising a P329G, P329R mutation.

[0135] In one exemplary embodiment, the mutated Fc region of any IgG isotype comprises one or more mutations at positions 234, 235, 236, 237, 297, 318, 320, 322 (as described in WO1988007089, incorporated by reference in its entirety herein). Other possible mutations in the Fc region, including substitutions, deletions and additions are also described in, for example, US20140170140, WO2009100309, US20090136494 and US8969526, incorporated by reference in their entireties herein.

[0136] *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction or ablation of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, RII and RIII. Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see, *e.g.* Hellstrom, I., *et al.*, Proc. Nat'l Acad. Sci. USA 83 (1986) 7059-7063) and Hellstrom, I., *et al.*, Proc. Nat'l Acad. Sci. USA 82 (1985) 1499-1502; U.S. Pat. No. 5,821,337 (see Bruggemann, M., *et al.*, J. Exp. Med. 166 (1987) 1351-1361). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI.TM. non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96.RTM. non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (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. Nat'l Acad. Sci. USA 95 (1998) 652-656. C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro, *et al.*, J. Immunol. Methods 202

(1996) 163; Cragg, M. S., *et al.*, Blood 101 (2003) 1045-1052; and Cragg, M. S., and Glennie, M. J., Blood 103 (2004) 2738-2743). FcRn binding and in vivo clearance/half- life determinations can also be performed using methods known in the art (see, *e.g.*, Petkova, S. B., *et al.*, Int'l. Immunol. 18(12) (2006) 1759-1769).

[0137] In some embodiments, the mutated Fc region of any IgG isotype comprises a mutation at position L328, such as L328M, L328D, L328E, L328N, L328Q, L328F, L328I, L328V, L328T, L328H, L328A (*see e.g.*, US20050054832)

[0138] In one embodiment, antibodies, or antigen-binding fragments thereof, of the disclosure exhibit reduced or ablated ADCC effector function as compared to unmodified antibodies. In another embodiment, antibodies, or antigen-binding fragments thereof, of the disclosure exhibit reduced ADCC effector function that is at least 2 fold, or at least 3 fold, or at least 5 fold or at least 10 fold or at least 50 fold or at least 100 fold less than that of an unmodified antibody. In still another embodiment, antibodies of the disclosure exhibit ADCC effector function that is reduced by at least 10%, or at least 20%, or by at least 30%, or by at least 40%, or by at least 50%, or by at least 60%, or by at least 70%, or by at least 80%, or by at least 90%, or by at least 100%, relative to an unmodified antibody. In a further aspect of the disclosure the reduction or down-modulation of ADCC effector function induced by the antibodies, or antigen-binding fragments thereof, of the present disclosure, is a reduction to 0, 2.5, 5, 10, 20, 50 or 75% of the value observed for induction of ADCC by unmodified antibodies. In certain embodiments, the reduction and/or ablation of ADCC activity may be attributed to the reduced affinity of the antibodies, or antigen-binding fragments thereof, of the disclosure for Fc ligands and/or receptors.

CDR substitutions that modulate pH-dependent TM-4SF1 binding of an anti-TM4SF1 antibody or antigen binding fragment thereof

[0139] One embodiment of the disclosure provides ADCs comprising an anti-TM4SF1 antibody or an antigen binding fragment thereof linked to a therapeutic molecule or a payload, wherein the anti-TM4SF1 antibody or antigen binding fragment thereof exhibit pH dependent binding affinity to TM4SF1. In some instances, an anti-TM4SF1 antibody or antigen binding fragment thereof binds to TM4SF1 with higher affinity at certain pH range as compared to other pH ranges. For example, an anti-TM4SF1 antibody or antigen binding fragment thereof may bind to TM4SF1 with different affinity at an acidic pH than at a neutral pH or a basic pH. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof binds to TM4SF1 with higher affinity at an acidic pH than at a neutral or basic pH. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof binds to TM4SF1 with lower affinity at an acidic pH than at a neutral or basic pH. In some embodiments, an anti-TM4SF1 antibody or antigen

binding fragment thereof binds to TM4SF1 at acidic pH and dissociates from TM4SF1 at neutral or basic pH. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof binds to TM4SF1 at pH7 or higher and detaches from TM4SF1 at pH6 or lower. In subcellular compartments such as plasma, cytosol, and nucleus, the pH is neutral or basic. In lysosomes or endosomes, the pH is acidic. Without being bound by any theory, an anti-TM4SF1 antibody or antigen binding fragment thereof bind to the antigen and subsequently internalized in the membrane of an endosome. A pH-dependent anti-TM4SF1 antibody or antigen binding fragment thereof can detach from TM4SF1 in an endosome and bind to FcRn receptors within the endosome, and can be recycled by the FcRn receptor back into circulation rather than degraded in a lysosome that the endosome progresses to. Accordingly, a pH dependent anti-TM4SF1 antibody or antigen binding fragment thereof can bind to TM4SF1 antigen multiple times. Accordingly, a pH dependent anti-TM4SF1 antibody and the associated therapeutic molecule or payload therewith can be recycled by FcRn receptors, without releasing the payload in the lysosome.

[0140] Target-mediated drug disposition, or TMDD, occurs when an antigen carries a bound antibody and/or any associated ADC payload to the lysosome, wherein the ADC is degraded and the payload is released. Lysosome toxicity related to TMDD as described in Grimm et al., J. Pharmacokinet. Pharmacodyn. 36(5): 407-20 (2009) is incorporated herein by reference in its entirety. In some embodiments, provided herein are ADCs comprising an anti-TM4SF1 antibody or antigen binding fragment thereof linked to a therapeutic molecule that exhibit reduced vascular toxicity, increased serum half-life, and/or improved therapeutic margin. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine amino acid residue substitutions in CDR residues. Not intended to be bound by any particular theory, the introduction of a histidine residue at a suitable position of an anti-TM4SF1 antibody may allow pH-regulatable binding affinity to TM4SF1. For example, an ADC with a pH-dependent anti-TM4SF1 antibody may dissociate from TM4SF1 in acidic lysosome or endosome environment, and subsequently be recycled into circulation via FcRn binding. As compared to an otherwise comparable wild type anti-TM4SF1 antibody or antigen binding fragment thereof, a pH-dependent ant-TM4SF1 antibody may exhibit increased serum half-life and reduced degradation rate or payload release rate in lysosomes. In some cases, the ADCs comprising a pH-dependent anti-TM4SF1 antibody or antigen binding fragment thereof may demonstrate increased half-life, reduced vascular toxicity, improved therapeutic window, and/or improved or at least about equivalent *in vivo* potency.

[0141] Disclosed herein are methods of making an ADC comprising an anti-TM4SF1 antibody or antigen binding fragment thereof that has increased half-life and/or pharmacodynamic effect

by regulating antibody-TM4SF1 binding affinity in a pH dependent manner, comprising selecting for antibody CDR histidine residues or other residues that optimize the microenvironment affecting pKa of the antibody, such that the antibody-TM4SF1 binding has a Kd ratio and/or Koff ratio at pH6.0/pH7.4 that is at least 2, 3, 4, 8, 10, 16, or more, or ranges between 2, 3, 4, 8, 10, 16, or more. In some embodiments, the method comprises introducing amino acid substitutions into an anti-TM4SF1 antibody or antigen binding fragment thereof to achieve TM4SF1 affinity with a KD at pH 7.4 of at least 100 nM as measured at 25 °C. In certain embodiments, said method comprises generating an antibody library enriched for histidines in CDR residues or other residues that optimize the microenvironment affecting pKa. In some embodiments, the antibody library comprises anti-TM4SF1 antibodies or antigen binding fragments thereof with histidine residues introduced into a CDR position. In some embodiments, the antibody library comprises a series of anti-TM4SF1 antibodies or antigen binding fragments thereof, wherein each anti-TM4SF1 antibody in the antibody library comprises a single histidine substitution at a different CDR position. In some embodiments, the antibody library comprises a series of anti-TM4SF1 antibodies or antigen binding fragments thereof, each comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 mutations to histidine residues. In some embodiments, every CDR position is mutated to histidine in at least one of the TM4SF1 antibodies or antigen fragments of the antibody library.

[0142] In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises 1, 2, 3, 4, 5, or more histidine substitutions in a CDR region. A histidine residue can be engineered into different positions of an anti-TM4SF1 antibody light chain (LC) or heavy chain (HC) for pH dependent binding affinity. Accordingly, in some embodiments, provided herein are ADCs with histidine engineered anti-TM4SF1 antibody or antigen binding fragment thereof. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR1, CDR2, and/or CDR3 of the light chain variable region (VL). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR1 of the light chain variable region (VL). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR2 of the light chain variable region (VL). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR3 of the light chain variable region (VL). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR1, CDR2, and/or CDR3 of the heavy chain variable region (VH). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR1 of the heavy chain variable region (VH). In some

embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR2 of the heavy chain variable region (VH). In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR3 of the heavy chain variable region (VH). Accordingly, in some embodiments, the ADCs of the present disclosure comprise a histidine engineered anti-TM4SF1 antibody or antigen binding fragment thereof.

[0143] In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR1, CDR2, and/or CDR3 of the light chain, for instance, in one or more of positions 30 (S30H), 92 (S92H), and 93 (N93H) of SEQ ID No. 101 or SEQ ID No. 131. In some embodiments, an anti-TM4SF1 antibody or antigen binding fragment thereof comprises one or more histidine residues in CDR1, CDR2, and/or CDR3 of the heavy chain, for instance in one or more of positions 28 (T28H), 31 (N31H), 32 (Y32H), 52 (N52H), 54 (Y54H), 57 (N57H), 100 (Q100H), and 101 (Y101H), of SEQ ID No. 92 or SEQ ID No. 130.

Substitution at position N297(Asn 297) and conjugation of one or more therapeutic molecules to an anti-TM4SF1 antibody or antigen binding fragment thereof

[0144] Human IgG molecules have a conserved glycosylation site at each N297 residue in the CH2 domain, making these pendant N-glycans a convenient target for site-specific conjugation. This glycosylation site is sufficiently far from the variable region that conjugation of drug moieties to attached glycans should not impact antigen binding. In some embodiments of this disclosure, therapeutic molecules are linked to the glycans, using exemplary methods that include oxidative cleavage of the vicinal diol moieties contained in these glycans with periodate to generate aldehydes that can be reductively aminated and conjugated to hydrazide and aminooxy compounds. (*See, e.g.*, O' Shannessy, *et al.* (1984) Immunol. Lett. 8:273-77).

[0145] Another method may include increasing the fucosylation of the N-acetylglucosamine residues in these glycans. Oxidation of these fucose residues can produce carboxylic acid and aldehyde moieties that can be used to link drugs and fluorophores to these specific sites on the antibody (*See, e.g.*, Zuberbuhler, *et al.* (2012) Chem. Commun. 48:7100-02). Another method may include modifying sialic acid in these glycans (as well as increasing the sialic acid content in these glycans) followed by oxidation of the sialic acid and conjugation with aminooxy-drugs to form oxime-linked conjugates (*See, e.g.*, Zhou, et al. (2014) Bioconjugate Chem. 25:510-20).

[0146] Alternatively, a sialyltransferase may be used to incorporate a modified sialic acid residue containing a bioorthogonal functional group into these glycans. The bioorthogonal functional group may then be modified to attach therapeutic molecules to the site of the glycan (*See, e.g.*Li, *et al.* (2014) Angew. Chem. Int. 53 :7179-82). Another approach to modifying these glycan sites

is the use of glycosyltransferases to link galactose, or galactose analogues containing ketones or azides, to the N-acetylglucosamine in these glycans, and linking drugs or radionucleotides to the galactose molecules (*See, e.g.*Khidekel, et al., (2003) J. Am. Chem. Soc. 125: 16162-63; Clark, *et al.*, (2008) J. Am. Chem. Soc. 130: 11576- 77; Boeggeman, *et al.* (2007) Bioconjugate Chem. 18:806-14). Another approach relies on the introduction of modified sugars into these glycans at the time of expression of the antibody by metabolic oligosaccharide engineering (*See, e.g.*Campbell, *et al.* (2007) Mol. BioSyst. 3: 187-94; Agard, *et al.*, (2009) Acc. Chem. Res. 42:788-97).

[0147] In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof is conjugated to a therapeutic molecule, by site-specific conjugation. Several native or engineered amino acids, including cysteines and glutamines, can be selected as the sites for conjugation.

[0148] In some instances, a cysteine residue can be engineered into different positions of antibody heavy chain (HC) or light chain (LC) for coupling, such as at position N297, *i.e.*, N297C. Thus, in some embodiments, the ADCs of the present disclosure comprise a cysteine engineered anti-TM4SF1 antibody or an antigen binding fragment thereof.

[0149] The introduction of a cysteine residue at a suitable position of the anti-TM4SF1 antibody may allow control of the site of conjugation and the obtained site- specific conjugates may be more homogeneous than the conjugates obtained *via* wild-type conjugation, *i.e.* conjugation *via* reduced interchain cysteines. In some cases, the ADCs comprising at least one conjugation via cysteine may demonstrate at least equivalent *in vivo* potency, improved pharmacokinetics (PK), and an expanded therapeutic window compared to wild-type conjugates. The ADC, in some embodiments, comprises a cleavable dipeptide linker (*i.e.*, valine-alanine) and a DNA-cross-linking pyrrolobenzodiazepine (PBD) dimer as the drug, which is linked to a cysteine at heavy chain position N297C in the Fc part of the anti-TM4SF1 antibody or antigen binding fragment thereof. In some cases, the ADCs have an average drug-to-antibody ratio (DAR) of greater than or equal to 1, such as a DAR of about 2, 6, 10 etc.

[0150] Without being bound by any particular theory, it is contemplated that site-specific conjugation through unpaired cysteine can be relatively simple and scalable. For instance, the therapeutic molecule coupling can be done without the need of special reagents. In some cases, ADCs prepared through site-specific cysteines can show stronger *in vivo* antitumor activities and could be better tolerated than the conventional conjugates. In some embodiments, position N297 of the anti-TM4SF1 antibody or an antigen binding fragment thereof can be mutated to cysteine, *i.e.*, N297C, and the cysteine residue can be conjugated to a therapeutic molecule. In some instances, the N297C mutation is combined with additional mutations in nearby residues, to add stabilizing residues (*e.g.*, arginine, lysine) and/or remove glutamic acid. In some cases, one or

more positions from residue 292-303 are modified, in addition to N297C. The sequence for positions 292-303 can be REEQYCSTYRVV (in IgG1), and REEQFCSTYRVV (in IgG4).

[0151] In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof is conjugated to a therapeutic molecule, by site-specific conjugation through a glutamine residue. In some cases, microbial transglutaminase (mTG) can be used to transfer an amine containing drug-linker or a reactive spacer into Q295 residue in the heavy chain of an anti-TM4SF1 antibody or an antigen binding fragment thereof, for example, a deglycosylated anti-TM4SF1 antibody or an antigen binding fragment thereof. The conjugation can be optimized using a two-step chemoenzymatic approach whereby a reactive spacer containing a bioorthogonal azido or thiol functional linker is attached to the antibody by mTG and subsequently reacted with either dibenzocyclooctynes (DBCO) or maleimide containing MMAE. By using strain-promoted azide-alkyne cycloaddition (SPAAC) or thiol-maleimide chemistry, ADCs can be generated with DAR, for example, at about 2.

[0152] In some instances, the anti-TM4SF1 antibody or antigen binding fragment thereof is conjugated to a therapeutic molecule, by site-specific conjugations through a glutamine residue (*e.g.*, Q295) as well as cysteine at position 297, N297C. This combination of mutations can open up two conjugation handles in the anti-TM4SF1 antibody or an antigen binding fragment thereof, and ADCs of highers DAR can be obtained. Thus, in some embodiments of this disclosure, ADCs are provided wherein more than one therapeutic molecules (*e.g.*, two therpeutic molecules) are conjugated to an anti-TM4SF1 antibody or antigen-binding fragment thereof via site specific conjugations at N297C and Q295. The cysteine conjugation can be, for example, to maleimide, haloacetamide, or another partner.

[0153] Increased DAR could lead to efficient ADC construction, minimal destabilization of the antibody structure, and enhanced ADC efficacy. A cysteine conjugation-based dual-loading linker enabling modular payload installation was recently developed (Levengood et al., 2017). Thus, there remains a need for ADCs capable of delivering multiple payloads.

[0154] In addition, the ADC linker structure and antibody–payload conjugation modality impact ADC homogeneity, cytotoxic potency, tolerability, and pharmacokinetics (PK). These key parameters may critically contribute to overall *in vivo* therapeutic efficacy (*See, e.g.*, Lu *et al.*, 2016, Hamblett *et al.*, 2004, Junutula *et al.*, 2008, and Behrens *et al.*, 2015). Thus, refining linker and conjugation chemistries is of crucial importance to maximize the therapeutic potential and safety profiles of ADCs.

[0155] Bioconjugation modality and method may be optimized for improved ADC stability and efficacy. In some embodiments, one or more therapeutic agents and/or diagnostic agents are conjugated to anti-TM4SF1 antibodies or antigen binding fragments via maleimide, *e.g.*,

cysteine-maleimide conjugation. Other functional groups besides maleimide, which in some instances are reactive with an anti-TM4SF1 antibody, such as a thiol group of a cysteine engineered anti-TM4SF1 antibody, include iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate. In some embodiments, the therapeutic agents and/or diagnostic agents are conjugated to anti-TM4SF1 antibodies or antigen binding fragments thereof via acetamide. For example, a therapeutic agent may be conjugated to an anti-TM4SF1 antibody or antigen binding fragment thereof via bromoacetamide conjugation. In some cases, an ADC comprising a bromoacetamide conjugated anti-TM4SF1 antibody or antigen binding fragment thereof exhibits increased stability, increased half-life, reduced toxicity, and/or improved therapeutic margin. Exemplary ADC structures are provided in **FIGS. 1** and **2**.

III. Anti-TM4SF1 Antibody or Antigen Binding Fragments thereof

[0156] TM4SF1 is a small plasma membrane glycoprotein (NCBI Ref Seq No. N P_055035.1) with tetraspanin topology but not homology (Wright *et al.* Protein Sci. 9: 1594-1600, 2000). It forms TM4SF1 -enriched domains (TMED) on plasma membranes, where, like genuine tetraspanins, it serves as a molecular facilitator that recruits functionally related membrane and cytosolic molecules (Shih *et al.* Cancer Res. 69: 3272-3277, 2009; Zukauskas *et al.*, Angiogenesis. 14: 345-354, 201 1), and plays important roles in cancer cell growth (Hellstrom *et al.* Cancer Res. 46: 391 7-3923, 1986), motility (Chang *et al.* Int J Cancer. 1 16: 243-252, 2005), and metastasis (Richman *et al.* Cancer Res. 5916s-5920s, 1995). The amino acid sequence of human TM4SF1 protein (NCBI RefSeq No. NP_055035.1) is shown below as SEQ ID NO: 134.

MCYGKCARCI GHSLVGLALL CIAANILLYF PNGETKYASE NHLSRFVWFF
SGIVGGGLLM LLPAFVFIGL EQDDCCGCCG HENCGKRCAM LSSVLAALIG
IAGSGYCVIV
AALGLAEGPLCLDSLGQWNYTFASTEGQYLLDTSTWSECTEPKHIVEWNVSLFSILLALG
GIEFILCLIQVINGVLGGIC GFCCSHQQQY DC (SEQ ID NO: 91)

[0157] In some embodiments, the anti-TM4SF1 antibodies and antigen binding fragments thereof, of the disclosure are specific to the ECL2 domain of TM4SF1. The amino acid sequence of human TM4SF1 ECL2 domain is
EGPLCLDSLGQWNYTFASTEGQYLLDTSTWSECTEPKHIVEWNVSLFS (SEQ ID NO: 92).

[0158] As described in **Table 1** below, included in the disclosure are novel antibodies that are specific to TM4SF1. The antibodies described in Table 1 are monoclonal murine antibodies AGX-A03, AGX-A04, AGX-A05, AGX-A07, AGX-A08, AGX-A09, and AGX-A11, each of

which were identified in the screen described in the Examples and bind the ECL2 region of TM4SF1. Further provided in **Table 1** below are humanized antibodies h AGX-A07 and h AGX-A01.

[0159] In some embodiments, the anti-TM4SF1 antibodies or antigen-binding fragments thereof, comprise an IgG heavy chain constant region comprising an amino acid sequence set forth in SEQ ID NO: 87 or 88, or a sequence that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SEQ ID NO: 73 or 74.

[0160] In another embodiment, the anti-TM4SF1 antibody or antigen-binding fragment thereof, comprises a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 89, or a sequence that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identical, or 100% identical to SEQ ID NO: 89.

[0161] In another embodiment, the anti-TM4SF1 antibody or antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 3, 15, 27, 39, 51, 63, or 75, or a sequence that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identical, or 100% identical to SEQ ID NO: 3, 15, 27, 39, 51, 63, or 75.

[0162] In another embodiment, the anti-TM4SF1 antibody or antigen-binding fragment thereof is humanized and, comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 90 or 92 or a sequence that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identical, or 100% identical to SEQ ID NO: 90 or 92.

[0163] In another embodiment, the anti-TM4SF1 antibody or antigen-binding fragment thereof is humanized and, comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 112 or 114, or a sequence that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identical, or 100% identical to SEQ ID NO: 112 or114.

[0164] In another embodiment, the anti-TM4SF1 antibody or antigen-binding fragment thereof, comprises a light chain variable domain comprising the amino acid sequence set forth in SEQ ID

NO: 9, 21, 33, 45, 57, 69, or 81, or a sequence that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identical, or 100% identical to SEQ ID NO: 9, 21, 33, 45, 57, 69, or 81.

[0165] In another embodiment, the anti-TM4SF1 antibody or antigen-binding fragment thereof is humanized and, comprises a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 97, 99, 101, 103, or 105 or a sequence that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identical, or 100% identical to SEQ ID NO: 97, 99, 101, 103 or 105. In another embodiment, the antibody or antigen-binding fragment thereof is humanized and, comprises a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 97, 99, or 101 or a sequence that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identical, or 100% identical to SEQ ID NO: 97, 99, or 101.

[0166] In another embodiment, the anti-TM4SF1 antibody or antigen-binding fragment thereof is humanized and, comprises a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 122, or a sequence that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identical, or 100% identical to SEQ ID NO: 122.

[0167] In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof comprises a heavy chain CDR1 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 6, 18, 30, 42, 54, 66, or 78. In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof comprises a heavy chain CDR2 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at

least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 7, 19, 31, 43, 55, 67, or 79. In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof comprises a heavy chain CDR3 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 8, 20, 32, 44, 56, 68, or 80.

[0168] In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof comprises a light chain CDR1 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 12, 24, 36, 48, 60, 72, or 84. In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof comprises a light chain CDR2 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 13, 25, 37, 49, 61, 73, or 85. In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof comprises a light chain CDR3 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at

least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 14, 26, 38, 50, 62, 74, or 86.

[0169] In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof is humanized and comprises a heavy chain CDR1 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 94 or SEQ ID NO: 115. In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof is humanized and comprises a heavy chain CDR2 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 95, SEQ ID NO: 116, or SEQ ID NO: 117. In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof is humanized and comprises a heavy chain CDR3 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 96, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, or SEQ ID NO: 121.

[0170] In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof is humanized and comprises a light chain CDR1 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at

least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, or SEQ ID NO: 127. In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof is humanized comprises a light chain CDR2 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 109 or SEQ ID NO: 128. In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof is humanized and comprises a light chain CDR3 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 110, SEQ ID NO: 111, or SEQ ID NO: 129. In some embodiments, the anti-TM4SF1 antibody or antigen binding fragment thereof is humanized and comprises a light chain CDR3 comprising an amino acid sequence that is from at least about 80% to at least about 85%, from at least about 85% to at least about 90%, from at least about 90% to at least about 91%, from at least about 91% to at least about 92%, from at least about 92% to at least about 93%, from at least about 93% to at least about 94%, from at least about 94% to at least about 95%, from at least about 95% to at least about 96%, from at least about 96% to at least about 97%, from at least about 97% to at least about 98%, from at least about 98% to at least about 99%, or from at least about 99% to 100% identical to SEQ ID NO: 110, or SEQ ID NO: 129.

[0171] The amino acid sequences of murine monoclonal antibody AGX-A03 are described in Table 1. Specifically, the heavy chain CDR sequences are set forth in SEQ ID Nos: 6, 7, and 8 (CDR1, CDR2, and CDR3), and the light chain CDR amino acid sequences are set forth in SEQ ID Nos: 12, 13, and 14 (CDR1, CDR2, and CDR3). Included in the disclosure are anti-TM4SF1 antibodies, or antigen binding fragments comprising a heavy chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 6, 7, and 8 and/or a light chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 12,

13, and 14. Included in the disclosure are humanized antibodies or antigen binding fragments comprising the CDRs of AGX-A03. Further, the heavy chain variable amino acid sequences and the light chain variable amino acid sequences of AGX-A03 are described in SEQ ID NOS: 3 and 9, respectively.

[0172] The amino acid sequences of murine monoclonal antibody AGX-A04 are described in Table 1. Specifically, the heavy chain CDR sequences are set forth in SEQ ID Nos: 18, 19, and 20 (CDR1, CDR2, and CDR3), and the light chain CDR amino acid sequences are set forth in SEQ ID Nos: 24, 25, and 26 (CDR1, CDR2, and CDR3). Included in the disclosure are anti-TM4SF1 antibodies, or antigen binding fragments comprising a heavy chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 18, 19, and 20 and/or a light chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 24, 25, and 26. Included in the disclosure are humanized antibodies or antigen binding fragments comprising the CDRs of AGX-A04. Further, the heavy chain variable amino acid sequences and the light chain variable amino acid sequences of AGX-A04 are described in SEQ ID NOS: 15 and 21, respectively.

[0173] The amino acid sequences of murine monoclonal antibody AGX-A05 are described in Table 1. Specifically, the heavy chain CDR sequences are set forth in SEQ ID Nos: 30, 31, and 32 (CDR1, CDR2, and CDR3), and the light chain CDR amino acid sequences are set forth in SEQ ID Nos: 36, 37, and 38 (CDR1, CDR2, and CDR3). Included in the disclosure are anti-TM4SF1 antibodies, or antigen binding fragments comprising a heavy chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 30, 31, and 32 and/or a light chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 36, 37, and 38. Included in the disclosure are humanized antibodies or antigen binding fragments comprising the CDRs of AGX-A05. Further, the heavy chain variable amino acid sequences and the light chain variable amino acid sequences of AGX-A05 are described in SEQ ID NOS: 27 and 33, respectively. The amino acid sequences of murine monoclonal antibody AGX-A07 are described in Table 1. Specifically, the heavy chain CDR sequences are set forth in SEQ ID Nos: 42, 43, and 44 (CDR1, CDR2, and CDR3), and the light chain CDR amino acid sequences are set forth in SEQ ID Nos: 48, 49, and 50 (CDR1, CDR2, and CDR3). Included in the disclosure are anti-TM4SF1 antibodies, or antigen binding fragments comprising a heavy chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 42, 43, and 44 and/or a light chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 48, 49, and 50. Included in the disclosure are humanized antibodies or antigen binding fragments comprising the CDRs of AGX-A07. Further, the heavy

chain variable amino acid sequences and the light chain variable amino acid sequences of AGX-A07 are described in SEQ ID NOs: 39 and 45, respectively.

[0174] In one embodiment, a humanized AGX-A07 (h AGX-A07) antibody or antigen binding fragments thereof is provided, comprising a heavy chain sequence as forth in the amino acid sequence of SEQ ID NO: 90. In some embodiments, the humanized AGX-A07 antibody or antigen binding fragments thereof is a humanized mutated AGX-A07 (hm AGX-A07) antibody or antigen binding fragments thereof, comprising a heavy chain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 90. As shown in **Table 6**, the heavy chain sequence set forth in SEQ ID NO: 90 is also referred to herein as AGX-A07 H2. In some embodiments, the humanized AGX-A07 antibody or antigen binding fragments thereof is a humanized mutated AGX-A07 antibody or antigen binding fragments thereof, comprising a heavy chain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 90, wherein the one or more substitutions are in amino acid positions 1, 44, and 80 of SEQ ID NO: 90. In some cases, the humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprises an E1Q (glutamic acid to glutamine substitution at position 1 of the heavy chain, SEQ ID NO: 90). In some cases, the humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprises a D44G (aspartate to glycine substitution at position 44 of the heavy chain, SEQ ID NO: 90). In some cases, the humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprises a F80Y (phenyl alanine to tyrosine substitution at position 80 of the heavy chain, SEQ ID NO: 90). In some embodiments, a humanized mutated AGX-A07 antibody or antigen binding fragments is provided, comprising a heavy chain sequence as forth in the amino acid sequence of SEQ ID NO: 92. As shown in **Table 6**, the heavy chain sequence set forth in SEQ ID NO: 92 is also referred to herein as AGX-A07 H2v1. In some embodiments, humanized AGX-A07 antibodies or antigen binding fragments are provided, comprising a light chain sequence as forth in the amino acid sequence of SEQ ID NO: 97. As shown in **Table 6**, the light chain sequence set forth in SEQ ID NO: 97 is also referred to herein as AGX-A07 L5. In some embodiments, the humanized AGX-A07 antibody or antigen binding fragments thereof is a humanized mutated AGX-A07 antibody or antigen binding fragments thereof, comprising a light chain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 97. In some embodiments, the humanized AGX-A07 antibodies or antigen binding fragments thereof is a humanized mutated AGX-A07 antibody or antigen binding fragments thereof, comprising a light chain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 97, wherein the one or more substitutions are in amino acid positions 3, 26, 62, and 90 of SEQ ID NO: 97. In

some cases, the humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprises an I3V (isoluecine to valine substitution at position 3 of the light chain, SEQ ID NO: 97). In some cases, the humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprises a N26Q (asparagine to glutamine substitution at position 26 of the light chain, SEQ ID NO: 97). In some cases, the humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprises a N26S (asparagine to serine substitution at position 26 of the light chain, SEQ ID NO: 97). In some cases, the humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprises a G62S (glycine to serine substitution at position 62 of the light chain, SEQ ID NO: 97). In some cases, the humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprises a W90Y (tryptophan to tyrosine substitution at position 90 of the light chain, SEQ ID NO: 97). In some embodiments, humanized mutated AGX-A07 antibodies or antigen binding fragments are provided, comprising a light chain sequence as forth in an amino acid sequence selected from the group consisting of SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, and SEQ ID NO: 105. As shown in **Table 6**, the light chain sequence set forth in SEQ ID NO: 99 is also referred to herein as AGX-A07 L5v1, the light chain sequence set forth in SEQ ID NO: 101 is also referred to herein as AGX-A07 L5v2, the light chain sequence set forth in SEQ ID NO: 103 is also referred to herein as AGX-A07 L5v3, and the light chain sequence set forth in SEQ ID NO: 105 is also referred to herein as AGX-A07 L5v4. Exemplary coding sequence for the heavy chain of a humanized AGX-A07 antibody or antigen binding fragment thereof is provided in SEQ ID NO: 91. Exemplary coding sequence for the heavy chain of a humanized mutated AGX-A07 antibody or antigen binding fragment thereof is provided in SEQ ID NO: 93. Exemplary coding sequence for the light chain of a humanized AGX-A07 antibody or antigen binding fragment thereof is provided in SEQ ID NO: 98 (AGX-A07 L5). Exemplary coding sequences for the light chain of a humanized mutated AGX-A07 antibody or antigen binding fragment thereof are provided in SEQ ID NO: 100 (AGX-A07 L5v1), SEQ ID NO: 102 (AGX-A07 L5v2), SEQ ID NO: 104 (AGX-A07 L5v3), and SEQ ID NO: 106 (AGX-A07 L5v4).

[0175] In one embodiment, a humanized AGX-A07 antibody or antigen binding fragments thereof is provided, comprising a heavy chain variable domain sequence as forth in the amino acid sequence of SEQ ID NO: 130 or SEQ ID NO: 132. In some embodiments, the humanized AGX-A07 antibody or antigen binding fragments thereof is a humanized mutated AGX-A07 antibody or antigen binding fragments thereof, comprising a heavy chain variable domain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 130 or SEQ ID NO: 132. In one embodiment, a humanized AGX-A07 antibody or antigen binding fragments thereof is provided, comprising a light chain variable

domain sequence as forth in the amino acid sequence of SEQ ID NO: 131 or SEQ ID NO: 133. In some embodiments, the humanized AGX-A07 antibody or antigen binding fragments thereof is a humanized mutated AGX-A07 antibody or antigen binding fragments thereof, comprising a light chain variable domain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 131 or SEQ ID NO: 133.

[0176] In some embodiments, the humanized AGX-A07 antibody or antigen binding fragment thereof is a humanized mutated AGX-A07 antibody or antigen binding fragment thereof comprising a light chain variable domain sequence comprising the sequence as set forth in the amino acid sequence of SEQ ID NO: 131 and a heavy chain variable domain sequence comprising the sequence as set forth in the amino acid sequence of SEQ ID NO: 130. In some embodiments, the humanized AGX-A07 antibody or antigen binding fragment thereof is a humanized mutated AGX-A07 antibody or antigen binding fragments thereof, comprising a light chain variable domain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 131 and a heavy chain variable domain sequence comprises one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 130. In some embodiments, the humanized AGX-A07 antibody or antigen binding fragments thereof is a humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprising a light chain variable domain sequence comprising the sequence as set forth in the amino acid sequence of SEQ ID NO: 133 and a heavy chain variable domain sequence comprising the sequence as set forth in the amino acid sequence of SEQ ID NO: 132. In some embodiments, the humanized AGX-A07 antibody or antigen binding fragments thereof is a humanized mutated AGX-A07 antibody or antigen binding fragments thereof, comprising a light chain variable domain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 133 and a heavy chain variable domain sequence comprises one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 132. In some embodiments, the humanized AGX-A07 antibody or antigen binding fragments thereof is a humanized mutated AGX-A07 antibody or antigen binding fragments thereof comprising a heavy chain sequence comprising the sequence as set forth in the amino acid sequence of SEQ ID NO: 156, or a sequence comprising one of more substitutions in the amino acid sequence of SEQ ID NO: 156.

[0177] In some cases, the humanized AGX-A07 antibodies or antigen binding fragments thereof comprise heavy chain CDR sequences as set forth in SEQ ID Nos: 94, 95, and 96 (CDR1, CDR2, and CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 94, 95, and 96 (CDR1, CDR2, and CDR3). In some cases, the humanized mutated AGX-A07 antibodies or antigen binding fragments thereof comprises heavy chain CDR

sequences as set forth in SEQ ID Nos: 94, 95, and 96 (CDR1, CDR2, and CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 94, 95, and 96 (CDR1, CDR2, and CDR3).

[0178] In some cases, the humanized mutated AGX-A07 antibodies or antigen binding fragments thereof comprise heavy chain CDR1 sequence as set forth in SEQ ID NO: 94, or a heavy chain CDR1 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID NO: 94. In some cases, the humanized mutated AGX-A07 antibodies or antigen binding fragments thereof comprise a heavy chain CDR2 sequence as set forth in SEQ ID NO: 95, or a heavy chain CDR2 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID NO: 95. In some cases, the humanized mutated AGX-A07 antibodies or antigen binding fragments thereof comprise a heavy chain CDR3 sequence as set forth in SEQ ID NO: 96, or a heavy chain CDR3 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID NO: 96.

[0179] In some cases, the humanized AGX-A07 antibodies or antigen binding fragments thereof comprise light chain CDR sequences as set forth in SEQ ID Nos: 107, 109, and 110 (CDR1, CDR2, and CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 107, 109, and 110 (CDR1, CDR2, and CDR3). In some cases, the humanized AGX-A07 antibodies or antigen binding fragments thereof comprise light chain CDR sequences as set forth in SEQ ID Nos: 107, 109, and 111 (CDR1, CDR2, and CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 107, 109, and 111 (CDR1, CDR2, and CDR3). In some cases, the humanized AGX-A07 antibodies or antigen binding fragments thereof comprise light chain CDR sequences as set forth in SEQ ID Nos: 108, 109, and 110 (CDR1, CDR2, and CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 108, 109, and 110 (CDR1, CDR2, and CDR3). In some cases, the humanized AGX-A07 antibodies or antigen binding fragments thereof comprise light chain CDR sequences as set forth in SEQ ID Nos: 108, 109, and 111 (CDR1, CDR2, and CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 108, 109, and 111 (CDR1, CDR2, and CDR3).

[0180] In some cases, the humanized mutated AGX-A07 antibodies or antigen binding fragments thereof comprise light chain CDR1 sequence as set forth in SEQ ID Nos: 107 or 108, or light chain CDR1 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 107 or 108. In some cases, the humanized mutated AGX-A07 antibodies or antigen binding fragments thereof comprise light chain CDR2 sequence as set forth in SEQ ID NO: 109, or light chain CDR2 sequence comprising one or more substitutions in the sequences as set forth

in SEQ ID NO: 109. In some cases, the humanized mutated AGX-A07 antibodies or antigen binding fragments thereof comprise light chain CDR3 sequence as set forth in SEQ ID Nos: 110 or 111, or light chain CDR1 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 110 or 111. In some cases, the humanized mutated AGX-A07 antibodies or antigen binding fragments thereof comprise light chain CDR3 sequence as set forth in SEQ ID NO: 110, or light chain CDR1 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 110.

[0181] In some embodiments, the humanized mutated AGX-A07 comprises a heavy chain variable region comprising the following amino acid substitutions: Q1E, D44G, F80Y in SEQ ID NO: 132 (also referred to herein as AGX-A07 H2), and a light chain variable region comprising the following amino acid substitutions: I3V, N26Q, G62S in SEQ ID NO: 133 (also referred to herein as AGX-A07 L5). In some embodiments, the humanized mutated AGX-A07 comprises a heavy chain variable region comprising the following amino acid substitutions: Q1E, D44G, F80Y in SEQ ID NO: 132, and a light chain variable region comprising the following amino acid substitutions: I3V, N26Q, G62S in SEQ ID NO: 133, wherein the heavy chain comprises CDR1 (SEQ ID NO: 94), CDR2 (SEQ ID NO: 95), and CDR3 (SEQ ID NO: 96), and the light chain comprises CDR1 (SEQ ID NO: 108), CDR2 (SEQ ID NO: 109), and CDR3 (SEQ ID NO: 110). In some embodiments, the humanized mutated AGX-A07 is AGX-A07 H2v1L5v2 and comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO: 130 (also referred to herein as AGX-A07 H2v1), and a light chain comprising the amino acid sequence as set forth in SEQ ID NO: 131 (also referred to herein as AGX-A07 L5v2). In some embodiments, the humanized mutated AGX-A07 comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO: 92, and a light chain comprising the amino acid sequence as set forth in SEQ ID NO: 101.

[0182] The amino acid sequences of murine monoclonal antibody AGX-A08 are described in Table 1. Specifically, the heavy chain CDR sequences are set forth in SEQ ID Nos: 54, 55, and 56 (CDR1, CDR2, and CDR3), and the light chain CDR amino acid sequences are set forth in SEQ ID Nos: 60, 61, and 62 (CDR1, CDR2, and CDR3). Included in the disclosure are anti-TM4SF1 antibodies, or antigen binding fragments comprising a heavy chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 54, 55, and 56 and/or a light chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 60, 61, and 62. Included in the disclosure are humanized antibodies or antigen binding fragments comprising the CDRs of AGX-A08. Further, the heavy chain variable amino acid sequences and the light chain variable amino acid sequences of AGX-A08 are described in SEQ ID NOs: 51 and 57, respectively.

[0183] The amino acid sequences of murine monoclonal antibody AGX-A09 are described in Table 1. Specifically, the heavy chain CDR sequences are set forth in SEQ ID Nos: 66, 67, and 68 (CDR1, CDR2, and CDR3), and the light chain CDR amino acid sequences are set forth in SEQ ID Nos: 72, 73, and 74 (CDR1, CDR2, and CDR3). Included in the disclosure are anti-TM4SF1 antibodies, or antigen binding fragments comprising a heavy chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 66, 67, and 68 and/or a light chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 72, 73, and 74. Included in the disclosure are humanized antibodies or antigen binding fragments comprising the CDRs of AGX-A09. Further, the heavy chain variable amino acid sequences and the light chain variable amino acid sequences of AGX-A09 are described in SEQ ID NOs: 63 and 69, respectively.

[0184] The amino acid sequences of murine monoclonal antibody AGX-A11 are described in Table 1. Specifically, the heavy chain CDR sequences are set forth in SEQ ID Nos: 78, 79, and 80 (CDR1, CDR2, and CDR3), and the light chain CDR amino acid sequences are set forth in SEQ ID Nos: 84, 85, and 86 (CDR1, CDR2, and CDR3). Included in the disclosure are anti-TM4SF1 antibodies, or antigen binding fragments comprising a heavy chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 78, 79, and 80 and/or a light chain variable region comprising CDRs as set forth in the amino acid sequences of SEQ ID Nos: 84, 85, and 862. Included in the disclosure are humanized antibodies or antigen binding fragments comprising the CDRs of AGX-A11. Further, the heavy chain variable amino acid sequences and the light chain variable amino acid sequences of AGX-A11 are described in SEQ ID NOS: 75 and 81, respectively.

[0185] The amino acid sequences of a humanized antibody AGX-A01 (h AGX-A01) are described in **Table 6**. As shown in **Table 6**, the heavy chain sequence set forth is SEQ ID NO: 112 is also referred to herein as AGX-A01 H1. Specifically, the heavy chain CDR sequences are set forth in SEQ ID Nos: 115, 116, and 118 (CDR1, CDR2, and CDR3) and the light chain CDR amino acid sequences are set forth in SEQ ID Nos: 124, 128, and 129 (CDR1, CDR2, and CDR3). Further, exemplary heavy chain amino acid sequence and the light chain amino acid sequence of the humanized AGX-A01 are described in SEQ ID Nos: 112 and 122, respectively. Exemplary coding sequences for the heavy chain and the light chain of the humanized AGX-A01 are described in SEQ ID Nos: 113 and 123, respectively

[0186] In some embodiments, the humanized AGX-A01 antibody or antigen binding fragments thereof is a humanized mutated AGX-A01 (hm AGX-A01) antibody or antigen binding fragments thereof, comprising a heavy chain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 112. In some embodiments,

the humanized AGX-A01 antibody or antigen binding fragments thereof is a humanized mutated AGX-A01 antibody or antigen binding fragments thereof, comprising a heavy chain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 112, wherein the one or more substitutions are in amino acid positions 63 and 106 of SEQ ID NO: 112. In some cases, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof comprises a G63S (glycine to serine substitution at position 63 of the heavy chain, SEQ ID NO: 112). In some cases, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof comprises a D106E (aspartate to glutamic acid substitution at position 106 of the heavy chain, SEQ ID NO: 112). In some cases, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof comprises a D106S (aspartate to serine substitution at position 106 of the heavy chain, SEQ ID NO: 112). In some embodiments, a humanized mutated AGX-A01 antibody or antigen binding fragments is provided, comprising a heavy chain sequence as forth in the amino acid sequence of SEQ ID NO: 114. As shown in **Table 6**, the heavy chain sequence set forth is SEQ ID NO: 114 is also referred to herein as AGX-A01 H1v1.

[0187] In some embodiments, humanized AGX-A01 antibodies or antigen binding fragments are provided, comprising a light chain sequence as forth in the amino acid sequence of SEQ ID NO: 122. As shown in **Table 6**, the light chain sequence set forth is SEQ ID NO: 122 is also referred to herein as AGX-A01 L10. In some embodiments, the humanized AGX-A01 antibody or antigen binding fragments thereof is a humanized mutated AGX-A01 antibody or antigen binding fragments thereof, comprising a light chain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 122. In some embodiments, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof is a humanized mutated AGX-A01 antibody or antigen binding fragments thereof, comprising a light chain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 122, wherein the one or more substitutions are in one or more amino acid positions selected from amino acid positions 1, 33, 42, 51, 86, and 90 of SEQ ID NO: 122. In some embodiments, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof is a humanized mutated AGX-A01 antibody or antigen binding fragments thereof, comprising a light chain sequence comprising one or more substitutions in the sequence as set forth in the amino acid sequence of SEQ ID NO: 122, wherein the one or more substitutions are in one or more amino acid positions selected from amino acid positions 1, 33, 42, 51, and 86 of SEQ ID NO: 122. In some cases, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof comprises an A1E (alanine to glutamic acid substitution at position 1 of the light chain, SEQ ID NO: 122). In some cases, the humanized mutated AGX-A01 antibody or

antigen binding fragments thereof comprises a N33S (asparagine to serine substitution at position 33 of the light chain, SEQ ID NO: 122). In some cases, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof comprises a M42Q (methionine to glutamine substitution at position 42 of the light chain, SEQ ID NO: 122). In some cases, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof comprises a V51L (valine to leucine substitution at position 51 of the light chain, SEQ ID NO: 122). In some cases, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof comprises a D86E (aspartate to glutamic acid substitution at position 86 of the light chain, SEQ ID NO: 122). In some cases, the humanized mutated AGX-A01 antibody or antigen binding fragments thereof comprises an I90V (isoleucine to valine substitution at position 90 of the light chain, SEQ ID NO: 122).

[0188] In some cases, the humanized AGX-A01 antibodies or antigen binding fragments thereof comprise heavy chain CDR sequences as set forth in SEQ ID Nos: 115 (CDR1); 116 (CDR2); and 118 (CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 115 (CDR1); 116 (CDR2); and 118 (CDR3). In some cases, the humanized mutated AGX-A01 antibodies or antigen binding fragments thereof comprise heavy chain CDR sequences as set forth in SEQ ID Nos: 115 (CDR1); 116 or 117 (CDR2); and 118, 119, 120, or 121 (CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 115 (CDR1); 116 or 117 (CDR2); and 118, 119, 120, or 121 (CDR3).

[0189] In some cases, the humanized mutated AGX-A01 antibodies or antigen binding fragments thereof comprise heavy chain CDR1 sequence as set forth in SEQ ID NO: 115, or a heavy chain CDR1 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID NO: 115. In some cases, the humanized mutated AGX-A01 antibodies or antigen binding fragments thereof comprise a heavy chain CDR2 sequence as set forth in SEQ ID NO: 116, or a heavy chain CDR2 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID NO: 116. In some cases, the humanized mutated AGX-A01 antibodies or antigen binding fragments thereof comprise a heavy chain CDR2 sequence as set forth in SEQ ID NO: 117, or a heavy chain CDR2 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID NO: 117. In some cases, the humanized mutated AGX-A01 antibodies or antigen binding fragments thereof comprise a heavy chain CDR3 sequence as set forth in a sequence selected from SEQ ID Nos: 118, 119, 120 and 121, or a heavy chain CDR3 sequence comprising one or more substitutions in a sequence selected from SEQ ID Nos: 118, 119, 120, and 121.

[0190] In some cases, the humanized AGX-A01 antibodies or antigen binding fragments thereof comprise light chain CDR sequences as set forth in SEQ ID Nos: 124 (CDR1); 128 (CDR2); and 129 (CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 124 (CDR1); 128 (CDR2); and 129 (CDR3). In some cases, the humanized mutated AGX-A01 antibodies or antigen binding fragments thereof comprise light chain CDR sequences as set forth in SEQ ID Nos: 124, 125, 126, or 127 (CDR1); 128 (CDR2); and 129 (CDR3), or CDR sequences comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 124, 125, 126, or 127 (CDR1); 128 (CDR2); and 129 (CDR3).

[0191] In some cases, the humanized mutated AGX-A01 antibodies or antigen binding fragments thereof comprise light chain CDR1 sequence as set forth in SEQ ID Nos: 125, 126, 127, or 128, or light chain CDR1 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 125, 126, 127, or 128. In some cases, the humanized mutated AGX-A01 antibodies or antigen binding fragments thereof comprise light chain CDR2 sequence as set forth in SEQ ID NO: 129, or light chain CDR2 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID NO: 129. In some cases, the humanized mutated AGX-A01 antibodies or antigen binding fragments thereof comprise light chain CDR3 sequence as set forth in SEQ ID Nos: 130, or light chain CDR1 sequence comprising one or more substitutions in the sequences as set forth in SEQ ID Nos: 130.

[0192] In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 3, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 9. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 15, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 21 In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 27, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 33. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 39, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 45. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 51, and a light chain variable domain encoded by a nucleic acid sequence as set forth in

SEQ ID NO: 57. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 63, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 69. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 75, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 81. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 90, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 97. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 90, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 99. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 90, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 101. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 90, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 103. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 90, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 105. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 92, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 97. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 92, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 99. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 92, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 101. In one embodiment, the disclosure provides an

anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 92, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 103. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 92, and a light chain variable domain encoded by a nucleic acid sequence as set forth in SEQ ID NO: 105.

[0193] In one embodiment, the present disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that has a heavy chain variable domain sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to an amino acid sequence selected from SEQ ID NO: 3, SEQ ID NO: 15, SEQ ID NO: 27, SEQ ID NO: 39, SEQ ID NO: 51, SEQ ID NO: 63, SEQ ID NO: 75, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 112, or SEQ ID NO: 114; and that has a light chain variable domain sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to an amino acid sequence selected from SEQ ID NO: 9, SEQ ID NO: 21, SEQ ID NO: 33, SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 81, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, or SEQ ID NO: 122. In one embodiment, the present disclosure provides an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that has a heavy chain variable domain sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to an amino acid sequence selected from SEQ ID NO: 3, SEQ ID NO: 15, SEQ ID NO: 27, SEQ ID NO: 39, SEQ ID NO: 51, SEQ ID NO: 63, SEQ ID NO: 75, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 112, or SEQ ID NO: 114; and that has a light chain variable domain sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to an amino acid sequence selected from SEQ ID NO: 9, SEQ ID NO: 21, SEQ ID NO: 33, SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 81, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, or SEQ ID NO: 122.

[0194] In one embodiment, the disclosure includes an anti-TM4SF1 antibody which is an IgG and comprises four polypeptide chains including two heavy chains each comprising a heavy chain variable domain and heavy chain constant regions CH1, CH2 and CH3, and two light chains each comprising a light chain variable domain and a light chain constant region (CL). In certain embodiments, the antibody is a human IgG1, IgG2, or an IgG4. In certain embodiments, the antibody is a human IgG1. In other embodiments, the antibody is an IgG2. The heavy and light chain variable domain sequences may contain CDRs as set forth in **Table 6**.

[0195] Complementarity determining regions (CDRs) are known as hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). CDRs and framework regions (FR) of a given antibody may be identified using the system described by Kabat et al. supra; Lefranc et al., supra and/or Honegger and Pluckthun, supra. Also familiar to those in the art is the numbering system described in Kabat et al. (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, Va.). In this regard Kabat et al. defined a numbering system for variable domain sequences, including the identification of CDRs, that is applicable to any antibody.

[0196] One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein.

[0197] An antigen binding protein may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest. The CDR3, in particular, is known to play an important role in antigen binding of an antibody or antibody fragment.

[0198] In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or an antigen-binding fragment thereof, comprising a heavy chain comprising a CDR3 domain as set forth in any one of SEQ ID NO: 8, SEQ ID NO: 20, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 56, SEQ ID NO: 68, or SEQ ID NO: 80 and comprising a variable domain comprising an amino acid sequence that has at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to a sequence as set forth in any one of SEQ ID NO: 3, SEQ ID NO: 15, SEQ ID NO: 27, SEQ ID NO: 39, SEQ ID NO: 51, SEQ ID NO: 63, or SEQ ID NO: 75. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or an antigen-binding fragment thereof, comprising a light chain comprising a CDR3 domain as set forth in any one of SEQ ID NO: 14, SEQ ID NO: 26, SEQ ID NO: 38, SEQ ID NO: 50, SEQ ID NO: 62, SEQ ID NO: 74, or SEQ ID NO: 86, and having a light chain variable domain comprising an amino acid sequence that has at least at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, or 100% identical to a sequence as set forth in any one of SEQ ID NO: 9, SEQ ID NO: 21, SEQ ID NO: 33, SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 69, or SEQ ID NO: 81. Thus, in certain embodiments, the CDR3 domain is held constant, while variability may be introduced into the remaining CDRs and/or framework regions of the heavy and/or light chains, while the antibody, or antigen binding fragment thereof, retains the ability to

bind to TM4SF1 and retains the functional characteristics, *e.g.*, binding affinity, of the parent, or has improved functional characteristic, *e.g.*, binding affinity, compared to the parent.

[0199] In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or an antigen-binding fragment thereof, comprising a heavy chain comprising a CDR2 domain as set forth in any one of SEQ ID NO: 7, SEQ ID NO: 19, SEQ ID NO: 31, SEQ ID NO: 43, SEQ ID NO: 55, SEQ ID NO: 67, or SEQ ID NO: 79 and comprising a variable domain comprising an amino acid sequence that has at least at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, or 100% identical to a sequence as set forth in any one of SEQ ID NO: 3, SEQ ID NO: 15, SEQ ID NO: 27, SEQ ID NO: 39, SEQ ID NO: 51, SEQ ID NO: 63, or SEQ ID NO: 75. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or an antigen-binding fragment thereof, comprising a light chain comprising a CDR2 domain as set forth in any one of SEQ ID NO: 13, SEQ ID NO: 25, SEQ ID NO: 37, SEQ ID NO: 49, SEQ ID NO: 61, SEQ ID NO: 73, or SEQ ID NO: 85, and having a light chain variable domain comprising an amino acid sequence that has at least at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, or 100% identical to a sequence as set forth in any one of SEQ ID NO: 9, SEQ ID NO: 21, SEQ ID NO: 33, SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 69, or SEQ ID NO: 81. Thus, in certain embodiments, the CDR2 domain is held constant, while variability may be introduced into the remaining CDRs and/or framework regions of the heavy and/or light chains, while the antibody, or antigen binding fragment thereof, retains the ability to bind to TM4SF1 and retains the functional characteristics, *e.g.*, binding affinity, of the parent, or has improved functional characteristic, *e.g.*, binding affinity, compared to the parent.

[0200] In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or an antigen-binding fragment thereof, comprising a heavy chain comprising a CDR1 domain as set forth in any one of SEQ ID NO: 6, SEQ ID NO: 18, SEQ ID NO: 30, SEQ ID NO: 42, SEQ ID NO: 54, SEQ ID NO: 66, or SEQ ID NO: 78 and comprising a variable domain comprising an amino acid sequence that has at least at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, or 100% identical to a sequence as set forth in any one of SEQ ID NO: 3, SEQ ID NO: 15, SEQ ID NO: 27, SEQ ID NO: 39, SEQ ID NO: 45, SEQ ID NO: 69, or SEQ ID NO: 81. In one embodiment, the disclosure provides an anti-TM4SF1 antibody, or an antigen-binding fragment thereof, comprising a light

chain comprising a CDR1 domain as set forth in any one of SEQ ID NO: 12, SEQ ID NO: 24, SEQ ID NO: 36, SEQ ID NO: 48, SEQ ID NO: 60, SEQ ID NO: 72, or SEQ ID NO: 84, and having a light chain variable domain comprising an amino acid sequence that has at least at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, or 100% identical to a sequence a set forth in any one of SEQ ID NO: 9, SEQ ID NO: 21, SEQ ID NO: 33, SEQ ID NO: 45, SEQ ID NO: 57, SEQ ID NO: 69, or SEQ ID NO: 81. Thus, in certain embodiments, the CDR1 domain is held constant, while variability may be introduced into the remaining CDRs and/or framework regions of the heavy and/or light chains, while the antibody, or antigen binding fragment thereof, retains the ability to bind to TM4SF1 and retains the functional characteristics, *e.g.,* binding affinity, of the parent.

[0201] In some embodiments, an anti-TM4SF1 antibody of this disclosure comprises a heavy chain comprising an Fc region, wherein said Fc region comprises a sequence selected from the group consisting of: SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 151, SEQ ID NO: 152, and SEQ ID NO: 153; or wherein said Fc region comprises a sequence comprising one or more substitutions in a sequence selected from the group consisting of: SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 151, SEQ ID NO: 152, and SEQ ID NO: 153. For instance, in some embodiments, an anti-TM4SF1 antibody of this disclosure comprises an Fc region, wherein said Fc region comprises a sequence that is at least about 70% to about 100%, such as at least about 70%, at least about 75%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 151, SEQ ID NO: 152, and SEQ ID NO: 153.

[0202] In some embodiments, an anti-TM4SF1 antibody of this disclosure comprises a heavy chain comprising a sequence selected from the group consisting of: SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 154, SEQ ID NO: 155, and SEQ ID NO: 156; or wherein said heavy chain comprises a sequence comprising one or

more substitutions in a sequence selected from the group consisting of: SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 154, SEQ ID NO: 155, and SEQ ID NO: 156. For instance, in some embodiments, an anti-TM4SF1 antibody of this disclosure comprises a heavy chain comprising a sequence that is at least about 70% to about 100%, such as at least about 70%, at least about 75%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 154, SEQ ID NO: 155, and SEQ ID NO: 156.

[0203] The anti-TM4SF1 antibodies and fragments described in Table 1 may also be humanized. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization may be performed, for example, following the method of Jones et al., 1986, Nature 321:522-25; Riechmann *et al.*, 1988, Nature 332:323-27; and Verhoeyen *et al.*, 1988, Science 239:1534-36), by substituting hypervariable region sequences for the corresponding sequences of a human antibody.

[0204] In some cases, the humanized antibodies are constructed by CDR grafting, in which the amino acid sequences of the six CDRs of the parent non-human antibody (*e.g.*, rodent) are grafted onto a human antibody framework. For example, Padlan *et al.* determined that only about one third of the residues in the CDRs actually contact the antigen, and termed these the "specificity determining residues," or SDRs (Padlan et al., 1995, FASEB J. 9:133-39). In the technique of SDR grafting, only the SDR residues are grafted onto the human antibody framework (*See, e.g.*, Kashmiri et al., 2005, Methods 36:25-34).

[0205] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies can be important to reduce antigenicity. For example, according to the so-called "best-fit" method, the sequence of the variable domain of a non-human (e.g., rodent) antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the rodent may be selected as the human framework for the humanized antibody (Sims et al., 1993, J. Immunol. 151:2296-308; and Chothia *et al.*, 1987, J. Mol. Biol. 196:901-17). Another method uses a particular framework derived from the

consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:4285-89; and Presta *et al.*, 1993, J. Immunol. 151:2623-32). In some cases, the framework is derived from the consensus sequences of the most abundant human subclasses, VL6 subgroup I (VL6 I) and VH subgroup III (VHIII). In another method, human germline genes are used as the source of the framework regions.

[0206] It is further generally desirable that antibodies be humanized with retention of their affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. These include, for example, WAM (Whitelegg and Rees, 2000, Protein Eng. 13:819-24), Modeller (Sali and Blundell, 1993, J. Mol. Biol. 234:779-815), and Swiss PDB Viewer (Guex and Peitsch, 1997, Electrophoresis 18:2714-23). Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, e.g., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0207] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, *e.g.*, Sims, *et al.*, J. Immunol. 151 (1993) 2296); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, *e.g.*, Carter, *et al.*, Proc. Natl. Acad. Sci. USA, 89 (1992) 4285; and Presta, *et al.*, J. Immunol., 151 (1993) 2623); human mature (somatically mutated) framework regions or human germline framework regions (see, *e.g.*, Almagro, and Fransson, Front. Biosci. 13 (2008) 1619-1633); and framework regions derived from screening FR libraries (see, *e.g.*, Baca, *et al.*, J. Biol. Chem. 272 (1997) 10678-10684 and Rosok, *et al.*, J. Biol. Chem. 271 (1996) 22611-22618).

[0208] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro, and Fransson, Front. Biosci. 13 (2008) 1619-1633, and are further described, *e.g.*, in Riechmann, *et al.*, Nature 332 (1988) 323-329; Queen, *et al.*, Proc. Nat'l Acad. Sci. USA 86 (1989) 10029-10033; U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri, *et al.*, Methods

36 (2005) 25-34 (describing SDR (a-CDR) grafting); Padlan, Mol. Immunol. 28 (1991) 489-498 (describing "resurfacing"); Dall'Acqua, *et al.*, Methods 36 (2005) 43-60 (describing "FR shuffling"); and Osbourn, *et al.*, Methods 36 (2005)61-68 and Klimka, *et al.*, Br. J. Cancer, 83 (2000) 252-260 (describing the "guided selection" approach to FR shuffling).

[0209] In one embodiment, an anti-TM4SF1 antibody, or antigen-binding fragment thereof, of the disclosure binds to cynomolgus TM4SF1 with a K_D about 1 x 10^{-6} M or less.

[0210] An anti-TM4SF1 antibody, or antigen-binding fragment thereof, of the disclosure, in certain embodiments, binds to an epitope on the ECL2 loop of human TM4SF1 with a K_D about 5 x 10^{-8} M or less as determined in a standard flow cytometry assay using HUVEC cells.

[0211] An anti-TM4SF1 antibody, or antigen-binding fragment thereof, of the disclosure, in certain embodiments, binds to human TM4SF1 with a K_D of about 1 x 10^{-8} M or less in a standard flow cytometry assay using HUVEC cells.

[0212] An anti-TM4SF1 antibody, or antigen-binding fragment thereof, of the disclosure, in certain embodiments, binds to human TM4SF1 with a K_D of about 1 x 10^{-3} M to about 1 x 10^{-4} M, about 1 x 10^{-4} M to about 1 x 10^{-5} M, about 1 x 10^{-5} M to about 1 x 10^{-6} M, about 1 x 10^{-6} to about 1 x 10^{-7} M, about 1 x 10^{-7} to about 1 x 10^{-8} M, about 1 x 10^{-8} M to about 1 x 10^{-9} M, about 1 x 10^{-9} M to about 1 x 10^{-10} M, about 1 x 10^{-10}M to about 1 x 10^{-11} M, about 1 x 10^{-11} M to about 1 x 10^{-12} M, about 2 x 10^{-3} M to about 2 x 10^{-4} M, about 2 x 10^{-4} M to about 2 x 10^{-5} M, about 2 x 10^{-5} M to about 2 x 10^{-6} M, about 2 x 10^{-6} to about 2 x 10^{-7} M, about 2 x 10^{-7} to about 2 x 10^{-8} M, about 2 x 10^{-8} M to about 2 x 10^{-9} M, about 2 x 10^{-9} M to about 2 x 10^{-10} M, about 2 x 10^{-10}M to about 2 x 10^{-11} M, about 2 x 10^{-11} M to about 2 x 10^{-12} M, about 3 x 10^{-3} M to about 3 x 10^{-4} M, about 3 x 10^{-4} M to about 3 x 10^{-5} M, about 3 x 10^{-5} M to about 3 x 10^{-6} M, about 3 x 10^{-6} to about 3 x 10^{-7} M, about 3 x 10^{-7} to about 3 x 10^{-8} M, about 3 x 10^{-8} M to about 3 x 10^{-9} M, about 3 x 10^{-9} M to about 3 x 10^{-10} M, about 3 x 10^{-10}M to about 3 x 10^{-11} M, about 3 x 10^{-11} M to about 3 x 10^{-12} M, about 4 x 10^{-3} M to about 4 x 10^{-4} M, about 4 x 10^{-4} M to about 4 x 10^{-5} M, about 4 x 10^{-5} M to about 4 x 10^{-6} M, about 4 x 10^{-6} to about 4 x 10^{-7} M, about 4 x 10^{-7} to about 4 x 10^{-8} M, about 4 x 10^{-8} M to about 4 x 10^{-9} M, about 4 x 10^{-9} M to about 4 x 10^{-10} M, about 4 x 10^{-10} M to about 4 x 10^{-11} M, about 4 x 10^{-11} M to about 4 x 10^{-12} M, about 5 x 10^{-3} M to about 5 x 10^{-4} M, about 5 x 10^{-4} M to about 5 x 10^{-5} M, about 5 x 10^{-5} M to about 5 x 10^{-6} M, about 5 x 10^{-6} to about 5 x 10^{-7} M, about 5 x 10^{-7} to about 5 x 10^{-8} M, about 5 x 10^{-8} M to about 5 x 10^{-9} M, about 5 x 10^{-9} M to about 5 x 10^{-10} M, about 5 x 10^{-10} M to about 5 x 10^{-11} M, about 5 x 10^{-11} M to about 5 x 10^{-12} M, about 5 x 10^{-7} M to about 5 x 10^{-11} M, about 5 x 10^{-7} M, about 1 x 10^{-7} M, about 5 x 10^{-8} M, about 1 x 10^{-8} M, about 5 x 10^{-9} M, about 1 x 10^{-9} M, about 5 x 10^{-10} M , about 1 x 10^{-10} M, about 5 x 10^{-11} M or about 1 x 10^{-11} M. In some embodiments, the K_D is determined in a standard flow cytometry assay using HUVEC cells.

[0213] An anti-TM4SF1 antibody, or antigen-binding fragment thereof, of the disclosure, in certain embodiments, binds to human TM4SF1 with a K_D of about 5×10^{-10} M or less in a standard flow cytometry assay using HUVEC cells.

[0214] An anti-TM4SF1 antibody, or antigen-binding fragment thereof, of the disclosure, in certain embodiments, binds to cynomolgus TM4SF1 with a K_D about 1×10^{-6} M or less in a standard flow cytometry assay using HEK293 overexpressing cells. In one embodiment, the HEK293 cells are transfected to express cynomolgus TM4SF1. In a further embodiment, HEK293 cells express cynomolgus TM4SF1 at about 600 mRNA copies per 10^6 copies 18S rRNA.

[0215] Methods of determining the K_D of an antibody or antibody fragment are known in the art. For example, surface plasmon resonance may be used to determine the K_D of the antibody to the antigen (*e.g.,* using a BIACORE 2000 or a BIACORE 3000 (BIAcore, Inc., Piscataway, N.J.) at 25°C with immobilized antigen or Fc receptor CM5 chips at about 10 response units (RU)). In certain embodiments FACS or flow cytometry is used to determine the K_D, whereby cells, such as HEK293 cells or HUVEC cells, that express TM4SF1 are used to bind the antibody or fragment and measure the K_D according to standard methods. Affinity determination of antibodies using flow cytometry is described, for example, in Geuijen *et al* (2005) *J Immunol Methods*.302(1-2):68-77. In certain embodiments, FACS is used to determine affinity of antibodies.

[0216] In one embodiment, the disclosure features an anti-TM4SF1 antibody or antigen binding fragment thereof, having CDR amino acid sequences described herein with conservative amino acid substitutions, such that the anti-TM4SF1 antibody or antigen binding fragment thereof comprises an amino acid sequence of a CDR that is at least 95% identical (or at least 96% identical, or at least 97% identical, or at least 98% identical, or at least 99% identical) to a CDR amino acid sequence set forth in Table 1. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine;

(3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine.

[0217] The disclosure further features in one aspect an anti-TM4SF1 antibody, or antigen-binding fragment thereof, that binds to an epitope on the ECL2 loop of human TM4SF1 with a K_D of about 5 x 10^{-8} M or less as determined in a standard flow cytometry assay using HUVEC cells, wherein the anti-TM4SF1 antibody, or antigen-binding fragment thereof, comprises a light chain variable region comprising a human IgG framework region and comprises a heavy chain variable region comprising a human IgG framework region. In one embodiment, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, is humanized. In one embodiment, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, cross reacts with cynomolgus TM4SF1.

[0218] In another aspect of the disclosure, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, is a humanized anti-TM4SF1 antibody, or antigen-binding fragment thereof, that binds to an epitope on the ECL2 loop of human TM4SF1 with a K_D about 5 x 10^{-8} M or less as determined in a standard flow cytometry assay using HUVEC cells. In one embodiment, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, binds to cynomolgus TM4SF1 with a K_D about 1 x 10^{-6} M or less in a standard flow cytometry assay using HEK293 overexpressing cells. In one embodiment, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, binds to human TM4SF1 with a K_D of about 1 x 10^{-8} M or less in a standard flow cytometry assay using HUVEC cells. In one embodiment, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, binds to human TM4SF1 with a K_D of 1 x 10^{-3} M to about 1 x 10^{-4} M, about 1 x 10^{-4} M to about 1 x 10^{-5} M, about 1 x 10^{-5} M to about 1 x 10^{-6} M, about 1 x 10^{-6} to about 1 x 10^{-7} M, about 1 x 10^{-7} to about 1 x 10^{-8} M, about 1 x 10^{-8} M to about 1 x 10^{-9} M, about 1 x 10^{-9} M to about 1 x 10^{-10} M, about 1 x 10^{-10}M to about 1 x 10^{-11} M, about 1 x 10^{-11} M to about 1 x 10^{-12} M, about 2 x 10^{-3} M to about 2 x 10^{-4} M, about 2 x 10^{-4} M to about 2 x 10^{-5} M, about 2 x 10^{-5} M to about 2 x 10^{-6} M, about 2 x 10^{-6} to about 2 x 10^{-7} M, about 2 x 10^{-7} to about 2 x 10^{-8} M, about 2 x 10^{-8} M to about 2 x 10^{-9} M, about 2 x 10^{-9} M to about 2 x 10^{-10} M, about 2 x 10^{-10}M to about 2 x 10^{-11} M, about 2 x 10^{-11} M to about 2 x 10^{-12} M, about 3 x 10^{-3} M to about 3 x 10^{-4} M, about 3 x 10^{-4} M to about 3 x 10^{-5} M, about 3 x 10^{-5} M to about 3 x 10^{-6} M, about 3 x 10^{-6} to about 3 x 10^{-7} M, about 3 x 10^{-7} to about 3 x 10^{-8} M, about 3 x 10^{-8} M to about 3 x 10^{-9} M, about 3 x 10^{-9} M to about 3 x 10^{-10} M, about 3 x 10^{-10}M to about 3 x 10^{-11} M, about 3 x 10^{-11} M to about 3 x 10^{-12} M, about 4 x 10^{-3} M to about 4 x 10^{-4} M, about 4 x 10^{-4} M to about 4 x 10^{-5} M, about 4 x 10^{-5} M to about 4 x 10^{-6} M, about 4 x 10^{-6} to about 4 x 10^{-7} M, about 4 x 10^{-7} to about 4 x 10^{-8} M, about 4 x

10^{-8} M to about 4 x 10^{-9} M, about 4 x 10^{-9} M to about 4 x 10^{-10} M, about 4 x 10^{-10} M to about 4 x 10^{-11} M, about 4 x 10^{-11} M to about 4 x 10^{-12} M, about 5 x 10^{-3} M to about 5 x 10^{-4} M, about 5 x 10^{-4} M to about 5 x 10^{-5} M, about 5 x 10^{-5} M to about 5 x 10^{-6} M, about 5 x 10^{-6} to about 5 x 10^{-7} M, about 5 x 10^{-7} to about 5 x 10^{-8} M, about 5 x 10^{-8} M to about 5 x 10^{-9} M, about 5 x 10^{-9} M to about 5 x 10^{-10} M, about 5 x 10^{-10}M to about 5 x 10^{-11} M, about 5 x 10^{-11} M to about 5 x 10^{-12} M, about 5 x 10^{-7} M to about 5 x 10^{-11} M, about 5 x 10^{-7} M, about 1 x 10^{-7} M, about 5 x 10^{-8} M, about 1 x 10^{-8} M, about 5 x 10^{-9} M, about 1 x 10^{-9} M, about 5 x 10^{-10} M , about 1 x 10^{-10} M, about 5 x 10^{-11} M or about 1 x 10^{-11} M. In some embodiments, the K_D is determined in a standard flow cytometry assay using HUVEC cells. In one embodiment, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, binds to human TM4SF1 with a K_D of about 5 x 10^{-10} M or less in a standard flow cytometry assay using TM4SF1 expressing HUVEC cells.

[0219] In one embodiment, binding of an anti-TM4SF1 antibody, or antigen binding fragment, of the disclosure to human TM4SF1 is not dependent on glycosylation of the ECL2 loop of human TM4SF1, *i.e.*, binding of the antibody is independent of glycosylation of TM4SF1 within the ECL2 loop (SEQ ID NO: 77).

[0220] The anti-TM4SF1 antibodies, or antigen-binding fragments thereof, of the disclosure may be any of any isotype (for example, but not limited to IgG, IgM, and IgE). In certain embodiments, antibodies, or antigen-binding fragments thereof, of the disclosure are IgG isotypes. In a specific embodiment, antibodies, or antigen-binding fragments thereof, of the disclosure are of the IgG1, IgG2 or IgG4 isotype. In certain embodiments, the anti-TM4SF1 antibody, or antigen-binding fragment thereof, are human IgG1, human IgG2, or human IgG4 isotype.

[0221] IgG2 is naturally the lowest in ADCC and/or CDC activity (An et al., MAbs. 2009 Nov-Dec; 1(6): 572–579). Accordingly, in certain embodiments it IgG2 is advantageously used. However, IgG2 has two extra cysteines (leading to 4 inter-hinge disulfide bonds) which make it prone to aggregation via formation of inter-antibody disulfide bonds. In a related embodiment, mutations to the IgG2 cysteines are made to decrease aggregation.

[0222] The present disclosure provides antibody fragments that bind to TM4SF1. In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to cells, tissues, or organs. For a review of certain antibody fragments, see Hudson et al., 2003, Nature Med. 9:129-34.

[0223] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., 1992, J. Biochem. Biophys. Methods 24:107-17; and Brennan et al., 1985,

Science 229:81-83). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv, and scFv antibody fragments can all be expressed in and secreted from E. coli or yeast cells, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form F(ab')2 fragments (Carter et al., 1992, Bio/Technology 10:163-67). According to another approach, F(ab')2 fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')2 fragment with increased in vivo half-life comprising salvage receptor binding epitope residues are described in, for example, U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv) (see, e.g., WO 93/16185; U.S. Pat. Nos. 5,571,894 and 5,587,458). Fv and scFv have intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv (See, e.g., Borrebaeck ed., supra). The antibody fragment may also be a "linear antibody," for example, as described in the references cited above. Such linear antibodies may be monospecific or multi-specific, such as bispecific.

[0224] In certain embodiments, the antigen binding fragment is selected from the group consisting of a Fab, a Fab', a F(ab')2, an Fv, and an scFv.

[0225] Anti-TM4SF1 antibodies (and fragments) that, for example, have a high affinity for human TM4SF1, can be identified using screening techniques known in the art. For example, monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., 1975, Nature 256:495-97, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567).

[0226] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized using, for example, the ECL2 loop of human TM4SF1 or cells expressing TM4SF1 (whereby the ECL2 loop is expressed on the cell surface), to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice 59-103 (1986)).

[0227] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium which, in certain embodiments, contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells (also referred to as fusion partner). For example,

if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which prevent the growth of HGPRT-deficient cells.

[0228] Exemplary fusion partner myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Exemplary myeloma cell lines are murine myeloma lines, such as SP-2 and derivatives, for example, X63-Ag8-653 cells available from the American Type Culture Collection (Manassas, Va.), and those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center (San Diego, Calif.). Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, 1984, Immunol. 133:3001-05; and Brodeur et al., Monoclonal Antibody Production Techniques and Applications 51-63 (1987)).

[0229] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. The binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as RIA or ELISA. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson et al., 1980, Anal. Biochem. 107:220-39.

[0230] Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, supra). Suitable culture media for this purpose include, for example, DMEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal, for example, by i.p. injection of the cells into mice.

[0231] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (e.g., using protein A or protein G-Sepharose) or ion-exchange chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, etc.

[0232] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells can serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells, such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody

protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., 1993, Curr. Opinion in Immunol. 5:256-62 and Pluckthun, 1992, Immunol. Revs. 130:151-88.

[0233] In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in, for example, Antibody Phage Display: Methods and Protocols (O'Brien and Aitken eds., 2002). In principle, synthetic antibody clones are selected by screening phage libraries containing phages that display various fragments of antibody variable region (Fv) fused to phage coat protein. Such phage libraries are screened against the desired antigen. Clones expressing Fv fragments capable of binding to the desired antigen are adsorbed to the antigen and thus separated from the non-binding clones in the library. The binding clones are then eluted from the antigen and can be further enriched by additional cycles of antigen adsorption/elution.

[0234] Variable domains can be displayed functionally on phage, either as single-chain Fv (scFv) fragments, in which VH and VL are covalently linked through a short, flexible peptide, or as Fab fragments, in which they are each fused to a constant domain and interact non-covalently, as described, for example, in Winter et al., 1994, Ann. Rev. Immunol. 12:433-55.

[0235] Repertoires of VH and VL genes can be separately cloned by PCR and recombined randomly in phage libraries, which can then be searched for antigen-binding clones as described in Winter et al., supra. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned to provide a single source of human antibodies to a wide range of non-self and also self- antigens without any immunization as described by Griffiths et al., 1993, EMBO J 12:725-34. Finally, naive libraries can also be made synthetically by cloning the unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro as described, for example, by Hoogenboom and Winter, 1992, J. Mol. Biol. 227:381-88.

[0236] Screening of the libraries can be accomplished by various techniques known in the art. For example, TM4SF1 (e.g., a soluble form of the ECL2 loop or cells expressing said loop) can be used to coat the wells of adsorption plates, expressed on host cells affixed to adsorption plates or used in cell sorting, conjugated to biotin for capture with streptavidin-coated beads, or used in any other method for panning display libraries. The selection of antibodies with slow dissociation kinetics (e.g., good binding affinities) can be promoted by use of long washes and monovalent phage display as described in Bass et al., 1990, Proteins 8:309-14 and WO 92/09690,

and by use of a low coating density of antigen as described in Marks et al., 1992, Biotechnol. 10:779-83.

[0237] Anti-TM4SF1 antibodies can be obtained by designing a suitable antigen screening procedure to select for the phage clone of interest followed by construction of a full length anti-TM4SF1 antibody clone using VH and/or VL sequences (*e.g.,* the Fv sequences), or various CDR sequences from VH and VL sequences, from the phage clone of interest and suitable constant region (e.g., Fc) sequences described in Kabat et al., supra.

[0238] Screening of anti-TM4SF1 antibodies can be performed using binding assays known in the art and described herein for determining whether the antibody has a therapeutic affinity for the ECL2 loop of TM4SF1. The ability of the antibody to inhibit or decrease metastatic cell activity can be measured using standard assays in the art, as well as those described herein. Preclinical assays require use of an animal model of metastasis, commonly of one of three types: (i) injection of metastatic mouse tumor cells such as B16F10 melanoma TCs into mice, commonly via tail vein injection to generate lung metastases, via portal vein or intrasplenic injection to generate liver metastases, or via left ventricular cardiac injection to generate bone and other metastases; (ii) orthotopic transplantation of metastatic tumor cells or intact tumor fragments into mice, which methods often require later surgical resection of the primary tumor to prevent morbidity associated with primary tumor growth; and (iii) genetically engineered mouse models of spontaneous metastasis, of which the most common is the MMTV-Pyt (mouse mammary tumor virus-polyomavirus middle T Antigen) mouse mammary carcinoma model which provides a highly realistic mouse model of human cancer metastasis; greater than 85% of hemizygous MMTV-PyMT females spontaneously develop palpable mammary tumors which metastasize to the lung at age to 8-16 weeks. Quantifying the metastatic burden in the lung, either by live animal imaging or direct counting of metastatic nodules in the lungs of sacrificed animals, as a function of the degree of TM4SF1 immunoblockade and achieving a therapeutic level, *e.g.,* at least a 50% reduction in lung metastasis, would be indicative, for example, of a therapeutic antibody that could be used in the methods of the disclosure. Further, cross-species reactivity assays are known in the art. Examples of assays that can be used are described, for example, in Khanna and Hunter (Carcinogenesis. 2005 Mar; 26(3):513-23) and Saxena and Christofori (Mol Oncol. 2013 Apr;7(2):283-96), incorporated by reference in their entireties herein.

[0239] In some embodiments, the anti-TM4SF1 antibodies and antigen binding fragments thereof can be used, *e.g.,* to treat or prevent cancer. In certain embodiments, the anti-TM4SF1 antibodies and antigen binding fragments of the disclosure can be used to prevent tumor cells from metastasizing. The anti-TM4SF1 antibodies and antigen binding fragments thereof, of this

disclosure, in some examples, prevent tumor cell metastasis by interfering with the interaction between tumor cells and blood vessel endothelial cells.

IV. Therapeutic molecules in the ADCs

[0240] In some embodiments, the ADCs of this disclosure comprise one or more therapeutic (also referred to herein as a therapeutic molecule or a therapeutic agent) conjugated to an anti-TM4SF1 antibody or an antigen binding fragment thereof. In some embodiments, the agent is a therapeutic agent or a diagnostic agent. In some embodiments, the therapeutic agent is a biologically active moiety. In some embodiments, the biologically active moiety comprises a radioactive isotope, a cytotoxic agent, a chemotherapeutic agent, a protein, a peptide, an antibody, a growth inhibitory agent, a prodrug activating enzyme, and an anti-hormonal agent. In some embodiments, a therapeutic molecule can be a small molecule (*e.g.*, both for cancer and for non-cancer angiogenic indications); a V-ATPase inhibitor; a pro-apoptotic agent; a Bcl2 inhibitor; an MCL1 inhibitor; a HSP90 inhibitor; an IAP inhibitor; an mTor inhibitor; a microtubule stabilizer; a microtubule destabilizer; an auristatin; a dolastatin; a maytansinoid; a MetAP (methionine aminopeptidase); an inhibitor of nuclear export of proteins CRM1; a DPPIV inhibitor; proteasome inhibitors; inhibitors of phosphoryl transfer reactions in mitochondria; a protein synthesis inhibitor; a kinase inhibitor (such as, a CDK2 inhibitor, a CDK9 inhibitor); a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder, a DHFR inhibitor, a nucleic acid, a CRISPR enzyme; degraders (such as agents that induce protein degradation, (*e.g.*, HSP90 inhibitor, selective estrogen receptor degraders (SERDs), selective androgen receptor degraders (SARDs); hydrophobic tags that can be used to recruit chaperones to a protein of interest, *e.g.*, Adamantane, Arg-Boc3; E3 ligase recruiting ligands, *e.g.*, Nutlin-3a (MDM2 ligand), Bestatin (cIAP ligand), VHL ligand, Pomalidomide (CRBN ligand); proteolysis-targeting chimeras (PROTACs) that may utilitize different D3 ligases to target a protein of interest for degradation)) (*see, e.g.*, Lai AC, Crews CM. Induced protein degradation: an emerging drug discovery paradigm. Nat Rev Drug Discov. 2016;16(2):101-114); antisense oligonucleotides; RNAi agents (such as siRNA), CRISPR-Cas9 gene editing systems; RNA molecules; DNA *e.g.*, plasmids; an anti-cancer agent, an anti-inflammatory agent, an anti-infective agent (*e.g.*, anti-fungal, antibacterial, anti-parasitic, anti-viral), an anesthetic agent; RNA polymerase II inhibitor; a DNA intercalating agent, a DNA cross-linking agent; an anti-tubulin agent; a cytotoxic drug, a tumor vaccine, an antibody, a peptide, pepti-bodies, a chemotherapeutic agent, a cytotoxic agent; a cytostatic agent; an immunological modifiers, an interferon, an interleukin, an immuno stimulatory growth hormone,

a cytokine, a vitamin, a mineral, an aromatase inhibitor, a Histone Deacetylase (HDAC), an HDAC inhibitor; a lipid nanoparticle to encapsulate one or more therapeutic molecule.

[0241] In some embodiments, the radioactive isotope may be one or more kinds selected from the group consisting of ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, and radioactive isotopes of Lu, but not limited thereto. In some embodiments, the prodrug-activating enzyme is one or more kinds selected from the group consisting of: an alkaline phosphatase, an arylsulfatase, a cytosine deaminase, a protease, a D-alanylcarboxy-peptidase, a carbohydrate-cleaving enzyme, a P-lactamase and a penicillin amidase, but not limited thereto.

[0242] The cytotoxic agent, ins some embodiments, comprises one or more selected from the group consisting of: ricin, saporin, gelonin, momordin, debouganin, diphtheria toxin, pseudomonas toxin, etc., but not limited thereto. The cytotoxic agent, in some instances is one or more kinds selected from the group consisting of: cisplatin, carboplatin, oxaliplatin, paclitaxel, melphalan, doxorubicin, methotrexate, 5-fluorouracil, etoposide, mechlorethamine, cyclophosphamide, bleomycin, a calicheamicin, a maytansine, a trichothene, CC1065, diphtheria A chain, Pseudomonas aeruginosa exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleuritesfordii proteins, dianthin proteins, Phytolaca americana proteins, momordica charantia inhibitors, curcin, crotin, sapaonaria officinalis inhibitors, gelonin, mitogellin, restrictocin, phenomycin, enomycin, tricothecenes, ribonucleases and deoxyribonucleases, but not limited thereto. In some embodiments, the cytotoxic agent is one or more kinds selected from the group consisting of: duocarmycin, monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), N2′-deacetyl-N2′-(3-mercapto-1-oxopropyl)maytansine (DM1), PBD (Pyrrolobenzodiazepine) dimer, duocarmycin, monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), but not limited thereto. In some embodiments, the cytotoxic agent comprises a ribosome inactivating protein, a histone deacetylase (HDAC) inhibitor, a tubulin inhibitor, an alkylating agent, an antibiotic, an antineoplastic agent, an antiproliferative agent, an antimetabolite, a topoisomerase I or II inhibitor, a hormonal agonist or antagonist, an immunomodulator, a DNA minor groove binder, and a radioactive agent. In certain embodiments, the ribosome inactivating protein is saporin. In some embodiments, the diagnostic agent is a label. In some embodiments, the label is a fluorescent label, a chromogenic label, or a radiolabel. In some embodiments, the agent is directly conjugated to the anti-TM4SF1 antibody or antigen binding fragment thereof. In other embodiments, the agent is indirectly conjugated to the anti-TM4SF1 antibody or antigen binding fragment thereof, optionally by a linker.

[0243] In some embodiments, an ADC of this disclosure comprises an anti-TM4SF1 antibody or antigen binding fragment thereof and one or more agents (*e.g.*, 1 , 2, 3, or 4 or more agents), such

as therapeutic agents, that act additively or synergistically with the anti-TM4SF1 antibody or antigen binding fragment thereof, for example, to kill or inhibit tumor cells (TCs) and/or tumor vasculature endothelial cells (ECs) in the treatment of a disorder associated with pathological angiogenesis, such as cancer. The therapeutic agent, for example, can be a biologically active moiety, such as a cytotoxic agent, a chemotherapeutic agent, a protein, a peptide, an antibody, a growth inhibitory agent, and/or an anti-hormonal agent.

[0244] Examples of tubulin inhibitors that can be conjugated, either directly or indirectly, to an anti-TM4SF1 antibody or antigen binding fragment thereof, can include, without limitation, polymerization inhibitors (*e.g.*, vinblastine, vincristine, vinorelbine, vinflunine, cryptophycin 52, hallchondrins, dolastatins, hemiasterlins that can bind to the vinca domain of tubulin; colchine, combretastatins, 2-methoxy-estradiol, E7010 that can bind to the cholchicine domain of tubulin; depolymerization inhibitors, such as paclitaxel, docetaxel, epothilon, discodermolide that can bind to the taxane site).

[0245] Exemplary chemotherapeutic agents include, but are not limited to, methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents; enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0246] In addition, a variety of radionuclides can be used for conjugation of the anti-TM4SF1 antibodies or antigen binding fragments to the therapeutic agents, to generate the ADCs of this disclosure. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Sm^{153}, Bi^{212}, P^{32}, and radioactive isotopes of Lu. Alternatively, the anti-TM4SF1 antibodies or antigen binding fragments can be conjugated to one or more smaller molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, aurostatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein. Other therapeutic agents that can be conjugated to TM4SF1 binding protein of the disclosure include, in various examples, BCNU, streptozoicin, vincristine and 5-fluorouracil etc.

[0247] The diagnostic agent for conjugation, in some embodiments, is a label, such as a fluorescent label, a chromogenic label, or a radiolabel. Accordingly, the label may be used for

detection purposes, and may be a fluorescent compound, an enzyme, a prosthetic group, a luminescent material, a bioluminescent material, or a radioactive material. The radiolabel, for example, may comprise a radioactive atom for scintigraphic studies, for example Tc^{99m} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123 again, iodine-131 , indium-111 , fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium , manganese or iron.

[0248] The one or more agents (*e.g.*, therapeutic agents and/or diagnostic agents) may be directly conjugated to anti-TM4SF1 antibodies or antigen binding fragments (*e.g.*, by way of a direct covalent or non-covalent interaction), such that the agent is immediately conjugated to the protein. An agent may be directly conjugated to a binding protein of the disclosure, for example, by a direct peptide bond. In other instances, the direct conjugation is by way of a direct non-covalent interaction, such as an interaction between the anti-TM4SF1 antibodies or antigen binding fragments and an agent that specifically binds to the anti-TM4SF1 antibodies or antigen binding fragments.

V. Linkers

[0249] The one or more agents (*e.g.*, therapeutic agents and/or diagnostic agents) may be indirectly conjugated to anti-TM4SF1 antibodies or antigen binding fragments (e.g., by way of a linker with direct covalent or non-covalent interactions). Linkers can be chemical linking agents, such as homobifunctional and heterobifunctional cross-linkers, which are available from many commercial sources. Regions available for cross-linking may be found on the binding protein (*e.g.*, anti-TM4SF1 antibodies) of the disclosure. The linker may comprise a flexible arm, *e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 carbon atoms. Exemplary linkers include BS3 ([Bis(sulfosuccinimidyl)suberate];BS3 is a homobifunctional N-hydroxysuccinimideester that targets accessible primary amines), NHS/EDC (N-hydroxysuccinimide and N-ethyl-(dimethylaminopropyl)carbodimide; NHS/EDC allows for the conjugation of primary amine groups with carboxyl groups), sulfo-EMCS ([N-e-Maleimidocaproic acid]hydrazide; sulfo-EMCS are heterobifunctional reactive groups (maleimide and NHS-ester) that are reactive toward sulfhydryl and amino groups), hydrazide (most proteins contain exposed carbohydrates and hydrazide is a useful reagent for linking carboxyl groups to primary amines), and SATA (N-succinimidyl-S-acetylthioacetate; SATA is reactive towards amines and adds protected sulfhydryls groups). To form covalent bonds, a chemically reactive group a wide variety of active carboxyl groups (e.g., esters) where the hydroxyl moiety is physiologically acceptable at the levels required to modify the peptide. Particular agents include N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyryloxy succinimide ester (GMBS), maleimido propionic acid (MPA) maleimido

hexanoic acid (MHA), and maleimido undecanoic acid (MUA). Primary amines are the principal targets for NHS esters. Accessible a-amino groups present on the N-termini of proteins and the ε-amine of lysine react with NHS esters. An amide bond is formed when the NHS ester conjugation reaction reacts with primary amines releasing N-hydroxysuccinimide. These succinimide containing reactive groups are herein referred to as succinimidyl groups. In certain embodiments of the disclosure, the functional group on the protein will be a thiol group and the chemically reactive group will be a maleimido-containing group such as gamma-maleimide-butrylamide (GMBA or MPA). Such maleimide containing groups are referred to herein as maleido groups. The maleimido group is most selective for sulfhydryl groups on peptides when the pH of the reaction mixture is 6.5-7.4. At pH 7.0, the rate of reaction of maleimido groups with sulfhydryls (*e.g.*, thiol groups on proteins such as serum albumin or IgG) is 1000-fold faster than with amines. Thus, a stable thioether linkage between the maleimido group and the sulfhydryl can be formed.

[0250] Further exemplary linker/linker chemistry that in some embodiments is used for conjugation of an anti-TM4SF1 antibody or an antigen binding fragment thereof, as described herein, include moieties that can be used in a click conjugation, e.g., in a two-step conjugation in which a first moiety is conjugated to an engineered cysteine (e.g., at position N297 with an N297C mutation), said first moiety containing a reactive handle, and a second moiety containing the linker-payload which reacts with the first moiety. An example of a possible reaction between the first moiety's reactive handle and the second moiety is a metal free click reaction that utilizes strain-promoted azide-alkyne cycloaddition. Examples of moieties include, but are not limited to, bicyclononyne (BCN) reacting with an azide or tetrazine, dibenzocyclooctyne (DBCO) reacting with an azide, also denoted as aza-dibenzocyclooctyne (DIBAC), a transcyclooctene (TCO) reacting with a tetrazine (such as methyl tetrazine), or a methyl cycloprene click handle reacting with tetrazine. Specific examples of such moieties are as follows, but not limited to: dibenzylcyclooctyne-PegX-carboxylic acid, perfluorophenyl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate Chemical Formula: C16H12F5NO4 Molecular Weight: 377.27; 6-(3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid Chemical Formula: C10H11Br2NO4 Molecular Weight: 369.01; (2-methylcycloprop-2-en-1-yl)methyl carbamate (E)-cyclooct-4-en-1-yl (2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)carbamate 3-(5-methylpyridin-2-yl)-6-(pyridin-2-yl)-1,2,4,5-tetrazine; ((1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-yl)methyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate Chemical Formula: C17H28N2O4 Molecular Weight: 324.42; ((1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-yl)methyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate Chemical Formula: C17H28N2O4 Molecular Weight: 324.42.

[0251] In other embodiments, the linker includes at least one amino acid (e.g., a peptide of at least 2, 3, 4, 5, 6, 7, 10, 15, 20, 25, 40, or 50 amino acids). In certain embodiments, the linker is a single amino acid (*e.g.*, any naturally occurring amino acid such as Cys). In other embodiments, a glycine-rich peptide such as a peptide can be used. In some cases, the linker can be a single amino acid (e.g., any amino acid, such as Gly or Cys). Examples of suitable linkers are succinic acid, Lys, Glu, and Asp, or a dipeptide such as Gly-Lys. When the linker is succinic acid, one carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the other carboxyl group thereof may, for example, form an amide bond with an amino group of the peptide or substituent. When the linker is Lys, Glu, or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the amino group thereof may, for example, form an amide bond with a carboxyl group of the substituent. When Lys is used as the linker, a further linker may be inserted between the ε-amino group of Lys and the substituent. In one particular embodiment, the further linker is succinic acid which, e.g., forms an amide bond with the ε- amino group of Lys and with an amino group present in the substituent. In one embodiment, the further linker is Glu or Asp (e.g., which forms an amide bond with the ε-amino group of Lys and another amide bond with a carboxyl group present in the substituent), that is, the substituent is a NE-acylated lysine residue.

[0252] In some embodiments, the anti-TM4SF1 antibody or an antigen binding fragment thereof as described herein and an oligonucleotide (*e.g.*, a nucleic acid molecule, such as an RNA molecule or a DNA molecule) can be conjugated using various approaches, such as a genetic conjugation, an enzymatic conjugation, a chemical conjugation, or any combination thereof.

[0253] In some embodiments, the RNA molecules within the ADCs may be conjugated to the anti-TM4SF1 antibody or an antigen binding fragment thereof using an enzymatic site-specific conjugation method which involves the use of a mammalian or bacterial transglutaminase enzyme. Microbial transglutaminases (mTGs) are versatile tools in modern research and biotechnology. The availability of large quantities of relatively pure enzymes, ease of use, and lack of regulation by calcium and guanosine-5′-triphosphate (GTP) has propelled mTG to be the main cross-linking enzyme used in both the food industry and biotechnology. Currently, mTGs are used in many applications to attach proteins and peptides to small molecules, polymers, surfaces, DNA, as well as to other proteins. *See, e.g.*, Pavel Strp, Veracity of microbial transglutaminase, Bioconjugate Chem. 25, 5, 855-862).

[0254] In some embodiments, the RNA molecules within the conjugates may be conjugated to the anti-TM4SF1 antibody or an antigen binding fragment thereof by way of a linker with direct covalent or non-covalent interactions. Linkers can be amino acid or peptide based linkers, or chemical linking agents, such as homobifunctional and heterobifunctional cross-linkers, which

are available from many commercial sources. Regions available for cross-linking may be found on the anti-TM4SF1 antibody or an antigen binding fragment thereof of the disclosure. The linker may comprise a flexible arm, *e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 carbon atoms. Exemplary linkers include cleavable, non-cleavable, covalent, or non-covalent linkers, or any combinations thereof. The cleavable linker, in some embodiments, comprises an acid-labile linker, a protease-sensitive linker, a photo-labile linker, or a disulfide-containing linker. In some embodiments, the linker comprises a cysteine linker or a non-cysteine linker, such as a lysine linker. In some embodiments, the anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an unnatural amino acid, wherein the antibody or antibody fragment and the oligonucleotide are linked/conjugated *via* the unnatural amino acid.

[0255] In some embodiments, the anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a natural amino acid, wherein the antibody or antibody fragment and the oligonucleotide are linked/conjugated *via* the natural amino acid. The unnatural amino acid may be inserted between two naturally occurring amino acids in the antibody or antibody fragment. The one or more unnatural amino acids may replace one or more naturally occurring amino acids in the antibody or antibody fragment. The one or more unnatural amino acids may be incorporated at the N terminus of the antibody or antibody fragment. The one or more unnatural amino acids may be incorporated at the C terminus of the antibody or antibody fragment. The unnatural amino acid may be incorporated distal to the binding region of antibody or antibody fragment. The unnatural amino acid may be incorporated near the binding region of the antibody or antibody fragment. The unnatural amino acid may be incorporated in the binding region of the antibody or antibody fragment.

[0256] The one or more unnatural amino acids may be encoded by a codon that does not code for one of the twenty natural amino acids. The one or more unnatural amino acids may be encoded by a nonsense codon (stop codon). The stop codon may be an amber codon. The amber codon may comprise a UAG sequence. The stop codon may be an ochre codon. The ochre codon may comprise a UAA sequence. The stop codon may be an opal or umber codon. The opal or umber codon may comprise a UGA sequence. The one or more unnatural amino acids may be encoded by a four-base codon.

[0257] The one or more unnatural amino acids may be p-acetylphenylalanine (pAcF or pAcPhe). The one or more unnatural amino acids may be selenocysteine. The one or more unnatural amino acids may be p-fluorophenylalanine (pFPhe). The one or more unnatural amino acids may be selected from the group comprising p-azidophenylalanine (pAzF),p-azidomethylphenylalanine(pAzCH2F), p-benzoylphenylalanine (pBpF), p-propargyloxyphenylalanine (pPrF), p-iodophenylalanine (pIF), p-cyanophenylalanine (pCNF), p-

carboxylmethylphenylalanine (pCmF), 3-(2-naphthyl)alanine (NapA), p-boronophenylalanine (pBoF), o-nitrophenylalanine (oNiF), (8-hydroxyquinolin-3-yl)alanine (HQA), selenocysteine, and (2,2'-bipyridin-5-yl)alanine (BipyA).). The one or more unnatural amino acids may be 4-(6-methyl-s-tetrazin-3-yl)aminopheynlalanine.

[0258] The one or more unnatural amino acids may be β-amino acids (β3 and β2), homo-amino acids, proline and pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine and tyrosine derivatives, linear core amino acids, diamino acids, D-amino acids, N-methyl amino acids, or a combination thereof.

[0259] Additional examples of unnatural amino acids include, but are not limited to, 1) various substituted tyrosine and phenylalanine analogues such as O-methyl-L-tyrosine, p-amino-L-phenylalanine, 3-nitro-L-tyrosine, p-nitro-L-phenylalanine, m-methoxy-L-phenylalanine and p-isopropyl-L-phenylalanine; 2) amino acids with aryl azide and benzophenone groups that may be photo-cross-linked; 3) amino acids that have unique chemical reactivity including acetyl-L-phenylalanine and m-acetyl-L-phenylalanine, O-allyl-L-tyrosine, O-(2-propynyl)-L-tyrosine, p-ethylthiocarbonyl-L-phenylalanine and p-(3-oxobutanoyl)-L-phenylalanine; 4) heavy-atom-containing amino acids for phasing in X-ray crystallography including p-iodo and p-bromo-L-phenylalanine; 5) the redox-active amino acid dihydroxy-L-phenylalanine; 6) glycosylated amino acids including b-N-acetylglucosamine-O-serine and a-N-acetylgalactosamine-O-threonine; 7) fluorescent amino acids with naphthyl, dansyl, and 7-aminocoumarin side chains; 8) photocleavable and photoisomerizable amino acids with azobenzene and nitrobenzyl Cys, Ser, and Tyr side chains; 9) the phosphotyrosine mimetic p-carboxymethyl-L-phenylalanine; 10) the glutamine homologue homoglutamine; and 11) 2-aminooctanoic acid. The unnatural amino acid may be modified to incorporate a chemical group. The unnatural amino acid may be modified to incorporate a ketone group.

[0260] The one or more unnatural amino acids may comprise at least one oxime, carbonyl, dicarbonyl, hydroxylamine group or a combination thereof. The one or more unnatural amino acids may comprise at least one carbonyl, dicarbonyl, alkoxy-amine, hydrazine, acyclic alkene, acyclic alkyne, cyclooctyne, aryl/alkyl azide, norbornene, cyclopropene, trans-cyclooctene, or tetrazine functional group or a combination thereof.

[0261] The one or more unnatural amino acids may be incorporated into the antibody or antibody fragment by methods known in the art. Cell-based or cell-free systems may be used to alter the genetic sequence of antibody or antibody fragment, thereby producing the antibody or antibody fragment with one or more unnatural amino acids. Auxotrophic strains may be used in place of engineered tRNA and synthetase. The one or more unnatural amino acids may be produced through selective reaction of one or more natural amino acids. The selective reaction may be

mediated by one or more enzymes. In one non-limiting example, the selective reaction of one or more cysteines with formylglycine generating enzyme (FGE) may produce one or more formylglycines as described in Rabuka et al., Nature Protocols 7:1052-1067 (2012).

[0262] The one or more unnatural amino acids may take part in a chemical reaction to form a linker. The chemical reaction to form the linker may be a bioorthogonal reaction. The chemical reaction to form the linker may be click chemistry.

[0263] Additional unnatural amino acids are disclosed in Liu et al. (Annu Rev Biochem, 79:413-44, 2010), Wang et al. (Angew Chem Int Ed, 44:34-66, 2005) and PCT application numbers PCT/US2012/039472, PCT/US2012/039468, PCT/US2007/088009, PCT/US2009/058668, PCT/US2007/089142, PCT/US2007/088011, PCT/US2007/001485, PCT/US2006/049397, PCT/US2006/047822 and PCT/US2006/044682, all of which are incorporated by reference in their entireties.

[0264] The one or more unnatural amino acids may replace one or more amino acids in the antibody or antibody fragment. The one or more unnatural amino acids may replace any natural amino acid in the antibody or antibody fragment.

[0265] The one or more unnatural amino acids may be incorporated in a light chain of the antibody or antibody fragment. The one or more unnatural amino acids may be incorporated in a heavy chain of the antibody or antibody fragment. The one or more unnatural amino acids may be incorporated in a heavy chain and a light chain of antibody or antibody fragment. The one or more unnatural amino acids may replace an amino acid in the light chain of the antibody or antibody fragment. The one or more unnatural amino acids may replace an amino acid in a heavy chain of the antibody or antibody fragment. The one or more unnatural amino acids may replace an amino acid in a heavy chain and a light chain of the antibody or antibody fragment.

[0266] anti-TM4SF1 antibody or an antigen binding fragment thereof anti-TM4SF1 antibody or an antigen binding fragment thereof anti-TM4SF1 antibody or an antigen binding fragment thereof anti-TM4SF1 antibody or an antigen binding fragment thereof anti-TM4SF1 antibody or an antigen binding fragment thereof anti-TM4SF1 antibody or an antigen binding fragment thereof anti-TM4SF1 antibody or an antigen binding fragment thereof In some embodiments, the linker comprises a small molecule fragment, a spacer, a non-covalent linker, or a combination thereof. In some embodiments, the linker comprises one or more of small molecule fragments. In some embodiments, the linker comprises a spacer.

[0267] In some embodiments, a linker comprises one or more of reactive moieties. In some embodiments, a linker comprises a reactive moiety selected from a Michael acceptor moiety, a leaving group moiety, or a moiety capable of forming a covalent bond with the antibody fragment and/or the therapeutic agent.

[0268] In some embodiments, a small anti-TM4SF1 antibody or an antigen binding fragment thereof anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a reactive moiety. In some embodiments, a small molecule fragment comprises a reactive moiety selected from a Michael acceptor moiety, a leaving group moiety, or a moiety capable of forming a covalent bond with the thiol group of a cysteine residue.

[0269] In some embodiments, the Michael acceptor moiety comprises an alkene or an alkyne moiety. In some embodiments, a small molecule fragment is obtained from a compound library. In some embodiments, the compound library comprises ChemBridge fragment library, Pyramid Platform Fragment-Based Drug Discovery, Maybridge fragment library, FRGx from AnalytiCon, TCI-Frag from AnCoreX, Bio Building Blocks from ASINEX, BioFocus 3D from Charles River, Fragments of Life (FOL) from Emerald Bio, Enamine Fragment Library, IOTA Diverse 1500, BIONET fragments library, Life Chemicals Fragments Collection, OTAVA fragment library, Prestwick fragment library, Selcia fragment library, TimTec fragment-based library, Allium from Vitas-M Laboratory, or Zenobia fragment library.

[0270] In some embodiments, a small molecule fragment comprises a carbodiimide, N-hydroxysuccinimide (NHS) ester, imidoester, pentafluorophenyl ester, hydroxymethyl phosphine, maleimide, haloacetyl, pyridyl disulfide, thiosulfonate, vinylsulfone, hydrazide, alkoxyamine, alkyne, azide, or isocyanate group. In some embodiments, a small molecule fragment comprises an alkyne or an azide group. In some embodiments, a small molecule fragment comprises an alkyne group. In some embodiments, a small molecule fragment comprises an azide group.

[0271] In some embodiments, a small molecule fragment covalently interacts with a spacer. In some embodiments, the spacer comprises an amide moiety, an ester moiety, an ether moiety, substituted or unsubstituted C1-C6alkylene moiety, substituted or unsubstituted C1-C6haloalkylene moiety, substituted or unsubstituted C1-C6heteroalkylene moiety, substituted or unsubstituted C3-C8cycloalkylene moiety, substituted or unsubstituted C2-C7heterocycloalkylene moiety, substituted or unsubstituted arylene moiety, a substituted or unsubstituted heteroarylene moiety or any combination thereof.

[0272] In some embodiments, the linker comprises MC (6-maleimidocaproyl), MCC (a maleimidomethyl cyclohexane-1-carboxylate), MP (maleimidopropanoyl), val-cit (valine-citrulline), val-ala (valine-alanine), ala-phe (alanine-phenylalanine), PAB (p-aminobenzyloxycarbonyl), SPP (N-Succinimidyl 4-(2-pyridylthio) pentanoate), SMCC (N-Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 carboxylate), SIAB (N-Succinimidyl (4-iodo-acetyl)aminobenzoate. Further examples of linkers include: BS3 ([Bis(sulfosuccinimidyl)suberate]; BS3 is a homobifunctional N-hydroxysuccinimideester that

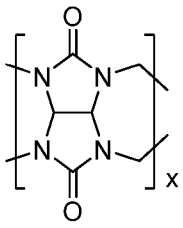
targets accessible primary amines), NHS/EDC (N-hydroxysuccinimide and N-ethyl-(dimethylaminopropyl)carbodimide; NHS/EDC allows for the conjugation of primary amine groups with carboxyl groups), sulfo-EMCS ([N-e-Maleimidocaproic acid]hydrazide; sulfo-EMCS are heterobifunctional reactive groups (maleimide and NHS-ester) that are reactive toward sulfhydryl and amino groups), hydrazide (most proteins contain exposed carbohydrates and hydrazide is a useful reagent for linking carboxyl groups to primary amines), and SATA (N-succinimidyl-S-acetylthioacetate; SATA is reactive towards amines and adds protected sulfhydryls groups). To form covalent bonds, a chemically reactive group a wide variety of active carboxyl groups (*e.g.*, esters) where the hydroxyl moiety is physiologically acceptable at the levels required to modify the peptide. Particular agents include N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyryloxy succinimide ester (GMBS), maleimido propionic acid (MPA) maleimido hexanoic acid (MHA), and maleimido undecanoic acid (MUA). Primary amines are the principal targets for NHS esters. Accessible a-amino groups present on the N-termini of proteins and the ε-amine of lysine react with NHS esters. An amide bond is formed when the NHS ester conjugation reaction reacts with primary amines releasing N-hydroxysuccinimide. These succinimide containing reactive groups are herein referred to as succinimidyl groups. In certain embodiments of the disclosure, the functional group on the protein will be a thiol group and the chemically reactive group will be a maleimido-containing group such as gamma-maleimide-butrylamide (GMBA or MPA). Such maleimide containing groups are referred to herein as maleido groups. The maleimido group is most selective for sulfhydryl groups on peptides when the pH of the reaction mixture is 6.5-7.4. At pH 7.0, the rate of reaction of maleimido groups with sulfhydryls (e.g., thiol groups on proteins such as serum albumin or IgG) is 1000-fold faster than with amines. Thus, a stable thioether linkage between the maleimido group and the sulfhydryl can be formed.

[0273] In other embodiments, the linker includes at least one amino acid (*e.g.*, a peptide of at least 2, 3, 4, 5, 6, 7, 10, 15, 20, 25, 40, or 50 amino acids). In certain embodiments, the linker is a single amino acid (*e.g.*, any naturally occurring amino acid such as Cys or Lys). In other embodiments, a glycine-rich peptide such as a peptide can be used. In some cases, the linker can be a single amino acid (*e.g.*, any amino acid, such as Gly or Cys or Lys). Examples of suitable linkers are succinic acid, Lys, Glu, and Asp, or a dipeptide such as Gly-Lys. When the linker is succinic acid, one carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the other carboxyl group thereof may, for example, form an amide bond with an amino group of the peptide or substituent. When the linker is Lys, Glu, or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue,

and the amino group thereof may, for example, form an amide bond with a carboxyl group of the substituent. When Lys is used as the linker, a further linker may be inserted between the ε-amino group of Lys and the substituent. In one particular embodiment, the further linker is succinic acid which, e.g., forms an amide bond with the ε- amino group of Lys and with an amino group present in the substituent. In one embodiment, the further linker is Glu or Asp (e.g., which forms an amide bond with the ε-amino group of Lys and another amide bond with a carboxyl group present in the substituent), that is, the substituent is a NE-acylated lysine residue. In some embodiments, a linker comprises a single-amino acid peptide consisting of a lysine. In some embodiments, a linker comprises a LysLys dipeptide. In some embodiments, a linker comprises a *Lys and/or Lys* dipeptide. In some embodiments, a linker comprises a LysLys* and/or*LysLys, Lys*Lys tripeptide. In some embodiments, a linker comprises a LysLysLys tripeptide.

[0274] In some embodiments, the conjugation of anti-TM4SF1 antibody or an antigen binding fragment thereof and the RNA molecules is carried out in a manner to produce a ring threaded molecule. In some embodiments, the spacer additionally comprises a macrocycle. In some embodiments, the macrocycle comprises a non-covalent macrocycle. In some embodiments, the macrocycle comprises a covalent macrocycle.

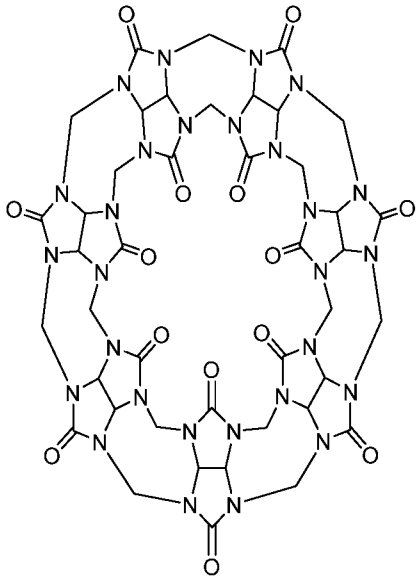
[0275] In some embodiments, the macrocycle comprises cucurbit[X]uril, wherein X is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, the macrocycle comprises cucurbit[X]uril, wherein X is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some embodiments, the macrocycle comprises cucurbit[X]uril, wherein X is 5, 6, 7, or 8. In some embodiments, the cucurbit[X]uril has a structure represented by:



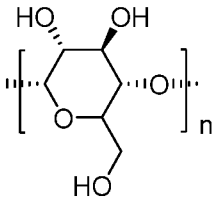
, wherein x is 5, 6, 7, or 8.

[0276] In some embodiments, x is 5. In some embodiments, x is 6. In some embodiments, x is 7. In some embodiments, x is 8.

[0277] In some embodiments, the macrocycle comprises cucurbit[6]uril (CB6). In some embodiments, the macrocycle comprises cucurbit[7]uril (CB7). In some embodiments, the cucurbit[7]uril has a structure represented by:

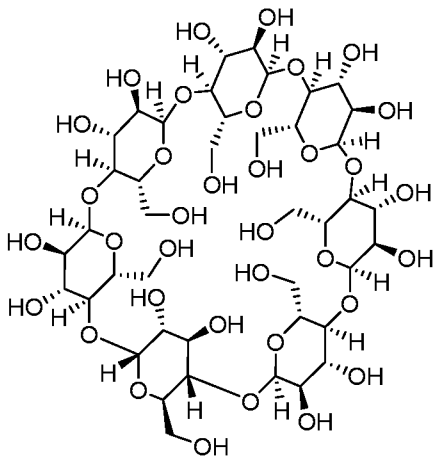


[0278] In some embodiments, the macrocycle comprises a cyclodextrin (CD). In some embodiments, the cyclodextrin has a structure represented by:

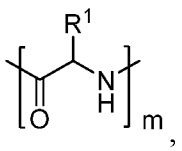


, wherein n is 5, 6, 7, or 8.

[0279] In some embodiments, the macrocycle comprises a beta-cyclodextrin (n = 7). In some embodiments, macrocycle comprises a gamma-cyclodextrin (n = 8). In some embodiments, the beta-cyclodextrin has a structure represented by:



[0280] In some embodiments, the macrocycle comprises a polypeptide. In some embodiments, the polypeptide has a structure represented by:

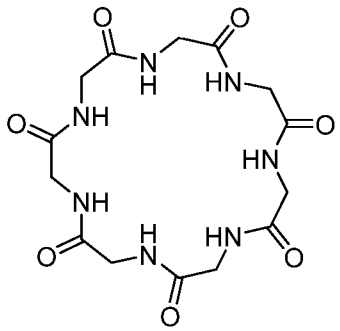


,

wherein

R^1 is H, D, F, -CN, substituted or unsubstituted C_1-C_6alkyl, substituted or unsubstituted C_1-C_6fluoroalkyl, substituted or unsubstituted C_1-C_6heteroalkyl, substituted or unsubstituted C_3-C_8cycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl; and m is 5, 6, 7, or 8.

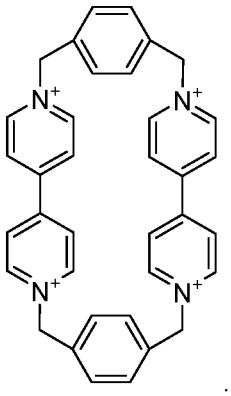
[0281] In some embodiments, the macrocycle comprises a cycloglycine. In some embodiments, the macrocycle comprises cyclo(glycylglycylglycylglycyglycyllglycyl). . In some embodiments, the macrocycle comprises cyclo(glycylglycylglycylglycylglycylglycylglycyl). In some embodiments, the cyclo(glycylglycylglycylglycylglycylglycylglycyl) has a structure represented by:

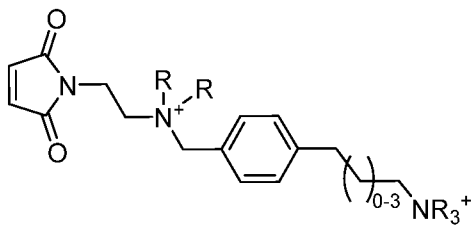


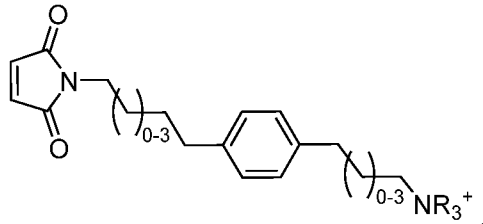
[0282] In some embodiments, the macrocycle comprises a crown ether. In some embodiments, the crown ether is a 15-crown-5, 18-crown-6, dibenzo-18-crown-6, or diaza-18-crown-6.

[0283] In some embodiments, the macrocycle comprises a cycloalkane. In some embodiments, the cycloalkane is a cyclopentadecane, cyclohexadecane, cycloheptadecane, or cyclooctadecane.

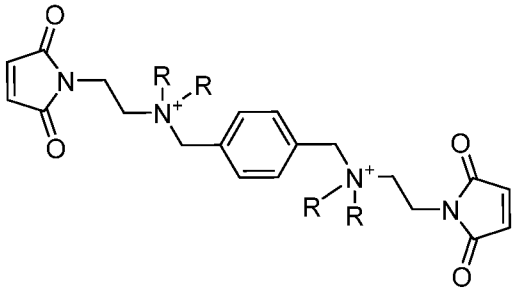
[0284] In some embodiments, the macrocycle comprises cyclobis(paraquat-p-phenylene) ($CBPQT^{4+}$). In some embodiments, the cyclobis(paraquat-p-phenylene) ($CBPQT^{4+}$) has a structure represented by:



[0285] In some embodiments, a linker comprises quaternary nitrogen. In some embodiments, the linker is:  , wherein each R is independently H or C1-C6

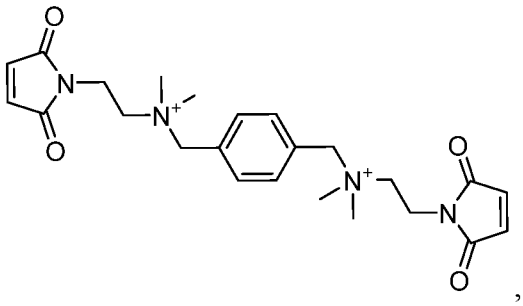


alkyl. In some embodiments, the linker is: , wherein each R is independently H or C1-C6 alkyl. In some embodiments, the linker is:

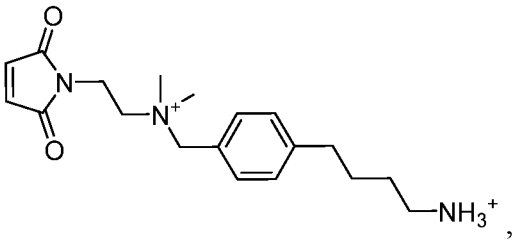


, wherein each R is independently H or C1-C6 alkyl.

[0286] In some embodiments, the linker is:



,



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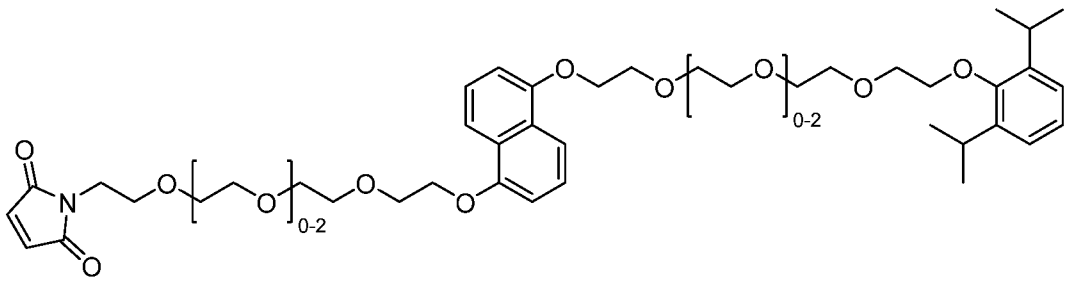
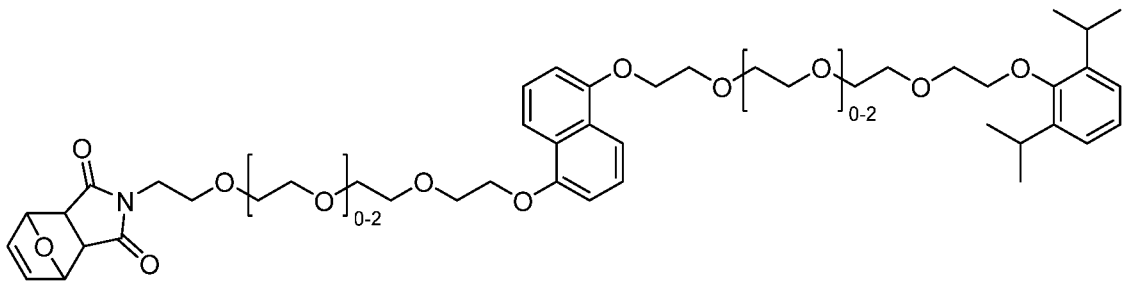
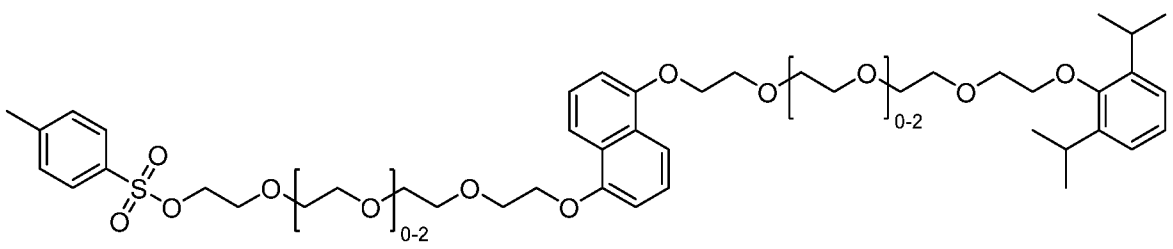


,



, or



.

[0287] In some embodiments, the conjugates are produced by linking a first portion of the linker to the anti-TM4SF1 antibody or an antigen binding fragment thereof and a second portion of the linker to the oligonucleotide. Conjugating the linker to anti-TM4SF1 antibody or an antigen binding fragment thereof or the therapeutic molecule may comprise production of an ionic bond, a covalent bond, a non-covalent bond or a combination thereof between the linker and the antibody, antigen binding fragment thereof or therapeutic agent. Conjugating the linker to the anti-TM4SF1 antibody or an antigen binding fragment thereof or the oligonucleotide may, in some cases, be performed as described in Roberts *et al.*, Advanced Drug Delivery Reviews 54:459-476 (2002). The linker may be selected from a bifunctional linker, a cleavable linker, a non-cleavable linker, an ethylene glycol linker, a bifunctional ethylene glycol linker, a flexible linker, or an inflexible linker. The linker may comprise a chemical group selected from a cyclooctyne, a cyclopropene, an aryl/alkyl azide, a trans-cyclooctene, a norborene, and a tetrazine. In some embodiments, a terminus of the linker comprises an alkoxy-amine. In some embodiments, a terminus of the linker comprises an azide or cyclooctyne group. In some embodiments, the antibody or antibody fragment or therapeutic agent may be coupled to the linker by a chemical group selected from a cyclooctyne, cyclopropene, aryl/alkyl azide, trans-cyclooctene, norborene, and tetrazine. Linking anti-TM4SF1 antibody or an antigen binding fragment thereof or an ologinucleotide to the linker may comprise conducting one or more copper-free reactions. Linking the antibody or antibody fragment or an ologinucleotide to the linker may comprise conducting one or more copper-containing reactions. Linking the anti-TM4SF1 antibody or an antigen binding fragment thereof or an ologinucleotide to the linker may comprise one or more cycloadditions. Linking anti-TM4SF1 antibody or an antigen binding fragment thereof or an ologinucleotide to the linker may comprise one or more Huisgen-cycloadditions. Linking the anti-TM4SF1 antibody or an antigen binding fragment thereof or an ologinucleotide to the linker may comprise one or more Diels Alder reactions. Linking anti-TM4SF1 antibody or an antigen binding fragment thereof or an ologinucleotide to the linker may comprise one or more Hetero Diels Alder reaction. In some embodiments, a terminus of the linker comprises a leaving group.

[0288] In some embodiments, a first portion of the linker covalently interacts with a cysteine containing anti-TM4SF1 antibody or an antigen binding fragment thereof, as described herein. In some embodiments, a first portion of the linker covalently interacts with a cysteine containing TM4SF1 antibody or an antigen binding fragment thereof, as described herein. In some embodiments, an oligonucleotide described herein covalently interacts with a second portion of the linker. In some embodiments, an oligonucleotide described herein non-covalently interacts with a second portion of the linker.

[0289] In some embodiments, a viral protein p19 based siRNA carrier is contemplated, which protein has been shown to have a high affinity for siRNA. *See, e.g.*, Yang *et al.* Cytosolic delivery of siRNA by ultra-high affinity dsRNA binding proteins, Nucleic Acids Res. 2017 Jul 27; 45(13): 7602–7614. In some examples, a p19-siRNA complex is generated and fused to an anti-TM4SF1 antibody or antigen-binding fragment thereof. In additional embodiments, a statistical or random conjugation methods via Cys, Lys, or Arginie residues within the antibody or antigen binding fragment thereof.

Synthesis of an ADC comprising an Anti-TM4SF1 antibody or an antigen binding fragment thereof and an siRNA

[0290] In one embodiment, a conjugate comprising an anti-TM4SF1 antibody or an antigen binding fragment thereof and an oligonucleotide is developed by covalent conjugation of the antibody or antigen binding fragment and the RNA molecule (*e.g.*, siRNA). As a first step of such an exemplary process, an engineered anti-TM4SF1 antibody is generated, in which a cysteine residue had been introduced in the heavy chain (thereby producing an anti-TM4SF1 HC THIOMAB). The anti-TM4SF1 thiomab, in some examples, provides at least two discrete positions for coupling with an RNA molecule, such as with an siRNA. For instance, one siRNA molecule can be coupled to each heavy chain of the anti-TM4SF1 thiomab. In a separate or subsequent step in the conjugation process, a chemically stabilized siRNA (synthesized, *e.g.*, using siSTABLE chemistry) modified with a 3'- amine for coupling to the passenger strand with a sequence targeting peptidlyprolyl isomerase B (PPIB, cyclophilin B) is generated. The conjugation, in some embodiments, further involves a reducible N-succinimidyl-4-(2-pyridyldithio)butyrate (SPDB) or a non-reducible succinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate) (SMCC) NHS (N-hydroxysuccinimide) linkers. In some embodiments, using the anti-TM4SF1 thiomab, an exemplary conjugate molecule according to this disclosure is generated in a multi step process involving at leats two primary steps: (i) reaction of an amine-tagged siRNA with an NHS-linker to form a thiol-reactive siRNA-linker adduct, and (ii) reacting the adduct with thiol groups on the THIOMAB to covalently link the siRNA via a thio-ester bond. The exemplary ADC is subsequently purified using anion exchange chromatography to remove free siRNA and then by size-exclusion chromatography to remove un-coupled antibody. Further techniques, such as gel electrophoresis and electrospray TOF mass spectrometry can then be used to assess the yield of the exemplary ADC, as well characteristics such as monomeric conjugates with one or two
linked siRNAs per antibody. Additional methods that can be employed for the conjugation involve the use or chemical or peptide based linkers, chemical or enzymatic conjugation methods (e.g., using mammalian or bacterial transglutaminase), or any combinations thereof. Any of the

linkers and/or methods described above can be used to couple the anti-TM4SF1 antibody or an antigen binding fragment thereof and the oligonucleotides of the conjugate.

[0291] Using appropriate coupling methods, it is possible to generate ADCs of this disclosure, which comprise, for example, an anti-TM4SF1 antibody or an antigen binding fragment thereof to oligonucleotide ratio of about 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10, or higher. In some embodiments, the ADC comprises an anti-TM4SF1 antibody or an antigen binding fragment thereof to oligonucleotide ratio of 1:1. This can be achieved, for example, by using an antigen binding fragment or a portion of an antibody, e.g., a half-antibody, Fab, or other fragments that comprise a THIOMAB engineered cysteine. In some examples, the ADC can be designed to comprise 1:1 ratios of an anti-TM4SF1 antibody or an antigen binding fragment thereof to oligonucleotide using a whole antibody which is conjugated to an oligonucleotide by a conjugation method that utilize a multimetallic protein (*e.g.*, a hexa-rhodium metallopeptide) to enable modification of proteins, on the basis of molecular recognition. For example, the anti-TM4SF1 antibody or an antigen binding fragment thereof and the oligonucleotide can be conjugated using a site-specific antibody functionalization, based on molecular recognition of the Fc domain constant region of the antibody by the multimetallic protein. In some embodiments, the multimetallic protein comprises three rhodium complexes attached to specific sites of a protein that binds to the Fc domain of an antibody. Upon binding, the multimetallic protein can catalzye site-specific conjugation of the oligonucleotide to the antibody. An advantage of using the multimetallic protein can be that the antibody is minimally disrupted, such as by avoiding engineering residues within the antibody, during the conjugation.

VI. Polynucleotides

[0292] Also provided, in some embodiments, are polynucleotides encoding an anti-TM4SF1 antibody or an antigen binding fragment thereof. In some embodiments, the polynucleotide molecules are provided as a DNA construct. In other embodiments, the polynucleotide molecules are provided as a messenger RNA transcript.

[0293] In some examples, an anti-TM4SF1 antibody of the present disclosure comprises a heavy chain variable domain encoded by a nucleic acid sequence as set forth in any one of SEQ ID NOs: 4, 16, 28, 40, 52, 64, or 76. In some examples, an anti-TM4SF1 antibody of the present disclosure comprises a light chain variable domain encoded by a nucleic acid sequence as set forth in any one of SEQ ID NOs: 10, 22, 34, 46, 58, 70, or 82.

[0294] In some embodiments are provided nucleic acid sequences that are codon optimized for expression in a host cell, *e.g.*, a bacterium, such as E. coli, or a eukaryotic cell, such as a CHO cell. In some examples, the nucleic acid sequences are codon optimized for expression in CHO cells. In some examples, an anti-TM4SF1 antibody of the present disclosure comprises a heavy

chain variable domain encoded by a codon optimized nucleic acid sequence as set forth in any one of SEQ ID NOs: 5, 17, 29, 41, 53, 65, or 77. In some examples, an anti-TM4SF1 antibody of the present disclosure comprises a light chain variable domain encoded by a codon optimized nucleic acid sequence as set forth in any one of SEQ ID NOs: 11, 23, 35, 47, 59, 71, or 83. In certain instances, the nucleic acid sequence of any one of SEQ ID NOs: 5, 17, 29, 41, 53, 65, or 77 is a nucleic acid sequence codon optimized for expression in CHO cell. In certain instances, the nucleic acid sequence of any one of SEQ ID NOs: 11, 23, 35, 47, 59, 71, or 83 is a nucleic acid sequence codon optimized for expression in CHO cell.

[0295] The polynucleotide molecules are constructed by known methods such as by incorporating the genes encoding the binding proteins into a genetic construct linked to a suitable promoter, and optionally a suitable transcription terminator, and expressing it in bacteria or other appropriate expression system such as, for example CHO cells. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. The promoter is selected such that it drives the expression of the polynucleotide in the respective host cell.

[0296] In some embodiments, a polynucleotide as described herein is inserted into a vector, preferably an expression vector, which represents a further embodiment. This recombinant vector can be constructed according to known methods. Vectors of particular interest include plasmids, phagemids, phage derivatives, virii (e.g., retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, lentiviruses, and the like), and cosmids.

[0297] A variety of expression vector/host systems may be utilized to contain and express the polynucleotide encoding the polypeptide of the described TM4SF1 binding protein. Examples of expression vectors for expression in E.coli are pSKK (Le Gall et al., J Immunol Methods. (2004) 285(1):111-27) or pcDNA5 (Invitrogen) for expression in mammalian cells.

[0298] Thus, the TM4SF1 binding proteins as described herein, in some embodiments, are produced by introducing a vector encoding the protein as described above into a host cell and culturing said host cell under conditions whereby the protein domains are expressed, may be isolated and, optionally, further purified.

VII. Methods of Treatment

[0299] The disclosure further provides a method for inhibiting cell-cell interactions that are endothelial cell (EC) specific, for example, but not limited to EC-EC, EC-mesenchymal stem cell, EC-fibroblast, EC-smooth muscle cell, EC-tumor cell, EC-leukocyte, EC-adipose cell and EC-neuronal cell interactions. In certain embodiments, the ADCs containing the anti-TM4SF1 antibodies and fragments of the present disclosure, can be used to treat any human disease or

disorder with a pathology that is characterized by abnormal EC-cell interactions. In certain embodiments, the EC-cell interaction is an EC-leukocyte interaction, where inhibition of the EC-leukocyte interaction is used to prevent inflammation.

[0300] In other embodiments, the disclosure features a method of treating or preventing a disease or disorder in a subject, wherein the disease or disorder is characterized by abnormal endothelial cell (EC)- cell interactions, said method comprising administering the antibody, or antigen-binding fragment thereof, as described herein. In certain embodiments, the EC-cell interactions include one or more of EC-mesenchymal stem cell, EC-fibroblast, EC-smooth muscle cell, EC-tumor cell, EC-leukocyte, EC-adipose cell and EC-neuronal cell interactions. In exemplary embodiments, the disease is an inflammatory disease or disorder, and the antibodies and fragments of the disclosure are used to inhibit EC-leukocyte interactions. In another exemplary embodiment, the disease or disorder is selected from an inflammatory disease or cancer. The adhesion of leukocytes to vascular endothelium is a hallmark of the inflammatory process. Accordingly, in one embodiment, an ADC containing an anti-TM4SF1 antibody, or an antigen binding fragment thereof, of the present disclosure is used to treat an inflammatory disease in which inhibiting leukocyte attachment to endothelial cells, or leukocyte transmigration across the endothelium is helpful for treatment (see, *e.g.* Rychly et al. ,Curr Pharm Des. 2006;12(29):3799-806, incorporated by reference in its entirety herein). Examples include, but are not limited to, sepsis, inflammatory bowel disease, psoriasis or multiple sclerosis.

[0301] Each year approximately half a million patients die from cancer in the United States alone. Tumor metastasis is responsible for ~90% of these deaths. No therapy that blocks metastasis is known. The present disclosure provides antibodies, and antigen-binding fragments thereof, that can treat cancer and inhibit metastatic cells based on immunoblockade of tumor cell (TC) - endothelial cell (EC) interactions mediated by a novel target, TM4SF1.

[0302] As described above, TM4SF1 is a small, tetraspanin-like, cell surface glycoprotein originally discovered as a TC antigen with roles in TC invasion and metastasis. TM4SF1 is selectively expressed by TCs and ECs. TM4SF1 is expressed at low levels on the vascular ECs supplying normal tissues in both mice and humans. It has been shown that TM4SF1 is expressed at ~10-20 fold higher levels on the vascular ECs lining the blood vessels supplying many human cancers, and at equivalent high levels on cultured ECs. TM4SF1-enriched microdomains (TMED) recruit cell surface proteins like integrins to assist the formation of nanopodia, thin membrane channels that extend from the cell surface and mediate cell-cell interactions. Thus, in certain instances, ADCs containing anti-TM4SF1 antibodies and fragments described herein interfere with nanopodia-mediated interactions and inhibit TC interactions with EC that are necessary for TC extravasation.

[0303] ADCs of this disclosure may be formulated for treating a subject (*e.g.*, a human) having a disorder associated with pathological angiogenesis (*e.g.*, cancer, such as breast cancer, ovarian cancer, renal cancer, colorectal cancer, liver cancer, gastric cancer, and lung cancer; obesity; macular degeneration; diabetic retinopathy; psoriasis; rheumatoid arthritis; cellular immunity; and rosacea.

[0304] TM4SF1 is highly expressed on the surface of most epithelial TCs, and, is also highly expressed on the EC lining tumor blood vessels and on cultured EC. It is expressed at ~10-20 fold lower levels on the surface of normal vascular ECs. In mouse models, tumor metastasis to lungs is related to TM4SF1 expression on both ECs and TCs. Metastasis requires initial attachment of TC to vascular EC and their subsequent migration across ECs to enter the lung or other metastatic sites. The examples below show that, in some instances, theanti-TM4SF1 antibodies of the present disclosure interfere with TC-EC interactions in culture and can also inhibit tumor metastasis *in vivo*.

[0305] Thus, the ADCs of the present disclosure can be used to block one or both of the earliest steps in metastasis, namely, TC attachment to vascular ECs and/or transmigration of TCs across ECs, and thereby prevent or substantially reduce the number of metastases in at risk cancer patients.

[0306] The present disclosure further provides a method for preventing metastasis. Human tumors typically shed TCs into the blood and lymphatics at early stages of growth; hence, early treatment of primary tumors provides no guarantee that metastasis has not already taken place. Thus, immunoblockade of TM4SF1 can be used to treat or prevent hematogenous metastases or to treat or prevent lymphatic metastases.

[0307] The methods of this disclosure are, in some embodiments, directed to inhibiting metastatic cells in a subject. In one embodiment, the subject has a cancer, *e.g.,* a cancer that is associated with metastasis or a cancer that has already metastasized. In other embodiments, the subject was already treated for cancer and is in remission or partial remission, wherein the benefits of administering ADCs containing the anti-TM4SF1 antibodies or fragments described herein are that they work to prevent metastasis and maintain remission or partial remission.

[0308] In certain embodiments, the disclosure provides a method of treating a person having a greater risk of developing metastasis, wherein administration of the ADCs containing the anti-TM4SF1 antibodies and fragments described herein can be used to inhibit or delay onset of metastasis.

[0309] Included in the disclosure is a method of blocking tumor metastasis, particularly metastasis to the lung, by administering an anti-TM4SF1 antibody to a subject in need thereof. In some examples, the anti-TM4SF1 antibody is a human anti-TM4SF1 antibody, also referred to

herein as anti-hTM4SF1. In certain embodiments, the methods can include administration of an effective amount of an ADC containing an anti-hTM4SF1 antibody to a subject in need thereof, wherein the effective amount of the antibody prevents tumor cell (TC) attachment to and migration across vascular endothelial cells (ECs).

[0310] In certain embodiments, an ADC containing an anti-TM4SF1 antibody is administered to a subject having cancer or at risk of having metastasis such that the dose amount and frequency maintains long term TM4SF1 immunoblockade. The dosing regimen will maximally inhibit TM4SF1-mediated metastasis by administering an ADC containing an anti-TM4SF1 antibody to a subject in an amount sufficient to saturate TM4SF1 expressed on normal vascular ECs of the subject.

[0311] In certain embodiments, the effective amount of an ADC containing an anti-TM4SF1 antibody, or an antigen binding fragment thereof, that is administered is an amount sufficient to, at one week, achieve circulating antibody concentrations > 1 μg/ml.

[0312] In certain embodiments, the effective amount of an ADC containing an anti-TM4SF1 antibody, or an antigen binding fragment thereof that is administered is an amount sufficient to maintain serum concentrations of the antibody at or above 1 μg/ml continuously for about 1 month.

[0313] In one embodiment, the disclosure provides a method of treating or preventing metastasis in a human subject comprising administering to the subject an effective amount of an ADC containing an anti-TM4SF1 antibody, or an antigen binding fragment thereof, wherein the effective amount of the antibody, or antigen binding fragment thereof, comprises 1 to 80 mg/kg of the amount of the antibody, or antigen binding fragment thereof.

[0314] The mode of administration for therapeutic use of the ADCs of the disclosure may be any suitable route that delivers the antibody to the host, such as parenteral administration, *e.g.*, intradermal, intramuscular, intraperitoneal, intravenous or subcutaneous, pulmonary, transmucosal (oral, intranasal, intravaginal, rectal), using a formulation in a tablet, capsule, solution, powder, gel, particle; and contained in a syringe, an implanted device, osmotic pump, cartridge, micropump; or other means appreciated by the skilled artisan, as well known in the art. Site specific administration may be achieved by for example intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intracardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravascular, intravesical, intralesional, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery.

[0315] In some embodiments, the ADCs of the disclosure may be administered to a subject by any suitable route, for example parentally by intravenous (i.v.) infusion or bolus injection, intramuscularly or subcutaneously or intraperitoneally. i.v. infusion may be given over for example 15, 30, 60, 90, 120, 180, or 240 minutes, or from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours. The dose given to a subject in some embodiments is about 0.005 mg to about 100 mg/kg, *e.g.*, about 0.05 mg to about 30 mg/kg or about 5 mg to about 25 mg/kg, or about 4 mg/kg, about 8 mg/kg, about 16 mg/kg or about 24 mg/kg, or for example about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg. In certain embodiments, the dose given to a subject is, for example about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90 or 100 mg/kg. In some instances, the dose of the antibodies of the disclosure given to a subject may be about 0.1 mg/kg to 10 mg/kg via intravenous administration. In some instances, the dose of the antibodies of the disclosure given to a subject is about 0.1 mg/kg to 10 mg/kg via subcutaneous administration. In some instances, the dose of the antibodies of the disclosure given to a subject is about 0.1 mg/kg via intravenous administration. In some instances, the dose of the antibodies of the disclosure given to a subject is about 0.1 mg/kg via subcutaneous administration. In some embodiments, the dose of the antibodies of the disclosure given to a subject is about 0.3 mg/kg via intravenous administration. In some examples, the dose of the antibodies of the disclosure given to a subject is about 0.3 mg/kg via subcutaneous administration. In some examples, the dose of the antibodies of the disclosure given to a subject is about 1.0 mg/kg via intravenous administration. In some examples, the dose of the antibodies of the disclosure given to a subject is about 1.0 mg/kg via subcutaneous administration. In some examples, the dose of the antibodies of the disclosure given to a subject is about 3.0 mg/kg via intravenous administration. In some examples, the dose of the antibodies of the disclosure given to a subject is about 3.0 mg/kg via subcutaneous administration. In some examples, the dose of the antibodies of the disclosure given to a subject may be about 10.0 mg/kg via intravenous administration. In some examples, the dose of the antibodies of the disclosure given to a subject is about 10.0 mg/kg via subcutaneous administration.

[0316] In certain embodiments, a fixed unit dose of the antibodies of the disclosure is given, for example, 50, 100, 200, 500 or 1000 mg, or the dose may be based on the patient's surface area, *e.g.*, 500, 400, 300, 250, 200, or 100 mg/m^2. In some instances, between 1 and 8 doses, (*e.g.*, 1, 2, 3, 4, 5, 6, 7 or 8) is administered to treat the patient, but 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more doses are given.

[0317] The administration of the ADCs of the disclosure described herein, in some embodiments, is repeated after one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, two months, three months,

four months, five months, six months or longer. Repeated courses of treatment are also possible, as is chronic administration. The repeated administration is at the same dose or at a different dose. In some examples, the ADCs of the disclosure described herein is administered at 8 mg/kg or at 16 mg/kg at weekly interval for 8 weeks, followed by administration at 8 mg/kg or at 16 mg/kg every two weeks for an additional 16 weeks, followed by administration at 8 mg/kg or at 16 mg/kg every four weeks by intravenous infusion. Alternatively, in some embodiments, the ADCs of the disclosure described herein are administered at between 0.1 mg/kg to about 10 mg/kg at weekly interval for 17 weeks. For example, in some cases the antibodies of the disclosure are provided as a daily dosage in an amount of about 0.1-100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses of every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof. In some embodiments, the antibodies of the disclosure described herein is administered prophylactically in order to reduce the risk of developing an inflammatory disease such as RA, psoriatic arthritis or psoriasis, delay the onset of the occurrence of an event in progression of the inflammatory disease such as RA, psoriatic arthritis or psoriasis. In some examples, the ADCs of the disclosure are lyophilized for storage and reconstituted in a suitable carrier prior to use. In some cases, the antibodies of the disclosure are supplied as a sterile, frozen liquid in a glass vial with stopper and aluminum seal with flip-off cap. In some examples, each vial migh contain ADC containing 3.3 mL of a 50 mg/mL solution of the antibody (including a 10% overfill) in a formulation of 10 mM histidine, 8.5% (w/v) sucrose, and 0.04% (w/v) Polysorbate 80 at pH 5.8. In some examples, the vials contain no preservatives and are for single use. Vials may be stored frozen and protected from light. To prepare for IV administration, the ADC formulations, in some examples, are filtered with a 0.22 micron filter before being diluted in sterile diluent. In some examples, diluted ADCs at volumes up to approximately 100 mL are administered by IV infusion over a period of at least 30 minutes using an in-line 0.22 micron filter. Alternatively, in some embodiments, the ADCs are administered as 1 or 2 subcutaneous injections containing about 50 mg/mL antibody in about 3.3 mL. The subcutaneous injection site may be, for example, within the abdominal area.

VIII. Pharmaceutical compositions

[0318] The ADCs of this disclosure, can, in some embodiments, be included in compositions (*e.g.*, pharmaceutical compositions). The pharmaceutical compositions of the disclosure may further include a pharmaceutically acceptable carrier, excipient, or diluent.

[0319] The term "pharmaceutical composition" as used herein refers to a composition containing a TM4SF1 binding protein described herein formulated with a pharmaceutically acceptable carrier, and manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (*e.g.*, a tablet, capsule, caplet, gel cap, or syrup); for topical administration (*e.g.*, as a cream, gel, lotion, or ointment) ; for intravenous administration (*e.g.*, as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); or in any other formulation described herein.

[0320] The term "pharmaceutically acceptable carrier" as used herein refers to a carrier which is physiologically acceptable to a treated mammal (*e.g.*, a human) while retaining the therapeutic properties of the protein with which it is administered. One exemplary pharmaceutically acceptable carrier is physiological saline. Other physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in Remington's Pharmaceutical Sciences (18th edition, A. Gennaro, 1990, Mack Publishing Company, Easton, PA), incorporated herein by reference.

[0321] Pharmaceutical compositions containing an ADC containing an TM4SF1 antibody or antigen-binding fragment thereof, are, in some embodiments, prepared as solutions, dispersions in glycerol, liquid polyethylene glycols, and any combinations thereof in oils, in solid dosage forms, as inhalable dosage forms, as intranasal dosage forms, as liposomal formulations, dosage forms comprising nanoparticles, dosage forms comprising microparticles, polymeric dosage forms, or any combinations thereof.

[0322] A pharmaceutically acceptable excipient is, in some examples, an excipient described in the Handbook of Pharmaceutical Excipients, American Pharmaceutical Association (1986). Non-limiting examples of suitable excipients include a buffering agent, a preservative, a stabilizer, a binder, a compaction agent, a lubricant, a chelator, a dispersion enhancer, a disintegration agent, a flavoring agent, a sweetener, a coloring agent.

[0323] In some embodiments an excipient is a buffering agent. Non-limiting examples of suitable buffering agents include sodium citrate, magnesium carbonate, magnesium bicarbonate, calcium carbonate, and calcium bicarbonate. As a buffering agent, sodium bicarbonate, potassium bicarbonate, magnesium hydroxide, magnesium lactate, magnesium glucomate, aluminium hydroxide, sodium citrate, sodium tartrate, sodium acetate, sodium carbonate, sodium polyphosphate, potassium polyphosphate, sodium pyrophosphate, potassium pyrophosphate,

disodium hydrogen phosphate, dipotassium hydrogen phosphate, trisodium phosphate, tripotassium phosphate, potassium metaphosphate, magnesium oxide, magnesium hydroxide, magnesium carbonate, magnesium silicate, calcium acetate, calcium glycerophosphate, calcium chloride, calcium hydroxide and other calcium salts or combinations thereof is used, in some embodiments, in a pharmaceutical composition of the present disclosure.

[0324] In some embodiments an excipient comprises a preservative. Non-limiting examples of suitable preservatives include antioxidants, such as alpha-tocopherol and ascorbate, and antimicrobials, such as parabens, chlorobutanol, and phenol. In some examples, antioxidants further include but are not limited to EDTA, citric acid, ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), sodium sulfite, p-amino benzoic acid, glutathione, propyl gallate, cysteine, methionine, ethanol and N- acetyl cysteine. In some instances preservatives include validamycin A, TL-3, sodium ortho vanadate, sodium fluoride, N-a-tosyl-Phe- chloromethylketone, N-a-tosyl-Lys-chloromethylketone, aprotinin, phenylmethylsulfonyl fluoride, diisopropylfluorophosphate, kinase inhibitor, phosphatase inhibitor, caspase inhibitor, granzyme inhibitor, cell adhesion inhibitor, cell division inhibitor, cell cycle inhibitor, lipid signaling inhibitor, protease inhibitor, reducing agent, alkylating agent, antimicrobial agent, oxidase inhibitor, or other inhibitor.

[0325] In some embodiments a pharmaceutical composition as described herein comprises a binder as an excipient. Non-limiting examples of suitable binders include starches, pregelatinized starches, gelatin, polyvinylpyrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinyloxoazolidone, polyvinylalcohols, C12-C18 fatty acid alcohol, polyethylene glycol, polyols, saccharides, oligosaccharides, and combinations thereof. The binders used in a pharmaceutical formulation are, in some examples, selected from starches such as potato starch, corn starch, wheat starch; sugars such as sucrose, glucose, dextrose, lactose, maltodextrin; natural and synthetic gums; gelatine; cellulose derivatives such as microcrystalline cellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, carboxymethyl cellulose, methyl cellulose, ethyl cellulose; polyvinylpyrrolidone (povidone); polyethylene glycol (PEG); waxes; calcium carbonate; calcium phosphate; alcohols such as sorbitol, xylitol, mannitol and water or any combinations thereof.

[0326] In some embodiments a pharmaceutical composition as described herein comprises a lubricant as an excipient. Non-limiting examples of suitable lubricants include magnesium stearate, calcium stearate, zinc stearate, hydrogenated vegetable oils, sterotex, polyoxyethylene monostearate, talc, polyethyleneglycol, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, and light mineral oil. The lubricants that are used in a pharmaceutical formulation,

in some embodiments, are be selected from metallic stearates (such as magnesium stearate, calcium stearate, aluminium stearate), fatty acid esters (such as sodium stearyl fumarate), fatty acids (such as stearic acid), fatty alcohols, glyceryl behenate, mineral oil, paraffins, hydrogenated vegetable oils, leucine, polyethylene glycols (PEG), metallic lauryl sulphates (such as sodium lauryl sulphate, magnesium lauryl sulphate), sodium chloride, sodium benzoate, sodium acetate and talc or a combination thereof.

[0327] In some embodiments a pharmaceutical formulation comprises a dispersion enhancer as an excipient. Non-limiting examples of suitable dispersants include, in some examples, starch, alginic acid, polyvinylpyrrolidones, guar gum, kaolin, bentonite, purified wood cellulose, sodium starch glycolate, isoamorphous silicate, and microcrystalline cellulose as high HLB emulsifier surfactants.

[0328] In some embodiments a pharmaceutical composition as described herein comprises a disintegrant as an excipient. In some embodiments a disintegrant is a non-effervescent disintegrant. Non-limiting examples of suitable non-effervescent disintegrants include starches such as corn starch, potato starch, pregelatinized and modified starches thereof, sweeteners, clays, such as bentonite, micro-crystalline cellulose, alginates, sodium starch glycolate, gums such as agar, guar, locust bean, karaya, pecitin, and tragacanth. In some embodiments a disintegrant is an effervescent disintegrant. Non-limiting examples of suitable effervescent disintegrants include sodium bicarbonate in combination with citric acid, and sodium bicarbonate in combination with tartaric acid.

[0329] In some embodiments an excipient comprises a flavoring agent. Flavoring agents incorporated into an outer layer are, in some examples, chosen from synthetic flavor oils and flavoring aromatics; natural oils; extracts from plants, leaves, flowers, and fruits; and combinations thereof. In some embodiments a flavoring agent can be selected from the group consisting of cinnamon oils; oil of wintergreen; peppermint oils; clover oil; hay oil; anise oil; eucalyptus; vanilla; citrus oil such as lemon oil, orange oil, grape and grapefruit oil; and fruit essences including apple, peach, pear, strawberry, raspberry, cherry, plum, pineapple, and apricot.

[0330] In some embodiments an excipient comprises a sweetener. Non-limiting examples of suitable sweeteners include glucose (corn syrup), dextrose, invert sugar, fructose, and mixtures thereof (when not used as a carrier); saccharin and its various salts such as a sodium salt; dipeptide sweeteners such as aspartame; dihydrochalcone compounds, glycyrrhizin; Stevia Rebaudiana (Stevioside); chloro derivatives of sucrose such as sucralose; and sugar alcohols such as sorbitol, mannitol, sylitol, and the like.

[0331] In some instances, a pharmaceutical composition as described herein comprises a coloring agent. Non-limiting examples of suitable color agents include food, drug and cosmetic colors (FD&C), drug and cosmetic colors (D&C), and external drug and cosmetic colors (Ext. D&C). A coloring agents can be used as dyes or their corresponding lakes.

[0332] In some instances, a pharmaceutical composition as described herein comprises a chelator. In some cases, a chelator is a fungicidal chelator. Examples include, but are not limited to: ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA); a disodium, trisodium, tetrasodium, dipotassium, tripotassium, dilithium and diammonium salt of EDTA; a barium, calcium, cobalt, copper, dysprosium, europium, iron, indium, lanthanum, magnesium, manganese, nickel, samarium, strontium, or zinc chelate of EDTA; trans-1,2-diaminocyclohexane-N,N,N',N'-tetraaceticacid monohydrate; N,N-bis(2-hydroxyethyl)glycine; 1,3-diamino-2-hydroxypropane-N,N,N',N'-tetraacetic acid; 1,3-diaminopropane-N,N,N',N'-tetraacetic acid; ethylenediamine-N,N'-diacetic acid; ethylenediamine-N,N'-dipropionic acid dihydrochloride; ethylenediamine-N,N'-bis(methylenephosphonic acid) hemihydrate; N-(2-hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid; ethylenediamine-N,N,N',N'-tetrakis(methylenephosponic acid); O,O'-bis(2-aminoethyl)ethyleneglycol-N,N,N',N'-tetraacetic acid; N,N-bis(2-hydroxybenzyl)ethylenediamine-N,N-diacetic acid; 1,6-hexamethylenediamine-N,N,N',N'-tetraacetic acid; N-(2-hydroxyethyl)iminodiacetic acid; iminodiacetic acid; 1,2-diaminopropane-N,N,N',N'-tetraacetic acid; nitrilotriacetic acid; nitrilotripropionic acid; the trisodium salt of nitrilotris(methylenephosphoric acid); 7,19,30-trioxa-1,4,10,13,16,22,27,33-octaazabicyclo[11,11,11] pentatriacontane hexahydrobromide; or triethylenetetramine-N,N,N',N",N'",N'"-hexaacetic acid.

[0333] Also contemplated are combination products that include an anti-TM4SF1 antibody as disclosed herein and one or more other antimicrobial or antifungal agents, for example, polyenes such as amphotericin B, amphotericin B lipid complex (ABCD), liposomal amphotericin B (L-AMB), and liposomal nystatin, azoles and triazoles such as voriconazole, fluconazole, ketoconazole, itraconazole, pozaconazole and the like; glucan synthase inhibitors such as caspofungin, micafungin (FK463), and V-echinocandin (LY303366); griseofulvin; allylamines such as terbinafine; flucytosine or other antifungal agents, including those described herein. In addition, it is contemplated that a peptide can be combined with topical antifungal agents such as ciclopirox olamine, haloprogin, tolnaftate, undecylenate, topical nysatin, amorolfine, butenafine, naftifine, terbinafine, and other topical agents. In some instances, a pharmaceutical composition comprises an additional agent. In some cases, an additional agent is present in a therapeutically effective amount in a pharmaceutical composition.

[0334] Under ordinary conditions of storage and use, the pharmaceutical compositions as described herein comprise a preservative to prevent the growth of microorganisms. In certain examples, the pharmaceutical compositions as described herein do not comprise a preservative. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The pharmaceutical compositions comprise a carrier which is a solvent or a dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and/or vegetable oils, or any combinations thereof. Proper fluidity is 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. The prevention of the action of microorganisms is brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, isotonic agents are included, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0335] For parenteral administration in an aqueous solution, for example, the liquid dosage form is suitably buffered if necessary and the liquid diluent rendered isotonic with sufficient saline or glucose. The liquid dosage forms are especially suitable for intravenous, intramuscular, subcutaneous, intratumoral, and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage is dissolved, in certain cases, in 1mL to 20 mL of isotonic NaCl solution and either added to 100 mL to 1000 mL of a fluid, e.g., sodium-bicarbonate buffered saline, or injected at the proposed site of infusion.

[0336] In certain embodiments, sterile injectable solutions is prepared by incorporating a immunotherapy agent, in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. The compositions disclosed herein are, in some instances, formulated in a neutral or salt form. Pharmaceutically-acceptable salts include, for example, the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups are, in some cases, derived from inorganic bases such as, for example, sodium, potassium, ammonium,

calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, the pharmaceutical compositions are administered, in some embodiments, in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

[0337] In certain embodiments, a pharmaceutical composition of this disclosure comprises an effective amount of an anti-TM4SF1 antibody, as disclosed herein, combined with a pharmaceutically acceptable carrier. "Pharmaceutically acceptable," as used herein, includes any carrier which does not interfere with the effectiveness of the biological activity of the active ingredients and/or that is not toxic to the patient to whom it is administered. Non-limiting examples of suitable pharmaceutical carriers include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents and sterile solutions. Additional non-limiting examples of pharmaceutically compatible carriers can include gels, bioadsorbable matrix materials, implantation elements containing the immunotherapeutic agents or any other suitable vehicle, delivery or dispensing means or material. Such carriers are formulated, for example, by conventional methods and administered to the subject at an effective amount.

IX. Combination therapies

[0338] In certain embodiments, the methods of this disclosure comprise administering an ADC as disclosed herein, followed by, preceded by or in combination with one or more further therapy. Examples of the further therapy can include, but are not limited to, chemotherapy, radiation, an anti-cancer agent, or any combinations thereof. The further therapy can be administered concurrently or sequentially with respect to administration of the immunotherapy. In certain embodiments, the methods of this disclosure comprise administering an immunotherapy as disclosed herein, followed by, preceded by, or in combination with one or more anti-cancer agents or cancer therapies. Anti-cancer agents include, but are not limited to, chemotherapeutic agents, radiotherapeutic agents, cytokines, immune checkpoint inhibitors, anti-angiogenic agents, apoptosis-inducing agents, anti-cancer antibodies and/or anti-cyclin-dependent kinase agents. In certain embodiments, the cancer therapies include chemotherapy, biological therapy, radiotherapy, immunotherapy, hormone therapy, anti-vascular therapy, cryotherapy, toxin therapy and/or surgery or combinations thereof. In certain embodiments, the methods of this disclosure include administering an immunotherapy, as disclosed herein, followed by, preceded by or in combination with one or more further immunomodulatory agents. An immunomodulatory agent includes, in some examples, any compound, molecule or substance capable of suppressing antiviral immunity associated with a tumor or cancer. Non-limiting examples of the further immunomodulatory agents include anti-CD33 antibody or variable region

thereof, an anti-CD11b antibody or variable region thereof, a COX2 inhibitor, e.g., celecoxib, cytokines, such as IL-12, GM-CSF, IL-2, IFN3 and 1FNy, and chemokines, such as MIP-1, MCP-1 and IL-8.

[0339] In certain examples, where the further therapy is radiation exemplary doses are 5,000 Rads (50 Gy) to 100,000 Rads (1000 Gy), or 50,000 Rads (500 Gy), or other appropriate doses within the recited ranges. Alternatively, the radiation dose are about 30 to 60 Gy, about 40 to about 50 Gy, about 40 to 48 Gy, or about 44 Gy, or other appropriate doses within the recited ranges, with the dose determined, example, by means of a dosimetry study as described above. "Gy" as used herein can refer to a unit for a specific absorbed dose of radiation equal to 100 Rads. Gy is the abbreviation for "Gray."

[0340] In certain examples, where the further therapy is chemotherapy, exemplary chemotherapeutic agents include without limitation alkylating agents (e.g., nitrogen mustard derivatives, ethylenimines, alkylsulfonates, hydrazines and triazines, nitrosureas, and metal salts), plant alkaloids (e.g., vinca alkaloids, taxanes, podophyllotoxins, and camptothecan analogs), antitumor antibiotics (e.g., anthracyclines, chromomycins, and the like), antimetabolites (e.g., folic acid antagonists, pyrimidine antagonists, purine antagonists, and adenosine deaminase inhibitors), topoisomerase I inhibitors, topoisomerase II inhibitors, and miscellaneous antineoplastics (e.g., ribonucleotide reductase inhibitors, adrenocortical steroid inhibitors, enzymes, antimicrotubule agents, and retinoids). Exemplary chemotherapeutic agents can include, without limitation, anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for

injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®), Ibrutinib, idelalisib, and brentuximab vedotin.

[0341] Exemplary alkylating agents include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen Mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

[0342] Exemplary anthracyclines can include, without limitation, e.g., doxorubicin (Adriamycin® and Rubex®); bleomycin (Lenoxane®); daunorubicin (dauorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetylravidomycin.

[0343] Exemplary vinca alkaloids include, but are not limited to, vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®).

[0344] Exemplary proteosome inhibitors can, but are not limited to, bortezomib (Velcade®); carfilzomib (PX-171-007, (S)-4-Methyl-N—((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoac etamido)-4-phenylbutanamido)-pentanamide); marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and O-Methyl-N-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-O-methyl-N-[(1S)-2-[(2R)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]-L-serinamide (ONX-0912).

[0345] "In combination with," as used herein, means that the anti-TM4SF1 antibody and the further therapy are administered to a subject as part of a treatment regimen or plan. In certain embodiments, being used in combination does not require that the anti-TM4SF1 antibody and the further therapy are physically combined prior to administration or that they be administered over the same time frame. For example, and not by way of limitation, the anti-TM4SF1 antibody and the one or more agents are administered concurrently to the subject being treated, or are administered at the same time or sequentially in any order or at different points in time.

X. Kits

[0346] In some embodiments, the disclosure provides kits that include a composition (*e.g.*, a pharmaceutical composition) of the disclosure (*e.g.*, a composition including an ADC containing an anti-TM4SF1 antibody or antigen binding fragment thereof). The kits include instructions to allow a clinician (*e.g.*, a physician or nurse) to administer the composition contained therein to a subject to treat a disorder associated with pathological angiogenesis (*e.g.*, cancer).

[0347] In certain embodiments, the kits include a package of a single-dose pharmaceutical composition(s) containing an effective amount of an antibody of the disclosure. Optionally, instruments or devices necessary for administering the pharmaceutical composition(s) may be included in the kits. For instance, a kit of this disclosure may provide one or more pre-filled syringes containing an effective amount of a vaccine, vector, stabilized trimer, or optimized viral polypeptide of the disclosure. Furthermore, the kits may also include additional components such as instructions regarding administration schedules for a subject having a disorder associated with pathological angiogenesis (*e.g.*, cancer) to use the pharmaceutical composition(s) containing a TM4SF1 binding protein or polynucleotide of the disclosure.

EXAMPLES

Example 1: Characterization of exemplary anti-TM4SF1 antibodies

[0348] *Affinity*

[0349] Antigen binding affinities of anti-TM4SF1 antibodies comprising various Fc mutations were tested, *via* a cell-based flow cytometry assay. Variants of an exemplary anti-TM4SF1 antibody AGX-A07, comprising Fc region mutation N297C (the "C" variant) or N297C in

combination with the mutations M252Y, S254T, and T256E (the "YTEC" variant), were tested using HUVEC cells (Primary Umbilical Vein Endothelial Cells; ATCC® PCS-100-010™) The EC_{50} values for binding are shown in **FIG. 3** (top left panel), where A07-wt corresponds to the AGX-A07 antibody without Fc region mutations. Similarly, a "C" variant and an "YTEC" variant of a murine surrogate (referred to as "MS" in the figures), were tested in immortalized mouse endothelial cell MS-1 cells (MILE SVEN 1; ATCC® CRL-2279™) The EC_{50} values for binding are shown in **FIG. 3** (top right panel and bottom right panel), where MS-wt corresponds to the murine surrogate antibody without the Fc region mutations.

[0350] *Tissue Distribution*

[0351] In this study, *in vivo* tissue distribution of the murine surrogate (MS) anti-TM4SF1 antibody "C" and "YTEC" variants was determined, in mice. Murine surrogate "C" variant conjugated to Alexa Fluor™ 647 (MS-C-647) and murine surrogate "YTEC" variant conjugated to Alexa Fluor™ 488 (MS-YTEC-488) were intraperitoneally co-injected to LLC (Lewis lung carcinoma) tumor bearing C57BL/6 (8 weeks old) mice, at a dose of 30 mg/kg (30 mpk). Major organs were harvest 24 or 48 hours after the injection and were fixed in 4% paraformaldehyde for embedding in OCT mounting media and sectioning. The MS-C-647 and MS-YTEC-488 antibody signals in tissue sections were captured *via* confocal microscope and the tissue distribution differences were examined.

[0352] All blood vessels were found to be positive for both MS-C-647 and MS-YTEC-488 signals. In some organs, tissue resident mast cells and/or pericytes also strongly interacted with the MS-YTEC-488 but not with the MS-C-647. These results suggested that the MS-YTEC-488 can readily be transcytosed from endothelium to tissues for their interaction with leukocyte *via* antibody constant region or pericytes *via* antigen binding. The overall tissue distribution observations are summarized in below table and also shown in **FIGS. 4** and **5** (bv = blood vessel).

[0353] **TABLE 1: Tissue distribution of MS-C and MS-YTEC**

Mouse organs	**Blood vessel staining**	**Tissue resident mast cell or pericyte staining**
Brain	Comparable between MS-C and MS-YTEC	no
Stomach		Yes for MS-YTEC
Small Intestine		Yes for MS-YTEC
Large Intestine		Yes for MS-YTEC
Eye		No
Female Reproductive System		Yes for MS-YTEC
Heart		No

Kidney		No
Liver		No
Lung		No
Pancreas		No
Skin+tumor		Yes for MS-YTEC

[0354] *Hydrophobicity*

[0355] The hydrophobicity of exemplary anti-TM4SF1 antibodies, and their Fc mutation containing variants were assessed in this study, using hydrophobic interaction chromatography. The tested antibodies were AGX-A07 (the "wt," "C," and "YTEC"); MS (the "wt," "C," and "YTEC"). An anti-Her2 antibody with an Fc mutation was used as a control. Results are plotted in **FIG. 6** and also summarized in **Table 2**. Both in case of AGX-A07 and the murine surrogate anti-TM4SF1 antibody, it was observed that the hydrophobicity increased with the "C" and "YTEC" mutations.

[0356] TABLE 2: Hydrophobicity summary

	Run 1	Run 2	Run 3	Average	StD
A07-wt	5.547	5.523	5.568	5.55	0.023
A07C	5.614	5.591	5.606	5.60	0.0012
A07-YTEC	5.781	5.77	5.888	5.81	0.065
MS-wt	5.697	5.7	5.797	5.73	0.057
MS-C	5.807	5.784	5.752	5.78	0.028
MS-YTEC	6.017	5.978	6.017	6.00	0.023
anti-Her2 K392C	5.496	5.467	5.532	5.5	0.033

Example 2: Antibody Drug Conjugates containing exemplary anti-TM4SF1 antibodies

[0357] Antibody drug conjugates (ADCs) containing exemplary anti-TM4SF1 antibodies were prepared and tested in *in vitro* and *in vivo* studies. **FIGS. 1** and **2** provide the structures of the ADCs, prepared using maleimide conjugation (**FIG. 2**) or bromoacetamide conjugation (**FIG. 1**); 1^4 S,1^6 S,3^2 S,3^3 S,2R,4S,10E,12E,14R)-8^6 -chloro-14 -hydroxy-8^5 ,14-dimethoxy-3^3 ,2,7,10-tetramethyl-1^2 ,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)-N-methyl-L-alaninate and 1^4 S,1^6 S,3^2 S,3^3 S,2R,4S,10E,12E,14R)-8^6 -chloro-1^4 -hydroxy-8^5 ,14-dimethoxy-3^3 ,2,7,10-tetramethyl-1^2 ,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-

oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-1-bromo-26,27-dimethyl-2,18,25-trioxo-6,9,12,15-tetraoxa-3,19,26-triazaoctacosan-28-oate.

[0358] *In vivo tolerance*

[0359] Eight weeks old C57Bl/6 mice were administered an ADC (MS-C-mc-DM1) containing the murine surrogate "C" variant (MS-C) conjugated to maytansine, prepared using maleimide conjugation, at various doses (40 mg/kg, 50 mg/kg, and 60 mg/kg). The DAR for the ADC was about 2.0 (deconvoluted spectrum shown in **FIG. 7**).

[0360] The ADC was tolerated at 40 mg/kg dose but not at the 50 mg/kg dose, as shown in **FIG. 8**. In addition, mice showed chest cavity fluid accumulation at day 10-12 with the 60 mg/kg dose of the MS-C-mc-DM1.

[0361] In further studies, mice of different ages were administered either (i) the MS-C-BA-DM1 ADC (containing the murine surrogate "C" variant conjugated to maytansine, prepared using bromoacetamide conjugation), or (ii) the MS-YTEC-BA-DM1 ADC (containing the murine surrogate "YTEC" variant conjugated to maytansine, prepared using bromoacetamide conjugation). It was observed that in general, the MS ADCs were better tolerated in the older mice (4-9 months) than in the younger mice (8 weeks). At a dose of 60 mg/kg, mice tolerated the MS-YTEC-BA-DM1 better than the MS-C-BA-DM1. Also, unlike the maleimide-conjugated ADCs, no obvious chest cavity fluid accumulation was observed with the BA-DM1 conjugated MS-C and MS-YTEC antibodies. This was an improvement over the maleimide-conjugated ADCs which had no survival at 60 mg/kg (*see* **FIG. 8**).

[0362] Results for the bromoacetamide-conjugated ADCs is shown in **FIG. 9**. Experiments 1 and 2 were carried out with two groups of animals. The survival rate at the 60 mg/kg dose is summarized in below table.

[0363] **TABLE 3: Survival rate**

	Survival Rate (%) at 60 mg/kg	
Age	MS-C-BA-DM1	MS-YTEC-BA-DM1
4-9 months	60	90
8 weeks	20	80

[0364] *Toxicity studies in cynomolgus monkeys*

[0365] In this study the animals were randomly divided into various groups and administered either (i) an ADC (AGX-A07-C-BA-DM1) containing the AGX-A07 "C" variant (A07-C) conjugated to maytansine, at 5 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg; or (ii) an ADC (AGX-

A07-BA-YTEC-DM1) containing the AGX-A07 "YTEC" (A07-YTEC) conjugated to maytansine, at 40 mg/kg. **Table 4** provides the details regarding animals tested.

[0366] TABLE 4: A non-GLP single dose study of ADCs containing exemplary anti-human TM4SF1 antibodies, by intravenous injection or infusion in cynomolgus monkeys

Group No.	Test Material	Dose Level (mg/kg)	Body weight (kg)	Amount Ab injected (mg)	No. of Female Animals (2 years 3 months to 3 years 1 month)
1	AGX-A07-C-BA-DM1	5	2.05	10.25	1
2		10	2.45	24.50	1
3		20	2.20	44.00	1
4		40	1.95	78.00	1
5	AGX-A07-YTEC-BA-DM1	40	2.40	96.00	1

[0367] Summary of macroscopic and microscopic pathological observations from the monkey study is provided in **Table 5**.

[0368] TABLE 5: Macroscopic/Microscopic pathology reports at termination (42 days after 1 injection of test article)

		A07-C-BA-DM1	A07-YTEC-BA-DM1
Macroscopic		There were no test article-related macroscopic findings at terminal euthanasia	
Microscopic	**pleura of the lungs** (thickening of lungs and expansion by fibrosis, multiple small vessels, mixed inflammatory cells, and proliferating fibroblasts; adhesion/inflammation/fibrosis)	mild at 40 mg/kg (also evidenced a mild edema of the alveoli and interstitium)	minimal at 40 mg/kg
	lung edema	mild 40 mg/kg	minimal at 40 mg/kg
	intimal proliferation of the aortic arch (with no other vessels affected)	(no mention)	minimal at 40 mg/kg
	endocardial hyperplasia (characterized by a loose expansion of the endocardium and sub-endocardium by fibrillar to homogenous amphophilic to lightly basophilic acellular material and slightly increased cellularity. When affected, this was seen in both ventricles, and in the most affected	in animals at all doses ≥10 mg/kg in a non-dose dependent pattern; moderate thickening seen at 10 mg/kg, mild at 20 mg/kg,	minimal thickening of the vascular endothelium was seen in the aorta, at the aortic arch.

	animal extended from the base to the apex of the heart, possibly extending into the atria or large vessels. However, valves were not seemingly affected.)	minimal at 40 mg/kg.	
	epicardial adhesion/inflammation/fibrosis (characterized by thickening of the atrial and heart base epicardium by fibrosis, fibroblasts, mixed inflammatory cells, and proliferating mesothelium)	minimal at 40 mg/kg	minimal at 40 mg/kg
	other microscopic observations (spontaneously occurring findings, they were low/isolated in frequency and/or distributed randomly among groups, or their appearance was similar to findings in controls from this and/or previous studies.)	considered incidental	considered incidental

[0369] *Pharmacokinetic studies in mice and cynomolgus monkeys*

[0370] In this study, various concentrations of exemplary anti-TM4SF1 antibodies and ADCs containing the same were assessed, in mouse and cynomolgus monkeys. Surrogate anti-mouse TM4SF1 antibodies (MS-C and MS-YTEC) cleared much faster in mice than the clearance of the anti-human TM4SF1 antibodies (A07-C and A07-YTEC) in monkey. The MS-YTEC cleared much faster than the MS-C in mice, when administered at the same dose of 60 mg/kg. In case of the exemplary anti-human TM4SF1 antibodies, A07-C-BA-DM1 and A07-YTEC-BA-DM1 were cleared in a similar pace in monkey, when administered at the same dose of 40 mg/kg.

[0371] Different injection route, intravenous (iv) and intraperitoneal (ip), showed very similar level of the murine surrogate antibodies in circulation in mice regardless whether the antibody was a naked antibody or conjugated with a DM1 payload. Results for this study are shown in **FIG. 10**.

[0372] *Efficacy*

[0373] In this study, the efficacy of murine surrogate anti-TM4SF1 antibodies conjugated to payload DM1, using bromoacetamide conjugation, MS-C-BA-DM1 and MS-YTEC-BA-DM1, in reducing tumor volume, were tested.

[0374] Briefly, eight weeks old C57/Bl6 mice that were previously implanted with B16-F10 tumor cells (ATCC® CRL-6475™ - mouse skin melanoma cells) were randomized into groups and injected with a control or ADCs as follows: (i) MS-C-BA-DM1 (DAR 2.2) at 12 mg/kg or 20 mg/kg; or (ii) MS-YTEC-BA-DM1 (DAR 2.1) at 12 mg/kg or 20 mg/kg.

[0375] Tumor volumes for a period of about 16 days, following injection, were measured and the results are shown in **FIG. 11**. At 12 mg/kg dosage, MS-C-BA-DM1 and MS-YTEC-BA-DM1 showed very similar B16-F10 tumor regression efficacy. Whereas, at 20 mg/kg dosage, MS-C-BA-DM1 showed better B16-F10 tumor regression efficacy than the MS-YTEC-BA-DM1.

[0376] Next, the tumor regression property of MS-YTEC-BA-DM1 (at DAR of about 2 or about 1) was assessed using a 24 mg/kg single injection, in eight weeks old C57/Bl6 mice that were previously implanted with B16-F10 tumor cells, as described above. The MS-YTEC-BA-DM1 at DAR1 and DAR2 conjugation showed very similar B16F10 tumor regression efficacy. Results are shown in **FIG. 12**.

[0377] A further efficacy study was carried out using a MiaPaca 2 (ATCC® CRL-1420™ - pancreatic carcinoma) xenograft tumor model. Briefly, eight weeks old athymic nude mice were randomized into groups and injected with a control or ADCs as follows: (i) MS-C-BA-DM1 at 12 mg/kg (single injection) or MS-YTEC-BA-DM1 at 12 mg/kg (single injection) (**FIG. 13** – top left panel); (ii) A07-C-BA-DM1 at 12 mg/kg (single injection) or A07-YTEC-BA-DM1 at 12 mg/kg (single injection) (**FIG. 13**- top right panel); (iii) MS-C-BA-DM1 at 12 mg/kg, single injection in combination with A07-C-BA-DM1 at 12 mg/kg, single injection or MS-YTEC-BA-DM1 at 12 mg/kg, single injection in combination with A07-YTEC-BA-DM1 at 12 mg/kg (**FIG. 13**- bottom left panel); (iv) MS-C-BA-DM1 at 3 mg/kg (q7d4- weekly for four times) in combination with A07-C-BA-DM1 at 3 mg/kg, q7d4 or MS-YTEC-BA-DM1 at 3 mg/kg, q7d4 in combination with A07-YTEC-BA-DM1 at 3 mg/kg, q7d4 (**FIG. 13**- bottom right panel).

[0378] MS-C-BA-DM1 and MS-YTEC-BA-DM1 show very similar efficacy of MiaPaca2 tumor regression. In combination therapy of MS+A07, MiaPaca2 tumor regression was better with the single injection of the higher dose (12 mg/kg), compared to the smaller dose (3 mg/kg) that was injected weekly for 4 times.

[0379] It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions, methods, and kits of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

TABLE 6. SEQUENCE DESCRIPTION

SEQ ID NO	Description	Sequence
Antibody AGX-A01		
1	AGX-A01 Variable heavy (VH) chain-amino acid	EVILVESGGGLVKPGGSLKLSCAASGFTFSSF AMSWVRQTPEKRLEWVATISSGSIYIYYTDG

SEQ ID NO	Description	Sequence
		VKGRFTISRDNAKNTVHLQMSSLRSEDTAM YYCARRGIYYGYDGYAMDYWGQGTSVTVS
2	AGX-A01 Variable light (VL) chain-amino acid	AVVMTQTPLSLPVSLGDQASISCRSSQSLVHS NGNTYLHWYMQKPGQSPKVLIYKVSNRFSG VPDRFSGSGSGTDFTLKISRVEADDLGIYFCS QSTHIPLAFGAGTKLELK
Antibody AGX-A03		
3	AGX-A03 Variable heavy (VH) chain-amino acid	QIQLVQSGPELKKPGETVKISCKASGYSFRDY GMNWVKQAPGRTFKWMGWINTYTGAPVY AADFKGRFAFSLDTSASAAFLQINNLKNEDT ATYFCARWVSYGNNRNWFFDFWGAGTTVT VSS
4	AGX-A03 Variable heavy (VH) chain-nucleic acid	CAGATCCAGTTGGTGCAGTCTGGACCTGAG CTGAAGAAGCCTGGAGAGACAGTCAAGAT CTCCTGCAAGGCTTCTGGGTATTCCTTCAG AGACTATGGAATGAACTGGGTGAAGCAGG CTCCAGGAAGGACTTTTAAGTGGATGGGCT GGATAAACACCTACACTGGAGCGCCAGTA TATGCTGCTGACTTCAAGGGACGGTTTGCC TTCTCTTTGGACACCTCTGCCAGCGCTGCC TTTTTGCAGATCAACAACCTCAAAAATGAA GACACGGCTACATATTTCTGTGCAAGATGG GTCTCCTACGGTAATAACCGCAACTGGTTC TTCGATTTTTGGGGCGCAGGGACCACGGTC ACCGTCTCCTCA
5	AGX-A03 Variable heavy (VH) chain-codon optimized nucleic acid	CAAATTCAGTTGGTTCAATCCGGCCCTGAG CTCAAGAAGCCTGGAGAGACAGTGAAGAT AAGTTGTAAGGCTAGTGGCTATTCATTTCG AGATTATGGGATGAATTGGGTCAAGCAGG CCCCAGGGCGGACCTTCAAATGGATGGGG TGGATCAATACTTACACTGGCGCACCAGTA TATGCAGCTGATTTTAAGGGTCGCTTTGCA TTTTCACTTGATACTTCAGCCAGTGCCGCT TTTTTGCAAATCAACAATCTCAAAAATGAA GACACTGCTACATATTTCTGCGCCAGGTGG GTGAGCTATGGCAATAACAGAAATTGGTT CTTTGACTTTTGGGGCGCAGGCACCACCGT CACTGTCTCATCA
6	VH- CDR1	GYSFRDYGMN
7	VH-CDR2	WINTYTGAPVYAADFKG
8	VH-CDR3	WVSYGNNRNWFFDF
9	AGX-A03	DVLMTQTPLSLPVRLGDQASISCRSSQTLVHS NGNTYLEWYLQKPGQSPKLLIYKVSNRLSG

SEQ ID NO	Description	Sequence
	Variable light (VL) chain- amino acid	VPDRFSGSGSGTDFTLKISRVETEDLGVYYCF QGSHGPWTFGGGTKLEIK
10	AGX-A03 Variable light (VL) chain- nucleic acid	GATGTTTTGATGACCCAAACTCCACTCTCC CTGCCTGTCCGTCTTGGAGATCAGGCCTCC ATCTCTTGTAGATCTAGTCAGACCCTTGTA CATAGTAATGGAAACACCTATTTAGAATG GTACCTGCAGAAACCAGGCCAGTCTCCAA AACTCTTGATCTACAAAGTTTCCAATCGAC TTTCTGGGGTCCCAGACAGGTTCAGTGGCA GTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGACTGAGGATCTGGG AGTTTATTACTGCTTTCAAGGTTCACATGG TCCGTGGACGTTCGGTGGAGGCACCAAGC TGGAAATCAAA
11	AGX-A03 Variable light (VL) chain- codon optimized nucleic acid	GACGTACTTATGACACAAACTCCCTTGAGC TTGCCAGTACGGCTTGGCGATCAAGCTTCA ATTTCATGTCGTTCTTCTCAAACACTTGTCC ACTCAAATGGGAATACATATTTGGAATGGT ATCTCCAAAAGCCCGGCCAATCCCCAAAA TTGTTGATTTACAAGGTGTCTAATCGACTC TCAGGCGTCCCCGACCGATTCTCCGGGAGC GGGTCCGGTACAGACTTCACCTTGAAAATC TCCAGGGTAGAAACTGAAGACCTCGGAGT CTACTATTGTTTCCAGGGGTCACACGGCCC CTGGACATTTGGAGGAGGAACTAAGCTCG AGATCAAA
12	VL- CDR1	RSSQTLVHSNGNTYLE
13	VL-CDR2	KVSNRLS
14	VL-CDR3	FQGSHGPWT
Antibody AGX-A04		
15	AGX-A04 Variable heavy (VH) chain- amino acid	EVQLQQSGPELVKPGASVKISCKTSGYTFTD YTMHWVRQSHGKSLEWIGSFNPNNGGLTNY NQKFKGKATLTVDKSSSTVYMDLRSLTSEDS AVYYCTRIRATGFDSWGQGTTLTVSS
16	AGX-A04 Variable heavy (VH) chain- nucleic acid	GAGGTCCAGCTGCAACAGTCTGGACCTGA GCTGGTGAAGCCTGGGGCTTCAGTGAAGA TATCCTGCAAGACTTCTGGATACACATTCA CTGATTACACCATGCACTGGGTGAGGCAG AGCCATGGAAAGAGCCTTGAGTGGATTGG AAGTTTTAATCCTAACAATGGTGGTCTTAC TAACTACAACCAGAAGTTCAAGGGCAAGG CCACATTGACTGTGGACAAGTCTTCCAGCA CAGTGTACATGGACCTCCGCAGCCTGACAT

SEQ ID NO	Description	Sequence
		CTGAGGATTCTGCAGTCTATTACTGTACAA GAATCCGGGCTACGGGCTTTGACTCCTGGG GCCAGGGCACCACTCTCACAGTCTCCTCA
17	AGX-A04 Variable heavy (VH) chain- codon optimized nucleic acid	GAGGTACAACTGCAACAGAGTGGACCTGA ACTTGTCAAACCTGGAGCAAGTGTGAAGA TTAGCTGTAAAACCAGTGGCTACACATTTA CCGATTATACTATGCACTGGGTAAGACAG AGCCACGGAAAATCACTGGAGTGGATTGG TAGTTTCAATCCTAACAACGGAGGATTGAC AAATTACAACCAGAAGTTCAAAGGGAAAG CCACCTTGACAGTTGATAAGTCCTCAAGTA CCGTGTATATGGATCTGCGTTCTCTCACAA GTGAAGATAGCGCAGTTTACTACTGTACCC GCATCCGAGCCACCGGGTTCGATTCATGGG GTCAGGGGACAACACTGACTGTTTCTTCT
18	VH- CDR1	GYTFTDYTMH
19	VH-CDR2	SFNPNNGGLTNYNQKFKG
20	VH-CDR3	IRATGFDS
21	AGX-A04 Variable light (VL) chain- amino acid	DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLN SRTRKNYLAWYQQKPGQSPKLLIYWASTRE SGVPDRFTGSGSGTDFTLTISNVQAEDLTVY YCKQSYNPPWTFGGGTKLEIK
22	AGX-A04 Variable light (VL) chain- nucleic acid	GACATTGTGATGTCACAGTCTCCATCCTCC CTGGCTGTGTCAGCAGGAGAGAAGGTCAC TATGAGCTGCAAATCCAGTCAGAGTCTGCT CAACAGTAGAACCCGAAAGAACTACTTGG CTTGGTACCAGCAGAAACCAGGGCAGTCT CCTAAACTGCTGATCTACTGGGCATCCACT AGGGAATCTGGGGTCCCTGATCGCTTCACA GGCAGTGGATCTGGGACAGATTTCACTCTC ACCATCAGCAATGTGCAGGCTGAAGACCT GACAGTTTATTACTGCAAGCAATCTTATAA TCCTCCGTGGACGTTCGGTGGAGGCACCAA GCTGGAAATCAAA
23	AGX-A04 Variable light (VL) chain- codon optimized nucleic acid	GACATAGTTATGTCCCAGTCTCCATCCAGC TTGGCTGTCAGCGCCGGAGAGAAAGTGAC TATGAGTTGTAAATCTTCCCAGTCCCTGCT TAACTCACGTACTCGGAAGAATTATCTTGC CTGGTATCAACAAAAGCCAGGTCAAAGTC CTAAGCTCCTTATTTACTGGGCCTCAACAC GGGAGTCAGGTGTCCCCGATCGCTTCACAG GTAGTGGGAGTGGTACTGACTTCACTCTCA CCATTTCAAATGTCCAAGCAGAAGACTTGA CTGTGTATTACTGTAAGCAGAGTTACAACC

SEQ ID NO	Description	Sequence
		CTCCTTGGACCTTTGGTGGGGGGACCAAAC TGGAGATCAAG
24	VL- CDR1	KSSQSLLNSRTRKNYLA
25	VL-CDR2	WASTRES
26	VL-CDR3	KQSYNPPWT
Antibody AGX-A05		
27	AGX-A05 Variable heavy (VH) chain- amino acid	EVQVQQSGPELVKPGASVKMSCKASGYTFT SYVMHWVKQKPGQGLEWIGYINPNNDNINY NEKFKGKASLTSDKSSNTVYMELSSLTSEDS AVYYCAGYGNSGANWGQGTLVTVSA
28	AGX-A05 Variable heavy (VH) chain- nucleic acid	GAGGTCCAGGTACAGCAGTCTGGACCTGA ACTGGTAAAGCCTGGGGCTTCAGTGAAGA TGTCCTGTAAGGCTTCTGGATACACATTCA CTAGCTATGTCATGCACTGGGTGAAGCAG AAGCCTGGGCAGGGCCTTGAGTGGATTGG ATATATTAATCCTAACAATGATAATATTAA CTACAATGAGAAGTTCAAAGGCAAGGCCT CACTGACTTCAGACAAATCCTCCAACACAG TCTACATGGAGCTCAGCAGCCTGACCTCTG AGGACTCTGCGGTCTATTACTGTGCAGGCT ATGGTAACTCCGGAGCTAACTGGGGCCAA GGGACTCTGGTCACTGTCTCTGCA
29	AGX-A05 Variable heavy (VH) chain- codon optimized nucleic acid	GAAGTTCAAGTTCAGCAAAGCGGGCCTGA GCTTGTCAAGCCAGGCGCATCAGTCAAAA TGAGCTGTAAGGCTTCCGGGTACACCTTCA CCAGTTATGTCATGCATTGGGTAAAACAAA AGCCAGGACAGGGACTCGAGTGGATAGGA TACATTAACCCAAATAACGACAACATTAA CTACAACGAGAAATTCAAGGGCAAAGCAT CATTGACTTCCGATAAATCCTCTAACACCG TGTACATGGAGCTGAGTTCATTGACCAGCG AGGATTCTGCCGTGTACTACTGTGCAGGTT ATGGCAACTCTGGTGCTAACTGGGGGCAG GGGACTCTGGTCACAGTCAGCGCA
30	VH- CDR1	GYTFTSYVMH
31	VH-CDR2	YINPNNDNINYNEKFKG
32	VH-CDR3	YGNSGAN
33	AGX-A05 Variable light (VL)chain- amino acid	DIQMTQSPASLSASVGETVTITCRTSKNIFNFL AWYHQKQGRSPRLLVSHTKTLAAGVPSRFS GSGSGTQFSLKINSLQPEDFGIYYCQHHYGTP WTFGGGTKLEIK

SEQ ID NO	Description	Sequence
34	AGX-A05 Variable light (VL) chain- nucleic acid	GACATCCAGATGACTCAGTCTCCAGCCTCC CTATCTGCATCTGTGGGAGAAACTGTCACC ATCACATGTCGAACAAGTAAAAATATTTTC AATTTTTTAGCATGGTATCACCAGAAACAG GGAAGATCTCCTCGACTCCTGGTCTCTCAT ACAAAAACCTTAGCAGCAGGTGTGCCATC AAGGTTCAGTGGCAGTGGCTCAGGCACAC AGTTTTCTCTGAAGATCAACAGCCTGCAGC CTGAAGATTTTGGGATTTATTACTGTCAAC ATCATTATGGTACTCCGTGGACGTTCGGTG GAGGCACCAAACTGGAAATCAAA
35	AGX-A05 Variable light (VL) chain- codon optimized nucleic acid	GACATTCAGATGACCCAGTCACCAGCATCT TTGAGCGCATCCGTTGGGGAGACTGTGAC AATCACATGCCGAACCAGTAAGAACATCT TCAACTTCCTCGCATGGTACCATCAAAAGC AGGGCAGGTCTCCCAGACTGCTTGTCTCTC ACACCAAGACACTGGCAGCAGGCGTCCCC AGCCGGTTTAGTGGTAGTGGATCTGGCACA CAGTTTAGTTTGAAAATCAATTCCCTGCAA CCCGAAGACTTCGGCATATACTATTGCCAG CACCACTATGGGACACCTTGGACTTTCGGA GGTGGTACTAAACTTGAGATTAAA
36	VL- CDR1	RTSKNIFNFLA
37	VL-CDR2	HTKTLAA
38	VL-CDR3	QHHYGTPWT
Antibody AGX-A07		
39	AGX-A07 Variable heavy (VH) chain- amino acid	QIQLVQSGPELKKPGETVKISCKASGYTFTNY GVKWVKQAPGKDLKWMGWINTYTGNPIYA ADFKGRFAFSLETSASTAFLQINNLKNEDTAT YFCVRFQYGDYRYFDVWGAGTTVTVSS
40	AGX-A07 Variable heavy (VH) chain- nucleic acid	CAGATCCAGTTGGTGCAGTCTGGACCTGAG CTGAAGAAGCCTGGAGAGACAGTCAAGAT CTCCTGCAAGGCTTCTGGGTATACCTTCAC AAACTATGGAGTGAAGTGGGTGAAGCAGG CTCCAGGAAAGGATTTAAAGTGGATGGGC TGGATAAACACCTACACTGGAAATCCAATT TATGCTGCTGACTTCAAGGGACGGTTTGCC TTCTCTTTGGAGACCTCTGCCAGCACTGCC TTTTTGCAGATCAACAACCTCAAAAATGAG GACACGGCTACATATTTCTGTGTAAGATTC CAATATGGCGATTACCGGTACTTCGATGTC TGGGGCGCAGGGACCACGGTCACCGTCTC CTCA

SEQ ID NO	Description	Sequence
41	AGX-A07 Variable heavy (VH) chain- codon optimized nucleic acid	CAAATCCAACTTGTCCAGAGCGGTCCCGA GTTGAAGAAGCCTGGCGAAACCGTGAAAA TCTCATGCAAGGCCAGTGGATATACATTTA CAAACTATGGCGTCAAGTGGGTGAAACAA GCCCCAGGTAAAGACTTGAAATGGATGGG ATGGATCAACACATACACAGGGAATCCTA TCTATGCAGCCGACTTTAAAGGCAGATTTG CCTTCAGTTTGGAGACATCTGCCTCCACCG CTTTCCTGCAAATAAATAACCTGAAAAATG AAGATACCGCTACATACTTCTGTGTACGGT TCCAGTACGGAGATTACCGCTATTTCGATG TGTGGGGCGCAGGTACCACAGTAACCGTC TCCTCA
42	VH- CDR1	GYTFTNYGVK
43	VH-CDR2	WINTYTGNPIYAADFKG
44	VH-CDR3	FQYGDYRYFDV
45	AGX-A07 Variable light (VL) chain- amino acid	QIILSQSPAILSASPGEKVTMTCRANSGISFIN WYQQKPGSSPKPWIYGTANLASGVPARFGG SGSGTSYSLTISRVEAEDAATYYCQQWSSNP LTFGAGTKLELR
46	AGX-A07 Variable light (VL) chain- nucleic acid	CAAATTATTCTCTCCCAGTCTCCAGCAATC CTGTCTGCATCTCCAGGGGAGAAGGTCAC GATGACTTGCAGGGCCAACTCAGGTATTA GTTTCATCAACTGGTACCAGCAGAAGCCA GGATCCTCCCCCAAACCCTGGATTTATGGC ACAGCCAACCTGGCTTCTGGAGTCCCTGCT CGCTTCGGTGGCAGTGGGTCTGGGACTTCT TACTCTCTCACAATCAGCAGAGTGGAGGCT GAAGACGCTGCCACTTATTACTGCCAGCAG TGGAGTAGTAACCCGCTCACGTTCGGTGCT GGGACCAAGCTGGAGTTGAGA
47	AGX-A07 Variable light (VL) chain- codon optimized nucleic acid	CAAATAATTCTGTCACAGTCCCCCGCTATA CTTAGTGCTTCACCAGGAGAAAAAGTGAC CATGACTTGTAGAGCTAATTCTGGCATATC ATTCATCAACTGGTATCAACAAAAGCCAG GTTCCTCCCCCAAGCCATGGATTTACGGGA CCGCCAACCTTGCTTCTGGGGTACCCGCTC GTTTCGGCGGATCAGGTTCAGGAACTTCCT ATAGCCTCACTATCAGTCGGGTTGAAGCTG AGGATGCCGCTACATATTACTGCCAGCAAT GGTCTAGTAATCCACTTACCTTTGGAGCTG GCACCAAATTGGAACTTCGT
48	VL- CDR1	RANSGISFIN
49	VL-CDR2	GTANLAS

SEQ ID NO	Description	Sequence
50	VL-CDR3	QQWSSNPLT
	Antibody AGX-A08	
51	AGX-A08 Variable heavy (VH) chain - amino acid	EVQLQQSGPELVKPGASVKLSCKASGYTVTS YVMHWVKQKPGQGLEWIGYINPYSDVTNC NEKFKGKATLTSDKTSSTAYMELSSLTSEDS AVYYCSSYGGGFAYWGQGTLVTVSA
52	AGX-A08 Variable heavy (VH) chain-nucleic acid	GAGGTCCAGCTGCAGCAGTCTGGACCTGA GCTGGTAAAGCCTGGGGCTTCAGTGAAGC TGTCCTGCAAGGCTTCTGGATACACAGTCA CTAGCTATGTTATGCACTGGGTGAAGCAGA AGCCTGGGCAGGGCCTTGAGTGGATTGGA TATATTAATCCTTACAGTGATGTTACTAAC TGCAATGAGAAGTTCAAAGGCAAGGCCAC ACTGACTTCAGACAAAACCTCCAGCACAG CCTACATGGAGCTCAGCAGCCTGACCTCTG AGGACTCTGCGGTCTATTACTGTTCCTCCT ACGGTGGGGGGTTTGCTTACTGGGGCCAA GGGACTCTGGTCACTGTCTCTGCA
53	AGX-A08 Variable heavy (VH) chain-codon optimized nucleic acid	GAAGTCCAGCTTCAGCAATCCGGCCCAGA ACTGGTAAAACCAGGCGCAAGTGTTAAGT TGAGTTGCAAAGCCAGTGGTTATACCGTTA CTTCATACGTCATGCATTGGGTAAAACAAA AGCCCGGCCAAGGGCTTGAATGGATCGGC TACATCAACCCTTACTCTGACGTCACCAAC TGCAACGAGAAATTCAAAGGGAAAGCCAC ATTGACCTCTGACAAGACAAGCAGTACCG CCTACATGGAGCTTTCTAGTTTGACTTCTG AAGACTCTGCTGTCTACTACTGTAGCAGCT ACGGCGGCGGCTTTGCTTACTGGGGCCAG GGTACATTGGTGACTGTGAGTGCA
54	VH- CDR1	GYTVTSYVMH
55	VH-CDR2	YINPYSDVTNCNEKFKG
56	VH-CDR3	YGGGFAY
57	AGX-A08 Variable light chain(VL) -amino acid	DIQMTQSPASLSASVGEPVTITCRASKNIYTY LAWYHQKQGKSPQFLVYNARTLAGGVPSRL SGSGSVTQFSLNINTLHREDLGTYFCQHHYD TPYTFGGGTNLEIK
58	AGX-A08 Variable light (VL) chain-nucleic acid	GACATCCAGATGACTCAGTCTCCAGCCTCC CTATCTGCATCTGTGGGAGAACCTGTCACC ATCACATGTCGAGCAAGTAAGAATATTTAC ACATATTTAGCATGGTATCACCAGAAACA GGGAAAATCTCCTCAGTTCCTGGTCTATAA TGCAAGAACCTTAGCAGGAGGTGTGCCAT

SEQ ID NO	Description	Sequence
		CAAGGCTCAGTGGCAGTGGATCAGTCACG CAGTTTTCTCTAAACATCAACACCTTGCAT CGAGAAGATTTAGGGACTTACTTCTGTCAA CATCATTATGATACTCCGTACACGTTCGGA GGGGGGACCAACCTGGAAATAAAA
59	AGX-A08 Variable light (VL) chain- codon optimized nucleic acid	GACATCCAGATGACACAGTCACCAGCATC CCTGTCCGCCTCAGTTGGGGAGCCTGTTAC CATAACTTGTCGGGCAAGCAAAAACATAT ACACCTATTTGGCTTGGTATCACCAAAAGC AAGGTAAGTCACCTCAGTTTCTTGTATATA ATGCCCGCACACTTGCTGGCGGAGTACCCT CTCGATTGTCTGGATCTGGCAGCGTTACCC AATTCAGCCTGAACATCAACACCCTCCATC GGGAAGATTTGGGTACCTATTTCTGTCAAC ATCACTACGACACCCCATACACCTTCGGAG GCGGCACAAATTTGGAAATTAAA
60	VL- CDR1	RASKNIYTYLA
61	VL-CDR2	NARTLAG
62	VL-CDR3	QHHYDTPYT
Antibody AGX-A09		
63	AGX-A09 Variable heavy (VH) chain- amino acid	EVQLQQSGPELVKPGASVKMSCKASGYTFSS YVMHWVKQKPGQGLEWIGYINPYSDVTNY NEKFKGKATLTSDRSSNTAYMELSSLTSEDS AVYYCARNYFDWGRGTLVTVSA
64	AGX-A09 Variable heavy (VH) chain- nucleic acid	GAGGTCCAGCTGCAGCAGTCTGGACCTGA GCTGGTAAAGCCTGGGGCTTCAGTGAAGA TGTCCTGCAAGGCTTCTGGATACACATTCT CTAGCTATGTTATGCACTGGGTGAAGCAGA AGCCTGGGCAGGGCCTTGAGTGGATTGGA TATATTAATCCTTACAGTGATGTCACTAAC TACAATGAGAAGTTCAAAGGCAAGGCCAC ACTGACTTCAGACAGATCCTCCAACACAGC CTACATGGAACTCAGCAGCCTGACCTCTGA GGACTCTGCGGTCTATTACTGTGCAAGAAA TTACTTCGACTGGGGCCGAGGGACTCTGGT CACAGTCTCTGCA
65	AGX-A09 Variable heavy (VH) chain- codon optimized nucleic acid	GAGGTACAGCTTCAGCAGAGTGGTCCAGA ACTCGTCAAGCCTGGGGCAAGCGTTAAGA TGAGTTGTAAAGCATCCGGTTACACATTCA GTAGCTATGTTATGCACTGGGTCAAACAGA AGCCTGGGCAGGGGTTGGAGTGGATCGGA TATATAAATCCCTATTCAGACGTAACTAAT TATAATGAAAAGTTCAAGGGGAAAGCAAC CTTGACAAGTGACCGGTCATCTAATACCGC ATACATGGAGCTGAGCTCATTGACAAGTG

SEQ ID NO	Description	Sequence
		AGGACTCTGCTGTGTATTACTGTGCCCGGA ACTACTTCGACTGGGGTAGGGGCACACTG GTAACTGTTAGTGCA
66	VH- CDR1	GYTFSSYVMH
67	VH-CDR2	YINPYSDVTNYNEKFKG
68	VH-CDR3	NYFD
69	AGX-A09 Variable light (VL) chain- amino acid	DIQMTQSPASLSASVGETVTITCRASKNVYS YLAWFQQKQGKSPQLLVYNAKTLAEGVPSR FSGGGSGTQFSLKINSLQPADFGSYYCQHHY NIPFTFGSGTKLEIK
70	AGX-A09 Variable light (VL) chain- nucleic acid	GACATCCAGATGACTCAGTCTCCAGCCTCC CTATCTGCATCTGTGGGAGAAACTGTCACC ATCACATGTCGAGCAAGTAAAAATGTTTAC AGTTATTTAGCATGGTTTCAACAGAAACAG GGGAAATCTCCTCAGCTCCTGGTCTATAAT GCTAAAACCTTAGCAGAAGGTGTGCCATC AAGGTTCAGTGGCGGGGGATCAGGCACAC AGTTTTCTCTGAAGATCAACAGCCTGCAGC CTGCAGATTTTGGGAGTTATTACTGTCAAC ATCATTATAATATTCCATTCACGTTCGGCT CGGGGACAAAGTTGGAAATAAAA
71	AGX-A09 Variable light (VL) chain- codon optimized nucleic acid	GACATACAAATGACACAAAGTCCCGCTAG TCTTTCAGCCAGTGTTGGTGAGACTGTGAC AATAACCTGTAGAGCTAGCAAAAATGTCT ACTCCTATCTGGCTTGGTTCCAGCAGAAAC AAGGAAAGAGTCCTCAGTTGCTCGTATATA ATGCTAAAACTTTGGCAGAAGGCGTCCCTT CTCGTTTCAGTGGCGGAGGAAGTGGGACT CAATTCTCACTGAAGATCAATAGCCTCCAG CCCGCCGACTTTGGGAGCTACTATTGCCAA CATCATTACAACATACCATTCACCTTTGGC TCAGGTACTAAACTCGAAATTAAA
72	VL- CDR1	RASKNVYSYLA
73	VL-CDR2	NAKTLAE
74	VL-CDR3	QHHYNIPFT
Antibody AGX-A11		
75	AGX-A11 Variable heavy (VH) chain- amino acid	QIQLVQSGPELKKPGETVKISCKASGFTFTNY PMHWVKQAPGKGLKWMGWINTYSGVPTY ADDFKGRFAFSLETSASTAYLQINNLKNEDM ATYFCARGGYDGSREFAYWGQGTLVTVS

SEQ ID NO	Description	Sequence
76	AGX-A11 Variable heavy (VH) chain- nucleic acid	CAGATCCAGTTGGTGCAGTCTGGACCTGAG CTGAAGAAGCCTGGAGAGACAGTCAAGAT CTCCTGCAAGGCTTCTGGGTTTACCTTCAC AAACTATCCAATGCACTGGGTGAAGCAGG CTCCAGGAAAGGGTTTAAAGTGGATGGGC TGGATAAACACCTACTCTGGAGTGCCAAC ATATGCAGATGACTTCAAGGGACGGTTTGC CTTCTCTTTGGAAACCTCTGCCAGCACTGC ATATTTGCAGATCAACAACCTCAAAAATG AGGACATGGCTACATATTTCTGTGCAAGAG GGGGCTACGATGGTAGCAGGGAGTTTGCT TACTGGGGCCAAGGGACTCTGGTCACTGTC TCT
77	AGX-A11 Variable heavy (VH) chain- codon optimized nucleic acid	CAGATACAACTCGTCCAGTCAGGTCCAGA GTTGAAGAAACCCGGAGAAACTGTGAAGA TATCCTGTAAAGCCAGCGGCTTTACTTTCA CAAACTACCCCATGCATTGGGTGAAGCAG GCCCCCGGAAAAGGACTCAAATGGATGGG ATGGATCAACACATACAGTGGGGTGCCTA CTTACGCAGACGATTTCAAAGGAAGGTTC GCATTTAGCTTGGAAACTAGCGCATCTACA GCATATCTCCAGATTAACAATCTTAAAAAT GAGGATATGGCAACATACTTCTGCGCTAG GGGAGGTTACGATGGGAGCAGGGAGTTCG CTTATTGGGGGCAAGGGACTCTTGTGACTG TAAGT
78	VH- CDR1	GFTFTNYPMH
79	VH-CDR2	WINTYSGVPTYADDFKG
80	VH-CDR3	GGYDGSREFAY
81	AGX-A11 Variable light (VL) chain- amino acid	DIVLTQSPASLAASLGQRATTSYRASKSVSTS GYSYMHWNQQKPGQPPRLLIYLVSNLESGV PARFSGSGSGTDFTLNIHPVEEEDAATYYCQ HIRELTTFGGGTKLEIK
82	AGX-A11 Variable light (VL) chain- nucleic acid	GACATTGTGCTGACACAGTCTCCTGCTTCC TTAGCTGCATCTCTGGGGCAGAGGGCCACC ACCTCATACAGGGCCAGCAAAAGTGTCAG TACATCTGGCTATAGTTATATGCACTGGAA CCAACAGAAACCAGGACAGCCACCCAGAC TCCTCATCTATCTTGTATCCAACCTAGAAT CTGGGGTCCCTGCCAGGTTCAGTGGCAGTG GGTCTGGGACAGACTTCACCCTCAACATCC ATCCTGTGGAGGAGGAGGATGCTGCAACC TATTACTGTCAGCACATTAGGGAGCTTACC

SEQ ID NO	Description	Sequence
		ACGTTCGGAGGGGGGACCAAGCTGGAAAT AAAA
83	AGX-A11 Variable light (VL) chain- codon optimized nucleic acid	GACATAGTGCTCACTCAGAGCCCTGCATCC CTTGCCGCCTCCCTCGGACAACGAGCTACT ACAAGCTACCGGGCATCAAAGTCCGTTAG CACATCAGGATACAGCTATATGCACTGGA ATCAGCAAAAGCCAGGCCAACCACCCCGT CTTCTCATCTACCTCGTAAGTAATCTGGAA TCAGGCGTGCCAGCCCGATTCAGTGGGTCA GGGTCTGGGACAGATTTCACCCTCAACATC CATCCAGTAGAGGAAGAGGACGCAGCAAC ATATTACTGCCAACACATTAGAGAACTTAC CACTTTCGGAGGAGGAACTAAATTGGAGA TCAAA
84	VL- CDR1	RASKSVSTSGYSYMH
85	VL-CDR2	LVSNLES
86	VL-CDR3	QHIRELTT
Constant Region Sequences		
87	IgG1 G1m17* (heavy chain constant region) * with L234A/L235A/G237A mutations SEQ ID NO: 88 is sequence without the terminal lysine	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPEAAGAPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
88	IgG1 G1m17* (heavy chain constant region) * with L234A/L235A/G237A mutations	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPEAAGAPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPG
89	IgG1 Km3 (light chain constant region)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
Humanized AGX-A07 sequences		

SEQ ID NO	Description	Sequence
90	AGX-A07 (humanized) H2 Heavy chain amino acid	QVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQDLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAFMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPEAAGAPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
91	AGX-A07 (humanized) H2 Heavy chain nucleic acid	TCTACCGGACAGGTGCAGTTGGTTCAGTCT GGCGCCGAAGTGAAGAAACCTGGCGCTTC TGTGAAGGTGTCCTGCAAGGCCTCTGGCTA CACCTTTACCAACTACGGCGTGAAATGGGT CCGACAGGCTCCTGGACAGGATCTGGAAT GGATGGGCTGGATCAACACCTACACCGGC AATCCTATCTACGCCGCCGACTTCAAGGGC AGAGTGACCATGACCACCGACACCTCTAC CTCCACCGCCTTCATGGAACTGCGGTCCCT GAGATCTGACGACACCGCCGTGTACTACTG CGTGCGGTTTCAGTACGGCGACTACCGGTA CTTTGATGTGTGGGGCCAGGGCACACTGGT CACCGTTTCTTCCGCTTCTACCAAGGGACC CAGCGTGTTCCCTCTGGCTCCTTCCTCTAA ATCCACCTCTGGCGGAACCGCTGCTCTGGG CTGTCTGGTCAAGGATTACTTCCCTGAGCC TGTGACCGTGTCCTGGAACTCTGGTGCTCT GACATCCGGCGTGCACACCTTTCCAGCTGT GCTGCAGTCCTCTGGCCTGTACTCTCTGTC CTCTGTCGTGACCGTGCCTTCTAGCTCTCT GGGCACCCAGACCTACATCTGCAACGTGA ACCACAAGCCTTCCAACACCAAGGTGGAC AAGAAGGTGGAACCCAAGTCCTGCGACAA GACCCACACCTGTCCTCCATGTCCTGCTCC AGAAGCTGCTGGCGCTCCCTCTGTGTTCCT GTTTCCTCCAAAGCCTAAGGACACCCTGAT GATCTCTCGGACCCCTGAAGTGACCTGCGT GGTGGTGGATGTGTCTCACGAGGACCCAG AAGTGAAGTTCAATTGGTACGTGGACGGC GTGGAAGTGCACAACGCCAAGACCAAGCC TAGAGAGGAACAGTACAACTCCACCTACA GAGTGGTGTCCGTGCTGACCGTGCTGCACC AGGATTGGCTGAACGGCAAAGAGTACAAG TGCAAGGTGTCCAACAAGGCACTGCCCGC

SEQ ID NO	Description	Sequence
		TCCTATCGAAAAGACCATCTCCAAGGCTAA GGGCCAGCCTCGGGAACCTCAGGTTTACA CCCTGCCTCCATCTCGGGAAGAGATGACCA AGAACCAGGTGTCCCTGACCTGCCTCGTGA AGGGCTTCTACCCTTCCGATATCGCCGTGG AATGGGAGTCCAATGGCCAGCCTGAGAAC AACTACAAGACAACCCCTCCTGTGCTGGAC TCCGACGGCTCATTCTTCCTGTACTCCAAG CTGACAGTGGACAAGTCTCGGTGGCAGCA GGGCAACGTGTTCTCCTGTTCTGTGATGCA CGAGGCCCTGCACAACCACTACACACAGA AGTCCCTGTCTCTGTCCCCTGGCAAGTGA
92	AGX-A07 H2v1 Heavy chain amino acid	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPEAAGAPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
93	AGX-A07 H2v1 Heavy chain nucleic acid	GAAGTGCAGTTGGTGCAGTCTGGCGCCGA AGTGAAGAAACCTGGCGCTTCTGTGAAGG TGTCCTGCAAGGCCTCTGGCTACACCTTTA CCAACTACGGCGTGAAATGGGTCCGACAG GCTCCTGGACAAGGCCTGGAATGGATGGG CTGGATCAACACCTACACCGGCAATCCTAT CTACGCCGCCGACTTCAAGGGCAGAGTGA CCATGACCACCGACACCTCTACCTCCACCG CCTACATGGAACTGCGGTCCCTGAGATCTG ACGACACCGCCGTGTACTACTGCGTGCGGT TTCAGTACGGCGACTACCGGTACTTTGATG TGTGGGGCCAGGGCACACTGGTCACCGTTT CTTCCGCTTCTACCAAGGGACCCAGCGTGT TCCCTCTGGCTCCTTCCTCTAAATCCACCTC TGGCGGAACCGCTGCTCTGGGCTGTCTGGT CAAGGATTACTTCCCTGAGCCTGTGACCGT GTCCTGGAATTCTGGTGCTCTGACATCCGG CGTGCACACCTTTCCAGCTGTGCTGCAGTC CTCTGGCCTGTACTCTCTGTCCTCTGTCGTG ACCGTGCCTTCTAGCTCTCTGGGCACCCAG ACCTACATCTGCAACGTGAACCACAAGCCT TCCAACACCAAGGTGGACAAGAAGGTGGA

SEQ ID NO	Description	Sequence
		ACCCAAGTCCTGCGACAAGACCCACACCT GTCCTCCATGTCCTGCTCCAGAAGCTGCTG GCGCTCCCTCTGTGTTCCTGTTTCCTCCAAA GCCTAAGGACACCCTGATGATCTCTCGGAC CCCTGAAGTGACCTGCGTGGTGGTGGATGT GTCTCACGAGGACCCAGAAGTGAAGTTCA ATTGGTACGTGGACGGCGTGGAAGTGCAC AACGCCAAGACCAAGCCTAGAGAGGAACA GTACAACTCCACCTACAGAGTGGTGTCCGT GCTGACCGTGCTGCACCAGGATTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTGTCC AACAAGGCACTGCCCGCTCCTATCGAAAA GACCATCTCCAAGGCTAAGGGCCAGCCTC GGGAACCTCAGGTTTACACCCTGCCTCCAT CTCGGGAAGAGATGACCAAGAACCAGGTG TCCCTGACCTGCCTCGTGAAGGGCTTCTAC CCTTCCGATATCGCCGTGGAATGGGAGTCC AATGGCCAGCCTGAGAACAACTACAAGAC AACCCCTCCTGTGCTGGACTCCGACGGCTC ATTCTTCCTGTACTCCAAGCTGACAGTGGA CAAGTCTCGGTGGCAGCAGGGCAACGTGT TCTCCTGTTCTGTGATGCACGAGGCCCTGC ACAACCACTACACACAGAAGTCCCTGTCTC TGTCCCCTGGCAAGTGA
94	VH-CDR1	GYTFTNYGVK
95	VH-CDR2	WINTYTGNPIYAADFK
96	VH-CDR3	FQYGDYRYFDV
97	AGX-A07 L5 Light chain amino acid	EIILTQSPATLSLSPGERATLSCRANSGISFIN WYQQKPGQAPRLLIYGTANLASGIPARFGGS GSGRDFTLTISSLEPEDFAVYYCQQWSSNPLT FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
98	AGX-A07 L5 Light chain nucleic acid	AAGCTTGCCACCATGGAAACCGACACACT GCTGCTGTGGGTGCTGTTGTTGTGGGTGCC AGGATCTACCGGAGAGATCATCCTGACAC AGAGCCCCGCCACATTGTCTCTGAGTCCTG GCGAGAGAGCTACCCTGTCCTGTAGAGCC AACTCCGGCATCTCCTTCATCAACTGGTAT CAGCAGAAGCCCGGCCAGGCTCCTAGACT GCTGATCTATGGCACCGCTAACCTGGCCTC TGGCATCCCTGCTAGATTTGGCGGCTCTGG CTCTGGCAGAGACTTCACCCTGACCATCTC TAGCCTGGAACCTGAGGACTTCGCCGTGTA CTACTGCCAGCAGTGGTCTAGCAACCCTCT GACCTTTGGCGGAGGCACCAAGGTGGAAA TCAAGAGAACCGTGGCCGCTCCTTCCGTGT

SEQ ID NO	Description	Sequence
		TCATCTTCCCACCATCTGACGAGCAGCTGA AGTCTGGCACAGCCTCTGTCGTGTGCCTGC TGAACAACTTCTACCCTCGGGAAGCCAAG GTGCAGTGGAAGGTGGACAATGCCCTGCA GTCCGGCAACTCCCAAGAGTCTGTGACCG AGCAGGACTCCAAGGACTCTACCTACAGC CTGTCCTCCACACTGACCCTGTCTAAGGCC GACTACGAGAAGCACAAGGTGTACGCCTG TGAAGTGACCCACCAGGGACTGTCTAGCC CCGTGACCAAGTCTTTCAACCGGGGCGAGT GCTGA
99	AGX-A07 L5v1 Light chain amino acid	EIVLTQSPATLSLSPGERATLSCRANSGISFIN WYQQKPGQAPRLLIYGTANLASGIPARFSGS GSGRDFTLTISSLEPEDFAVYYCQQWSSNPLT FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
100	AGX-A07 L5v1 Light chain nucleic acid	TCTACAGGCGAGATCGTGCTGACCCAGTCT CCTGCCACATTGTCTCTGAGTCCTGGCGAG AGAGCTACCCTGTCCTGTAGAGCCAACTCC GGCATCTCCTTCATCAACTGGTATCAGCAG AAGCCCGGCCAGGCTCCTAGACTGCTGATC TATGGCACCGCTAACCTGGCCTCTGGCATC CCTGCTAGATTTTCCGGCTCTGGCTCTGGC AGAGACTTCACCCTGACCATCTCTAGCCTG GAACCTGAGGACTTCGCCGTGTACTACTGC CAGCAGTGGTCTAGCAACCCTCTGACCTTT GGCGGAGGCACCAAGGTGGAAATCAAGAG AACCGTGGCCGCTCCTTCCGTGTTCATCTT CCCACCATCTGACGAGCAGCTGAAGTCTG GCACAGCCTCTGTCGTGTGCCTGCTGAACA ACTTCTACCCTCGGGAAGCCAAGGTGCAGT GGAAGGTGGACAATGCCCTGCAGTCCGGC AACTCCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACTCTACCTACAGCCTGTCCTC CACACTGACCCTGTCTAAGGCCGACTACGA GAAGCACAAGGTGTACGCCTGTGAAGTGA CCCACCAGGGACTGTCTAGCCCCGTGACCA AGTCTTTCAACCGGGGCGAGTGCTGA
101	AGX-A07 L5v2 Light chain amino acid	EIVLTQSPATLSLSPGERATLSCRAQSGISFIN WYQQKPGQAPRLLIYGTANLASGIPARFSGS GSGRDFTLTISSLEPEDFAVYYCQQWSSNPLT FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO	Description	Sequence
102	AGX-A07 L5v2 Light chain nucleic acid	TCTACAGGCGAGATCGTGCTGACCCAGTCT CCTGCCACATTGTCTCTGAGTCCTGGCGAG AGAGCTACCCTGTCTTGTAGAGCCCAGTCC GGCATCTCCTTCATCAACTGGTATCAGCAG AAGCCCGGCCAGGCTCCTAGACTGCTGATC TATGGCACCGCTAACCTGGCCTCTGGCATC CCTGCTAGATTTTCCGGCTCTGGCTCTGGC AGAGACTTCACCCTGACCATCTCTAGCCTG GAACCTGAGGACTTCGCCGTGTACTACTGC CAGCAGTGGTCTAGCAACCCTCTGACCTTT GGCGGAGGCACCAAGGTGGAAATCAAGAG AACCGTGGCCGCTCCTTCCGTGTTCATCTT CCCACCATCTGACGAGCAGCTGAAGTCTG GCACAGCCTCTGTCGTGTGCCTGCTGAACA ACTTCTACCCTCGGGAAGCCAAGGTGCAGT GGAAGGTGGACAATGCCCTGCAGTCTGGC AACTCCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACTCTACCTACAGCCTGTCCTC CACACTGACCCTGTCTAAGGCCGACTACGA GAAGCACAAGGTGTACGCCTGTGAAGTGA CCCACCAGGGACTGTCTAGCCCCGTGACCA AGTCTTTCAACCGGGGCGAGTGCTGA
103	AGX-A07 L5v3 Light chain amino acid	EIVLTQSPATLSLSPGERATLSCRANSGISFIN WYQQKPGQAPRLLIYGTANLASGIPARFSGS GSGRDFTLTISSLEPEDFAVYYCQQYSSNPLT FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
104	AGX-A07 L5v3 Light chain nucleic acid	TCTACAGGCGAGATCGTGCTGACCCAGTCT CCTGCCACATTGTCTCTGAGTCCTGGCGAG AGAGCTACCCTGTCCTGTAGAGCCAACTCC GGCATCTCCTTCATCAACTGGTATCAGCAG AAGCCCGGCCAGGCTCCTAGACTGCTGATC TATGGCACCGCTAACCTGGCCTCTGGCATC CCTGCTAGATTTTCCGGCTCTGGCTCTGGC AGAGACTTCACCCTGACCATCTCTAGCCTG GAACCTGAGGACTTCGCCGTGTACTACTGC CAGCAGTACAGCAGCAACCCTCTGACCTTT GGCGGAGGCACCAAGGTGGAAATCAAGAG AACCGTGGCCGCTCCTTCCGTGTTCATCTT CCCACCATCTGACGAGCAGCTGAAGTCTG GCACAGCCTCTGTCGTGTGCCTGCTGAACA ACTTCTACCCTCGGGAAGCCAAGGTGCAGT GGAAGGTGGACAATGCCCTGCAGTCCGGC AACTCCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACTCTACCTACAGCCTGTCCTC CACACTGACCCTGTCTAAGGCCGACTACGA GAAGCACAAGGTGTACGCCTGTGAAGTGA

SEQ ID NO	Description	Sequence
		CCCACCAGGGACTGTCTAGCCCCGTGACCA AGTCTTTCAACCGGGGCGAGTGCTGA
105	AGX-A07 L5v4 Light chain amino acid	EIVLTQSPATLSLSPGERATLSCRAQSGISFIN WYQQKPGQAPRLLIYGTANLASGIPARFSGS GSGRDFTLTISSLEPEDFAVYYCQQYSSNPLT FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
106	AGX-A07 L5v4 Light chain nucleic acid	TCTACAGGCGAGATCGTGCTGACCCAGTCT CCTGCCACATTGTCTCTGAGTCCTGGCGAG AGAGCTACCCTGTCTTGTAGAGCCCAGTCC GGCATCTCCTTCATCAACTGGTATCAGCAG AAGCCCGGCCAGGCTCCTAGACTGCTGATC TATGGCACCGCTAACCTGGCCTCTGGCATC CCTGCTAGATTTTCCGGCTCTGGCTCTGGC AGAGACTTCACCCTGACCATCTCTAGCCTG GAACCTGAGGACTTCGCCGTGTACTACTGC CAGCAGTACAGCAGCAACCCTCTGACCTTT GGCGGAGGCACCAAGGTGGAAATCAAGAG AACCGTGGCCGCTCCTTCCGTGTTCATCTT CCCACCATCTGACGAGCAGCTGAAGTCTG GCACAGCCTCTGTCGTGTGCCTGCTGAACA ACTTCTACCCTCGGGAAGCCAAGGTGCAGT GGAAGGTGGACAATGCCCTGCAGTCTGGC AACTCCCAAGAGTCTGTGACCGAGCAGGA CTCCAAGGACTCTACCTACAGCCTGTCCTC CACACTGACCCTGTCTAAGGCCGACTACGA GAAGCACAAGGTGTACGCCTGTGAAGTGA CCCACCAGGGACTGTCTAGCCCCGTGACCA AGTCTTTCAACCGGGGCGAGTGCTGA
107	VL-CDR1 (variant 1)	RANSGISFIN
108	VL-CDR1 (variant 2)	RAQSGISFIN
109	VL-CDR2	GTANLAS
110	VL-CDR3 (variant 1)	QQWSSNPLT
111	VL-CDR3 (variant 2)	QQYSSNPLT
Humanized AGX-A01 sequences		
112	AGX-A01 H1 Heavy chain amino acid	EVQLVESGGGLVKPGGSLRLSCAASGFTFSS FAMSWVRQAPGKGLEWVSTISSGSIYIYYTD GVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARRGIYYGYDGYAMDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPEAAGAPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPE

SEQ ID NO	Description	Sequence
		VKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
113	AGX-A01 H1 Heavy chain nucleic acid	GAGGTGCAGCTGGTTGAATCTGGCGGAGG ACTTGTGAAGCCTGGCGGCTCTCTGAGACT GTCTTGTGCCGCCTCTGGCTTCACCTTCTCC AGCTTTGCCATGTCCTGGGTCCGACAGGCT CCTGGCAAAGGACTGGAATGGGTGTCCAC CATCTCCTCCGGCTCCATCTACATCTACTA CACCGACGGCGTGAAGGGCAGATTCACCA TCAGCAGAGACAACGCCAAGAACTCCCTG TACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTATTGTGCCAGACG GGGCATCTACTATGGCTACGACGGCTACGC TATGGACTATTGGGGACAGGGCACACTGG TCACCGTGTCCTCTGCTTCTACCAAGGGAC CCAGCGTGTTCCCTCTGGCTCCTTCCTCTA AATCCACCTCTGGCGGAACCGCTGCTCTGG GCTGTCTGGTCAAGGATTACTTCCCTGAGC CTGTGACCGTGTCCTGGAACTCTGGTGCTC TGACATCCGGCGTGCACACCTTTCCAGCTG TGCTGCAGTCCTCTGGCCTGTACTCTCTGT CCTCTGTCGTGACCGTGCCTTCTAGCTCTCT GGGCACCCAGACCTACATCTGCAACGTGA ACCACAAGCCTTCCAACACCAAGGTGGAC AAGAAGGTGGAACCCAAGTCCTGCGACAA GACCCACACCTGTCCTCCATGTCCTGCTCC AGAAGCTGCTGGCGCTCCCTCTGTGTTCCT GTTTCCTCCAAAGCCTAAGGACACCCTGAT GATCTCTCGGACCCCTGAAGTGACCTGCGT GGTGGTGGATGTGTCTCACGAGGACCCAG AAGTGAAGTTCAATTGGTACGTGGACGGC GTGGAAGTGCACAACGCCAAGACCAAGCC TAGAGAGGAACAGTACAACTCCACCTACA GAGTGGTGTCCGTGCTGACCGTGCTGCACC AGGATTGGCTGAACGGCAAAGAGTACAAG TGCAAGGTGTCCAACAAGGCACTGCCCGC TCCTATCGAAAAGACCATCTCCAAGGCTAA GGGCCAGCCTCGGGAACCTCAGGTTTACA CCCTGCCTCCATCTCGGGAAGAGATGACCA AGAACCAGGTGTCCCTGACCTGCCTCGTGA AGGGCTTCTACCCTTCCGATATCGCCGTGG AATGGGAGTCCAATGGCCAGCCTGAGAAC AACTACAAGACAACCCCTCCTGTGCTGGAC TCCGACGGCTCATTCTTCCTGTACTCCAAG CTGACAGTGGACAAGTCTCGGTGGCAGCA

SEQ ID NO	Description	Sequence
		GGGCAACGTGTTCTCCTGTTCTGTGATGCA CGAGGCCCTGCACAACCACTACACACAGA AGTCCCTGTCTCTGTCCCCTGGCAAGTGA
114	AGX-A01 H1v1 Heavy chain amino acid	EVQLVESGGGLVKPGGSLRLSCAASGFTFSS FAMSWVRQAPGKGLEWVSTISSGSIYIYYTD SVKGRFTISRDNAKNSLYLQMNSLRAEDTAV YYCARRGIYYGYEGYAMDYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPEAAGAPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
115	VH- CDR1	GFTFSSFAMS
116	VH-CDR2 (variant 1)	TISSGSIYIYYTDGVKG
117	VH-CDR2 (variant 2)	TISSGSIYIYYTDSVKG
118	VH-CDR3 (variant 1)	RGIYYGYDGYAMDY
119	VH-CDR3 (variant 2)	RGIYYGYEGYAMDY
120	VH-CDR3 (variant 3)	RGIYYGYSGYAMDY
121	VH-CDR3 (variant 4)	RGIYYGYAGYAMDY
122	AGX-A01 L10 Light chain amino acid	AIVLTQSPGTLSLSPGERATLSCRSSQSLVHS NGNTYLHWYMQKPGQAPRVLIYKVSNRFSG IPDRFSGSGSGTDFTLTISRLEPDDFAIYYCSQ STHIPLAFGQGTKLEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
123	AGX-A01 L10 Light chain nucleic acid	GCCATCGTGTTGACCCAGTCTCCAGGCACA TTGTCTCTGAGCCCTGGCGAGAGAGCTACC CTGTCCTGCAGATCTTCTCAGTCCCTGGTG CACTCCAACGGCAACACCTACCTGCACTGG TACATGCAGAAGCCCGGACAGGCTCCCAG AGTGCTGATCTACAAGGTGTCCAACCGGTT CTCTGGCATCCCCGACAGATTTTCCGGCTC TGGCTCTGGCACCGACTTCACCCTGACCAT CTCTAGACTGGAACCCGACGACTTCGCCAT CTACTACTGCTCCCAGTCCACACACATCCC TCTGGCTTTTGGCCAGGGCACCAAGCTGGA AATCAAGAGAACCGTGGCCGCTCCTTCCGT GTTCATCTTCCCACCATCTGACGAGCAGCT

SEQ ID NO	Description	Sequence
		GAAGTCCGGCACAGCTTCTGTCGTGTGCCT GCTGAACAACTTCTACCCTCGGGAAGCCA AGGTGCAGTGGAAGGTGGACAATGCCCTG CAGTCCGGCAACTCCCAAGAGTCTGTGACC GAGCAGGACTCCAAGGACTCTACCTACAG CCTGTCCTCCACACTGACCCTGTCTAAGGC CGACTACGAGAAGCACAAGGTGTACGCCT GTGAAGTGACCCACCAGGGCCTGTCTAGC CCTGTGACCAAGTCTTTCAACCGGGGCGAG TGTTGA
124	VL- CDR1 (variant 1)	RSSQSLVHSNGNTYLH
125	VL-CDR1 (variant 2)	RSSQSLVHSSGNTYLH
126	VL-CDR1 (variant 3)	RSSQSLVHSTGNTYLH
127	VL-CDR1 (variant 4)	RSSQSLVHSQGNTYLH
128	VL-CDR2	KVSNRFS
129	VL-CDR3	SQSTHIPLA
Humanized AGX-A07 H2v1L5v2		
130	AGX-A07 H2v1 Heavy chain variable region amino acid	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SS
131	AGX-A07 H2v1L5v2 Light chain variable region amino acid	EIVLTQSPATLSLSPGERATLSCRAQSGISFIN WYQQKPGQAPRLLIYGTANLASGIPARFSGS GSGRDFTLTISSLEPEDFAVYYCQQWSSNPLT FGGGTKVEIK
Humanized AGX-A07 H2L5		
132	AGX-A07 H2 Heavy chain variable region amino acid	QVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQDLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAFMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SS
133	AGX-A07 L5 Light chain variable region amino acid	EIILTQSPATLSLSPGERATLSCRANSGISFIN WYQQKPGQAPRLLIYGTANLASGIPARFGGS GSGRDFTLTISSLEPEDFAVYYCQQWSSNPLT FGGGTKVEIK
Fc Region Sequences		
135	IgG1 L234A/L235A/G237A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPE*AA*G*A*PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRV

SEQ ID NO	Description	Sequence
		VSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
136	IgG1 L234A/L235A/G237A+N297C	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPE***AA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQY***C***STYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
137	IgG1 L234A/L235A/G237A + P331G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPE***AA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPA***G***I EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
138	IgG1 L234A/L235A/G237A + N297C/P331G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPE***AA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQY***C***STYRV VSVLTVLHQDWLNGKEYKCKVSNKALPA***G***I EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
139	IgG1 L234A/L235A/G237A + K322A/P331G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPE***AA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKC***A***VSNKALPA***G***I EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT

SEQ ID NO	Description	Sequence
		PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
140	IgG1 L234A/L235A/G237A + E233P/P331G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAP***PAA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPA***G***I EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
141	IgG1 L234A/L235A/G237A + E233P/N297C	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAP***EAA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQY***C***STYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
142	IgG1 L234A/L235A/G237A + N297C/K322A/P331G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAP***EAA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQY***C***STYRV VSVLTVLHQDWLNGKEYKC***A***VSNKALPA***G***I EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
143	IgG1 L234A/L235A/G237A + E233P/N297C/P331G	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAP***EAA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQY***C***STYRV VSVLTVLHQDWLNGKEYKCKVSNKALPA***G***I EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
144	IgG1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL

SEQ ID NO	Description	Sequence
	L234A/L235A/G237A + E233P/D265A/N297C/K322A/P331G	YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAP***EAA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVAVSHEDPEV KFNWYVDGVEVHNAKTKPREEQY***C***STYRV VSVLTVLHQDWLNGKEYKC***A***VSNKALPA***G***I EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
145	IgG1 L234A/L235A/G237A + E233P/D265A/N297C/K322A/P331G-PGKKP	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAP***EAA***G***A***PSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQY***C***STYRV VSVLTVLHQDWLNGKEYKC***A***VSNKALPA***G***I EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGKKP
146	IgG4 S228P (sequence includes AGX-A07 H2v1 heavy chain variable region amino acid)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCP***P***CPAPEFLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK
147	IgG4 S228P/L235E (sequence includes AGX-A07 H2v1 heavy chain variable region amino acid)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCP***P***CPAPEF***E***GGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPP

SEQ ID NO	Description	Sequence
		VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK
148	IgG4 S228P/L235E/N297C (sequence includes AGX-A07 H2v1 heavy chain variable region amino acid)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCP***P***CPAPEF***E***GGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQF***C***STYRVVSV LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGK
149	IgG4 S228P/F234A/L235E/N297C (sequence includes AGX-A07 H2v1 heavy chain variable region amino acid)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCP***P***CPAPE***AE***GGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKTKPREEQF***C***STYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK
150	IgG4 S228P/L235E/N297C-LGKKP (sequence includes AGX-A07 H2v1 heavy chain variable region amino acid)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCP***P***CPAPEF***E***GGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQF***C***STYRVVSV LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGKKP

SEQ ID NO	Description	Sequence
151	IgG1 M252Y/S254T/T256E	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTL***Y***I***T***R***E***PEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
152	IgG1 T252Q/M428L	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKD***Q***LMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS V***L***HEALHNHYTQKSLSLSPGK
153	IgG1 M428L/N434S	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS V***L***HEALH***S***HYTQKSLSLSPGK

SEQ ID NO	Description	Sequence
154	IgG4 T250Q/M428L (sequence includes AGX-A07 H2v1 heavy chain variable region amino acid)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCPSCPAPEFLGGPSVFLFP PKPKD***Q***LMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV ***L***HEALHNHYTQKSLSLSLGK
155	IgG4 M428L/N434S (sequence includes AGX-A07 H2v1 heavy chain region amino acid)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCPSCPAPEFLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV ***L***HEALH***S***HYTQKSLSLSLGK
156	IgG1 M252Y/S254T/T256E	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGVKWVRQAPGQGLEWMGWINTYTGNPI YAADFKGRVTMTTDTSTSTAYMELRSLRSD

SEQ ID NO	Description	Sequence
	(sequence includes AGXA07 H2v1 heavy chain variable region amino acid)	DTAVYYCVRFQYGDYRYFDVWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLYITREPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK

[0380] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat.

2. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises said mutation at position N297.

3. The antibody-drug conjugate of claim 2, wherein said mutation at position N297 comprises N297C.

4. The antibody-drug conjugate of any one of claims 1-3, wherein said IgG Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

5. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

6. The antibody-drug conjugate of claim 5, wherein said IgG Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, T356, M428, and N434; as numbered by the EU index as set forth in Kabat.

7. The antibody-drug conjugate of claim 6, wherein said IgG Fc region comprises said mutation at position N297.

8. The antibody-drug conjugate of claim 7, wherein said mutation at position N297 comprises N297C.

9. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising a cysteine residue at position N297, as numbered by the EU index as set forth in Kabat.

10. The antibody-drug conjugate of claim 9, wherein said IgG Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat.

11. The antibody-drug conjugate of claim 9 or 10, wherein said IgG Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

12. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising a human IgG1 Fc region comprising a cysteine residue at position N297 and a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat.

13. The antibody-drug conjugate of claim 12, wherein said IgG Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

14. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising a cysteine residue at position N297, as numbered by the EU index as set forth in Kabat, wherein said antibody-drug conjugate comprises a drug to antibody ratio (DAR) of greater than or equal to about 1.

15. The antibody-drug conjugate of claim 14, wherein said IgG Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat.

16. The antibody-drug conjugate of claim 14 or 15, wherein said IgG Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

17. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1

antibody or an antigen binding fragment thereof comprises an IgG Fc region comprising a cysteine residue at position N297 and an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, wherein numbering is according to the EU index as set forth in Kabat.

18. The antibody-drug conjugate of claim 17, wherein said IgG Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434; as numbered by the EU index as set forth in Kabat.

19. The antibody-drug conjugate of any one of claims 4, 5-8, 11, 13, and 16-18, wherein said one or more amino acid residues after position K447 are independently selected from the group consisting of: a lysine, a proline, an arginine, or any combinations thereof.

20. The antibody-drug conjugate of claim 19, wherein said one or more amino acid residues after position K447 are independently selected from the group consisting of: said lysine and said proline.

21. The antibody-drug conjugate of any one of claims 1-20, wherein said IgG Fc region comprises said mutation at position E233.

22. The antibody-drug conjugate of claim 21, wherein said mutation at position E233 comprises E233P.

23. The antibody-drug conjugate of any one of claims 1-22, wherein said IgG Fc region comprises said mutation at position L234.

24. The antibody-drug conjugate of claim 23, wherein said mutation at position L234 comprises L234A.

25. The antibody-drug conjugate of any one of claims 1-24, wherein said IgG Fc region comprises said mutation at position L235.

26. The antibody-drug conjugate of any one of claims 1-25, wherein said mutation at position L235 comprises L235A.

27. The antibody-drug conjugate of any one of claims 1-26, wherein said IgG Fc region comprises said mutation at position G237.

28. The antibody-drug conjugate of claim 27, wherein said mutation at position G237 comprises G237A.

29. The antibody-drug conjugate of any one of claims 1-28, wherein said IgG Fc region comprises said mutation at position M252.

30. The antibody-drug conjugate of claim 29, wherein said mutation at position M252 comprises M252Y.

31. The antibody-drug conjugate of any one of claims 1-30, wherein said IgG Fc region comprises said mutation at position S254.

32. The antibody-drug conjugate of claim 31, wherein said mutation at position S254 comprises S254T.

33. The antibody-drug conjugate of any one of claims 1-32, wherein said IgG Fc region comprises said mutation at position T256.

34. The antibody-drug conjugate of claim 33, wherein said mutation at position T256 comprises T256E.

35. The antibody-drug conjugate of any one of claims 1-34, wherein said IgG Fc region comprises said mutation at position M428.

36. The antibody-drug conjugate of claim 35, wherein said mutation at position M428 comprises M428L.

37. The antibody-drug conjugate of any one of claims 1-36, wherein said IgG Fc region comprises said mutation at position N434.

38. The antibody-drug conjugate of claim 37, wherein said mutation at position N434 comprises N434S or N434A.

39. The antibody-drug conjugate of any one of claims 1-38, wherein said IgG Fc region comprises said mutation at position T250.

40. The antibody-drug conjugate of claim 39, wherein said mutation at position T250 comprises T250Q.

41. The antibody-drug conjugate of any one of claims 1-40, wherein said IgG Fc region comprises said mutation at position D265.

42. The antibody-drug conjugate of claim 41, wherein said mutation at position D265 comprises D265A.

43. The antibody-drug conjugate of any one of claims 1-42, wherein said IgG Fc region comprises said mutation at position K322.

44. The antibody-drug conjugate of claim 43, wherein said mutation at position K322 comprises K322A.

45. The antibody-drug conjugate of any one of claims 1-44, wherein said IgG Fc region comprises said mutation at position P331.

46. The antibody-drug conjugate of claim 45, wherein said mutation at position P331 comprises P331G.

47. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises T250Q and M428L.

48. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises M428L.

49. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises M428L and N434S.

50. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises N434A.

51. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises L234A, L235A, and G237A.

52. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises L234A, L235A, G237A, and P331G.

53. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises L234A, L235A, G237A, N297C, and P331G.

54. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises L234A, L235A, G237A, K322A, and P331G.

55. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises E233P, L234A, L235A, G237A, and P331G.

56. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises E233P, L234A, L235A, G237A, and N297C.

57. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises E233P, L234A, L235A, G237A, and N297C.

58. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises L234A, L235A, G237A, N297C, K322A, and P331G.

59. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises E233P, L234A, L235A, G237A, D265A, N297C, K322A, and P331G.

60. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises E233P, L234A, L235A, G237A, D265A, N297C, K322A, and P331G.

61. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises E233P and D265A.

62. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises M252Y, S254T, and T256E.

63. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises M252Y, S254T, T256E, and N297C.

64. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises K322A and P331G, and wherein said IgG Fc region further comprises an extended C-terminus

that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447.

65. The antibody-drug conjugate of claim 1, wherein said IgG Fc region comprises an amino acid sequence selected from the group consisting of SEQ ID Nos. 87-88, 135-145, and 151-153.

66. The antibody-drug conjugate of any one of claims 1-65, wherein said IgG Fc region exhibits reduced or ablated binding with C1q.

67. The antibody-drug conjugate of any one of claims 1-66, wherein said IgG Fc region exhibits reduced or ablated binding to an Fc receptor.

68. The antibody-drug conjugate of any one of claims 1-67, wherein said anti-TM4SF1 antibody exhibits reduced or ablated ADCC or CDC effector function.

69. The antibody-drug conjugate of any one of claims 1-68, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises:

(a) a heavy chain comprising a CDR3 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 8, 20, 32, 44, 56, 68, 80, 96, 118, 119, 120, or 121; a CDR2 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 7, 19, 31, 43, 55, 67, 79, 95, 116, or 117; and a CDR1 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 6, 18, 30, 42, 54, 66, 78, 94, or 115; and

(b) a light chain comprising a CDR3 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 14, 26, 38, 50, 62, 74, 86, 110, or 129; a CDR2 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 13, 25, 37, 49, 61, 73, 85, 109, or 128; and a CDR1 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 12, 24, 36, 48, 60, 72, or 84, 107, 108, 124, 125, 126, or 127.

70. The antibody-drug conjugate of claim 69, wherein said heavy chain comprises an amino acid sequence that has at least 75% identity to SEQ ID NO: 3, 15, 27, 39, 51, 63, 75, 90, 92, 112, 114, 130, or 132, and a light chain comprises an amino acid sequence that has at least 75% identity to SEQ ID NO: 9, 21, 33, 45, 57, 69, 81, 97, 99, 101, 122, 131, or 133.

71. The antibody-drug conjugate of claim 70, wherein said heavy chain comprises a sequence as set forth in SEQ ID NO: 3, 15, 27, 39, 51, 63, 75, 90, 92, 112, 114, 130, or 132, and wherein said light chain variable domain comprises a sequence as set forth in SEQ ID NO: 9, 21, 33, 45, 57, 69, 81, 97, 99, 101, 122, 131, or 133.

72. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 6; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 14, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 13, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 12.

73. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 20, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 19, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 18; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 26, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 25, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 24.

74. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 32, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 31, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 30; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 38, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 37, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 36.

75. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 44, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 43, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 42; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 50, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 49, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 48.

76. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 56, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 55, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 54; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 62, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 61, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 60.

77. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 68, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 67, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 66; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 74, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 73, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 72.

78. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 80, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 79, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 78; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 86, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 85, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 84.

79. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 111 or SEQ ID NO: 110, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 107 or SEQ ID NO: 108.

80. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 110, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 107.

81. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ

ID NO: 110, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 108.

82. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 111, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 107.

83. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 111, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 108.

84. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 118, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 116, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 115; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 129, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 128, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 124.

85. The antibody-drug conjugate of any one of claims 69-71, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 118, SEQ ID NO: 119, SEQ IN NO: 120, or SEQ ID NO: 121, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 116 or SEQ ID NO: 117, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 115; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 129, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 128, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, or SEQ ID NO: 127.

86. The antibody-drug conjugate of any one of claims 0-85, wherein said antigen-binding fragment comprises an Fab, an Fab', an $F(ab')_2$, an Fv, or an scFv.

87. The antibody-drug conjugate of any one of claims 1-85, wherein said therapeutic molecule comprises at least one of: a small molecule, a degrader, a nucleic acid molecule, a CRISPR-Cas9 gene editing system, and a lipid nanoparticle, or any combinations thereof.

88. The antibody-drug conjugate of any one of claims 1-86, wherein said therapeutic molecule comprises at least one of: a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder, a DHFR inhibitor, a nucleic acid, a CRISPR enzyme, or any combinations thereof.

89. The antibody-drug conjugate of claim 87, wherein said degrader comprises an agent that induces protein degradation.

90. The antibody-drug conjugate of claim 89, wherein said agent that induces protein degradation comprises a hydrophobic tag, a proteolysis inducing chimera, an HSP90 inhibitor, a selective estrogen receptor degrader (SERD), and a selective androgen receptor degrader (SARD), or any combinations thereof.

91. The antibody-drug conjugate of claim 87, wherein said lipid nanoparticle encapsulates one or more therapeutic molecules.

92. The antibody-drug conjugate of claim 87, wherein said nucleic acid molecule comprises an RNA molecule or a DNA molecule.

93. The antibody-drug conjugate of claim 92, wherein said RNA molecule comprises an siRNA, an antisense-RNA, an miRNA, an antisense miRNA, an antagomir (anti-miRNA), an shRNA, or an mRNA.

94. The antibody-drug conjugate of any one of claims 87-93, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof and said therapeutic molecule are conjugated by a linker in a single or a multistep protocol.

95. The antibody-drug conjugate of claim 94, wherein said linker comprises a cleavable linker, a non-cleavable linker, a hydrophilic linker, a pro-charged linker, or a dicarboxylic acid based linker.

96. The antibody-drug conjugate of claim 95, wherein said cleavable linker comprises a cleavable covalent or non-covalent linker.

97. The antibody-drug conjugate of claim 94, wherein said linker comprises a non-cleavable covalent or non-covalent linker.

98. The antibody-drug conjugate of claim 95 or 96, wherein said cleavable linker comprises an acid-labile linker, a protease-sensitive linker, a photo-labile linker, or a disulfide-containing linker.

99. The antibody-drug conjugate of claim 94, wherein said linker comprises a cysteine linker or a non-cysteine linker.

100. The antibody-drug conjugate of claim 99, wherein said non-cysteine linker comprises a lysine linker.

101. The antibody-drug conjugate of claim 94, wherein said linker comprises a MC (6-maleimidocaproyl), a MCC (a maleimidomethyl cyclohexane-1-carboxylate), a MP (maleimidopropanoyl), a val-cit (valine-citrulline), a val-ala (valine-alanine), an ala-phe (alanine-phenylalanine), a PAB (p-aminobenzyloxycarbonyl), a SPP (N-Succinimidyl 4-(2-pyridylthio) pentanoate), 2,5-dioxopyrrolidin-1-yl 4-(pyridin-2-ylthio)hexanoate, 2,5-dioxopyrrolidin-1-yl 5-methyl-4-(pyridin-2-ylthio)hexanoate, 2,5-dioxopyrrolidin-1-yl 5-methyl-4-(pyridin-2-ylthio)heptanoate, 2,5-dioxopyrrolidin-1-yl 5-ethyl-4-(pyridin-2-ylthio)heptanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclopentyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclohexyl-4-(pyridin-2-ylthio)butanoate, a SMCC (N-Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 carboxylate), or a SIAB (N-Succinimidyl (4-iodo-acetyl)aminobenzoate).

102. The antibody-drug conjugate of claim 94, wherein said linker is derived from a cross-linking reagent, wherein the cross-linking reagent comprises N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), 2,5-dioxopyrrolidin-1-yl 3-cyclopropyl-3-(pyridin-2-yldisulfaneyl)propanoate, 2,5-dioxopyrrolidin-1-yl 3-cyclobutyl-3-(pyridin-2-yldisulfaneyl)propanoate, N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-yldisulfaneyl)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-yldisulfaneyl)butanoate, N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-yldisulfaneyl)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-yldisulfaneyl)butanoate, N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC), or 2,5-

dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazaheptadecan-1-oate (CX1-1).

103. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297, as numbered by the EU index as set forth in Kabat.

104. The antibody-drug conjugate of claim 103, wherein said human IgG4 Fc region comprises said mutation at position N297.

105. The antibody-drug conjugate of claim 104, wherein said mutation at position N297 comprises N297C.

106. The antibody-drug conjugate of any one of claims 103-105, wherein said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

107. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

108. The antibody-drug conjugate of claim 107, wherein said human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297, as numbered by the EU index as set forth in Kabat.

109. The antibody-drug conjugate of any one of claims 107 or 108, wherein said human IgG4 Fc region comprises said mutation at position N297.

110. The antibody-drug conjugate of claim 109, wherein said mutation at position N297 comprises N297C.

111. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1

antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a cysteine residue at position N297, as numbered by the EU index as set forth in Kabat.

112. The antibody-drug conjugate of claim 111, wherein said human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, and H435, , as numbered by the EU index as set forth in Kabat.

113. The antibody-drug conjugate of claim 111 or 112, wherein said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

114. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a cysteine residue at position N297 and a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, and H435, , as numbered by the EU index as set forth in Kabat.

115. The antibody-drug conjugate of claim 114, wherein said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

116. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a cysteine residue at position N297, as numbered by the EU index as set forth in Kabat, wherein said antibody-drug conjugate comprises a drug to antibody ratio (DAR) of greater than or equal to 1.

117. The antibody-drug conjugate of claim 116, wherein said human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, and H435, as numbered by the EU index as set forth in Kabat.

118. The antibody-drug conjugate of claim 116 or 117, wherein said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

119. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises a human IgG4 Fc region comprising a cysteine residue at position N297 and an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, wherein numbering is according to the EU index as set forth in Kabat.

120. The antibody-drug conjugate of claim 119, wherein said human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, and H435, as numbered by the EU index as set forth in Kabat.

121. The antibody-drug conjugate of any one of claims 106-110, 113, 115, 118-120, wherein said one or more amino acid residues after position K447 is independently selected from the group consisting of: a lysine, a proline, an arginine, or any combinations thereof.

122. The antibody-drug conjugate of claim 121, wherein said one or more amino acid residues after position K447 is independently selected from the group consisting of: said lysine and said proline.

123. The antibody-drug conjugate of any one of claims 103-106, 108-110, 112-115, 117-118, and 120-122, wherein said human IgG4 Fc region comprises said mutation at position S228.

124. The antibody-drug conjugate of claim 123, wherein said mutation at position S228 comprises S228P.

125. The antibody-drug conjugate of any one of claims 92-95, 97-99, 101-104, 106-107, and 109-113, wherein said human IgG4 Fc region comprises said mutation at position F234.

126. The antibody-drug conjugate of claim 114, wherein said mutation at position F234 comprises F234A.

127. The antibody-drug conjugate of any one of claims 103-106, 108-110, 112-115, 117-118, and 120-126, wherein said human IgG4 Fc region comprises said mutation at position L235.

128. The antibody-drug conjugate of claim 127, wherein said mutation at position L235 comprises L235E.

129. The antibody-drug conjugate of claim 128, wherein said human IgG4 Fc region comprises S228P and L235E.

130. The antibody-drug conjugate of claim 129, wherein said human IgG4 Fc region comprises S228P, L235E, and N297C.

131. The antibody-drug conjugate of claim 130, wherein said human IgG4 Fc region comprises S228P, F234A, L235E, and N297C.

132. The antibody-drug conjugate of claim 130, wherein said human IgG4 Fc region comprises S228P, L235E, and N297C, and wherein said human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447.

133. The antibody-drug conjugate of claim 103, wherein said human IgG4 Fc region comprises M428L and N434S.

134. The antibody-drug conjugate of claim 103, wherein said human IgG4 Fc region comprises mutations at L235 and F234.

135. The antibody-drug conjugate of claim 103, wherein said human IgG4 Fc region comprises mutations at positions L328, A330, and T299.

136. The antibody-drug conjugate of claim 103, wherein said human IgG4 Fc region comprises S228P, F234A, L235A, G237A, and P238S.

137. The antibody-drug conjugate of claim 103, wherein said human IgG4 Fc region comprises F243A and V264A.

138. The antibody-drug conjugate of claim 103, wherein said human IgG4 Fc region comprises S228P and L235A.

139. The antibody-drug conjugate of claim 103, wherein said human IgG4 Fc region comprises M252Y and M428L; D259I and V308F; or N434S.

140. The antibody-drug conjugate of claim 103, wherein said human IgG4 Fc region comprises T307Q and N434S; M428L and V308F; Q311V and N434S; H433K and N434F; E258F and V427T; or T256D, Q311V, and A378V.

141. The antibody-drug conjugate of any one of claims 103-140, wherein said human IgG4 Fc region comprises one or more of the following properties: (i) reduced or ablated binding with C1q; (ii) reduced or ablated binding to an Fc receptor; and (iii) reduced or ablated ADCC or CDC effector function.

142. The antibody-drug conjugate of any one of claims 103-141, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprises:

(a) a heavy chain comprising a CDR3 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ

ID NO: 8, 20, 32, 44, 56, 68, 80, 96, 118, 119, 120, or 121; a CDR2 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 7, 19, 31, 43, 55, 67, 79, 95, 116, or 117; and a CDR1 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 6, 18, 30, 42, 54, 66, 78, 94, or 115; and

(b) a light chain comprising a CDR3 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 14, 26, 38, 50, 62, 74, 86, 110, or 129; a CDR2 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 13, 25, 37, 49, 61, 73, 85, 109, or 128; and a CDR1 domain comprising an amino acid sequence that has at least 75% identity to a sequence selected from the group consisting of SEQ ID NO: 12, 24, 36, 48, 60, 72, or 84, 107, 108, 124, 125, 126, or 127.

143. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 6; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 14, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 13, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 12.

144. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 20, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 19, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 18; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 26, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 25, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 24.

145. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 32, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 31, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 30; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 38, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 37, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 36.

146. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 44, a CDR2

domain comprising the amino acid sequence as set forth in SEQ ID NO: 43, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 42; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 50, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 49, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 48.

147. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 56, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 55, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 54; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 62, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 61, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 60.

148. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 68, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 67, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 66; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 74, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 73, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 72.

149. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 80, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 79, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 78; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 86, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 85, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 84.

150. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 110, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 107.

151. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 96, a CDR2

domain comprising the amino acid sequence as set forth in SEQ ID NO: 95, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 94; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 110, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 109, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 108.

152. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 118, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 116, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 115; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 129, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 128, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 124.

153. The antibody-drug conjugate of claim 142, wherein said heavy chain comprises a CDR3 domain comprising the amino acid sequence se set forth in SEQ ID NO: 118, SEQ ID NO: 119, SEQ IN NO: 120, or SEQ ID NO: 121, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 116 or SEQ ID NO: 117, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 115; and wherein said light chain comprises a CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 129, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO: 128, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, or SEQ ID NO: 127.

154. The antibody-drug conjugate of any one of claims 142-153, wherein said heavy chain comprises an amino acid sequence that has at least 75% identity to SEQ ID NO: 3, 15, 27, 39, 51, 63, 75, 90, 92, 112, or 114, and a light chain comprising an amino acid sequence that has at least 75% identity to SEQ ID NO: 9, 21, 33, 45, 57, 69, 81, 97, 99, 101, or 122.

155. The antibody-drug conjugate of claim 154, wherein said heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 3, 15, 27, 39, 51, 63, 75, 90, 92, 112, 114, 130, or 132, and wherein said light chain comprises an amino acid sequence as set forth in SEQ ID NO: 9, 21, 33, 45, 57, 69, 81, 97, 99, 101, 122, 131, or 133.

156. The antibody-drug conjugate of any one of claims 103-155, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof comprising said human IgG4 Fc region comprises an amino acid sequence selected from the group consisting of SEQ ID Nos. 146-150, and 154-155.

157. The antibody-drug conjugate of any one of claims 103-156, wherein said antigen-binding fragment comprises an Fab, an Fab', an $F(ab')_2$, an Fv, or an scFv.

158. The antibody-drug conjugate of any one of claims 103-157, wherein said therapeutic molecule comprises at least one of: a small molecule, a degrader, a nucleic acid molecule, a CRISPR-Cas9 gene editing system, and a lipid nanoparticle, or any combinations thereof.

159. The antibody-drug conjugate of any one of claims 103-158, wherein said therapeutic molecule comprises at least one of: a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder, a DHFR inhibitor, a nucleic acid, a CRISPR enzyme, or any combinations thereof.

160. The antibody-drug conjugate of claim 158, wherein said degrader comprises an agent that induces protein degradation.

161. The antibody-drug conjugate of claim 160, wherein said agent that induces protein degradation comprises a hydrophobic tag, a proteolysis inducing chimera, an HSP90 inhibitor, a selective estrogen receptor degrader (SERD), a selective androgen receptor degrader (SARD), or any combinations thereof.

162. The antibody-drug conjugate of claim 158, wherein said lipid nanoparticle encapsulates one or more therapeutic molecules.

163. The antibody-drug conjugate of claim 158, wherein said nucleic acid molecule comprises an RNA molecule or a DNA molecule.

164. The antibody-drug conjugate of claim 163, wherein said RNA molecule comprises an siRNA, an antisense-RNA, an miRNA, an antisense miRNA, an antagomir (anti-miRNA), an shRNA, or an mRNA.

165. The antibody-drug conjugate of any one of claims 158-164, wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof and said therapeutic molecule are conjugated by a linker in a single or multistep protocol.

166. The antibody-drug conjugate of claim 165, wherein said linker comprises a cleavable linker, a non-cleavable linker, a hydrophilic linker, a pro-charged linker, or a dicarboxylic acid based linker.

167. The antibody-drug conjugate of claim 166, wherein said cleavable linker comprises a cleavable covalent or non-covalent linker.

168. The antibody-drug conjugate of claim 165, wherein said linker comprises a non-cleavable covalent or non-covalent linker.

169. The antibody-drug conjugate of claim 166 or 167, wherein said cleavable linker comprises an acid-labile linker, a protease-sensitive linker, a photo-labile linker, or a disulfide-containing linker.

170. The antibody-drug conjugate of claim 165, wherein said linker comprises a cysteine linker or a non-cysteine linker.

171. The antibody-drug conjugate of claim 170, wherein said non-cysteine linker comprises a lysine linker.

172. The antibody-drug conjugate of claim 165, wherein said linker comprises a MC (6-maleimidocaproyl), a MCC (a maleimidomethyl cyclohexane-1-carboxylate), a MP (maleimidopropanoyl), a val-cit (valine-citrulline), a val-ala (valine-alanine), an ala-phe (alanine-phenylalanine), a PAB (p-aminobenzyloxycarbonyl), a SPP (N-Succinimidyl 4-(2-pyridylthio) pentanoate), 2,5-dioxopyrrolidin-1-yl 4-(pyridin-2-ylthio)hexanoate, 2,5-dioxopyrrolidin-1-yl 5-methyl-4-(pyridin-2-ylthio)hexanoate, 2,5-dioxopyrrolidin-1-yl 5-methyl-4-(pyridin-2-ylthio)heptanoate, 2,5-dioxopyrrolidin-1-yl 5-ethyl-4-(pyridin-2-ylthio)heptanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclopentyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclohexyl-4-(pyridin-2-ylthio)butanoate, a SMCC (N-Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 carboxylate), or a SIAB (N-Succinimidyl (4-iodo-acetyl)aminobenzoate).

173. The antibody-drug conjugate of claim 165, wherein said linker is derived from a cross-linking reagent, wherein the cross-linking reagent comprises N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), 2,5-dioxopyrrolidin-1-yl 3-cyclopropyl-3-(pyridin-2-yldisulfaneyl)propanoate, 2,5-dioxopyrrolidin-1-yl 3-cyclobutyl-3-(pyridin-2-yldisulfaneyl)propanoate, N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-yldisulfaneyl)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-yldisulfaneyl)butanoate, N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-yldisulfaneyl)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-yldisulfaneyl)butanoate, N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC), or 2,5-

dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazaheptadecan-1-oate (CX1-1).

174. A method of treating or preventing a disease or disorder in a subject, wherein the disease or disorder is characterized by abnormal endothelial cell (EC)- cell interaction, said method comprising administering to the subject an antibody-drug conjugate according to any one of claims 1-102.

175. A method of treating or preventing a disease or disorder in a subject, wherein the disease or disorder is characterized by abnormal endothelial cell (EC)- cell interaction, said method comprising administering to the subject an antibody-drug conjugate according to any one of claims 103-173.

176. The method of claim 174 or 175, wherein the EC-cell interaction comprises one or more of EC-mesenchymal stem cell, EC-fibroblast, EC-smooth muscle cell, EC-tumor cell, EC-leukocyte, EC-adipose cell, and EC-neuronal cell interactions.

177. The method of any one of claims 174-176, wherein the disease or disorder comprises an inflammatory disease or a cancer.

178. A method of treating or preventing inflammation in a subject, said method comprising administering to the subject an antibody-drug conjugate according to any one of claims 1-102.

179. A method of treating or preventing inflammation in a subject, said method comprising administering to the subject an antibody-drug conjugate according to any one of claims 103-173.

180. A method of treating or preventing metastasis in a subject, said method comprising administering to the subject an antibody-drug conjugate according to any one of claims 1-102, wherein the subject is in partial or complete remission from a cancer.

181. A method of treating or preventing metastasis in a subject, said method comprising administering to the subject an antibody-drug conjugate according to any one of claims 103-173, wherein the subject is in partial or complete remission from a cancer.

182. A method of treating a subject having a cancer which is associated with a high risk of metastasis, said method comprising administering an antibody-drug conjugate according to any one of claims 1-102, to the subject having the cancer which is associated with the high risk of metastasis.

183. A method of treating a subject having a cancer which is associated with a high risk of metastasis, said method comprising administering an antibody-drug conjugate according to any one of claims 103-173, to the subject having the cancer which is associated with the high risk of metastasis.

184. A method of treating or preventing metastasis in a subject having a cancer, said method comprising administering an antibody-drug conjugate according to any one of claims 1-102, to the subject having the cancer.

185. A method of treating or preventing metastasis in a subject having a cancer, said method comprising administering an antibody-drug conjugate according to any one of claims 103-173, to the subject having the cancer.

186. The method of any one of claims 180-185, wherein the subject is undergoing a treatment which may induce metastasis.

187. The method of claim 186, wherein the treatment comprises surgery, radiation treatment and chemotherapy.

188. The method of any one of claims 180-187, wherein the subject is a human.

189. The method of any one of claims 177 and 180-188, wherein the cancer is a carcinoma or a sarcoma.

190. The method of claim 189, wherein the carcinoma comprises breast cancer, lung cancer, colon cancer, prostate cancer, pancreatic cancer, liver cancer, gastric cancer, renal cancer, bladder cancer, uterine cancer, cervical cancer, ovarian cancer.

191. The method of claim 189, wherein the sarcoma comprises an angiosarcoma, an osteosarcoma, or a soft tissue sarcoma.

192. The method of claim any one of claims 177 and 180-188, wherein the cancer is a glioblastoma.

193. A method of treating or preventing lymphatic or hematogenous metastasis in a human subject comprising administering to the human subject an antibody-drug conjugate according to any one of claims 1-102.

194. A method of treating or preventing lymphatic or hematogenous metastasis in a human subject comprising administering to the human subject an antibody-drug conjugate according to any one of claims 103-173.

195. A pharmaceutical composition comprising (i) an antibody-drug conjugate according to any one of claims 1-173 and (ii) a pharmaceutically acceptable carrier.

196. An anti-TM4SF1 binding protein comprising a modified human IgG1 Fc region, wherein said modified human IgG1 Fc region comprises one or more amino acid substitutions selected from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434, as numbered by the EU index as set forth in Kabat, wherein said anti-TM4SF1 binding protein demonstrates improved vascular safety compared to an otherwise identical binding protein that does not comprise an amino acid substitution selected

from the group consisting of E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434.

197. An anti-TM4SF1 binding protein comprising a modified human IgG4 Fc region, wherein said modified human IgG4 Fc region comprises one or more amino acid substitutions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297, as numbered by the EU index as set forth in Kabat, wherein said anti-TM4SF1 binding protein demonstrates improved vascular safety compared to an otherwise identical binding protein that does not comprise an amino acid substitutions selected from the group consisting of S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297.

198. The anti-TM4SF1 binding protein of claim 196, wherein the modified human IgG1 Fc region comprises a mutation at one or more positions selected from the group consisting of T250, M252, S254, T256, M428, and N434 as numbered by the EU index as set forth in Kabat.

199. The anti-TM4SF1 binding protein of claim 198, wherein the modified human IgG1 Fc region comprises a mutation selected from the group consisting of T250Q, M252Y, S254T, T256E, M428L, and N434S, as numbered by the EU index as set forth in Kabat.

200. The anti-TM4SF1 binding protein of claim 198, wherein the modified human IgG1 Fc region comprises mutations T250Q and M428L.

201. The anti-TM4SF1 binding protein of claim 199, wherein the modified human IgG1 Fc region comprises mutations M252Y, S254T, and T256E.

202. The anti-TM4SF1 binding protein of claim 199, wherein the modified human IgG1 Fc region comprises mutations M428L and N434S.

203. The anti-TM4SF1 binding protein of claim 197, wherein the modified human IgG4 Fc region comprises a mutation at one or more positions selected from the group consisting of T250, M428, and N434 as numbered by the EU index as set forth in Kabat.

204. The anti-TM4SF1 binding protein of claim 203, wherein the modified human IgG4 Fc region comprises a mutation selected from the group consisting of T250Q, M428L, and N434S as numbered by the EU index as set forth in Kabat.

205. The anti-TM4SF1 binding protein of claim 204, wherein the modified human IgG4 Fc region comprises mutations T250Q and M428L.

206. The anti-TM4SF1 binding protein of claim 204, wherein the modified human IgG4 Fc region comprises M428L and N434S.

207. The anti-TM4SF1 binding protein of any one of claims 196-206, wherein the binding protein exhibits increased affinity to FcRn as compared to a control anti-TM4SF1 binding protein comprising a wild type IgG1 Fc or IgG4 Fc.

208. The anti-TM4SF1 binding protein of any one of claims 196-207, wherein said anti-TM4SF1 binding protein comprises an anti-TM4SF1 antibody or an antigen binding fragment thereof.

209. The anti-TM4SF1 binding protein of claim 208, wherein the anti-TM4SF1 antibody or an antigen binding fragment thereof is conjugated to a therapeutic molecule, wherein said therapeutic molecule comprises at least one of: a small molecule, a degrader, a nucleic acid molecule, a CRISPR-Cas9 gene editing system, and a lipid nanoparticle, or any combinations thereof.

210. A pharmaceutical composition comprising the binding protein of any one of claims 196-209.

211. A method of treating or preventing a disease or disorder in a subject, wherein the disease or disorder is characterized by abnormal endothelial cell (EC)- cell interaction, said method comprising administering to the subject a binding protein according to any one of claims 196-209 or a pharmaceutical composition according to claim 210.

212. The method of claim 211, wherein the EC-cell interaction comprises one or more of EC-mesenchymal stem cell, EC-fibroblast, EC-smooth muscle cell, EC-tumor cell, EC-leukocyte, EC-adipose cell, and EC-neuronal cell interactions.

213. The method of claim 211 or 212, wherein the disease or disorder comprises an inflammatory disease or a cancer.

214. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or said antigen binding fragment thereof comprises a human IgG1 Fc region comprising a mutation at one or more positions selected from the group consisting of T250, M252, S254, T256, M428, and N434 as numbered by the EU index as set forth in Kabat.

215. The antibody drug conjugate of claim 214, wherein the human IgG1 Fc region comprises a mutation selected from the group consisting of T250Q, M252Y, S254T, T256E, M428L, and N434S, as numbered by the EU index as set forth in Kabat.

216. The antibody-drug conjugate of claim 215, wherein the human IgG1 Fc region comprises mutations at positions T250 and M428.

217. The antibody drug conjugate of claim 215, wherein the human IgG1 Fc region comprises mutations T250Q and M428L.

218. The antibody drug conjugate of claim 214, wherein the human IgG1 Fc region comprises mutations at positions M252, S254, and T256.

219. The antibody drug conjugate of claim 218, wherein the human IgG1 Fc region comprises mutations M252Y, S254T, and T256E.

220. The antibody drug conjugate of claim 214, wherein the human IgG1 Fc region comprises mutations at positions M428 and N434.

221. The antibody drug conjugate of claim 220, wherein the human IgG1 Fc region comprises mutations M428L and N434S.

222. The antibody drug conjugate of any one of claims 214-221, wherein the human IgG1 Fc region further comprises a mutation at position N297.

223. The antibody drug conjugate of claim 222, wherein the mutation at position N297 is N297C.

224. The antibody drug conjugate of any one of claims 214-223, wherein the human IgG1 Fc region further comprises an extended C-terminus that is positively charged, wherein said extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

225. The antibody drug conjugate of any one of claims 214-224, wherein the human IgG1 Fc region further comprises a mutation at one or more positions selected from the group consisting of E233, L234, L235, G237, D265, N297, K322, and P331; as numbered by the EU index as set forth in Kabat.

226. The antibody drug conjugate of claim 225, wherein the human IgG1 Fc region comprises a mutation selected from the group consisting of E233P, L234A, L235A, G237A, D265A, N297C, K322A, and P331G.

227. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises 2, 3, 4, 5, 6, or 7 mutations selected from the group consisting of E233P, L234A, L235A, G237A, D265A, N297C, K322A, and P331G.

228. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises mutations L234A, L235A, and G237A.

229. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises mutations L234A, L235A, G237A, and P331G.

230. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises mutations L234A, L235A, G237A, K322A, and P331G.

231. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises mutations L234A, L235A, G237A, E233P, and P331G.

232. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises mutations L234A, L235A, G237A, and N297C.

233. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises mutations L234A, L235A, G237A, N297C, and P331G.

234. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises mutations L234A, L235A, G237A, N297C, K322A, and P331G.

235. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises mutations L234A, L235A, G237A, N297C, E233P, and P331G.

236. The antibody drug conjugate of claim 226, wherein the human IgG1 Fc region comprises mutations L234A, L235A, G237A, D265A, N297C, K322A, and P331G.

237. An antibody-drug conjugate comprising (i) an anti-TM4SF1 antibody or an antigen binding fragment thereof and (ii) a therapeutic molecule, wherein said anti-TM4SF1 antibody or said antigen binding fragment thereof comprises a human IgG4 Fc region comprising a mutation at one or more positions selected from the group consisting of T250, M428, and N434 as numbered by the EU index as set forth in Kabat.

238. The antibody drug conjugate of claim 237, wherein the human IgG4 Fc region comprises a mutation selected from the group consisting of T250Q, M428L, and N434S as numbered by the EU index as set forth in Kabat.

239. The antibody-drug conjugate of claim 238, wherein the human IgG4 Fc region comprises mutations at positions T250 and M428.

240. The antibody drug conjugate of claim 239, wherein the human IgG4 Fc region comprises mutations T250Q and M428L.

241. The antibody drug conjugate of claim 238, wherein the human IgG4 Fc region comprises mutations at positions M428 and N434.

242. The antibody drug conjugate of claim 241, wherein the human IgG4 Fc region comprises mutations M428L and N434S.

243. The antibody drug conjugate of any one of claims 237-242, wherein the human IgG4 Fc region further comprises a mutation at position N297.

244. The antibody drug conjugate of claim 243, wherein the mutation at position N297 is N297C.

245. The antibody drug conjugate of any one of claims 237-244, wherein the human IgG4 Fc region further comprises an extended C-terminus that is positively charged, wherein said

extended C-terminus comprises one or more amino acid residues after position K447, as numbered by the EU index as set forth in Kabat.

246. The antibody drug conjugate of any one of claims 237-244, wherein the human IgG4 Fc region further comprises a mutation at one or more positions selected from the group consisting of S228, F234, and L235 as numbered by the EU index as set forth in Kabat.

247. The antibody drug conjugate of claim 246, wherein the human IgG4 Fc region comprises a mutation selected from the group consisting of S228P, F234A, L235E, and N297C as numbered by the EU index as set forth in Kabat.

248. The antibody drug conjugate of claim 247, wherein the human IgG4 Fc region comprises 2, 3,or 4, mutations selected from the group consisting of S228P, F234A, L235E, and N297C.

249. The antibody drug conjugate of claim 246, wherein the IgG4 Fc region comprises a mutation at position S228.

250. The antibody drug conjugate of claim 249, wherein the mutation at position S228 is S228P.

251. The antibody drug conjugate of claim 246, wherein the IgG4 Fc region comprises mutations at positions S228 and L235.

252. The antibody drug conjugate of claim 251, wherein the IgG4 Fc region comprises mutations S228P and L235E.

253. The antibody drug conjugate of claim 246, wherein the IgG4 Fc region comprises mutations at positions S228, L235, and N297.

254. The antibody drug conjugate of claim 253, wherein the IgG4 Fc region comprises mutations S228P, L235E, and N297C.

255. The antibody drug conjugate of any one of claims 214-254, wherein the antibody drug conjugate exhibits increased affinity to FcRn as compared to a control antibody drug conjugate comprising a wild type IgG1 Fc or IgG4 Fc.

256. The antibody drug conjugate of any one of claims 214-255, wherein said therapeutic molecule comprises at least one of: a small molecule, a degrader, a nucleic acid molecule, a CRISPR-Cas9 gene editing system, and a lipid nanoparticle, or any combinations thereof.

257. The antibody-drug conjugate of any one of claims 214-255, wherein said therapeutic molecule comprises at least one of: a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV

inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder, a DHFR inhibitor, a nucleic acid, a CRISPR enzyme, or any combinations thereof.

258. The antibody-drug conjugate of claim 256, wherein said degrader comprises an agent that induces protein degradation.

259. The antibody-drug conjugate of claim 258, wherein said agent that induces protein degradation comprises a hydrophobic tag, a proteolysis inducing chimera, an HSP90 inhibitor, a selective estrogen receptor degrader (SERD), a selective androgen receptor degrader (SARD) or any combinations thereof.

260. The antibody-drug conjugate of claim 256, wherein said lipid nanoparticle encapsulates one or more therapeutic molecules.

261. The antibody-drug conjugate of claim 260, wherein said nucleic acid molecule comprises an RNA molecule or a DNA molecule.

262. The antibody-drug conjugate of claim 261, wherein said RNA molecule comprises an siRNA, an antisense-RNA, an miRNA, an antisense miRNA, an antagomir (anti-miRNA), an shRNA, or an mRNA.

263. The antibody-drug conjugate of any one of claims 214-262 , wherein said anti-TM4SF1 antibody or an antigen binding fragment thereof and said therapeutic molecule are conjugated by a linker in a single or multistep protocol.

264. The antibody-drug conjugate of claim 263, wherein said linker comprises a cleavable linker, a non-cleavable linker, a hydrophilic linker, a pro-charged linker, or a dicarboxylic acid based linker.

265. The antibody-drug conjugate of claim 263, wherein said linker comprises a cleavable covalent or non-covalent linker.

266. The antibody-drug conjugate of claim 263, wherein said linker comprises a non-cleavable covalent or non-covalent linker.

267. The antibody-drug conjugate of claim 265, wherein said cleavable linker comprises an acid-labile linker, a protease-sensitive linker, a photo-labile linker, or a disulfide-containing linker.

268. The antibody-drug conjugate of claim 263, wherein said linker comprises a cysteine linker or a non-cysteine linker.

269. The antibody-drug conjugate of claim 268, wherein said non-cysteine linker comprises a lysine linker.

270. The antibody-drug conjugate of claim 263, wherein said linker comprises a MC (6-maleimidocaproyl), a MCC (a maleimidomethyl cyclohexane-1-carboxylate), a MP (maleimidopropanoyl), a val-cit (valine-citrulline), a val-ala (valine-alanine), an ala-phe (alanine-phenylalanine), a PAB (p-aminobenzyloxycarbonyl), a SPP (N-Succinimidyl 4-(2-pyridylthio) pentanoate), 2,5-dioxopyrrolidin-1-yl 4-(pyridin-2-ylthio)hexanoate, 2,5-dioxopyrrolidin-1-yl 5-methyl-4-(pyridin-2-ylthio)hexanoate, 2,5-dioxopyrrolidin-1-yl 5-methyl-4-(pyridin-2-ylthio)heptanoate, 2,5-dioxopyrrolidin-1-yl 5-ethyl-4-(pyridin-2-ylthio)heptanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclopentyl-4-(pyridin-2-ylthio)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclohexyl-4-(pyridin-2-ylthio)butanoate, a SMCC (N-Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 carboxylate), or a SIAB (N-Succinimidyl (4-iodo-acetyl)aminobenzoate).

271. The antibody-drug conjugate of claim 263, wherein said linker is derived from a cross-linking reagent, wherein the cross-linking reagent comprises N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), 2,5-dioxopyrrolidin-1-yl 3-cyclopropyl-3-(pyridin-2-yldisulfaneyl)propanoate, 2,5-dioxopyrrolidin-1-yl 3-cyclobutyl-3-(pyridin-2-yldisulfaneyl)propanoate, N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-yldisulfaneyl)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-yldisulfaneyl)butanoate, N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), 2,5-dioxopyrrolidin-1-yl 4-cyclopropyl-4-(pyridin-2-yldisulfaneyl)butanoate, 2,5-dioxopyrrolidin-1-yl 4-cyclobutyl-4-(pyridin-2-yldisulfaneyl)butanoate, N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC), or 2,5-dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazaheptadecan-1-oate (CX1-1).

272. A method of treating or preventing a disease or disorder in a subject, wherein the disease or disorder is characterized by abnormal endothelial cell (EC)- cell interaction, said method comprising administering to the subject an antibody-drug conjugate according to any one of claims 214-271.

273. The method of claim 272, wherein the EC-cell interaction comprises one or more of EC-mesenchymal stem cell, EC-fibroblast, EC-smooth muscle cell, EC-tumor cell, EC-leukocyte, EC-adipose cell, and EC-neuronal cell interactions.

274. The method of claims 271 or 272, wherein the disease or disorder comprises an inflammatory disease or a cancer.

275. A method of treating or preventing inflammation in a subject, said method comprising administering to the subject an antibody-drug conjugate according to any one of claims 214-271.

276. A method of treating or preventing metastasis in a subject, said method comprising administering to the subject an antibody-drug conjugate according to any one of claims 214-271, wherein the subject is in partial or complete remission from a cancer.

277. A method of treating a subject having a cancer which is associated with a high risk of metastasis, said method comprising administering an antibody-drug conjugate according to any one of claims 214-271, to the subject having the cancer which is associated with the high risk of metastasis.

278. A method of treating or preventing metastasis in a subject having a cancer, said method comprising administering an antibody-drug conjugate according to any one of claims 214-271, to the subject having the cancer.

279. The method of any one of claims 272-278, wherein the subject is undergoing a treatment which may induce metastasis.

280. The method of claim 279, wherein the treatment comprises surgery, radiation treatment and chemotherapy.

281. The method of any one of claims 272-280, wherein the subject is a human.

282. The method of any one of claims 274 and 276-281, wherein the cancer is a carcinoma or a sarcoma.

283. The method of claim 282, wherein the carcinoma comprises breast cancer, lung cancer, colon cancer, prostate cancer, pancreatic cancer, liver cancer, gastric cancer, renal cancer, bladder cancer, uterine cancer, cervical cancer, ovarian cancer.

284. The method of claim 282, wherein the sarcoma comprises an osteosarcoma, angiosarcoma, or a soft tissue sarcoma.

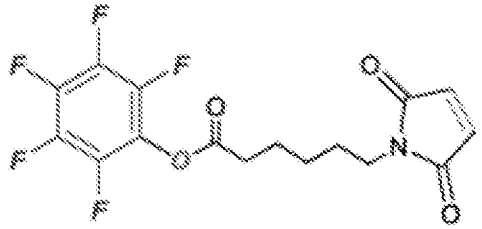
285. The method of claim any one of claims 274 and 276-281, wherein the cancer is a glioblastoma.

286. A method of treating or preventing lymphatic or hematogenous metastasis in a human subject comprising administering to the human subject an antibody-drug conjugate according to any one of claims 214-271.

287. The method of any one of claims 272-286, wherein the antibody drug conjugate exhibits longer serum half-life after administration as compared to a control antibody drug conjugate comprising a wild type IgG1 Fc or IgG4 Fc.

288. A pharmaceutical composition comprising (i) an antibody-drug conjugate according to any one of claims 214-271 and (ii) a pharmaceutically acceptable carrier.

289. An antibody drug conjugate comprising an anti-TM4SF1 antibody or an antigen binding fragment thereof comprising a modified IgG Fc region comprising one or more mutations selected from the group consisting of: (i) S228, F234, L235, G237, P238, F243, T250, M252, S254, T256, E258, D259, V264, D265, K288, T299, T307, V308, Q311, K322, L328, P329, A330, P331, T356, K370, A378, R409, V427, M428, H433, N434, H435, and N297; or (ii) E233, L234, L235, G237, M252, S254, T250, T256, D265, N297, K322, P331, M428, and N434, conjugated to a therapeutic molecule via a linker, wherein said linker is derived from a compound of Formula: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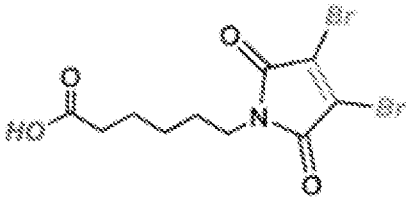
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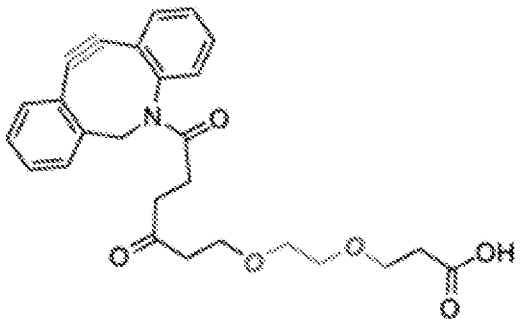
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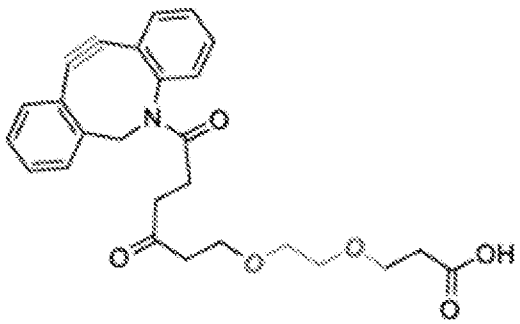
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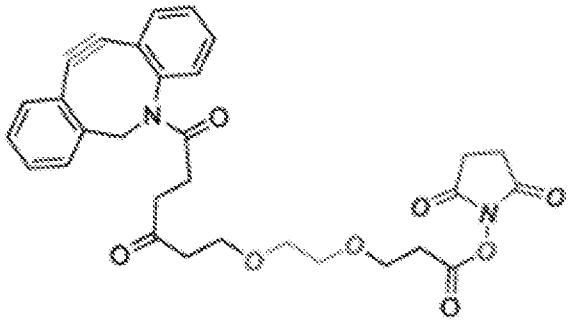
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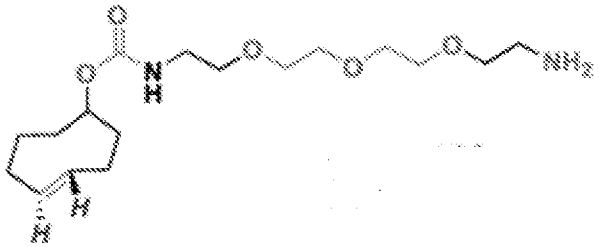
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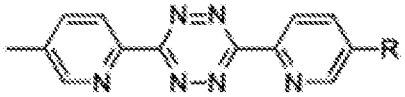
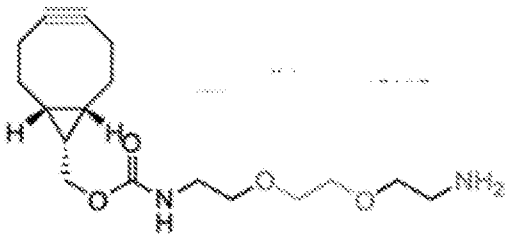

R = spacer/linker to mAb or Payload ;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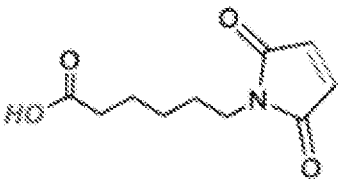
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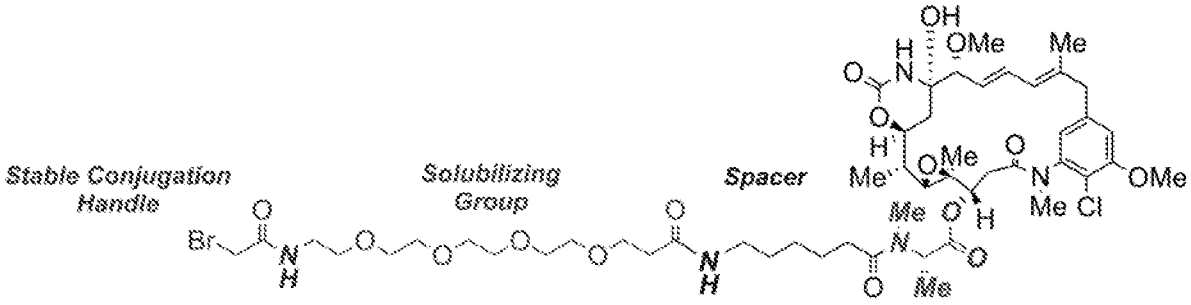
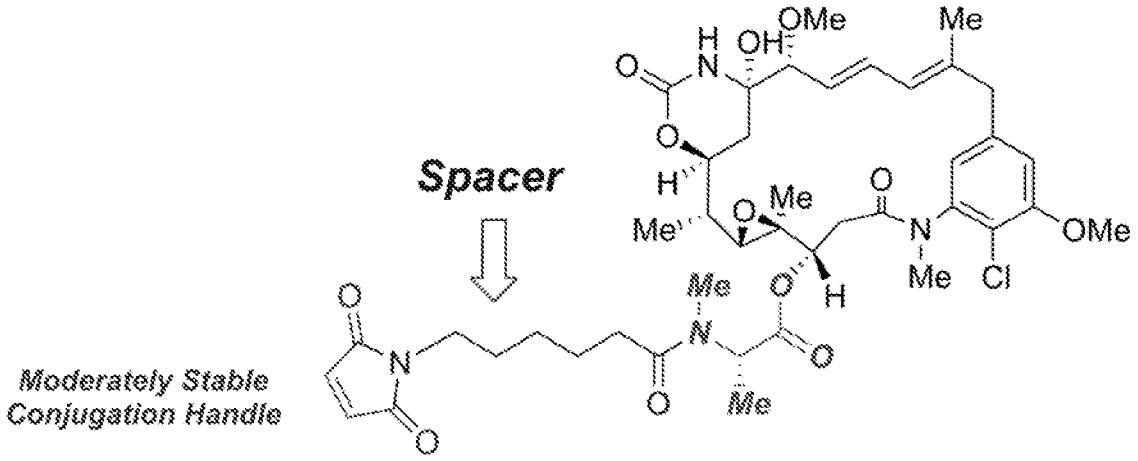
FIG. 1



Chemical Formula: $C_{51}H_{77}BrClN_5O_{16}$
Molecular Weight: 1131.6

BrAc-Peg4-Ahx-N-Methyl-Alanine-Maytansine

FIG. 2



Chemical Formula: $C_{42}H_{55}ClN_4O_{12}$
Molecular Weight: 843.4

Maleimide-Ahx-N-Methyl-Alanine-Maytansine
"MC-DM-1"

FIG. 3

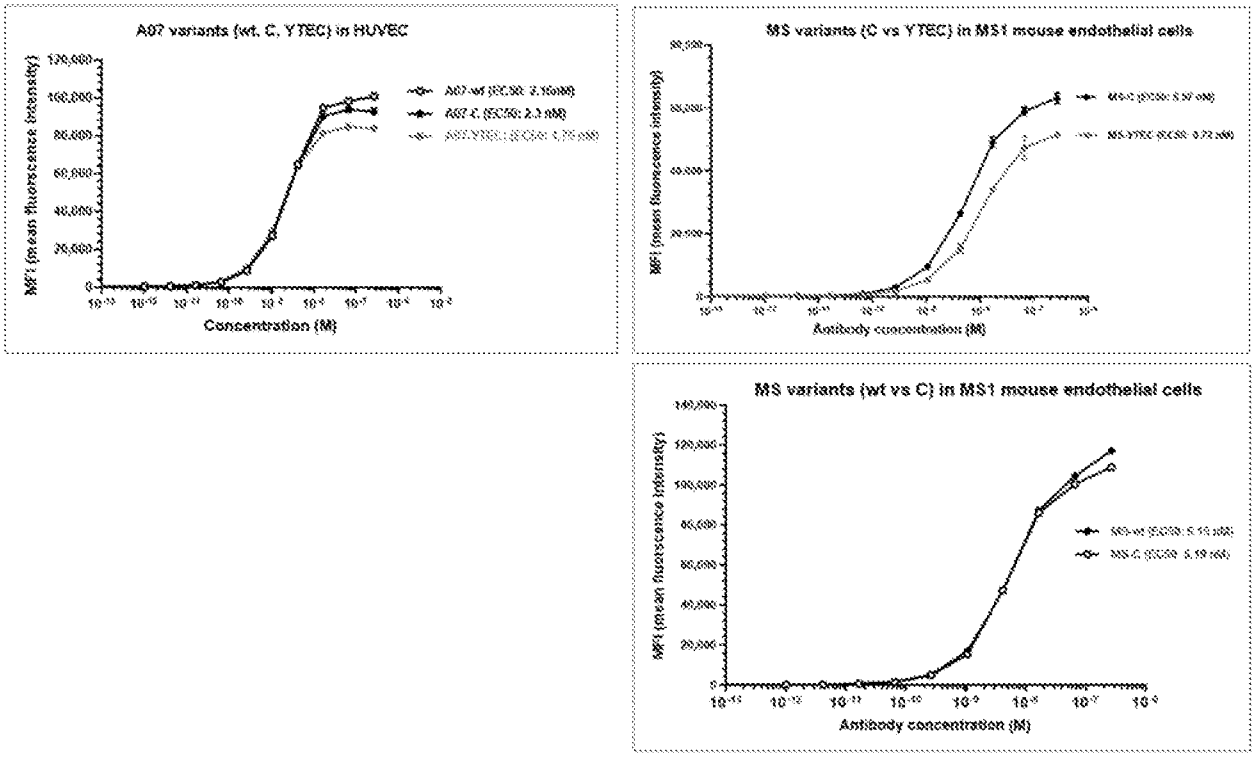

A07 variants (wt, C, YTEC) in HUVEC
MFI (mean fluorescence intensity)
Concentration (M)
MS variants (C vs YTEC) in MS1 mouse endothelial cells
MFI (mean fluorescence intensity)
Antibody concentration (M)
MS variants (wt vs C) in MS1 mouse endothelial cells
MFI (mean fluorescence intensity)
Antibody concentration (M)

FIG. 4

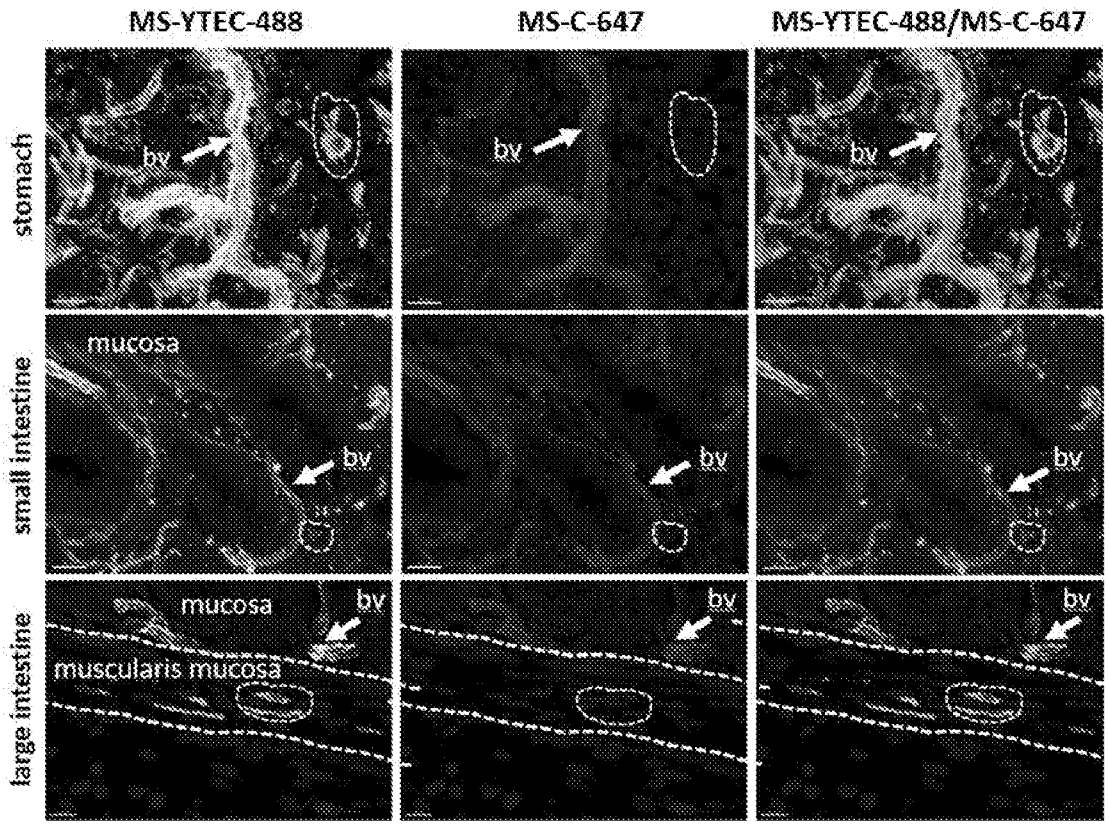

MS-YTEC-488
MS-C-647
MS-YTEC-488/MS-C-647
stomach
small intestine
large intestine
bv
mucosa
muscularis mucosa

FIG. 5

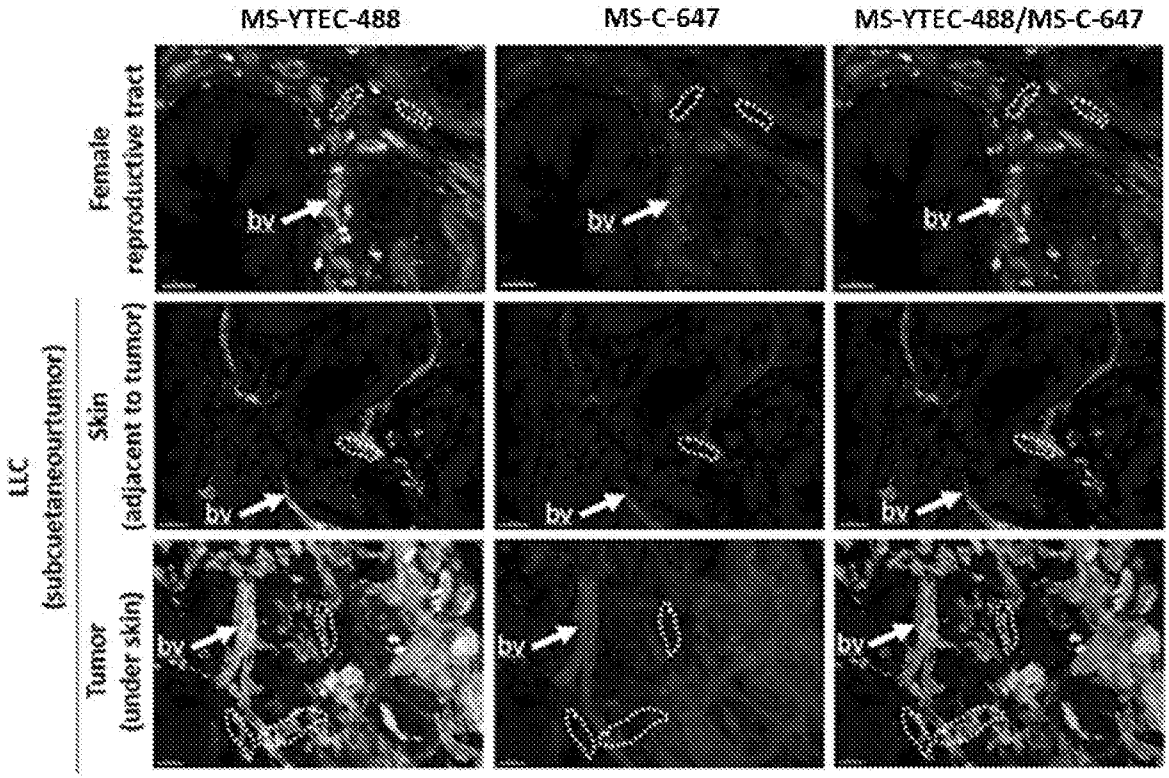

MS-YTEC-488
MS-C-647
MS-YTEC-488/MS-C-647
Female reproductive tract
LLC (subcuetaneourtumor)
Skin (adjacent to tumor)
Tumor (under skin)
by

FIG. 6

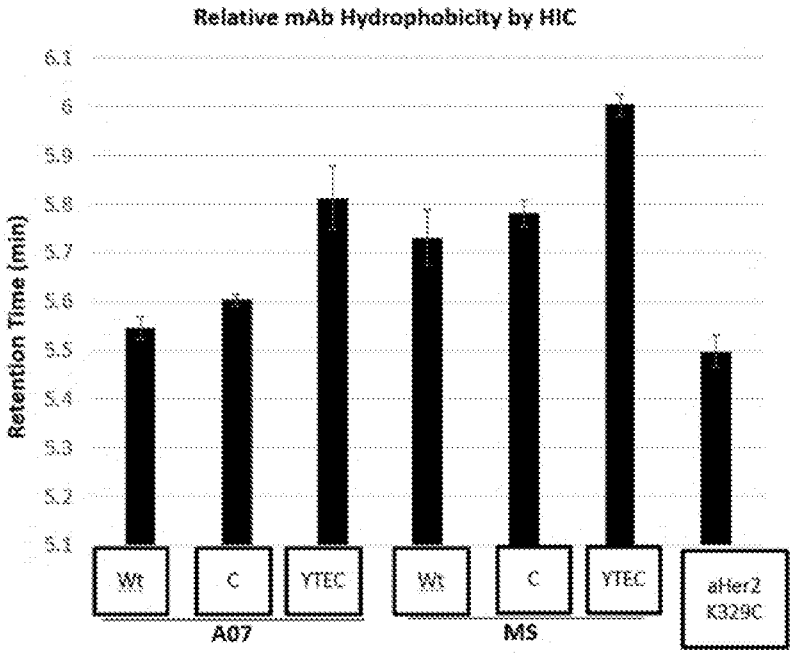

Relative mAb Hydrophobicity by HIC
Retention Time (min)
6.1
6
5.9
5.8
5.7
5.6
5.5
5.4
5.3
5.2
5.1
Wt
C
YTEC
Wt
C
YTEC
aHer2 K329C
A07
MS

FIG. 7

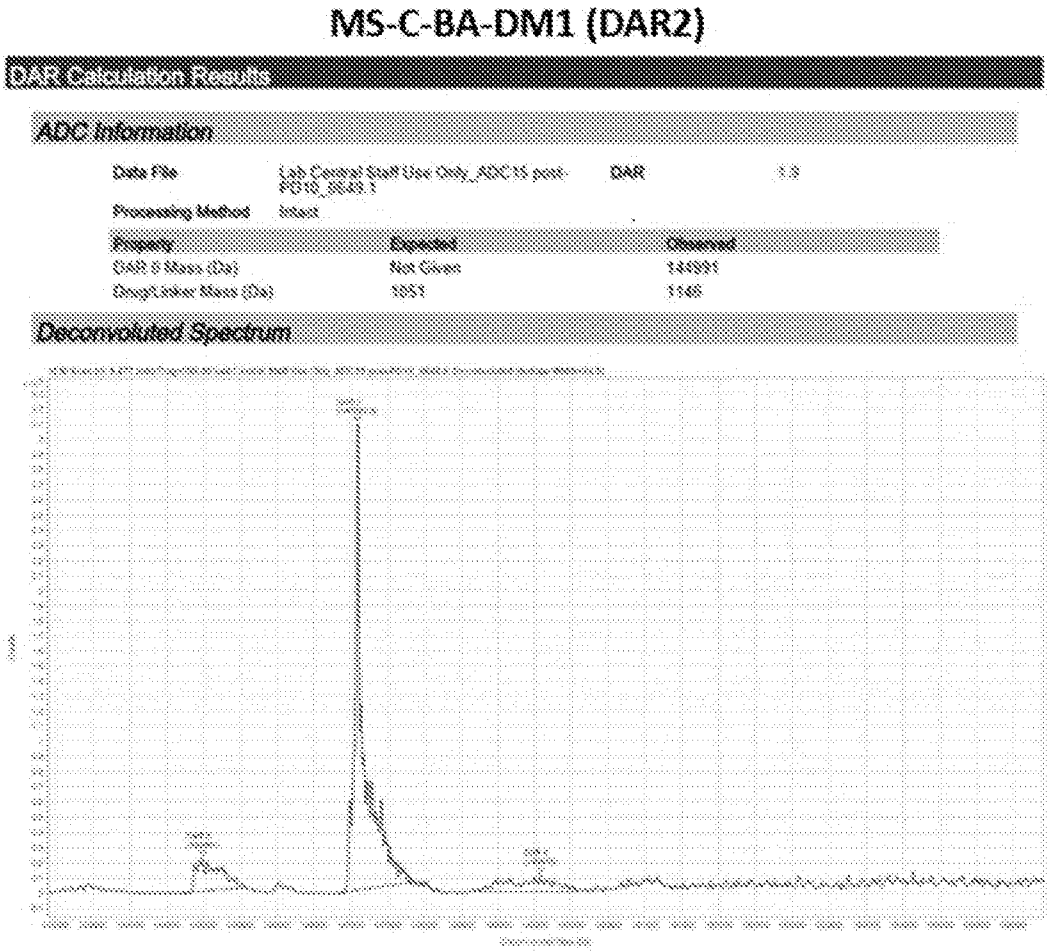

MS-C-BA-DM1 (DAR2)
ADC Information
Deconvoluted Spectrum

FIG. 8

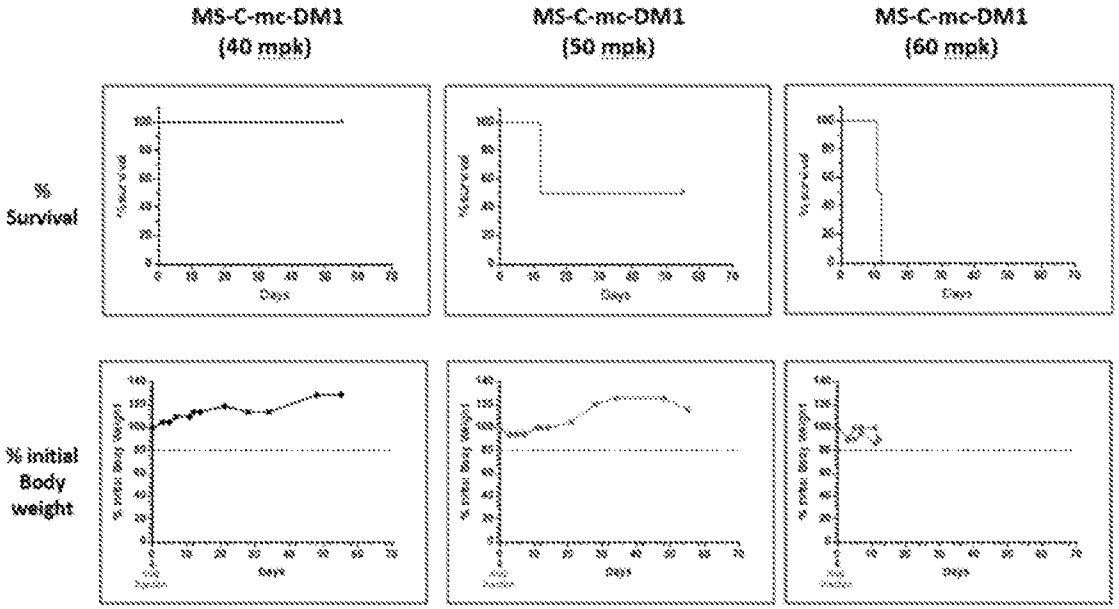

MS-C-mc-DM1 (40 mpk)
MS-C-mc-DM1 (50 mpk)
MS-C-mc-DM1 (60 mpk)
% Survival
% initial Body weight
Days

FIG. 9

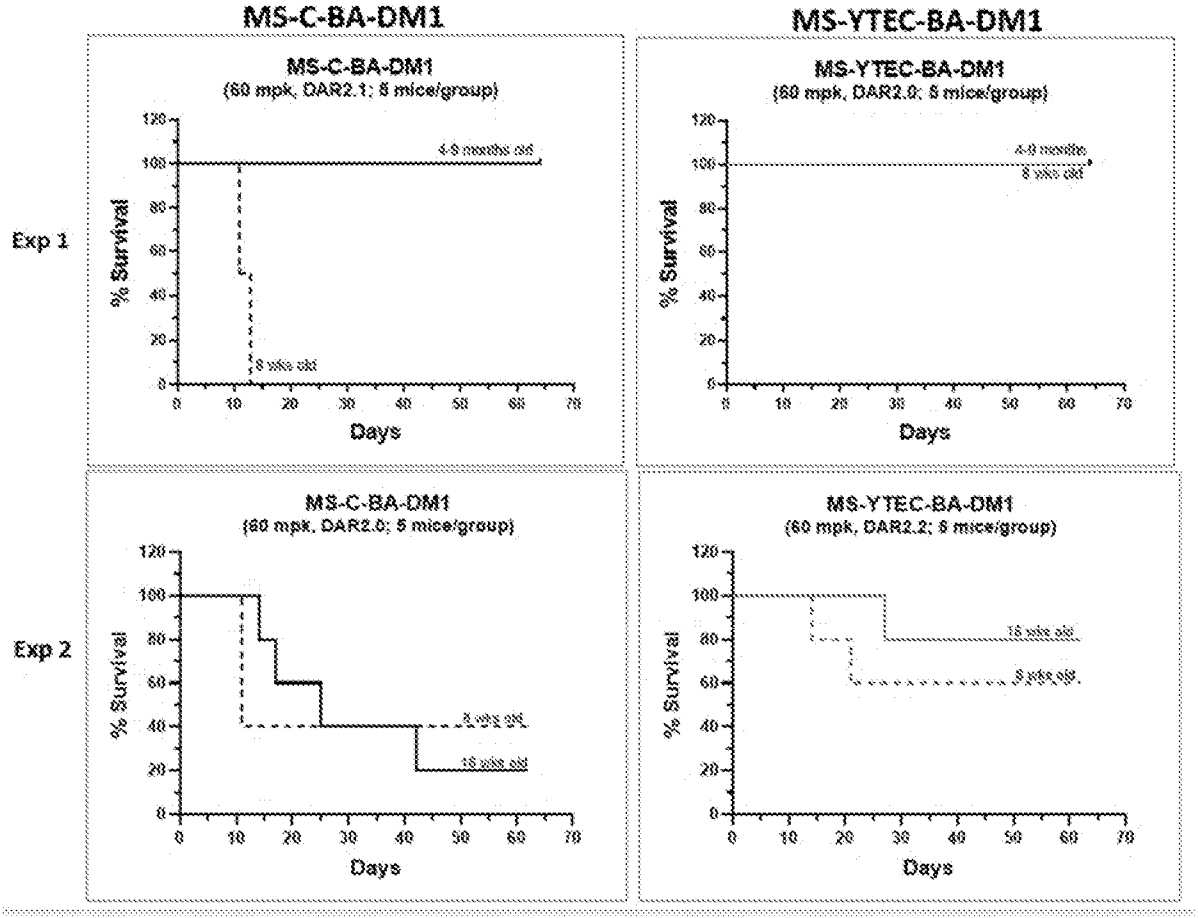

MS-C-BA-DM1
MS-YTEC-BA-DM1
Exp 1
Exp 2
MS-C-BA-DM1
(60 mpk, DAR2.1; 5 mice/group)
MS-YTEC-BA-DM1
(60 mpk, DAR2.0; 5 mice/group)
MS-C-BA-DM1
(60 mpk, DAR2.0; 5 mice/group)
MS-YTEC-BA-DM1
(60 mpk, DAR2.2; 5 mice/group)
% Survival
Days

FIG. 10

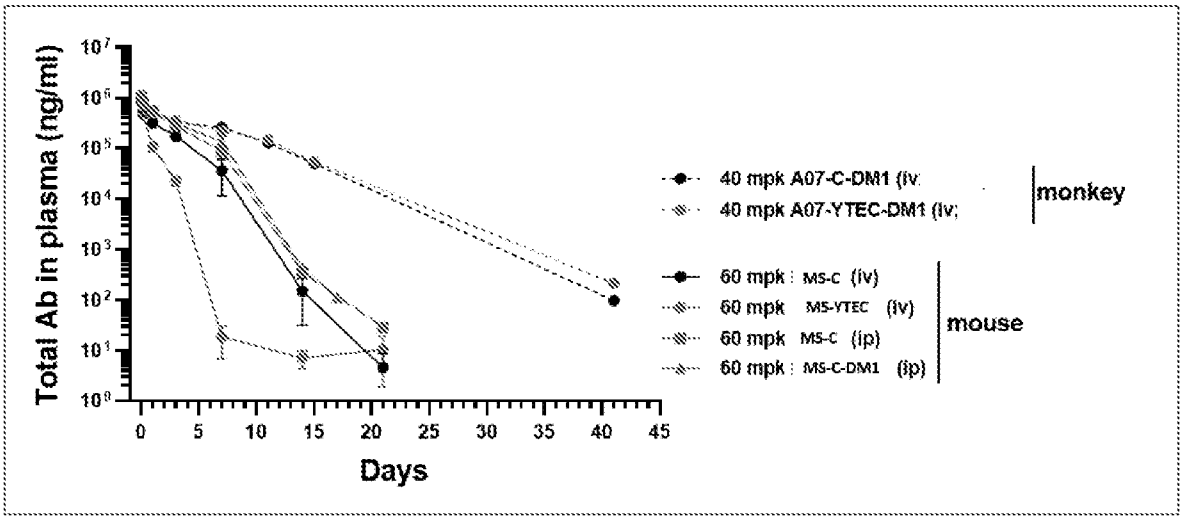

Total Ab in plasma (ng/ml)
Days
40 mpk A07-C-DM1 (iv)
40 mpk A07-YTEC-DM1 (iv)
monkey
60 mpk : MS-C (iv)
60 mpk MS-YTEC (iv)
60 mpk MS-C (ip)
60 mpk : MS-C-DM1 (ip)
mouse

FIG. 11

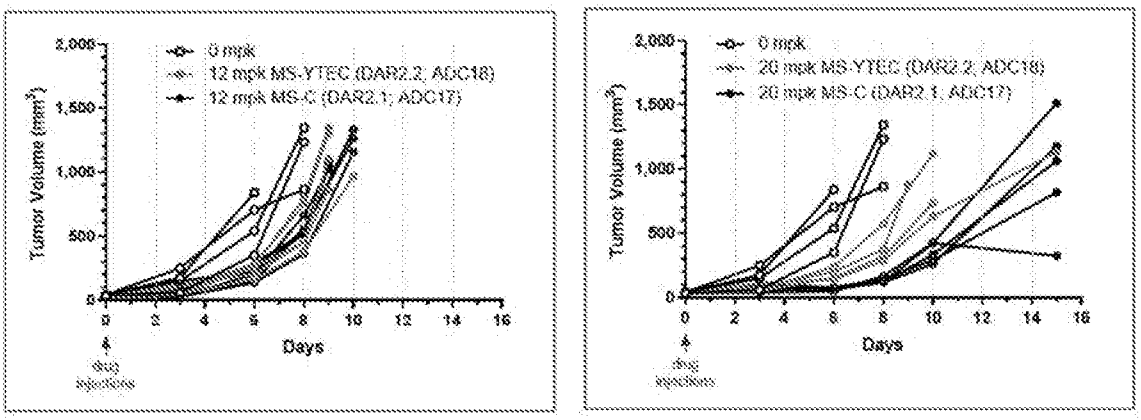

0 mpk
12 mpk MS-YTEC (DAR2.2; ADC18)
12 mpk MS-C (DAR2.1; ADC17)
Tumor Volume (mm³)
Days
drug injections
0 mpk
20 mpk MS-YTEC (DAR2.2; ADC18)
20 mpk MS-C (DAR2.1; ADC17)
Tumor Volume (mm³)
Days
drug injections

FIG. 12

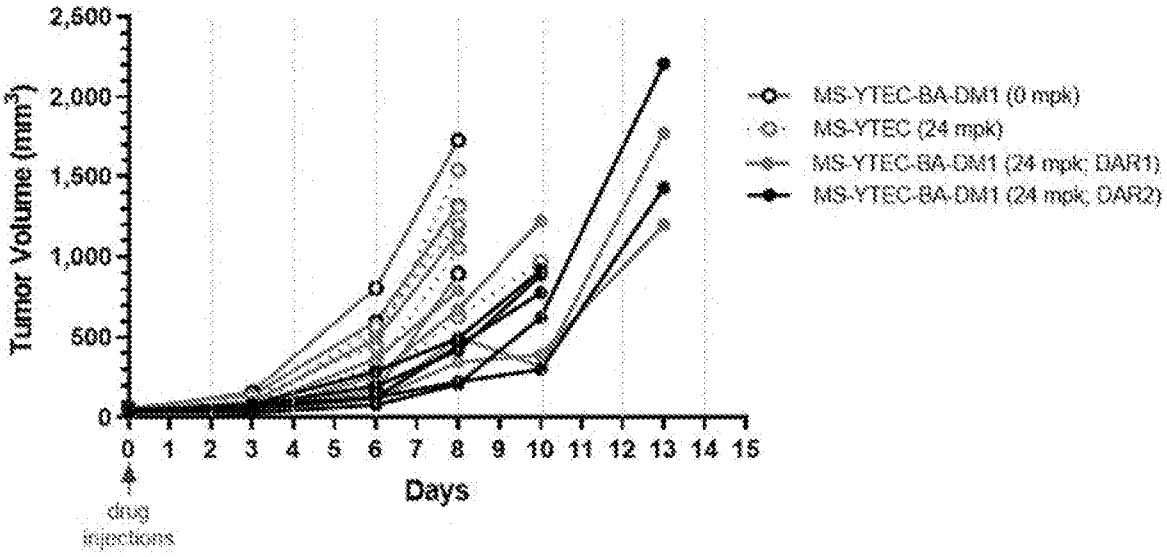

MS-YTEC-BA-DM1 (0 mpk)
MS-YTEC (24 mpk)
MS-YTEC-BA-DM1 (24 mpk; DAR1)
MS-YTEC-BA-DM1 (24 mpk; DAR2)
Tumor Volume (mm³)
Days
drug injections

FIG. 13

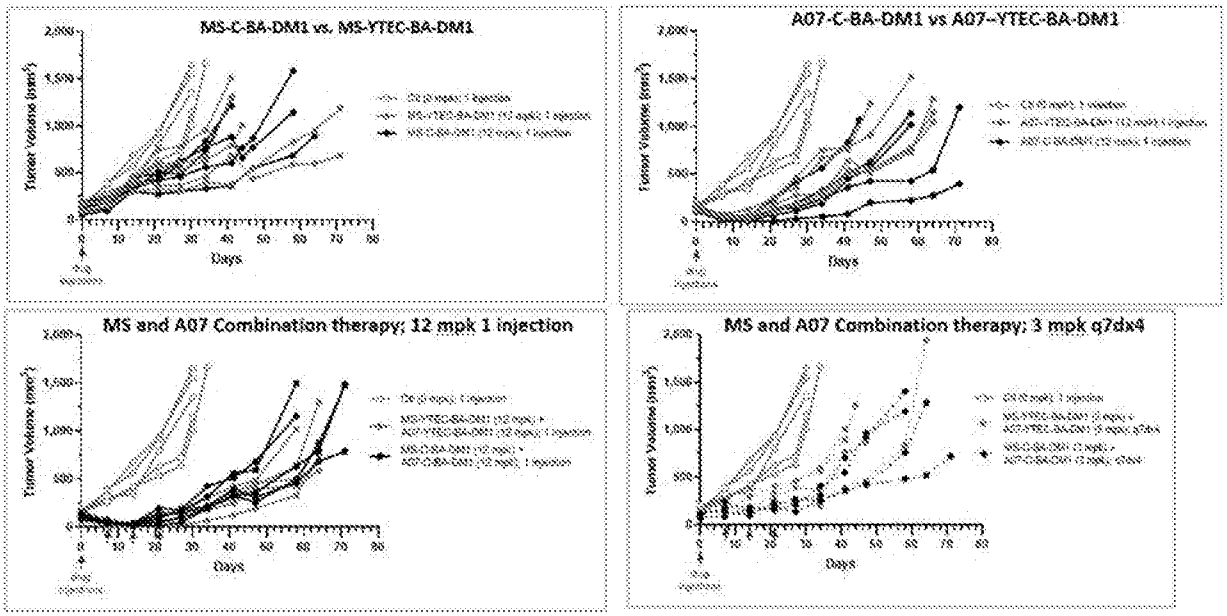
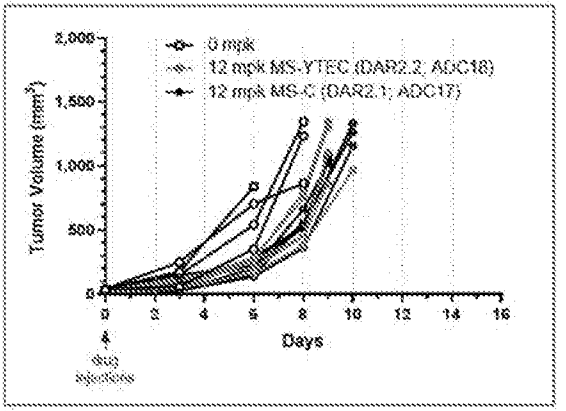
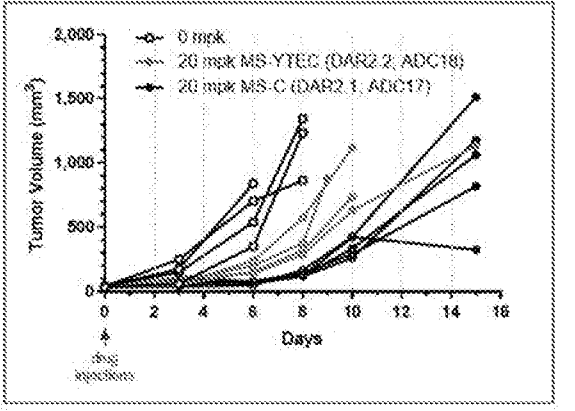

MS-C-BA-DM1 vs. MS-YTEC-BA-DM1
A07-C-BA-DM1 vs A07--YTEC-BA-DM1
MS and A07 Combination therapy; 12 mpk 1 injection
MS and A07 Combination therapy; 3 mpk q7dx4
Tumor Volume (mm³)
Days

FIG. 11


0 mpk
12 mpk MS-YTEC (DAR2.2; ADC18)
12 mpk MS-C (DAR2.1; ADC17)
Tumor Volume (mm³)
2,000
1,500
1,000
500
0
0
2
4
6
8
10
12
14
16
Days
4
dosing injections


0 mpk
20 mpk MS-YTEC (DAR2.2; ADC18)
20 mpk MS-C (DAR2.1; ADC17)
Tumor Volume (mm³)
2,000
1,500
1,000
500
0
0
2
4
6
8
10
12
14
16
Days
4
dosing injections