

and inhibits Ang1 signaling, leading to endothelial and vascular destabilization, breakdown of the blood-retinal barrier, and inflammation (Klaassen et al., 2013, Prog Retin Eye Res, 34:19-48, Saharinen et al., 2017, Nat Rev Drug Discov, 16:635-661).

Tie receptors, including Tie1 and Tie2, are type 1 transmembrane protein receptor
5 tyrosine kinases (RTKs) (Ramsauer, M. & D'Amore, P.A. J. Clin. Invest. (2002);110:1615–1617). Tie stands for Tyrosine kinase receptors with immunoglobulin and EGF homology domains. Tie2 is located in endothelial cells of all forming blood vessels and in the endocardium of mouse embryos (Korhonen et al., Blood (1992);80:2548- 2555). The ectodomains or extracellular domain (“ECD”) of Tie2 includes three Immunoglobulin (Ig)
10 domains (Ig1, Ig2 and Ig3), three epidermal growth factor (EGF) domains and a fibronectin type III domain (FNIII). The Ig-EGF region of Tie2 mediates angiopoietin recognition and binding (Fiedler, U. et al., J. Biol. Chem. (2003);278:1721–1727; Barton, W.A., et al., Structure (2005);13:825–832).

Two ligands for the Tie2 receptor have been identified: Angiopoietin-1 (Ang1) and
15 Angiopoietin-2 (Ang2). Ang-1, a Tie2 agonist, binds and induces the tyrosine phosphorylation of Tie2 and its expression in vivo is in close proximity with developing blood vessels (Davis et al., Cell (1996);87: 1161-1169). Mice lacking Ang-1 exhibit angiogenic deficits reminiscent of those seen in mice lacking Tie2, which supports that Ang-1 is a primary physiologic ligand for Tie2 and that Tie2 has critical in vivo angiogenic actions (Suri et al., Cell (1996);87:1171-
20 1180). Ang-1 is anti-inflammatory, and promotes vascular integrity, reduces vascular permeability. Ang2 was identified to be a naturally occurring antagonist for Tie2. Transgenic overexpression of Ang2 disrupts blood vessel formation in the mouse embryo (Maisonpierre et al., Science 277: 55-60,1997). Ang2 can be pro-inflammatory, disrupt EC quiescence, and increase vascular permeability. Together, studies support that the Ang1/Ang2/Tie2 system
25 plays a critical role in angiogenesis. Given the significant role for Tie2 in angiogenesis, agents that recognize Tie2, and methods of using such agents, are desired. Moreover, compositions which function as Tie2 agonists and which can reduce vascular permeability or enhance vascular integrity have great potential as therapeutics, especially for treatment of ocular disorders.

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SUMMARY

The ~~invention~~disclosure provides anti-Tie2 antibodies, compositions (e.g., conjugates) comprising the anti-Tie2 antibodies or fragments thereof, and methods of using the same.

The presently disclosed subject matter provides an isolated antibody that specifically binds Tie2, or an antigen-binding fragment thereof, a composition comprising at least one or more anti-Tie2 antibodies or antigen-binding fragments thereof, and methods of using the same. In an exemplary embodimentexample, the anti-Tie2 antibody is a Fab.

In one aspect, the present disclosure provides an isolated antibody or antigen-binding fragment thereof that specifically binds to Tie2, wherein the antibody or fragment thereof comprises: a heavy chain variable domain (VH) comprising (a) CDR-H1 comprising the amino acid sequence NTDIS (SEQ ID NO:3), (b) CDR-H2 comprising the amino acid sequence RISPSDGNTYYADSVKG (SEQ ID NO:4), and (c) CDR-H3 comprising the amino acid sequence RTRWASX1AX2DY (SEQ ID NO:5), where X1 is M, L, K, F, Y, R, N, Q, H or W and/or X2 is F, Y L, Q, I, K, or H, and a light chain variable domain (VL) comprising (d) CDR-L1 comprising the amino acid sequence RASQDVSTAVA (SEQ ID NO:8), (e) CDR-L2 comprising the amino acid sequence SASFLYS (SEQ ID NO:9), and (f) CDR-L3 comprising the amino acid sequence QQSYTTPPT (SEQ ID NO:10). In one aspectexample, an antibody that specifically binds Tie2 or an antigen-binding fragment thereof is provided, wherein the anti-Tie2 antibody comprises a heavy chain (HC) variable domain (VH domain) comprising a CDR-H1 comprising the amino acid sequence of NTDIS (SEQ ID NO:3), CDR-H2 comprising the amino acid sequence of RISPSDGNTYYADSVKG (SEQ ID NO:4), and CDR-H3 comprising the amino acid sequence of (a) RTRWASX1AX2DY (SEQ ID NO:5 wherein X1 is M, L, K, F, Y, R, N, Q, H or W and/or X2 is F, Y L, Q, I, K, or H), (b) RTRWASWAMDY (SEQ ID NO:6), or (c) RTRWASWAFDY (SEQ ID NO:7); and a light chain (LC) variable domain (VL domain) comprising CDR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO:8), CDR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO:9), and CDR-L3 comprising the amino acid sequence of QQSYTTPPT (SEQ ID NO:10). In some embodimentexamples, CDR-H3 comprises SEQ ID NO:6 or SEQ ID NO:7. In particular embodimentexamples, CDR-H3 comprises SEQ ID NO:7.

In some embodimentexamples, the anti-Tie2 antibody comprises a VH framework FR1 sequence of SEQ ID NO:11, a VH framework FR2 sequence of SEQ ID NO:12, a VH framework FR3 sequence of SEQ ID NO:13, and/or a VH framework FR4 sequence of SEQ ID NO:14. In other embodimentexamples, the anti-Tie2 antibody comprises a VL framework FR1 sequence of SEQ ID NO:15, a VL framework FR2 sequence of SEQ ID NO:16, a VL framework FR3 sequence of SEQ ID NO:17, and/or a VL framework FR3 sequence of SEQ ID NO:18.

In some embodimentexamples, the anti-Tie2 antibody comprises a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, and CDR-H3 comprising the amino acid sequence of (a) SEQ ID NO:5 wherein X1 is M, L, K, F, Y, R, N, Q, H or W and/or X2 is F, Y L, Q, I, K, or H, (b) SEQ ID NO:6, or (c) SEQ ID NO:7; and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:8, CDR-L2 comprising the amino acid sequence of SEQ ID NO:9, and CDR-L3 comprising the amino acid sequence of SEQ ID NO:10. In other embodimentexamples, CDR-H3 comprises SEQ ID NO:6 or SEQ ID NO:7. In particular embodimentexamples, CDR-H3 comprises SEQ ID NO:7. In still other embodimentexamples, the VH

domain is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:20 and the VL domain is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:21.

In some embodimentexamples, the anti-Tie2 antibody or antigen-binding fragment thereof comprises the VH domain sequence of SEQ ID NO:19 or SEQ ID NO:22 and the VL domain sequence of SEQ ID NO:21.

In some embodimentexamples, the anti-Tie2 antibody comprises a VH domain sequence of SEQ ID NO:20. In other embodimentexamples, the anti-Tie2 antibody comprises a VL domain sequence of SEQ ID NO:21. In still other embodimentexamples, the anti-Tie2 antibody comprises a VH domain sequence of SEQ ID NO:20 and a VL domain sequence of SEQ ID NO:21

In one aspect, the present disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to Tie-2 wherein the antibody comprises a HC sequence of SEQ ID NO:55 and a LC sequence of SEQ ID NO:25. In some embodimentexamples, the anti-Tie2 antibody or fragment thereof comprises a heavy chain (HC) domain sequence of SEQ ID NO:55 and a light chain (LC) domain sequence of SEQ ID NO:25. In another aspect, the present disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to Tie-2 wherein the antibody comprises a HC sequence of SEQ ID NO:23 and a LC sequence of SEQ ID NO:56. In other embodimentexamples, the anti-Tie2 antibody or fragment thereof comprises a heavy chain (HC) domain sequence of SEQ ID NO:23 and a light chain (LC) domain sequence of SEQ ID NO:56.

In some aspectexamples, an antibody that specifically binds Tie2, or antigen-binding fragment thereof, is provided, which comprises a VH domain comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and CDR-H3 comprising the amino acid sequence of SEQ ID NO:30, and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:8, CDR-L2 comprising the amino acid sequence of SEQ ID NO:9, and CDR-L3 comprising the amino acid sequence of SEQ ID NO:10. In some embodimentexamples, the VH domain comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:31 and the VL domain comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:21. In still other embodimentexamples, the VH domain comprises SEQ ID NO:31 and the VL domain comprises SEQ ID NO:21. In other embodimentexamples, the HC comprises SEQ ID NO:32 and the LC comprises SEQ ID NO:25.

In some aspectexamples, an antibody that specifically binds Tie2, or antigen-binding fragment thereof, is provided, which comprises a VH domain comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO:33, CDR-H2 comprising the amino acid sequence of SEQ ID NO:34, and CDR-H3 comprising the amino acid sequence of SEQ ID NO:35, and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:8, CDR-L2 comprising the amino acid sequence of SEQ ID NO:9, and CDR-L3 comprising the amino acid sequence of SEQ ID NO:10. In some embodimentexamples, the VH domain comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:36 and

the VL domain comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:21. In still other embodimentexamples, the VH domain comprises SEQ ID NO:36 and the VL domain comprises SEQ ID NO:21. In other embodimentexamples, the HC comprises SEQ ID NO:37 and the LC comprises SEQ ID NO:25.

In some aspectexamples, an antibody that specifically binds Tie2, or antigen-binding fragment thereof, is provided, which comprises a VL domain comprising CDR-L1 comprising SEQ ID NO:8, CDR-L2 comprising SEQ ID NO:9, and CDR-L3 comprising SEQ ID NO:10; and a VH domain comprising: (a) CDR-H1 comprising SEQ ID NO:38, CDR-H2 comprising SEQ ID NO:39, and CDR-H3 comprising SEQ ID NO:40; (b) CDR-H1 comprising SEQ ID NO:43, CDR-H2 comprising SEQ ID NO:44, and CDR-H3 comprising SEQ ID NO:45; or (c) CDR-H1 comprising SEQ ID NO:48, CDR-H2 comprising SEQ ID NO:49, and CDR-H3 comprising SEQ ID NO:50.. In other embodimentexamples, the VL domain comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:21; and the VH domain comprises of (a), (b), or (c) comprises an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to (d) SEQ ID NO:41; (e) SEQ ID NO:46; or (f) SEQ ID NO:51, respectively.

In some embodimentexamples, the antibody or antigen-binding fragment thereof comprises a LC of SEQ ID NO:21 and a HC comprising SEQ ID NO:42, SEQ ID NO:46, or SEQ ID NO:52.

In some aspectexamples, an antibody that specifically binds Tie2, or antigen-binding fragment thereof, is provided, which comprises a 2 VH domains comprising, in an N-terminal to C-terminal direction, a CDR-H1 comprising SEQ ID NO:6, CDR-H2 comprising SEQ ID NO:8, a CDR-H3 comprising SEQ ID NO:9, a CDR-H1 comprising SEQ ID NO:48, CDR-H2 comprising SEQ ID NO:49, a CDR-H3 comprising SEQ ID NO:50, and 2 VL domains comprising, in an N-terminal to C-terminal direction, CDR-L1 comprising the amino acid sequence of SEQ ID NO:8, CDR-L2 comprising the amino acid sequence of SEQ ID NO:9, CDR-L3 comprising the amino acid sequence of SEQ ID NO:10, CDR-L1 comprising the amino acid sequence of SEQ ID NO:8, CDR-L2 comprising the amino acid sequence of SEQ ID NO:9, and CDR-L3 comprising the amino acid sequence of SEQ ID NO:10. In some embodimentexamples, , the HC comprises SEQ ID NO:54 and the LC comprises SEQ ID NO:53.

In some embodimentexamples, the anti-Tie2 antibody or antigen-binding fragment thereof comprises an engineered cysteine, wherein the engineered cysteine is in a HC constant domain and/or in a LC constant domain. In other embodimentexamples, the engineered cysteine is selected from T120C, G166C, G178C, T187C, and T209C in the heavy chain, and Q124C, R142C,

Q155C, L201C, T206C, K107C, K126C and K149C in the light chain, wherein the residue number is according to EU numbering. In other embodimentexamples, the anti-Tie2 antibody or antigen-binding fragment thereof is a Fab wherein the HC of the Fab ends in the amino acids CDKTHTSPPC (SEQ ID NO:83). In some embodimentexamples, the Fab ends in the amino acid sequence of SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, or SEQ ID NO:86.

In some embodimentexamples, the anti-Tie2 antibody is a monoclonal antibody.

In some embodimentexamples, the anti-Tie2 antibody is a humanized, chimeric or human antibody.

In some embodimentexamples, the anti-Tie2 antibody is a full length IgG1 or full length IgM antibody.

In some embodimentexamples, the anti-Tie2 antibody or fragment thereof binds Tie2, wherein Tie2 is a protein having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:1.

In preferred embodimentexamples, the anti-Tie2 antibody or fragment thereof is a Fab.

In some embodimentexamples, the anti-Tie2 antibody is an antibody or Fab that competes with the anti-Tie2 antibody comprising a VH sequence of SEQ ID NO:22 and a VL sequence of SEQ ID NO:21.

In some embodimentexamples, the anti-Tie2 antibody or fragment thereof does not bind to the Ig1 domain of Tie2. In other embodimentexamples, the anti-Tie2 antibody or fragment thereof does not bind to an EGF domain of Tie2. In still other embodimentexamples, the anti-Tie2 antibody or fragment thereof does not bind to the Ig3 domain of Tie2. In yet other embodimentexamples, the anti-Tie2 antibody or fragment thereof does not bind to a FNIII domain of the Tie2 protein.

In some embodimentexamples, the antibody or antigen-binding fragment thereof binds to cynomolgus monkey Tie2. In other embodimentexamples, the cynomolgus monkey Tie2 comprises the amino acid sequence of SEQ ID NO:2 or variant thereof.

In some embodimentexamples, the antibody or antigen-binding fragment thereof that binds Tie2 is a multi-specific antibody. In other embodimentexamples, the multi-specific antibody binds Tie2 and VEGF. In other embodimentexamples, the multi-specific antibody binds Tie2 and Factor D. In still other embodimentexamples, the multi-specific antibody binds Tie2 and Ang2.

In some embodimentexamples, the anti-Tie2 antibody is a multi-specific antibody which activates Tie2. In other embodimentexamples, the multi-specific antibody binds Tie2 and VEGF, wherein the multi-specific antibody can activate Tie2. In other embodimentexamples, the multi-specific antibody binds Tie2 and Factor D, wherein the multi-specific antibody can activate Tie2. In

still other embodimentexamples, the multi-specific antibody binds Tie2 and Ang2, wherein the multi-specific antibody can activate Tie2.

In one aspect, the present disclosure provides an isolated nucleic acid encoding the antibody or antigen-binding fragment thereof as disclosed herein. In one aspectexample, an isolated nucleic acid encoding the anti-Tie2 antibody or antigen-binding fragment thereof is provided.

In one aspect, the present disclosure provides a host cell comprising the nucleic acid as disclosed herein. In one aspectexample, a host cell comprising the isolated nucleic acid encoding the anti-Tie2 antibody or antigen-binding fragment thereof is provided.

In one aspectexample, a method of producing an antibody or antigen-binding fragment thereof that binds Tie2 is provided. In some embodimentexamples, the method comprises culturing the host cell comprising the nucleic acid encoding the anti-Tie2 antibody under conditions suitable for expression of the anti-Tie2 antibody. In other embodimentexamples, the method further comprises recovering the anti-Tie2 antibody from the host cell.

In one aspectexample, an anti-Tie2 antibody produced by the above method is provided.

In one aspect, the present disclosure provides a conjugate that binds to Tie2, wherein the conjugate comprises at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight antibodies or antigen-binding fragment thereof as disclosed herein, wherein each of the antibodies or antigen-binding fragment thereof is linked to a multimerizing moiety. In one aspectexample, a conjugate is provided, comprising at least two antibodies that specifically bind Tie2, or antigen-binding fragment thereof, according to the embodimentexamples disclosed herein, and a multi-armed moiety. In preferred embodimentexamples, each of the at least two anti-Tie2 antibodies is a Fab.

In some embodimentexamples, the conjugate comprises an anti-Tie2 Fab which binds Tie2, and a multi-armed moiety wherein the multi-armed moiety is linked to at least 2 anti-Tie2 Fabs. In some embodimentexamples, the multi-armed moiety is linked to at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 anti-Tie2 Fabs. In other embodimentexamples, the multi-armed moiety is linked to 2, 3, 4, 5, 6, 7, 8, 9 or 10 anti-Tie2 Fabs. In still other embodimentexamples, the multi-armed moiety is linked to 6 or 8 anti-Tie2 Fabs. In yet other embodimentexamples, the multi-armed moiety is linked to 8 anti-Tie2 Fabs. In preferred embodimentexamples, the multi-armed moiety is linked to 6 anti-Tie2 Fabs.

In one aspectexample, the conjugate binds to and activates Tie2 activity.

In some embodimentexamples, the conjugate which binds to Tie2 activates AKT phosphorylation. In other embodimentexamples, activation of AKT phosphorylation is demonstrated by an increase in phosphorylated AKT protein in an in vitro assay. In other embodimentexamples, the Tie2 binding agent activates phosphorylation of Tie2. In still other embodimentexamples, the activation of Tie2 phosphorylation is measured in vitro.

In some embodimentexamples, exposing Tie2-expressing cells to the conjugate does not reduce Tie2 protein levels in the cells. In other embodimentexamples, the exposing does not reduce Tie2 protein levels in the cells by more than 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, or 75%. In yet other embodimentexamples, the exposing reduces Tie2 protein levels in the cells by less than 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, or 75%. In still other embodimentexamples, the

exposing reduces Tie2 protein levels in the cell by more than about 25% and less than about 75%, by more than about 50% and less than about 75%, or by more than about 60% and less than about 80%. In some [embodimentexamples](#), the Tie2 protein levels are measured by western blot before and after in vitro incubation of the Tie2 binding agent with Tie2-expressing cells. In other [embodimentexamples](#), the incubation is at 37°C for 10 to 36 hours or from 12 to 24 hours.

In some [embodimentexamples](#), the anti-Tie2 antibody or antigen-binding fragment thereof has an equilibrium dissociation constant (Kd) ranging from 0.1 uM to 10 uM, from 0.01 uM to 10 uM, or from 0.1 to 100 uM.

In some [embodimentexamples](#), the conjugate comprising the multi-armed moiety and the anti-Tie2 antibody or antigen-binding fragment thereof increases translocation of Tie2 to cell-cell junctions.

In some [embodimentexamples](#), the conjugate reduces vascular permeability.

In some [embodimentexamples](#), the conjugate facilitates vascular stability, and/or increases vascular integrity.

In some [embodimentexamples](#), the conjugate does not inhibit or reduce binding of Ang1 to Tie2.

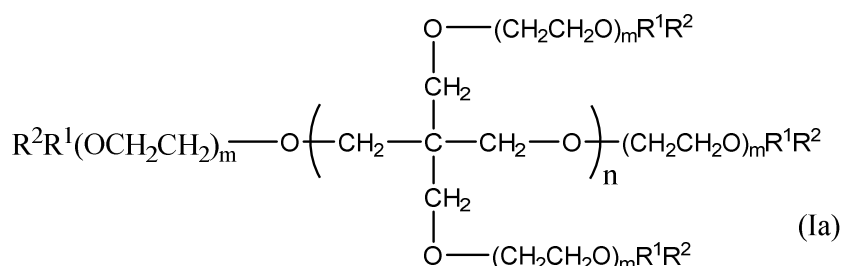
In some [embodimentexamples](#), the conjugate inhibits or reduces binding of Ang2 to Tie2.

In some [embodimentexamples](#), the conjugate activities are measured using an in vitro assay.

In some [embodimentexamples](#), the multi-armed polyol is selected from the group consisting of a dimer, a tetramer, a hexamer, and an octamer. In preferred [embodimentexamples](#), the multi-armed polyol is a hexamer or an octamer. In more preferred [embodimentexamples](#), the multi-armed polyol is a hexamer.

In some [embodimentexamples](#), the polyol is a poly(alkylene oxide) polymer. In other [embodimentexamples](#), the polyol is a poly(alkylene glycol). In yet other [embodimentexamples](#), the polyol is a polyethylene glycol (PEG). In some [embodimentexamples](#), the PEG is a functionalized multi-armed PEG.

In some [embodimentexamples](#), the PEG has the structure of general formula (Ia):

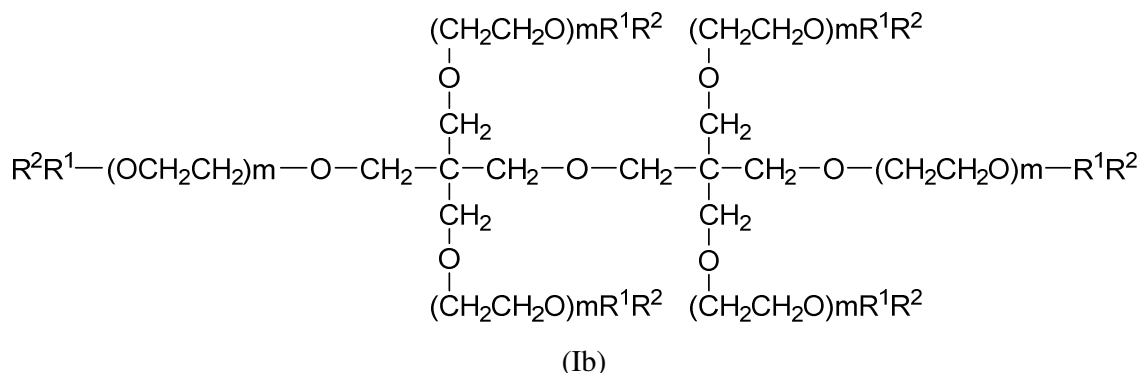


wherein each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of from about 45 to about 1000, about 20 to about 1000, about 10 to about 1000, about 3 to about 250, about 3 to about 200, about 3 to about 100, about 10 to about 50, about 10 to about 30, about 20 to about 30, about 50 to about 200, or from about 100 to about 150; and n is an integer from about 1 to about 10; each R¹ is independently either absent, or is a linking group; and each R² is independently either hydrogen or a terminal reactive group; wherein at least one R² is a terminal reactive group. In some embodimentexamples, R² is independently selected from a thiol reactive group, an amino reactive group, and combinations thereof.

In some embodimentexamples, the PEG has the structure of general formula (Ia), wherein n is an integer from 1 to 3.

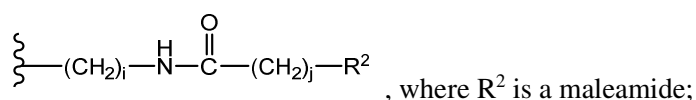
In some embodimentexamples, the PEG has the structure of general formula (Ia), wherein n is 1, and the multi-armed PEG is a tetramer.

In some embodimentexamples, the PEG has the structure of general formula (Ia), wherein n is 2, and the multi-armed PEG is a hexamer. In such embodimentexamples, the hexamer has the structure of general formula (Ib):

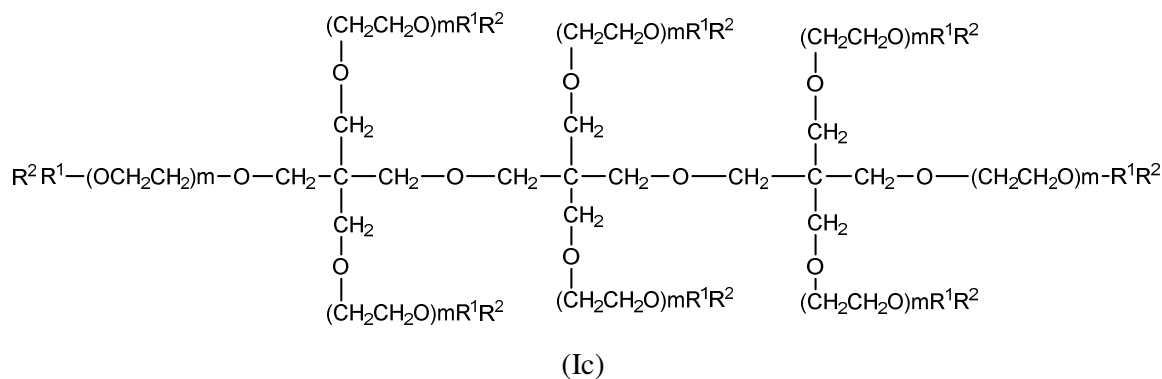


wherein each m is independently an integer of from about 45 to about 1000, about 20 to about 1000, about 10 to about 1000, about 3 to about 250, about 3 to about 200, about 3 to about 100, about 10 to about 50, about 10 to about 30, about 20 to about 30, about 50 to about 200, or from about 100 to about 150; each R¹ is independently either absent or is a linking group; and each R² is independently either hydrogen or a terminal reactive group; wherein at least one R² is a terminal reactive group and is covalently linked to an anti-Tie2 antibody fragment or Fab as described above. In some embodimentexamples, each m is independently an integer from about 15 to 35 or from about 20 to 30. In other embodimentexamples, each m is independently an integer of about 22.

In some [embodimentexamples](#), R^1 and R^2 taken together has the structure



In some [embodimentexamples](#), the PEG has the structure of general formula (Ia), wherein n is 3, and the multi-armed PEG is an octamer. In such [embodimentexamples](#), the octamer has the structure of general formula (Ic):



wherein each m is independently an integer from 3-250; each R^1 is independently either absent or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to an anti-Tie2 Fab as described above. In some [embodimentexamples](#), each m is independently an integer from 15 to 35. In other [embodimentexamples](#), each m is independently an integer of about 22.

In some [embodimentexamples](#), the at least two anti-Tie2 antibody fragments or Fabs described herein are covalently linked to the multi-armed polyol. In other [embodimentexamples](#), the multi-armed polyol of the conjugate is covalently linked to the at least two anti-Tie2 antibody fragments or Fabs through a free sulfhydryl group of a cysteine amino acid. In other [embodimentexamples](#), the cysteine amino acid is an engineered cysteine. In yet other [embodimentexamples](#), the cysteine amino acid is in the anti-Tie2 constant domain. In other [embodimentexamples](#), the cysteine amino acid is at the C-terminus of the heavy chain (HC) or the light chain (LC) of the anti-Tie2 Fab. In a preferred [embodimentexample](#), the cysteine amino acid is not at the N-terminus or the C-terminus of the HC or the LC.

In some [embodimentexamples](#), the conjugate comprising an anti-Tie2 antibody or antigen-binding fragment thereof which comprises an engineered cysteine in its HC and/or LC. In other [embodimentexamples](#), the engineered cysteine is selected from a T120C, G166C, G178C, T187C, and T209C in the HC; or the engineered cysteine is selected from Q124C, R142C, Q155C, L201C,

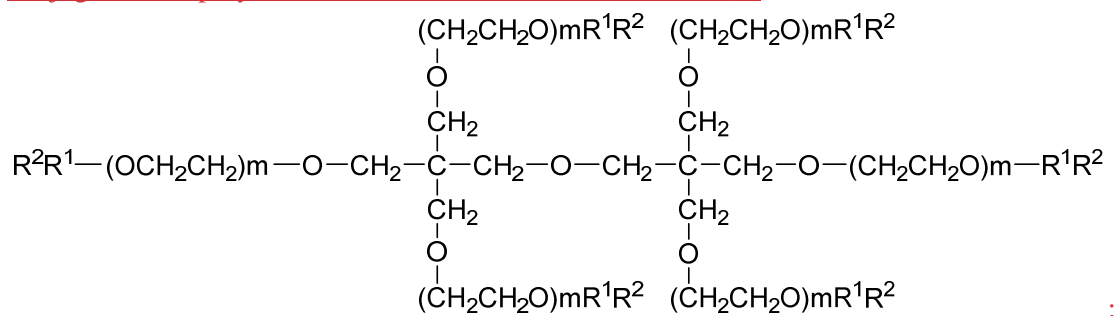
T206C, K107C, K126C, and K149C in the LC; wherein the residue number of the engineered cysteine is according to EU numbering.

In some [embodimentexamples](#), the conjugate comprises a multi-armed polyol that is covalently linked to the at least two anti-Tie2 antibody fragments or Fabs through a free amino group of a lysine amino acid. In other [embodimentexamples](#), the lysine amino acid is in the constant region of the anti-Tie2 antibody fragment or Fab. In still other [embodimentexamples](#), the lysine amino acid is at the C-terminus of the heavy chain or the light chain of the anti-Tie2 antibody fragment or Fab. In an alternative [embodimentexample](#), the Tie2 binding agent is not covalently linked to the at least two anti-Tie2 Fabs through a free amino group of a lysine.

In some [embodimentexamples](#), the conjugate deconjugates less than 20%, less than 15%, or less than 10% per month at physiological conditions in vitro. In other [embodimentexamples](#), the conjugate deconjugates less than 20%, less than 15%, or less than 10% per month at physiological conditions in vivo.

In some [embodimentexamples](#), the conjugate is stable over an extended period of time, with loss of Tie2 binding capacity of less than 20%, less than 15%, or less than 10% per month at physiological conditions.

In one aspect, the present disclosure provides a conjugate which binds Tie2 comprising: a Fab comprising a HC having SEQ ID NO:55 and a LC having SEQ ID NO:25; conjugated to a polyol which has the structure of Formula 1(b)



where each m is independently an integer from 20-30, where R¹ and R² taken together

has the structure $\text{S}-(\text{CH}_2)_i-\text{N}-\overset{\text{O}}{\parallel}{\text{C}}-(\text{CH}_2)_j-\text{R}^2$, where R² is a maleamide; and wherein the polyol is linked to the Fab HC at residue C209 (EU numbering).

In one [aspectexample](#), a conjugate comprising an anti-Tie2 antibody or antigen-binding fragment thereof and a multi-armed moiety is provided, wherein the multi-armed moiety comprises an IgM molecule. In other [embodimentexamples](#), the IgM molecule comprises a J chain and the multi-armed moiety comprises 5 anti-Tie2 Fabs, wherein approximately each of the 5 anti-Tie2 Fabs is linked to the IgM molecule. In still other [embodimentexamples](#), the IgM molecule does not comprise a J chain and the multi-armed moiety comprises 6 anti-Tie2 Fabs, wherein each of the 6 anti-Tie2 Fabs is linked to the IgM molecule.

In some [embodimentexamples](#), the conjugate comprises an IgM molecule which is an IgM variant that has an amino acid substitution whereby the IgM variant reduces or eliminates complement-dependent cytotoxicity (CDC) activity. In preferred [embodimentexamples](#), the IgM variant comprises the substitution P436G based on EU numbering.

In one [aspectexample](#), a conjugate comprising an anti-Tie2 antibody or antigen-binding fragment thereof and a multi-armed moiety is provided, wherein the multi-armed moiety comprises at least 2, 4, 6, 8 or 10 peptides, wherein approximately each of the peptides is covalently linked to an anti-Tie2 Fab. In some [embodimentexamples](#), the anti-Tie2 Fab sequence ends at residue 221, 222, 223, 224, or 225 (EU numbering). In other [embodimentexamples](#), each of the peptides is a nucleoside diphosphate kinase (NDK) peptide. In still other [embodimentexamples](#), each of

the NDK peptides comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:71.

In some [embodimentexamples](#), each of the NDK peptides is linked at its N-terminal end to the C-terminal end of the anti-Tie2 Fab heavy chain or light chain. In other [embodimentexamples](#), a linker is present between the Fab heavy chain or light chain and the peptide. In still other [embodimentexamples](#), the linker is an amino acid linker. In yet other [embodimentexamples](#), the amino acid linker comprises 2, 3, 4, 5, 6, 7, 8, 2 to 20, 5 to 10 or 4 to 10 amino acids. In other [embodimentexamples](#), the linker comprises glycines.

In some [embodimentexamples](#), the multi-armed moiety comprises 6 or 8 peptides. In other [embodimentexamples](#), the multi-armed moiety comprises 6 peptides. In a preferred [embodimentexample](#), the multi-armed moiety comprises 6 NDK peptides.

In one [aspectexample](#), a conjugate comprising an antibody or antigen-binding fragment thereof and a multi-armed moiety is provided, wherein the multi-armed moiety comprises an IgM molecule. In other [embodimentexamples](#), the IgM molecule comprises a J chain and the multi-armed moiety comprises 5 antibody fragments, Fabs, or antigen-binding fragments thereof, wherein approximately each of the 5 antibody fragments, Fabs, or antigen-binding fragments thereof is linked to the IgM molecule. In still other [embodimentexamples](#), the IgM molecule does not comprise a J chain and the multi-armed moiety comprises 6 antibody fragments, Fabs, or antigen-binding fragments thereof, wherein each of the 6 antibody fragments, Fabs, or antigen-binding fragments thereof is linked to the IgM molecule.

In some [embodimentexamples](#), the conjugate comprises an IgM molecule which is an IgM variant that has an amino acid substitution whereby the IgM variant reduces or eliminates complement-dependent cytotoxicity (CDC) activity. In preferred [embodimentexamples](#), the IgM variant comprises the substitution P436G based on EU numbering.

In one [aspectexample](#), a conjugate comprising an antibody, antibody fragments, Fabs, or antigen-binding fragments thereof and a multi-armed moiety is provided, wherein the multi-armed moiety comprises at least 2, 4, 6, 8 or 10 peptides, wherein approximately each of the peptides is covalently linked to the antibody, antibody fragments, Fabs, or antigen-binding fragments thereof. In some [embodimentexamples](#), the antibody, antibody fragments, Fabs, or antigen-binding fragments thereof sequence ends at residue 221, 222, 223, 224, or 225 (EU numbering). In other [embodimentexamples](#), each of the peptides is a nucleoside diphosphate kinase (NDK) peptide. In still other [embodimentexamples](#), each of the NDK peptides comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:71.

In some embodimentexamples, each of the NDK peptides is linked at its N-terminal end to the C-terminal end of the heavy chain or light chain of the antibody, antibody fragments, Fabs, or antigen-binding fragments thereof. In other embodimentexamples, a linker is present between the Fab heavy chain or light chain and the peptide. In still other embodimentexamples, the linker is an amino acid linker. In yet other embodimentexamples, the amino acid linker comprises 2, 3, 4, 5, 6, 7, 8, 2 to 20, 5 to 10 or 4 to 10 amino acids. In other embodimentexamples, the linker comprises glycines.

In some embodimentexamples, the multi-armed moiety comprises 6 or 8 peptides. In other embodimentexamples, the multi-armed moiety comprises 6 peptides. In a preferred embodimentexample, the multi-armed moiety comprises 6 NDK peptides.

In one aspect, the present disclosure provides a pharmaceutical composition comprising the conjugate as disclosed herein and a pharmaceutically acceptable carrier. In one aspectexample, a pharmaceutical composition is provided comprising an anti-Tie2 antibody or fragment thereof according to the present disclosure and a pharmaceutically acceptable carrier, excipient, or diluent. In some embodimentexamples, the pharmaceutical composition comprises an anti-Tie2 binding agent which comprises a plurality of anti-Tie2 Fabs linked to a multi-armed moiety as described herein, and a pharmaceutically acceptable carrier.

In some embodimentexamples, the pharmaceutical composition comprising the anti-Tie2 antibody or the Tie2 binding agent further comprises an additional therapeutic agent. In other embodimentexamples, the additional therapeutic agent is selected from the group consisting of an anti-VEGF antibody, an anti-Tie2 antibody, and an anti-Ang2 antibody. In some embodimentexamples, the additional therapeutic agent is selected from an Ang2 antagonist, a VEGF antagonist, a VEGF trap, an anti-VEGF antibody, an anti-Ang2 antibody, and a complement component antagonist.

In some embodimentexamples, any of the preceding pharmaceutical compositions can be used as a medicament.

In some embodimentexamples, any of the preceding pharmaceutical compositions can be used in the manufacture of a medicament for treating an ocular disorder in a subject.

In some embodimentexamples, any of the preceding pharmaceutical compositions can be used in reducing or inhibiting pathologic vascular permeability in a subject having an ocular disorder.

In another aspectexample, any of the preceding pharmaceutical compositions can be used in treating an ocular disorder in a subject.

In one aspect, the present disclosure provides a long acting delivery device for ocular delivery comprising the pharmaceutical composition as disclosed herein and a means for delivering the composition intravitreally to a patient, wherein the composition remains effective on site for a prolonged period of time.

In one aspect, the present disclosure provides use of the antibody or antigen-binding fragment thereof as disclosed herein, the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a Tie2 pathway-mediated disorder in a subject in need thereof.

In one aspect, the present disclosure provides the antibody as disclosed herein or the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, for use as a medicament.

In one aspect, the present disclosure provides the antibody as disclosed herein or the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, for use in treating a Tie2 pathway-mediated disorder and/or an ocular disorder in a subject in need thereof.

In one aspect, the present disclosure provides use of the antibody as disclosed herein or the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for the treatment of a Tie2 pathway-mediated disorder and/or an ocular disorder in a subject in need thereof.

In one aspectexample, a method for treating an individual in need thereof is provided comprising administering to the patient an anti-Tie2 antibody and/or a Tie2 binding agent as described herein. In some embodimentexamples, the method comprises administering to the individual a pharmaceutical composition as described herein comprising an anti-Tie2 conjugate as described herein.

In some embodimentexamples, the individual has been diagnosed with a vascular disorder. In other embodimentexamples, the individual has been diagnosed with a vascular disorder of the eye.

In another aspectexample, the inventiondisclosure features a method of inhibiting vascular permeability in a subject suffering from a disorder associated with undesirable vascular permeability, the method comprising administering to the subject an effective amount of any one of the preceding antibodies or conjugates, thereby inhibiting vascular permeability in the subject.

In another aspectexample, the inventiondisclosure features a method of treating a disorder associated with undesirable vascular permeability, the method comprising administering an effective amount of any one of the preceding antibodies or conjugates to a subject in need of such treatment.

In some embodimentexamples, the individual has been diagnosed with a disorder associated with the Tie2 pathway.

In some embodimentexamples, the individual has been diagnosed with a an eye disorder selected from the group consisting of diabetic macular edema (DME), age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, choroidal neovascularization (CNV), uveitis, diabetic retinopathy, ischemia-related retinopathy, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, glaucoma, retinopathy in the absence of edema, and retinal neovascularization.

In some embodimentexamples, the individual has received treatment with an anti-VEGF antibody. In other embodimentexamples, the individual was did not experience therapeutic efficacy of the anti-VEGF antibody, experiences reduced therapeutic efficacy of the anti-VEGF antibody, and/or stopped experiencing therapeutic efficacy of the anti-VEGF antibody.

In some embodimentexamples, the method further comprises administering to the individual a second therapeutic agent. In other embodimentexamples, the second therapeutic agent is selected from the group consisting of an anti-VEGF antibody, an anti-Ang2 antibody, an anti-VEGF/anti-Ang2 bispecific antibody, a VEGF antagonist and an Ang2 antagonist.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Provides a schematic illustration of Tie2 domains used in panning VH libraries.

FIGS. 2A-2B show results of an assay to assess binding by anti-Tie2 antibodies Tie2.1, Tie2.10, Tie2.11 and Tie2.12 to mouse Tie2 ECD5 domain (FIG. 2A) and to human Tie2 ECD5 domain (FIG. 2B). The ECD5 domain comprises the Ig1, Ig2, EGF and Ig3 domains of Tie2.

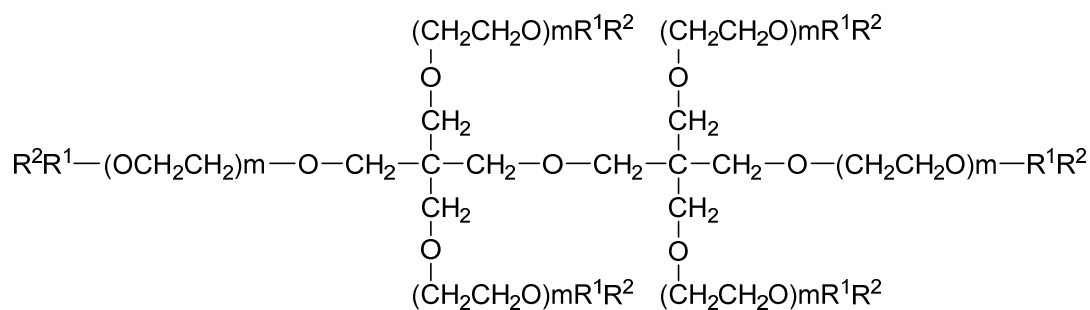
WHAT IS CLAIMED IS:

1. An isolated antibody or antigen-binding fragment thereof that specifically binds to Tie2, wherein the antibody or fragment thereof comprises:
a heavy chain variable domain (VH) comprising (a) CDR-H1 comprising the amino acid
5 sequence NTDIS (SEQ ID NO:3), (b) CDR-H2 comprising the amino acid sequence
RISPSDGNTYYADSVKG (SEQ ID NO:4), and (c) CDR-H3 comprising the amino acid
sequence RTRWASX1AX2DY (SEQ ID NO:5), where X1 is M, L, K, F, Y, R, N, Q, H or W
and/or or X2 is F, Y L, Q, I, K, or H, and a light chain variable domain (VL) comprising (d)
CDR-L1 comprising the amino acid sequence RASQDVSTAVA (SEQ ID NO:8), (e) CDR-L2
10 comprising the amino acid sequence SASFLYS (SEQ ID NO:9), and (f) CDR-L3 comprising
the amino acid sequence QQSYTTPPT (SEQ ID NO:10).
2. The antibody or antigen-binding fragment thereof according to claim 1, wherein the
CDR-H3 comprises the amino acid sequence RTRWASWAMDY (SEQ ID NO:6).
3. The antibody or antigen-binding fragment thereof according to claim 1 or 2, wherein
15 the CDR-H3 comprises the amino acid sequence RTRWASWAFDY (SEQ ID NO:7).
4. The antibody or antigen-binding fragment thereof of any one of the preceding claims,
which is a monoclonal antibody, a humanized antibody, an antibody fragment, and/or a
chimeric antibody.
5. The antibody or antigen-binding fragment thereof of any one of the preceding claims,
20 which is a Fab fragment that binds Tie2.
6. The antibody or antigen-binding fragment thereof of any one of claims 1 to 5,
comprising a VL domain comprising an amino acid sequence having at least 90% sequence
identity to the amino acid sequence of SEQ ID NO:21 and a VH domain comprising an amino
acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID
25 NO:20.
7. The antibody or antigen-binding fragment thereof of any one of claims 1 to 6,
comprising a VL domain comprising an amino acid sequence having at least 99% sequence
identity to the amino acid sequence of SEQ ID NO:21 and a VH domain comprising an amino
acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID
30 NO:20.
8. The antibody or antigen-binding fragment thereof of any one of the preceding claims,
wherein the antibody comprises the VL sequence of SEQ ID NO:21 and a VH sequence of
SEQ ID NO:20.

9. The antibody or antigen-binding fragment thereof of any one of claims 1 to 8, wherein the antibody comprises an engineered cysteine, wherein
the engineered cysteine is selected from a T120C, G166C, G178C, T187C, and T209C in the HC; or
5 the engineered cysteine is selected from Q124C, R142C, Q155C, L201C, T206C, K107C, K126C, and K149C in the LC;
wherein the residue number of the engineered cysteine is according to EU numbering.
10. The antibody or antigen-binding fragment thereof of claim 9, wherein the engineered cysteine is selected from T209C in the HC and T206C in the LC.
- 10 11. The antibody or antigen-binding fragment thereof of claim 9 or 10, wherein the engineered cysteine is T206C in the LC.
12. The antibody or antigen-binding fragment thereof of claim 9 or 10, wherein the engineered cysteine is T209C in the HC.
13. The antibody or antigen-binding fragment thereof of any one of the preceding claims,
15 comprising a LC comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:25 and a HC comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:55.
14. The antibody or antigen-binding fragment thereof of any one of the preceding claims, comprising a LC comprising an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO:25 and a HC comprising an amino acid sequence
20 having at least 99% sequence identity to the amino acid sequence of SEQ ID NO:55.
15. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody comprises a LC comprising the sequence of SEQ ID NO:25 and a HC comprising the sequence of SEQ ID NO:55.
- 25 16. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the HC constant regions terminates at position 221 (EU numbering).
17. An antibody or antigen-binding fragment thereof that specifically binds to Tie-2 wherein the antibody comprises a HC sequence of SEQ ID NO:55 and a LC sequence of SEQ ID NO:25.
- 30 18. An antibody or antigen-binding fragment thereof that specifically binds to Tie-2 wherein the antibody comprises a HC sequence of SEQ ID NO:23 and a LC sequence of SEQ ID NO:56.
19. An isolated nucleic acid encoding the antibody or antigen-binding fragment thereof of any of the preceding claims.

20. A host cell comprising the nucleic acid of claim 19.
21. A conjugate that binds to Tie2, wherein the conjugate comprises at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight antibodies or antigen-binding fragment thereof according to any one of claims 1 to 18, wherein each of the
- 5 antibodies or antigen-binding fragment thereof is linked to a multimerizing moiety.
22. The conjugate of claim 21, wherein the conjugate causes a reduction in Tie2 protein levels in an in vitro assay by less than 25%, less than 50% or less than 75%.
23. The conjugate of claim 21 or 22, wherein the conjugate does not cause a reduction in Tie-2 protein levels in an in vitro assay by more than 25%, more than 50%, or more than 75%.
- 10 24. The conjugate of any one of claims 21 to 23, wherein the multimerizing moiety comprises a polyol, a polypeptide, and/or a peptide.
25. The conjugate of any one of claims 21 to 24, wherein the multimerizing moiety comprising a polyol, wherein the polyol is a multi-armed polyol, selected from a dimer, a tetramer, a hexamer, and an octamer.
- 15 26. The conjugate of claim 25, wherein the multi-armed polyol is a hexamer.
27. The conjugate of any one of claims 24 to 26, wherein the polyol is a polyethylene glycol (PEG).
28. The conjugate of any one of claims 24 to 27, wherein the polyol is covalently linked to the at least two antibodies or antigen-binding fragment thereof through a free sulfhydryl group
- 20 of an engineered cysteine amino acid, wherein the engineered cysteine is selected from the group consisting of
- T120C, G166C, G178C, T187C, and T209C in the HC; or
- the engineered cysteine is selected from the group consisting of Q124C, R142C, Q155C, L201C, T206C, K107C, K126C, and K149C in the LC;
- 25 wherein the residue number is according to EU numbering.
29. The conjugate of claim 28, wherein the engineered cysteine is T206C in the LC, wherein the residue number is according to EU numbering.
30. The conjugate of claim 28, wherein the engineered cysteine is T209C in the HC, wherein the residue number is according to EU numbering.
- 30 31. The conjugate of any one of claims 25 to 27, wherein the polyol is covalently linked to the at least one antibody through a free amino group of a lysine amino acid.
32. The conjugate according to any one of claims 27 to 31, wherein the PEG has a weight average molecular weight of from about 500 Daltons (Da) to about 300,000 Da.

33. The conjugate according to any one of claims 27 to 32, wherein the PEG has the structure of general formula (Ib):

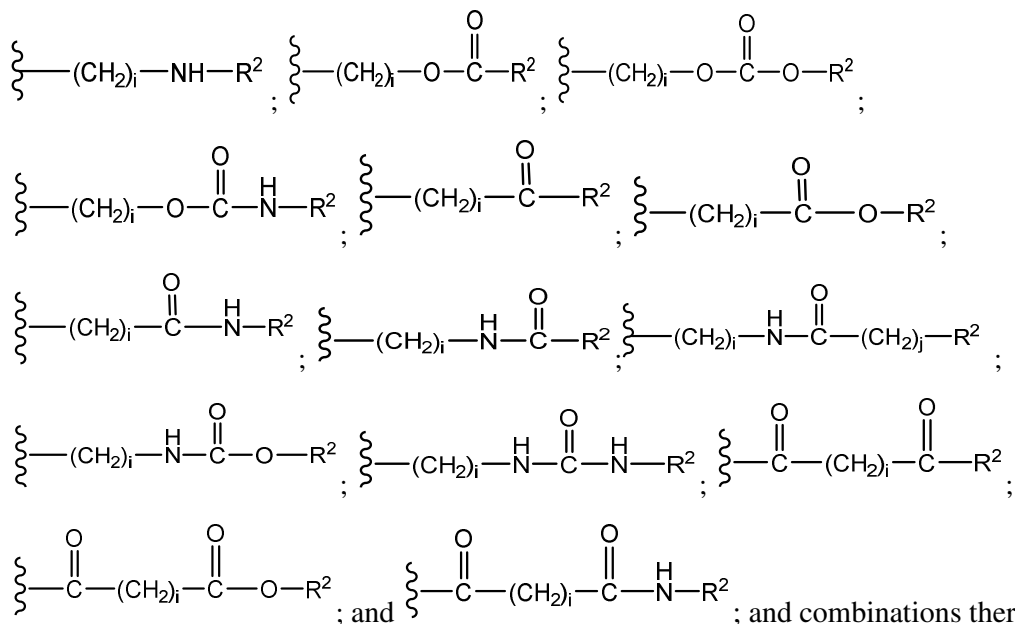


where each m is independently an integer from 3-250; each R¹ is independently either
 5 absent or is a linking group; and each R² is independently either hydrogen or a terminal reactive group;

where at least one R² is a terminal reactive group and is covalently linked to the antibody or antigen-binding fragment thereof of any one of claims 1 to 14.

34. The conjugate according to claim 33, wherein at least one R¹ is a linking group,

10 wherein R¹ and R² when taken together are selected from: $\zeta-(\text{CH}_2)-\text{R}^2$;



wherein each i is independently an integer of 0-10; j is an integer of 0-10; and R² is a terminal reactive group selected from the group consisting of a thiol reactive group, an amino reactive group, and combinations thereof.

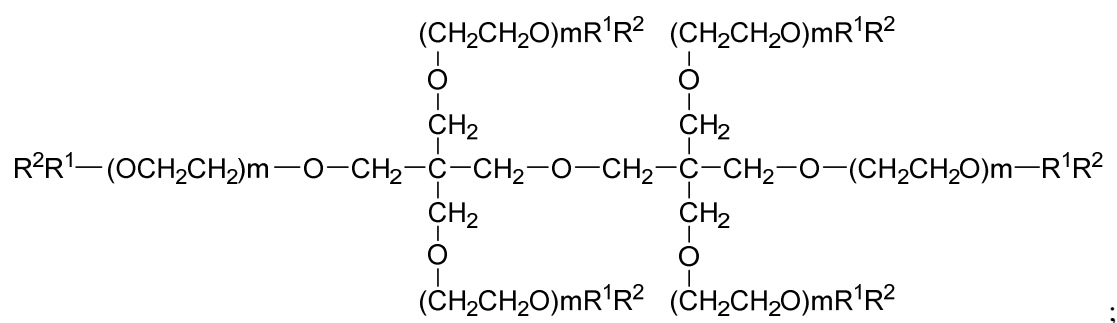
35. The conjugate according to claim 33 or claim 34, wherein at least 1 R² is a maleamide
 20 and is covalently linked to the antibody or antigen-binding fragment thereof.

36. The conjugate according to any one of claims 33 to 35, wherein each R² is a maleamide.

37. A conjugate which binds Tie2 comprising:

a Fab comprising a HC having SEQ ID NO:55 and a LC having SEQ ID NO:25;

5 conjugated to a polyol which has the structure of Formula 1(b)



where each m is independently an integer from 20-30, where R¹ and R² taken together

has the structure $\text{---}(\text{CH}_2)_i\text{---}\text{N}(\text{H})\text{---}\overset{\text{O}}{\parallel}\text{C}(\text{O})\text{---}(\text{CH}_2)_j\text{---}\text{R}^2$, where R² is a maleamide; and

wherein the polyol is linked to the Fab HC at residue C209 (EU numbering).

10 38. A pharmaceutical composition comprising the conjugate according to any one of claims 21 to 37 and a pharmaceutically acceptable carrier.

39. The pharmaceutical composition of claim 38, further comprising an additional therapeutic agent, wherein the additional therapeutic agent is selected from the group consisting of a VEGF antagonist, an Ang2 antagonist, an HtrA1 antagonist, and IL33

15 antagonist, a complement component antagonist, and a second Tie2 agonist.

40. A long acting delivery device for ocular delivery comprising the pharmaceutical composition of claim 38 or 39 and a means for delivering the composition intravitreally to a patient, wherein the composition remains effective on site for a prolonged period of time.

41. Use of the antibody or antigen-binding fragment thereof of any one of claims 1 to 18, the conjugate of any one of claims 21 to 37, or the pharmaceutical composition of claim 38 or 39, in the manufacture of a medicament for ~~A method of~~ treating a Tie2 pathway-mediated disorder in a subject in need thereof, ~~comprising administering to the subject an effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1 to 18, the conjugate of any one of claims 21 to 36, or the pharmaceutical composition of any one of~~ claims 37 to 38.

42. The ~~method-use~~ of claim 41, wherein the Tie2 pathway-mediated disorder is a vascular permeability disorder.

43. The ~~method-use~~ of claim 41 or 42, wherein the Tie2 pathway-mediated disorder is an eye condition.

44. The ~~method-use~~ of claim 43, wherein the eye condition is selected from diabetic macular edema (DME), diabetic retinopathy, age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, choroidal neovascularization (CNV), uveitis, ischemia-related retinopathy, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, glaucoma, and retinal neovascularization.

45. The ~~method-use~~ of claim 43 or 44, wherein the eye condition is DME.

46. The antibody of any one of claims 1 to 18 or the conjugate of any one of claims 21 to 3937, or the pharmaceutical composition of claim 38 or 39, for use as a medicament.

47. The antibody of any one of claims 1 to 18 or the conjugate of any one of claims 21 to 3937, or the pharmaceutical composition of claim 38 or 39, for use in treating a Tie2 pathway-mediated disorder and/or an ocular disorder in a subject in need thereof.

48. Use of the antibody of any one of claims 1 to 18 or the conjugate of any one of claims 21 to 3937, or the pharmaceutical composition of claim 38 or 39, in the manufacture of a medicament for the treatment of a Tie2 pathway-mediated disorder and/or an ocular disorder in a subject in need thereof.