therapeutic agent is relatively soluble. In addition, intraocular injections are highly unpleasant for the patient. Furthermore, as the intraocular injection itself may cause complications, such as endophthalmitis and retinal detachment, it is highly desirable to have the longest possible duration between injections, while retaining therapeutic levels of drug in the eye.

[6] In addition to the above, therapeutic agents delivered by intravitreal injections can lack duration of action since the agents can often rapidly disperse within the eye after injection. Such lack of duration is particularly undesirable since it can necessitate greater injection frequency. Ranibizumab and pegaptanib, for example, are administered to a patient via intraocular injection every 4 and 6 weeks, respectively, which is a highly unpleasant experience for the patient.

[7] Thus, there is widespread recognition that the field of ophthalmology would benefit from longer lasting formulations. They would benefit patient care and ocular health by providing extended delivery of therapeutic agents to the eye while minimizing the problems associated with patient compliance to prescribed therapeutic medical regimens.

[8] Expression of vascular endothelial growth factor (VEGF), a signal protein produced by cells that stimulates vasculogenesis and angiogenesis, plays an important role in various ocular conditions, such as in certain forms of macular degeneration and retinopathies.

[9] Various medicaments to treat such ocular conditions are on the market, such as ranibizumab, aflibercept and pegaptanib. Application to the patient occurs via intraocular injections every 4 and 8 weeks.

[10] In view of the above, there exists a need to provide a form of administration that overcomes these drawbacks at least partially.

SUMMARY

[11] The disclosure provides cross-linked hyaluronic acid (HA) drug conjugates, pharmaceutical compositions and methods of using such conjugates for treatment of ocular indications, and methods of making the conjugates.

[12] In certain examples the disclosure provides a cross-linked HA drug conjugate comprising a plurality of hyaluronic acid polymers 2A and a plurality of hyaluronic acid polymers 2B, wherein:

each 2A comprises a plurality of linearly connected units, the units consisting essentially of:

the number of Z^1 units in 2B is from about 0.75t to about 0.94t;

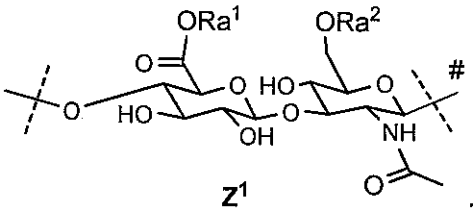
the combined number of Z^2 and Z^4 units is from about 0.25t to about 0.06t;

the number of Z^2 units is at least 0.01t; and

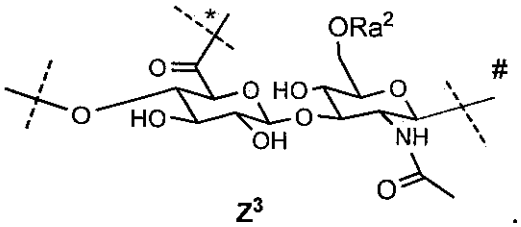
the number of Z^4 units is at least 0.01t.

[12a] In an aspect, there is provided a cross-linked HA drug conjugate comprising a plurality of hyaluronic acid polymers 2A and a plurality of hyaluronic acid polymers 2B, wherein:

each 2A comprises a plurality of linearly connected units, the units consisting essentially of:

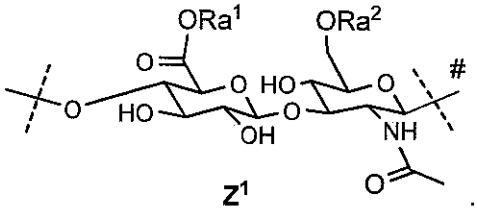


Z^1 ; and

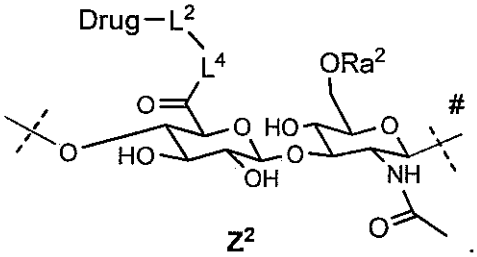


Z^3 ;

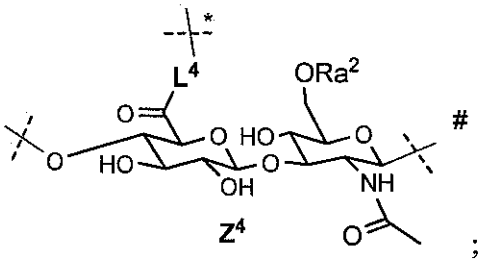
each 2B comprises a plurality of linearly connected units, the units consisting essentially of:



Z^1 ;



Z^2 ; and



Z^4 ;

wherein

an unmarked dashed line indicate a point of attachment to an adjacent unit at a dashed line marked with #, or to a hydrogen,

a dashed line marked with # indicate a point of attachment to an adjacent unit at an unmarked dashed line, or to a hydroxyl; and

a dashed line marked marked with * indicates a point of cross-linking attachment between a unit Z^3 of 2A and a unit Z^4 of 2B, such that at least one 2A is cross-linked to at least one 2B;

Drug is a therapeutic agent;

Ra^1 and Ra^2 are each independently is hydrogen; C_{1-4} alkyl; an alkali metal ion, an ammonium ion, or an alkaline earth metal ion;

L^2 is a reversible prodrug linker;

L^4 is an optionally biodegradable spacer and may be the same or different in Z^2 and Z^4;

2A comprises a total of s units wherein s is from 25 to 2500, wherein;

the number of Z^1 units in 2A is from about 0.8s to about 0.99s, and

the number of Z^3 units is from about 0.1s to about 0.01s;

2B comprising a total of t units wherein t is from 25 to 2500, wherein;

the number of Z^1 units in 2B is from about 0.75t to about 0.94t;

the combined number of Z^2 and Z^4 units is from about 0.14t to about 0.06t;

the number of Z^2 units is at least 0.01t; and

the number of Z^4 units is at least 0.01t.

[13] In certain examples, s is from 50 to 2000.

[14] In certain examples, s is from 75 to 1500.

[15] In certain examples, s is from 75 to 1000.

[16] In certain examples, s is from 80 to 500.

[17] In certain examples, s is from 100 to 250.

[18] In certain examples, s is from 100 to 200.

[19] In certain examples, s is from 200 to 800, or from 300 to 600.

[20] In certain examples, t is from 50 to 2000.

[21] In certain examples, t is from 75 to 1500.

[22] In certain examples, t is from 75 to 1000.

[23] In certain examples, t is from 80 to 500.

[24] In certain examples, t is from 100 to 250.

[25] In certain examples, t is from 100 to 200.

[26] In certain examples, t is from 200 to 800, or from 300 to 600.

[27] In certain examples, the number of Z^1 units in 2A is from about 0.91s to about 0.98s.

[28] In certain examples, the number of Z^1 units in 2A is from about 0.8s to about 0.99s.

[29] In certain examples, the number of Z^1 units in 2A is from about 0.82s to about 0.99s.

[30] In certain examples, the number of Z^1 units in 2A is from about 0.84s to about 0.99s.

[31] In certain examples, the number of Z^1 units in 2A is from about 0.86s to about 0.99s.

[32] In certain examples, the number of Z^1 units in 2A is from about 0.88s to about 0.99s.

[33] In certain examples, the number of Z^1 units in 2A is from about 0.9s to about 0.99s.In certain examples, the number of Z^1 units in 2A is from about 0.92s to about 0.97s.

[34] In certain examples, the number of Z^1 units in 2A is from about 0.93s to about 0.96s.

[35] In certain examples, the number of Z^1 units in 2A is from about 0.94s to about 0.95s.

[36] In certain examples, the number of Z^3 units in 2A is from about 0.2s to about 0.01s.

[37] In certain examples, the number of Z^3 units in 2A is from about 0.18s to about 0.01s.

[38] In certain examples, the number of Z^3 units in 2A is from about 0.16s to about 0.01s.

[39] In certain examples, the number of Z^3 units in 2A is from about 0.14s to about 0.01s.In certain examples, the number of Z^3 units in 2A is from about 0.12s to about 0.01s.

[40] In certain examples, the number of Z^3 units in 2A is from about 0.10s to about 0.01s.

[41] n certain examples, the number of Z^3 units is from about 0.09s to about 0.02s.

[42] In certain examples, the number of Z^3 units is from about 0.08s to about 0.03s.

[43] In certain examples, the number of Z^3 units is from about 0.07s to about 0.04s.

[44] In certain examples, the number of Z^1 units in 2B is from about 0.88s to about 0.92s.

[45] In certain examples, the number of Z^1 units in 2B is from about 0.86s to about 0.94s.

[46] In certain examples, the number of Z^1 units in 2B is from about 0.89s to about 0.91s.

[47] In certain examples, the number of Z^1 units in 2B is from about 0.86s to about 0.94s.

[48] In certain examples, the combined number of Z^2 and Z^4 units in 2B is from about 0.25t to about 0.05t.

[49] In certain examples, the combined number of Z^2 and Z^4 units in 2B is from about 0.23t to about 0.05t.

[50] In certain examples, the combined number of Z^2 and Z^4 units in 2B is from about 0.21t to about 0.05t.

[51] In certain examples, the combined number of Z^2 and Z^4 units in 2B is from about 0.19t to about 0.05t.

[52] In certain examples, the combined number of Z^2 and Z^4 units in 2B is from about 0.17t to about 0.05t.

[53] In certain examples, the combined number of Z^2 and Z^4 units in 2B is from about 0.15t to about 0.05t.

[54] In certain examples, the combined number of Z^2 and Z^4 units in 2B is from about 0.13t to about 0.05t.

[55] In certain examples, the combined number of Z^2 and Z^4 units is from about 0.12t to about 0.06t.

[56] In certain examples, the combined number of Z^2 and Z^4 units is from about 0.11t to about 0.07t.

[57] In certain examples, the combined number of Z^2 and Z^4 units is from about 0.25t to about 0.06t.

[58] In certain examples, the number of Z^2 units is from 0.02t to 0.12t.

[59] In certain examples, the number of Z^2 units is from 0.04t to 0.10t.

[60] In certain examples, the number of Z^2 units is from 0.06t to 0.08t.

[61] In certain examples, the number of Z^2 units is from 0.07t to 0.08t.

[62] In certain examples, the number of Z^2 units is from 0.075t to 0.08t.

[63] In certain examples, the number of Z^2 units is 0.077t.

[64] In certain examples, the number of Z^4 units is from 0.01t to 0.12t.

[65] In certain examples, the number of Z^4 units is from 0.02t to 0.12t.

[66] In certain examples, the number of Z^4 units is from 0.04t to 0.10t.

[67] In certain examples, the number of Z^4 units is from 0.06t to 0.08t.

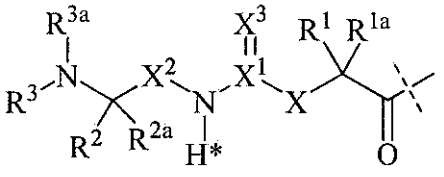
[68] In certain examples, the number of Z^4 units is from 0.01t to 0.04t.

[69] In certain examples, the number of Z^4 units is from 0.02t to 0.03t.

[70] In certain examples, the number of Z^4 units is from 0.02t to 0.025t.

[71] In certain examples, the number of Z^4 units is 0.023t.

[72] In certain examples, the reversible prodrug linker L^2 coupling the drug to spacer L^4 is of formula XIIa



XIIa

wherein

the dashed line indicates the attachment to a nitrogen of a drug compound (not shown) by forming an amide bond;

-X- is $-C(R^4R^{4a})-$; $-N(R^4)-$; -O-; $-C(R^4R^{4a})-C(R^5R^{5a})-$; $-C(R^5R^{5a})-C(R^4R^{4a})-$; $-C(R^4R^{4a})-N(R^6)-$; $-N(R^6)-C(R^4R^{4a})-$; $-C(R^4R^{4a})-O-$; $-O-C(R^4R^{4a})-$; or $-C(R^7R^{7a})-$;

X^1 is C; or S(O);

$-X^2-$ is $-C(R^8R^{8a})-$; or $-C(R^8R^{8a})-C(R^9R^{9a})-$;

$=X^3$ is =O; =S; or =N-CN;

$-R^1$, $-R^{1a}$, $-R^2$, $-R^{2a}$, $-R^4$, $-R^{4a}$, $-R^5$, $-R^{5a}$, $-R^6$, $-R^8$, $-R^{8a}$, $-R^9$, $-R^{9a}$ are independently selected from the group consisting of -H; and C_{1-6} alkyl;

$-R^3$, $-R^{3a}$ are independently selected from the group consisting of -H; and C_{1-6} alkyl, provided that in case one of $-R^3$, $-R^{3a}$ or both are other than -H they are connected to N to which they are attached through an SP^3-hybridized carbon atom;

$-R^7$ is $-N(R^{10}R^{10a})$; or $-NR^{10}-(C=O)-R^{11}$;

$-R^{7a}$, $-R^{10}$, $-R^{10a}$, $-R^{11}$ are independently of each other -H; or C_{1-10} alkyl;

optionally, one or more of the pairs $-R^{1a}/-R^{4a}$, $-R^{1a}/-R^{5a}$, $-R^{1a}/-R^{7a}$, $-R^{4a}/-R^{5a}$, $-R^{8a}/-R^{9a}$ form a chemical bond;

optionally, one or more of the pairs $-R^1/-R^{1a}$, $-R^2/-R^{2a}$, $-R^4/-R^{4a}$, $-R^5/-R^{5a}$, $-R^8/-R^{8a}$, $-R^9/-R^{9a}$ are joined together with the atom to which they are attached to form a C_{3-10} cycloalkyl; or 3- to 10-membered heterocyclyl;

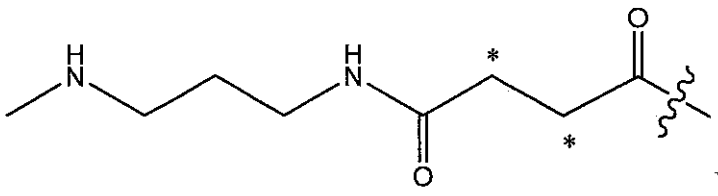
optionally, one or more of the pairs $-R^1/-R^4$, $-R^1/-R^5$, $-R^1/-R^6$, $-R^1/-R^{7a}$, $-R^4/-R^5$, $-R^4/-R^6$, $-R^8/-R^9$, $-R^2/-R^3$ are joined together with the atoms to which they are attached to form a ring A;

optionally, R^3/R^{3a} are joined together with the nitrogen atom to which they are attached to form a 3- to 10-membered heterocycle;

Ring A is selected from the group consisting of phenyl; naphthyl; indenyl; indanyl; tetralinyl; C_{3-10} cycloalkyl; 3- to 10-membered heterocyclyl; and 8- to 11-membered heterobicyclyl; and wherein the group of formula XIIa is substituted with $-L^4$ provided that the hydrogen marked with the asterisk in formula (XIIa) is not replaced by $-L^4$ or other substituent;

wherein $-L^4-$ is a single chemical bond or a spacer moiety as defined herein.

[73] In certain examples, the reversible prodrug linker L^2 is of the formula VIIa:

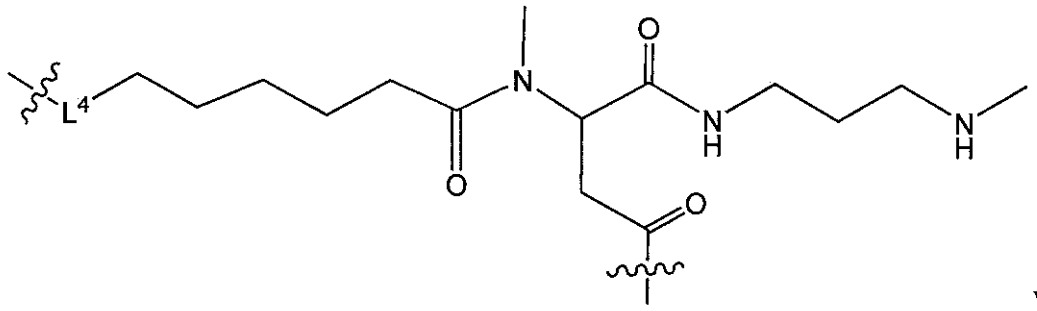


VIIb

wherein:

each asterisk represents an independent site of attachment to the spacer L^4.

[74] IIn certain examples the reversible prodrug linker L^2 together with spacer L^4 is of the formula VIIc:



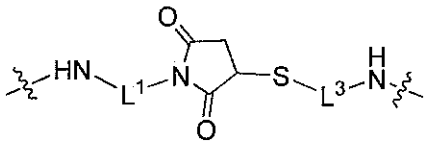
VIIc.

wherein:

the right-most wavy line represents the point of attachment to the nitrogen atom of Drug; and

the left-most wavy line represents the point of attachment to a unit Z^2 of hyaluronic acid 2B.

[75] In certain examples, the spacer L^4 connecting hyaluronic acid polymer 2A to hyaluronic acid polymer 2B (by connecting unit Z^3 of polymer 2A to unit Z^4 of polymer 2B) is of the formula:



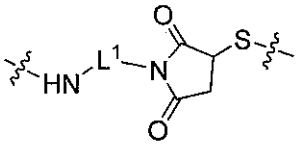
wherein:

the right-most wavy line represents the point of attachement to a unit Z^3 on hyaluronic acid polymer 2A; and

the left-most wavy line represents the point of attachment to a unit Z^4 on hyaluronic acid polymer 2B.

[76] The spacer L^4 connecting hyaluronic acid polymer 2A to hylauronic acid polymer 2B may, in certain examples, be reversed in orientation such that the right-most wavy line of the above formula represents the point of attachement to a unit Z^4 on hyaluronic acid polymer 2B; and the left-most wavy line represents the point of attachment to a unit Z^3 on hyaluronic acid polymer 2A.

[77] In certain examples, the spacer L_4 joining reversible prodrug linker L^2 to hyaluronic acid polymer 2B is of the formula



wherein:

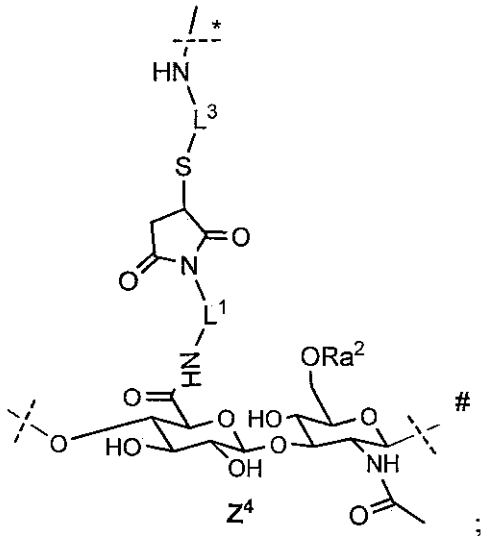
the right-most wavy line represents the point of attachement to L^2; and

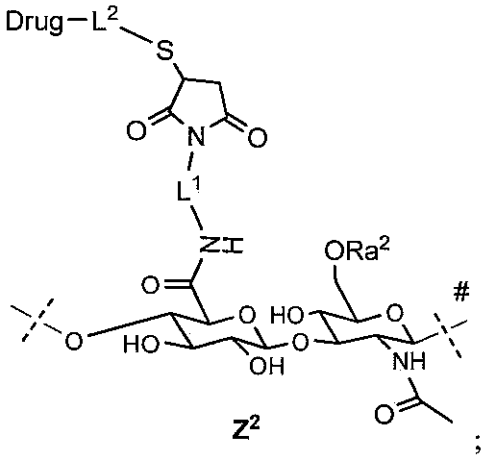
the left-most wavy line represents the point of attachment to a unit Z^2 on hyaluronic acid polymer 2B;

wherein:

L^1 is a spacer; and

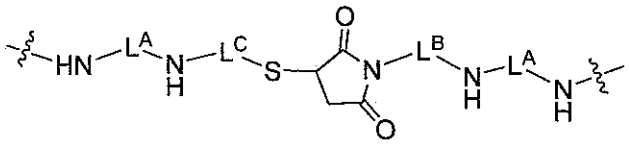
L^3 is a biodegradable spacer.

[78] In certain examples, the unit Z^4 is  ; wherein L^1, L^3 and Ra^2 are as defined herein.

[79] In certain examples the unit Z^2 is  ;

wherein L^1, L^2, L^3 and Ra^2 are as defined herein.

[80] In certain examples, the spacer L_4 connecting hyaluronic acid polymer 2A to Hylauronic acid polymer 2B is of the formula:



wherein:

the right-most wavy line represents the point of attachement to a unit Z^3 on hyaluronic acid polymer 2B; and

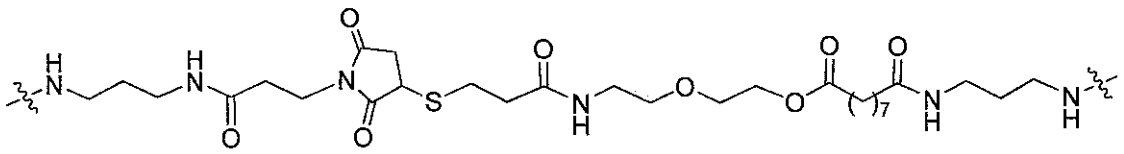
the left-most wavy line represents the point of attachment to a unit Z^4 on hyaluronic acid polymer 2A;

L^A is a spacer;

L^B is a spacer; and

L^C is a biodegradable spacer.

[81] In certain examples, the spacer L^4 connecting hyaluronic acid polymer 2A to hylauronic acid polymer 2B is of the formula:

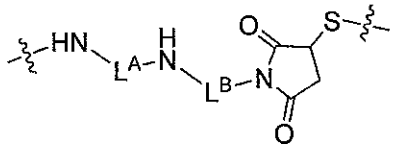


wherein:

the right-most wavy line represents the point of attachement to a unit Z^3 on hyaluronic acid polymer 2A; and

the left-most wavy line represents the point of attachment to a unit Z^4 on hyaluronic acid polymer 2B.

[82] In certain examples the spacer L^4 joining reversible prodrug linker L^2 to hyaluronic acid polymer 2A is of the formula



wherein:

the right-most wavy line represents the point of attachement to L^2; and

the left-most wavy line represents the point of attachment to a unit Z^2 on hyaluronic acid polymer 2B;

wherein:

L^A is a spacer;

L^B is a spacer; and

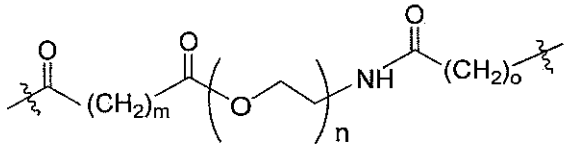
L^C is a biodegradable spacer.

[83] In certain examples, L^A is optionally substituted and/or optionally interrupted C_{1-10} alkylene.

[84] In certain examples, L^A is linear C_{2-4} alkylene.

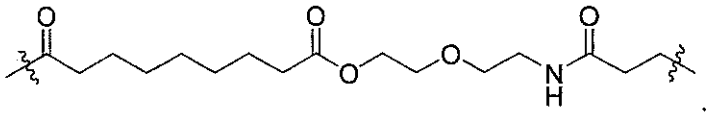
[85] In certain examples, L^B is linear -(O)-C_{1-5} alkylene.

[86] In certain examples, L^C is:

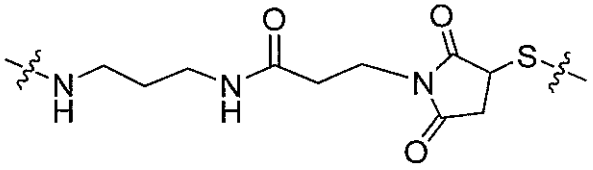


wherein m is from 0 to 10, n is from 1 to 4, and o is from 1 to 4.

[87] In certain examples, L^C is:



[88] In certain examples the spacer L_4 joining reversible prodrug linker L^2 to hyaluronic acid polymer 2B is of the formula

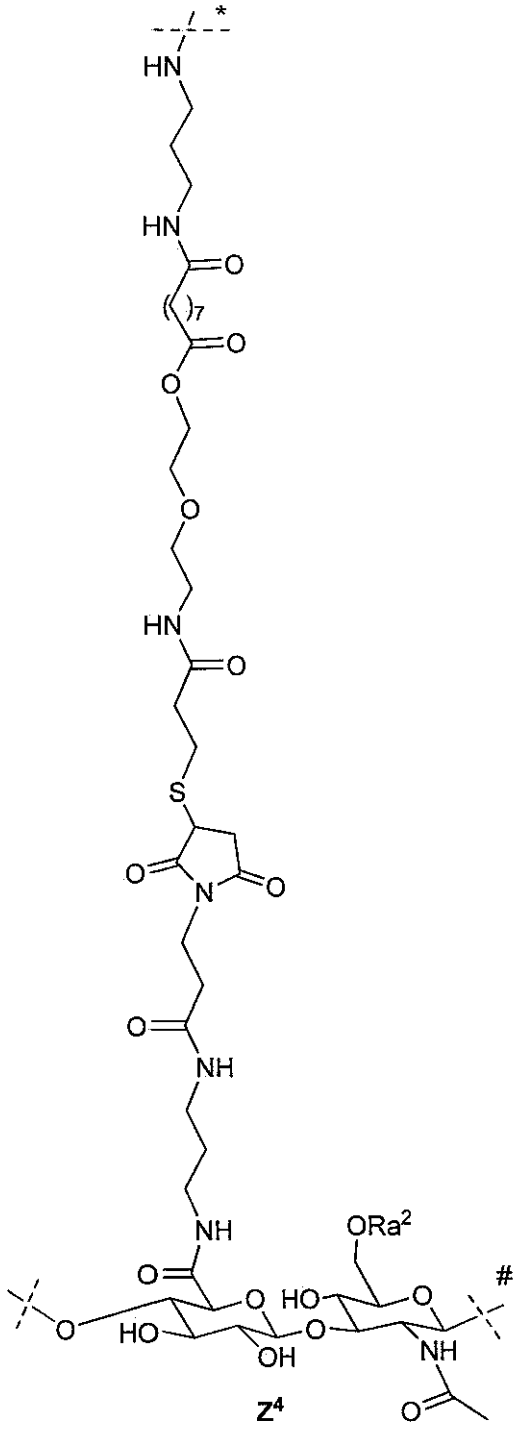


wherein:

the right-most wavy line represents the point of attachement to a unit Z^3 on hyaluronic acid polymer 2A; and

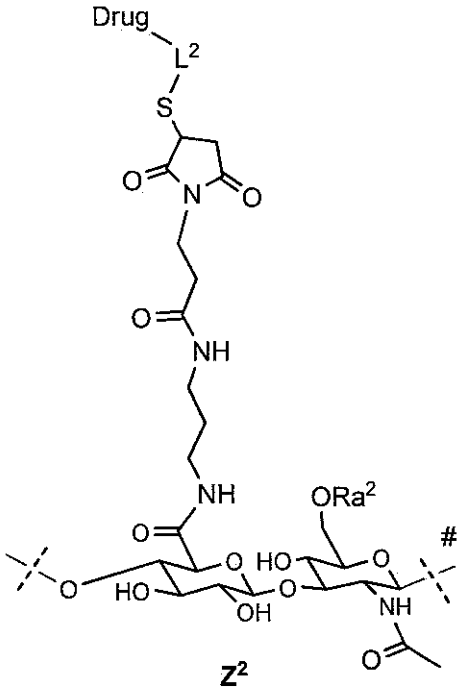
the left-most wavy line represents the point of attachment to a unit Z^4 on hyaluronic acid polymer 2B.

[89] In certain examples, the unit Z^4 is:



wherein Ra^2 is as defined herein..

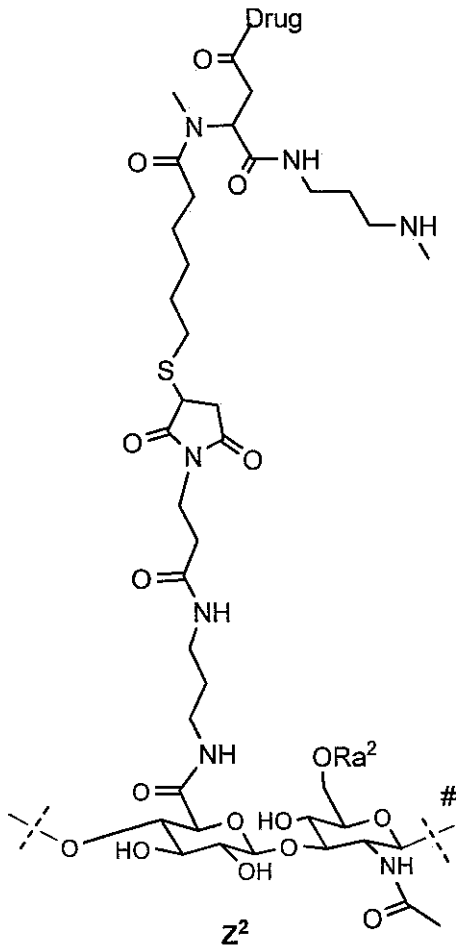
[90] In certain examples, the unit Z^2 is

[91] 

Z^2 ;

wherein L^2, Ra^2 and Drug are as defined herein.

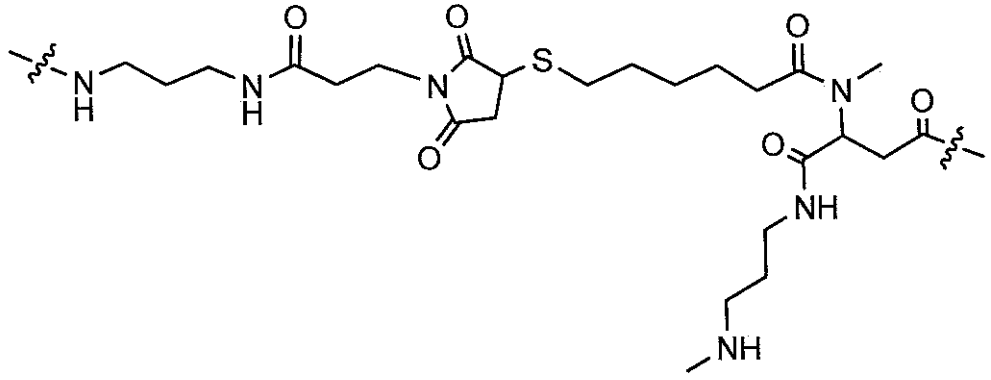
[92] In certain examples, the unit Z^2 is



Z^2

wherein Ra^2 and Drug are as defined herein.

[93] In certain examples, the combined reversible prodrug linker L^2 together with spacer L^4 is of the formula VIId



VIId

wherein:

the right-most wavy line represents the point of attachment to the nitrogen atom of Drug; and

the left-most wavy line represents the point of attachment to a unit Z^2 of hyaluronic acid 2B.

[94] In certain examples, drug is an antibody.

[95] In certain examples, the antibody is a VEGF antagonist.

[96] In certain examples, the antibody is an anti-VEGF antibody fragment.

[97] In certain examples, the antibody fragment is a Fab antibody fragment.

[98] In certain examples, the Fab antibody fragment is ranibizumab or LUCENTIS ®.

[99] In certain examples, the antibody comprises the following six hypervariable regions (HVRs):

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of $GX_1TPX_2GGX_3X_4X_5YX_6DSVX_7X_8$ (SEQ ID NO: 2), wherein X_1 is Ile or His, X_2 is Ala or Arg, X_3 is Tyr or Lys, X_4 is Thr or Glu, X_5 is Arg, Tyr, Gln, or Glu, X_6 is Ala or Glu, X_7 is Lys or Glu, and X_8 is Gly or Glu;

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQX$_1$VSTAVA (SEQ ID NO: 4), wherein X$_1$ is Asp or Arg;

(e) an HVR-L2 comprising the amino acid sequence of X$_1$ASFLYS (SEQ ID NO: 5), wherein X$_1$ is Ser or Met; and

(f) an HVR-L3 comprising the amino acid sequence of X$_1$QGYGX$_2$PFT (SEQ ID NO: 6), wherein X$_1$ is Gln, Asn, or Thr and X$_2$ is Ala, Asn, Gln, or Arg.

[100] In certain examples, the antibody comprises the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7), GITPAGGYEYYADSVKG (SEQ ID NO: 21), or GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10) or QQGYGNPFT (SEQ ID NO: 23).

[101] In certain examples, the antibody comprises the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10).

[102] In certain examples, the antibody further comprises the following heavy chain variable (VH) domain framework regions (FRs):

(a) an FR-H1 comprising the amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTIS (SEQ ID NO: 13);

(b) an FR-H2 comprising the amino acid sequence of WVRQAPGKGLEWVA (SEQ ID NO: 14);

(c) an FR-H3 comprising the amino acid sequence of RFTISADTSKNTAYLQMRSLRAEDTAVYYCAR (SEQ ID NO: 15); and

(d) an FR-H4 comprising the amino acid sequence of WGQGTLVTVSS (SEQ ID NO: 16).

[103] In certain examples, the antibody further comprises the following light chain variable (VL) domain FRs:

(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17);

(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18);

(c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19); and

(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

[104] In certain examples, the antibody comprises the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGNPFT (SEQ ID NO: 23).

[105] In certain examples, the antibody further comprises the following VL domain FRs:

(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17);

(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18);

(c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO: 24); and

(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

[106] In certain examples, the antibody comprises the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10).

[107] In certain examples, the antibody further comprises the following VL domain FRs:

(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17), DIQMTQSPESLSASVGDEVTITC (SEQ ID NO: 25), or DIQMTQSPSSLSASVGDEVTITC (SEQ ID NO: 26);

(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18) or WYQQKPGEAPKLLIY (SEQ ID NO: 27);

(c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19) or GVPSRFSGSGSGTDFTLTIESLQPEDAATYYC (SEQ ID NO: 28); and

(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

[108] In certain examples, the antibody further comprises the following VH domain FRs:

(a) an FR-H1 comprising the amino acid sequence of EEQLVEEGGGLVQPGESLELSCAASGFEIS (SEQ ID NO: 29) or EEQLVEEGGGLVQPGESLRLSCAASGFEIS (SEQ ID NO: 51);

(b) an FR-H2 comprising the amino acid sequence of WVRQEPGEGLEWVA (SEQ ID NO: 30);

(c) an FR-H3 comprising the amino acid sequence of RFTISADTSENTAYLQMNELRAEDTAVYYCAR (SEQ ID NO: 31); and

(d) an FR-H4 comprising the amino acid sequence of WGQGELVTVSS (SEQ ID NO: 32).

[109] In certain examples, the antibody further comprises the following VH domain FRs:

(e) an FR-H1 comprising the amino acid sequence of EEQLVEEGGGLVQPGESLELSCAASGFEIS (SEQ ID NO: 29) or EEQLVEEGGGLVQPGESLRLSCAASGFEIS (SEQ ID NO: 52);

(f) an FR-H2 comprising the amino acid sequence of WVRQEPGEGLEWVA (SEQ ID NO: 30);

(g) an FR-H3 comprising the amino acid sequence of RFTISADTSENTAYLQMNELRAEDTAVYYCAR (SEQ ID NO: 31); and

(h) an FR-H4 comprising the amino acid sequence of WGQGELVTVSS (SEQ ID NO: 32).

[110] In certain examples, the antibody comprises (a) a VH domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11, 40, or 42; (b) a VL domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 12, 41, or 46; or (c) a VH domain as in (a) and a VL domain as in (b).

[111] In certain examples, the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO:11, 40, or 42; (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 12, 41, or 46; or (c) a VH domain as in (a) and a VL domain as in (b).

[112] In certain examples, the antibody comprises a VH domain comprising the amino acid sequence of SEQ ID NO:11 and a VL domain comprising the amino acid sequence of SEQ ID NO:12.

[113] In certain examples, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:48 and a light chain comprising the amino acid sequence of SEQ ID NO:50.

[114] In certain examples, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:49 and a light chain comprising the amino acid sequence of SEQ ID NO:50.

[115] In certain examples, the antibody-hydrogel conjugate has an ocular effective half-life that is increased relative to a reference antibody that is not covalently attached to the hydrogel.

[116] In certain examples, the ocular effective half-life is increased at least about 2-fold relative to the reference antibody.

[117] In certain examples, the ocular effective half-life is increased at least about 5-fold relative to the reference antibody.

[118] In certain examples, the ocular effective half-life is increased at least about 8-fold relative to the reference antibody.

[119] In certain examples, the ocular effective half-life is increased at least about 10-fold relative to the reference antibody.

[120] In certain examples, the ocular effective half-life is increased at least about 12-fold relative to the reference antibody.

[121] In certain examples, the ocular effective half-life is increased at least about 15-fold relative to the reference antibody.

[122] In certain examples, the ocular effective half-life is increased at least about 16-fold relative to the reference antibody.

[123] In certain examples, the ocular effective half-life is a vitreal half-life.

[124] In certain examples, the reference antibody is identical to the antibody of the antibody conjugate.

[125] In certain examples, the disclosure provides a pharmaceutical composition for use as a medicament, for use in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject, for use in reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis, and/or for use in treating a disorder associated with pathological angiogenesis in a subject.

[126] In another aspect, there is provided a pharmaceutical composition comprising the hydrogel conjugate of the disclosure and a pharmaceutically acceptable carrier, excipient, or diluent.

[126a] In another aspect, there is provided the pharmaceutical composition of the disclosure for use as a medicament.

[126b] In a further aspect, there is provided the pharmaceutical composition of the disclosure for use in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject.

[126c] In another aspect, there is provided the pharmaceutical composition of the disclosure for use in reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

[126d] In another aspect, there is provided pharmaceutical composition of the disclosure for use in treating a disorder associated with pathological angiogenesis in a subject.

[127] In certain examples, the pharmaceutical composition further comprising a second agent, wherein the second agent is selected from the group consisting of an antibody, an anti-angiogenic agent, a cytokine, a cytokine antagonist, a corticosteroid, an analgesic, and a compound that binds to a second biological molecule.

[128] In certain examples, the pharmaceutical composition further comprising a second agent, wherein the second agent is selected from the group consisting of an antibody, an anti-angiogenic agent, a cytokine, a cytokine antagonist, a corticosteroid, an analgesic, and a compound that binds to a second biological molecule.

[129] In certain examples, the anti-angiogenic agent of the pharmaceutical composition is a VEGF antagonist.

[130] In certain examples, the VEGF antagonist of the pharmaceutical composition is an anti-VEGF antibody, an anti-VEGF receptor antibody, a soluble VEGF receptor fusion protein, an aptamer, an anti-VEGF DARPin®, or a VEGFR tyrosine kinase inhibitor.

[131] In certain examples, the anti-VEGF antibody of of the pharmaceutical composition is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody.

[132] In certain examples, the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody.

[133] In certain examples, the anti-VEGF/anti-Ang2 antibody is RG-7716.

[134] In certain examples, the soluble VEGF receptor fusion protein is aflibercept (EYLEA®).

[135] In certain examples, the aptamer is pegaptanib (MACUGEN®).

[136] In certain examples, the anti-VEGF DARPin® is abicipar pegol.

[137] In certain examples, the VEGFR tyrosine kinase inhibitor is selected from the group consisting of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416), and SUTENT® (sunitinib).

[138] In certain examples, the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to AMD risk.

[139] In certain examples, the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR.

[140] In certain examples, the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; IL-8; CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A.

[141] In certain examples, the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof.

[142] In certain examples, the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and (Fab')2 fragments.

[143] In certain examples, the disorder associated with pathological angiogenesis is an ocular disorder.

[144] In certain examples, the ocular disorder is selected from the group consisting of age-related macular degeneration (AMD), macular degeneration, macular edema, diabetic macular edema (DME) (including focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic retinopathy (DR) (including proliferative DR (PDR), non-proliferative DR (NPDR), and high-altitude DR), other ischemia-related retinopathies, retinopathy of prematurity (ROP), retinal vein occlusion (RVO) (including central (CRVO) and branched (BRVO) forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, familial exudative vitreoretinopathy (FEVR), Coats' disease, Norrie Disease, Osteoporosis-Pseudoglioma Syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, cystoid macular edema (CME), vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease.

[145] In certain examples, the ocular disorder is AMD, DME, DR, or RVO.

[146] In certain examples, the ocular disorder is AMD.

[147] In certain examples, AMD is wet AMD.

[148] In certain examples, the disclosure provides a method for treating an ocular indication, the method comprising administering a therapeutic amount of a solution of the pharmaceutical compositions described herein.

[148a] In another aspect, there is provided use of a solution of the composition of the disclosure in the manufacture of a medicament for treating an ocular indication in a subject in need of such treatment.

[149] In certain examples, the administration of the pharmaceutical composition is intraocular.

[150] In certain examples, the pharmaceutical composition is injected into the vitreum of the subject.

[151] In certain examples, the pharmaceutical composition is injected using a needle having a gauge of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, or 32.

[152] IIn certain examples, the ocular indication is selected from age-related macular degeneration (AMD), macular degeneration, macular edema, diabetic macular edema (DME) (including focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic retinopathy (DR) (including proliferative DR (PDR), non-proliferative DR (NPDR), and high-altitude DR), other ischemia-related retinopathies, retinopathy of prematurity (ROP), retinal vein occlusion (RVO) (including central (CRVO) and branched (BRVO) forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, familial exudative vitreoretinopathy (FEVR), Coats' disease, Norrie Disease, Osteoporosis-Pseudoglioma Syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, cystoid macular edema (CME), vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease.

[153] In certain examples, the disclosure provides a method for producing a hydrogel drug conjugate, the method comprising:

(a) providing a first hyaluronic acid, or an alkali metal salt or a derivative thereof, having at least three first reactive groups thereon;

(b) providing a second hyaluronic acid, or an alkali metal salt or a derivative thereof, having at least two second reactive group thereon, wherein the first and second reactive groups are capable reacting with each other to form a covalent bond;

(c) coupling at least one drug to one of the first reactive groups; and

(d) cross-linking the first and second hyaluronic acids by reaction of a first reactive group and a second reactive group to form a cross-linker and form the hydrogel conjugate.

[153a] In a further aspect, there is provided a method for producing a hydrogel drug conjugate, the method comprising:

(a) providing a first hyaluronic acid or a derivative thereof, having at least three first reactive groups thereon;

(b) providing a second hyaluronic acid or a derivative thereof, having at least two second reactive group thereon, wherein the first and second reactive groups are capable reacting with each other to form a covalent bond;

(c) coupling at least one drug to one of the first reactive groups; and

(d) cross-linking the first and second hyaluronic acids by reaction of a first reactive group and a second reactive group to form a cross-linker and form the hydrogel conjugate;

wherein events (b) and (c) may optionally be interchanged.

[154] In certain examples, the drug is coupled to the first hyaluronic acid via a reversible prodrug linker.

[155] In certain examples, the drug is coupled to the reversible prodrug linker and purified to form a purified drug-reversible prodrug linker conjugate prior to coupling with the first hyaluronic acid, wherein the drug-reversible prodrug linker conjugate is purified by:

(a) tagging the drug-reversible prodrug linker conjugate with a purification tag to form a tagged drug-reversible prodrug linker conjugate mixture;

(b) purifying a tagged drug-reversible prodrug linker monoconjugate from the mixture by chromatographic separation; and

(c) removing the purification tag from the tagged drug-reversible prodrug linker monoconjugate to form the purified drug-reversible prodrug linker conjugate.

[156] In certain examples, the cross-linker comprises a biodegradable spacer moiety.

[157] In certain examples, the cross-linker comprises an azelaic acid ester moiety.

BRIEF DESCRIPTION OF THE DRAWINGS

[158] Figure 1 is a cross-linked hyaluronic acid drug conjugate in accordance with the invention.

[159] Figure 2 is a reaction scheme according to one aspect of the present disclosure for the preparation of maleimide functionalized hyaluronic acid (HA) and maleimide functionalized hyaluronic acid having a conjugated drug.

[160] Figure 3 is a reaction scheme according to one aspect of the present disclosure for the preparation of protected thiol functionalized hyaluronic acid, thiol functionalized hyaluronic acid, and a cross-linked hyaluronic acid prodrug composition.

[161] Figure 4 is a reaction scheme according to one aspect of the present disclosure for the preparation of maleimide functionalized hyaluronic acid and protected disulfide functionalized hyaluronic acid.

[162] Figure 5 is a reaction scheme according to one aspect of the present disclosure for the preparation of thiol functionalized hyaluronic acid, thiol functionalized hyaluronic acid having a conjugated drug, and a cross-linked hyaluronic acid prodrug composition.

[163] Figure 6 is a reaction scheme according to one aspect of the present disclosure for the preparation of amine functionalized hyaluronic acid and the preparation of maleimide functionalized hyaluronic acid therefrom.

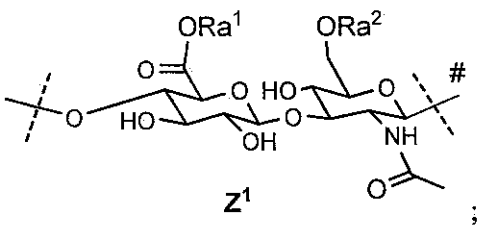
[164] Figure 7 is a reaction scheme according to one aspect of the present disclosure showing maleimide functionalized hyaluronic acid and maleimide functionalized hyaluronic acid having a conjugated drug.

[165] Figure 8 is a reaction scheme according to one aspect of the present disclosure for the preparation of amine functionalized hyaluronic acid, protected disulfide functionalized hyaluronic acid, and thiol functionalized hyaluronic acid.

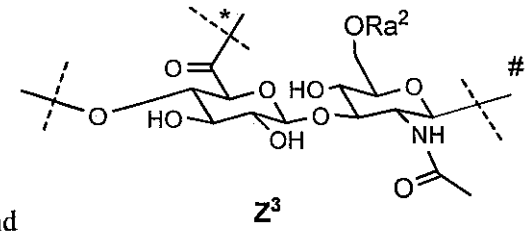
WHAT IS CLAIMED IS:

1. A cross-linked HA drug conjugate comprising a plurality of hyaluronic acid polymers 2A and a plurality of hyaluronic acid polymers 2B, wherein:

each 2A comprises a plurality of linearly connected units, the units consisting essentially of:

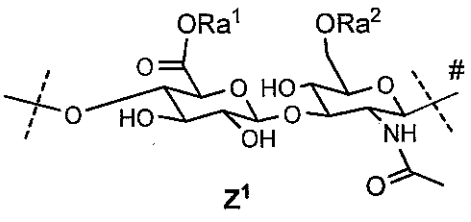


Z^1 ; and

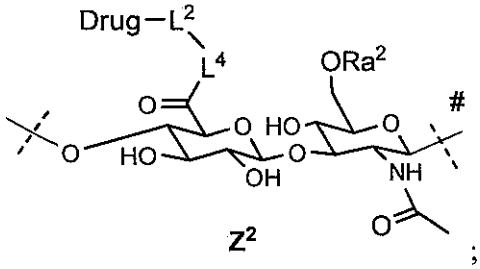


Z^3 ;

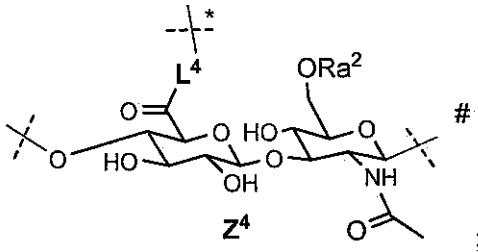
each 2B comprises a plurality of linearly connected units, the units consisting essentially of:



Z^1 ;



Z^2 ; and



Z^4 ;

wherein

an unmarked dashed line indicate a point of attachment to an adjacent unit at a dashed line marked with #, or to a hydrogen,

a dashed line marked with # indicate a point of attachment to an adjacent unit at an unmarked dashed line, or to a hydroxyl; and

a dashed line marked marked with * indicates a point of cross-linking attachment between a unit Z^3 of 2A and a unit Z^4 of 2B, such that at least one 2A is cross-linked to at least one 2B;

Drug is a therapeutic agent;

Ra^1 and Ra^2 are each independently is hydrogen; C_{1-4} alkyl; an alkali metal ion, an ammonium ion, or an alkaline earth metal ion;

L^2 is a reversible prodrug linker;

L^4 is an optionally biodegradable spacer and may be the same or different in Z^2 and Z^4;

2A comprises a total of s units wherein s is from 25 to 2500, wherein;

the number of Z^1 units in 2A is from about 0.8s to about 0.99s, and

the number of Z^3 units is from about 0.1s to about 0.01s;

2B comprising a total of t units wherein t is from 25 to 2500, wherein;

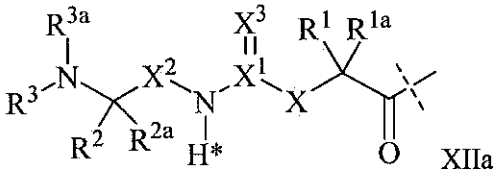
the number of Z^1 units in 2B is from about 0.75t to about 0.94t;

the combined number of Z^2 and Z^4 units is from about 0.14t to about 0.06t;

the number of Z^2 units is at least 0.01t; and

the number of Z^4 units is at least 0.01t.

2. The hydrogel conjugate of claim 1, wherein the reversible prodrug linker L^2 coupling the drug to spacer L^4 is of formula XIIa



XIIa

wherein:

the dashed line indicates the attachment to a nitrogen of a drug compound (not shown) by forming an amide bond;

-X- is $-C(R^4R^{4a})-$; $-N(R^4)-$; $-O-$; $-C(R^4R^{4a})-C(R^5R^{5a})-$; $-C(R^5R^{5a})-C(R^4R^{4a})-$; $-C(R^4R^{4a})-N(R^6)-$; $-N(R^6)-C(R^4R^{4a})-$; $-C(R^4R^{4a})-O-$; $-O-C(R^4R^{4a})-$; or $-C(R^7R^{7a})-$;

X^1 is C; or S(O);

$-X^2-$ is $-C(R^8R^{8a})-$; or $-C(R^8R^{8a})-C(R^9R^{9a})-$;

$=X^3$ is =O; =S; or =N-CN;

$-R^1$, $-R^{1a}$, $-R^2$, $-R^{2a}$, $-R^4$, $-R^{4a}$, $-R^5$, $-R^{5a}$, $-R^6$, $-R^8$, $-R^{8a}$, $-R^9$, $-R^{9a}$ are independently selected from the group consisting of -H; and C_{1-6} alkyl;

$-R^3$, $-R^{3a}$ are independently selected from the group consisting of -H; and C_{1-6} alkyl, provided that in case one of $-R^3$, $-R^{3a}$ or both are other than -H they are connected to N to which they are attached through an SP^3-hybridized carbon atom;

$-R^7$ is $-N(R^{10}R^{10a})$; or $-NR^{10}-(C=O)-R^{11}$;

$-R^{7a}$, $-R^{10}$, $-R^{10a}$, $-R^{11}$ are independently of each other -H; or C_{1-10} alkyl;

optionally, one or more of the pairs $-R^{1a}/-R^{4a}$, $-R^{1a}/-R^{5a}$, $-R^{1a}/-R^{7a}$, $-R^{4a}/-R^{5a}$, $-R^{8a}/-R^{9a}$ form a chemical bond;

optionally, one or more of the pairs $-R^1/-R^{1a}$, $-R^2/-R^{2a}$, $-R^4/-R^{4a}$, $-R^5/-R^{5a}$, $-R^8/-R^{8a}$,

$-R^9/-R^{9a}$ are joined together with the atom to which they are attached to form a C_{3-10} cycloalkyl; or 3- to 10-membered heterocyclyl;

optionally, one or more of the pairs $-R^1/-R^4$, $-R^1/-R^5$, $-R^1/-R^6$, $-R^1/-R^{7a}$, $-R^4/-R^5$, $-R^4/-R^6$, $-R^8/-R^9$, $-R^2/-R^3$ are joined together with the atoms to which they are attached to form a ring A;

optionally, R^3/R^{3a} are joined together with the nitrogen atom to which they are attached to form a 3- to 10-membered heterocycle;

Ring A is selected from the group consisting of phenyl; naphthyl; indenyl; indanyl; tetralinyl; C_{3-10} cycloalkyl; 3- to 10-membered heterocyclyl; and 8- to 11-membered heterobicyclyl; and wherein the group of formula XIIa is substituted with $-L^4$ provided that the hydrogen marked with the asterisk in formula (XIIa) is not replaced by $-L^4$ or other substituent;

wherein $-L^4-$ is a single chemical bond or a spacer moiety as defined herein..

3. The hydrogel conjugate of claim 2, wherein:

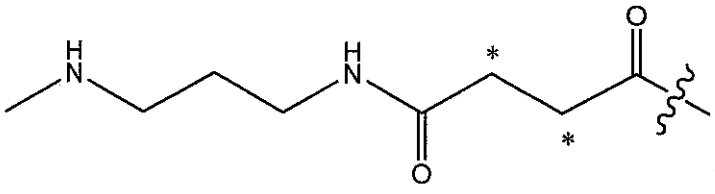
X^3 is =O;

$-R^1$ and $-R^{1a}$ are hydrogen;

$-R^3$ is methyl and R^{3a} is hydrogen; and

X is $-C(R^7R^{7a})-$; with R^7 being $NR^{10}-(C=O)-R^{11}$.

4. The hydrogel conjugate of any of claim 1 or 2 wherein the reversible prodrug linker L^2 is of the formula VIIa(i):

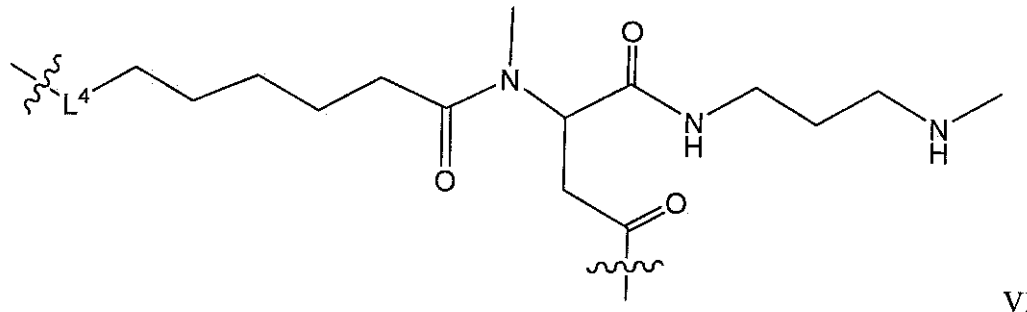


VIIa(i)

wherein:

each asterisk represents an independent site of attachment to the spacer L^4.

5. The hydrogel conjugate of any of claims 1 through 4 wherein (i) the reversible prodrug linker L^2 together with spacer L^4 is of the formula VIIc:



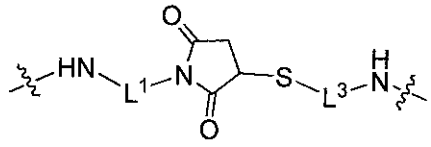
VIIc

wherein:

the right-most wavy line represents the point of attachment to the nitrogen atom of Drug; and

the left-most wavy line represents the point of attachment to a unit Z^2 of hyaluronic acid 2B; wherein optionally

(ii) the spacer L^4 connecting hyaluronic acid polymer 2A to hylauronic acid polymer 2B is of the formula:

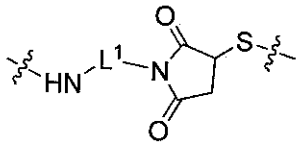


wherein:

the right-most wavy line represents the point of attachement to a unit Z^3 on hyaluronic acid polymer 2A; and

the left-most wavy line represents the point of attachment to a unit Z^4 on hyaluronic acid polymer 2B;

the spacer L_4 joining reversible prodrug linker L^2 to hyaluronic acid polymer 2B is of the formula



wherein:

the right-most wavy line represents the point of attachement to L^2; and

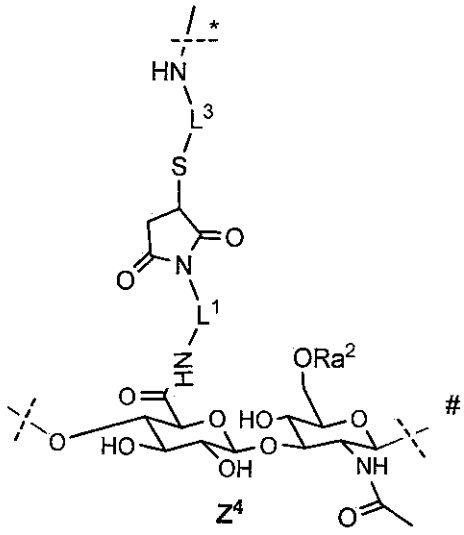
the left-most wavy line represents the point of attachment to a unit Z^2 on hyaluronic acid polymer 2B;

wherein:

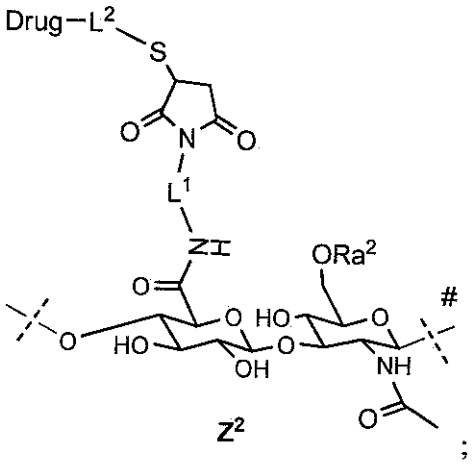
L^1 is a spacer; and

L^3 is abiodegradable spacer; and wherein optionally

(iii) unit Z^4 is



; and



unit Z^2 is ;

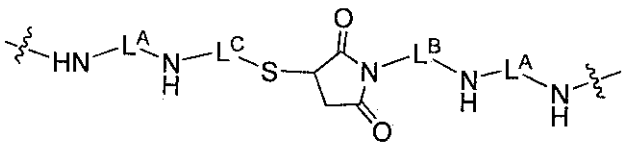
wherein:

L^1 is a spacer;

L^3 is abiodegradable spacer; and

2A, 2B, Drug, R^{a1}, R^{a2}, R^{a3}, R^{a4}, L_2, Z^1, Z^2, Z^3 and Z^4 are as recited in claim 1.

6. The hydrogel conjugate of any of claims 1 through 5(i) wherein the spacer L^4 connecting hyaluronic acid polymer 2A to Hylauronic acid polymer 2B is of the formula:

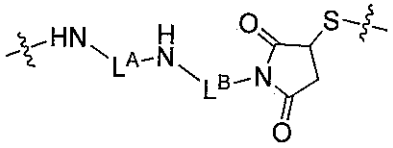


wherein:

the right-most wavy line represents the point of attachement to a unit Z^3 on hyaluronic acid polymer 2B; and

the left-most wavy line represents the point of attachment to a unit Z^4 on hyaluronic acid polymer 2A;

and the spacer L_4 joining reversible prodrug linker L^2 to hyaluronic acid polymer 2A is of the formula



wherein:

the right-most wavy line represents the point of attachement to L^2; and

the left-most wavy line represents the point of attachment to a unit Z^2 on hyaluronic acid polymer 2B;

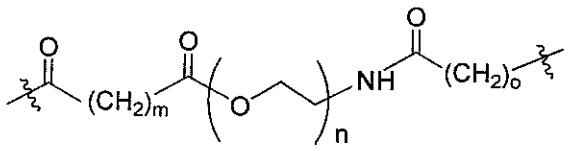
wherein:

L^A is a spacer;

L^B is a spacer; and

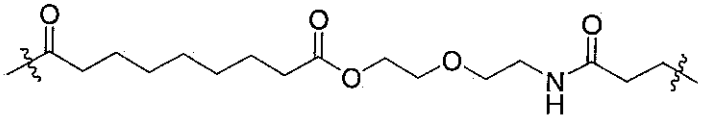
L^C is a biodegradable spacer.

7. The hydrogel conjugate of claim 6, wherein (i) L^A is optionally substituted and/or optionally interrupted C_{1-10} alkylene; wherein optionally
(ii) L^A is linear C_{2-4} alkylene; wherein optionally
(iii) L^B is linear -(O)-C_{1-5} alkylene; wherein optionally
(iv) L^C is:



wherein m is from 0 to 10, n is from 1 to 4, and o is from 1 to 4; or

L^C is:



; wherein optionally

(v) the drug is an antibody; wherein optionally

(vi) the antibody is a VEGF antagonist; wherein optionally

(vii) the antibody is an anti-VEGF antibody fragment; wherein optionally

(viii) the antibody fragment is a Fab antibody fragment; and wherein optionally

(ix) the Fab antibody fragment is ranibizumab.

8. The hydrogel conjugate of claim 7(vi), wherein

(i) the antibody comprises the following six hypervariable regions (HVRs):

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of $GX_1TPX_2GGX_3X_4X_5YX_6DSVX_7X_8$ (SEQ ID NO: 2), wherein X_1 is Ile or His, X_2 is Ala or Arg, X_3 is Tyr or Lys, X_4 is Thr or Glu, X_5 is Arg, Tyr, Gln, or Glu, X_6 is Ala or Glu, X_7 is Lys or Glu, and X_8 is Gly or Glu;

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of $RASQX_1VSTAVA$ (SEQ ID NO: 4), wherein X_1 is Asp or Arg;

(e) an HVR-L2 comprising the amino acid sequence of $X_1ASFLYS$ (SEQ ID NO: 5), wherein X_1 is Ser or Met; and

(f) an HVR-L3 comprising the amino acid sequence of X_1QGYGX_2PFT (SEQ ID NO: 6), wherein X_1 is Gln, Asn, or Thr and X_2 is Ala, Asn, Gln, or Arg; wherein optionally

(ii) the antibody comprises the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7), GITPAGGYEYYADSVKG (SEQ ID NO: 21), or GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10) or QQGYGNPFT (SEQ ID NO: 23); wherein optionally

(iii) the antibody comprises the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10); wherein optionally

(iv) the antibody further comprises the following heavy chain variable (VH) domain framework regions (FRs):

(a) an FR-H1 comprising the amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTIS (SEQ ID NO: 13);

(b) an FR-H2 comprising the amino acid sequence of WVRQAPGKGLEWVA (SEQ ID NO: 14);

(c) an FR-H3 comprising the amino acid sequence of RFTISADTSKNTAYLQMRSLRAEDTAVYYCAR (SEQ ID NO: 15); and

(d) an FR-H4 comprising the amino acid sequence of WGQGTLVTVSS (SEQ ID NO: 16); and wherein optionally

(v) the antibody further comprises the following light chain variable (VL) domain FRs:

(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17);

(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18);

(c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19); and

(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

9. The hydrogel conjugate of claim 8(i) or (ii), wherein (i) the antibody comprises the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGNPFT (SEQ ID NO: 23); and wherein optionally

(ii) the antibody further comprises the following VL domain FRs:

(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17);

(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18);

(c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO: 24); and

(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

10. The hydrogel conjugate of claim 8(i) or (ii), wherein (i) the antibody comprises the following six HVRs:

(a) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(b) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(c) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(d) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(e) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(f) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10); and wherein optionally

(ii) the antibody further comprises the following VL domain FRs:

(a) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17), DIQMTQSPESLSASVGDEVTITC (SEQ ID NO: 25), or DIQMTQSPSSLSASVGDEVTITC (SEQ ID NO: 26);

(b) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18) or WYQQKPGEAPKLLIY (SEQ ID NO: 27);

(c) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19) or GVPSRFSGSGSGTDFTLTIESLQPEDAATYYC (SEQ ID NO: 28); and

(d) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

11. The hydrogel conjugate of any one of claims 9-10, wherein the antibody further comprises the following VH domain FRs:

(a) an FR-H1 comprising the amino acid sequence of EEQLVEEGGGLVQPGESLELSCAASGFEIS (SEQ ID NO: 29) or EEQLVEEGGGLVQPGESLRLSCAASGFEIS (SEQ ID NO: 52);

(b) an FR-H2 comprising the amino acid sequence of WVRQEPGEGLEWVA (SEQ ID NO: 30);

(c) an FR-H3 comprising the amino acid sequence of RFTISADTSENTAYLQMNELRAEDTAVYYCAR (SEQ ID NO: 31); and

(d) an FR-H4 comprising the amino acid sequence of WGQGELVTVSS (SEQ ID NO: 32).

12. The hydrogel conjugate of claim 7(vi), wherein the antibody comprises (a) a VH domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11, 40, or 42; (b) a VL domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 12, 41, or 46; or (c) a VH domain as in (a) and a VL domain as in (b).

13. The hydrogel conjugate of claim 7(vii), wherein the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO:11, 40, or 42; (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 12, 41, or 46; or (c) a VH domain as in (a) and a VL domain as in (b).

14. The hydrogel conjugate of claim 7(viii), wherein the antibody comprises a VH domain comprising the amino acid sequence of SEQ ID NO:11 and a VL domain comprising the amino acid sequence of SEQ ID NO:12.

15. The hydrogel conjugate of claim 7(ix), wherein the antibody comprises: (i) a heavy chain comprising the amino acid sequence of SEQ ID NO:48 and a light chain comprising the amino acid sequence of SEQ ID NO:50; or (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO:49 and a light chain comprising the amino acid sequence of SEQ ID NO:50.

16. The hydrogel conjugate of any one of claims 1-15, wherein
(i) the antibody-hydrogel conjugate has an ocular effective half-life that is increased relative to a reference antibody that is not covalently attached to the hydrogel; wherein optionally

(ii) the ocular effective half-life is increased at least about 2-fold relative to the reference antibody; wherein optionally

(iii) the ocular effective half-life is increased at least about 2.5-fold relative to the reference antibody; wherein optionally

(iv) the ocular effective half-life is increased at least about 3-fold relative to the reference antibody; wherein optionally

(v) the ocular effective half-life is increased at least about 3.5-fold relative to the reference antibody; wherein optionally

(vi) the ocular effective half-life is increased at least about 4-fold relative to the reference antibody; wherein optionally

(vii) the ocular effective half-life is increased at least about 5-fold relative to the reference antibody; and wherein optionally

(viii) the ocular effective half-life is increased at least about 6-fold relative to the reference antibody.

17. The hydrogel conjugate of any one of claims 16(i)-(viii), wherein

(i) the ocular effective half-life is a vitreal effective half-life; and wherein optionally

(ii) the reference antibody is identical to the antibody of the hydrogel conjugate.

18. A pharmaceutical composition comprising the hydrogel conjugate of any one of claims 1-17 and a pharmaceutically acceptable carrier, excipient, or diluent.

19. The pharmaceutical composition of claim 18, (i) further comprising a second agent, wherein the second agent is selected from the group consisting of an antibody, an anti-angiogenic agent, a cytokine, a cytokine antagonist, a corticosteroid, an analgesic, and a compound that binds to a second biological molecule; wherein optionally

(ii) the anti-angiogenic agent is a VEGF antagonist; wherein optionally

(iii) the VEGF antagonist is an anti-VEGF antibody, an anti-VEGF receptor antibody, a soluble VEGF receptor fusion protein, an aptamer, an anti-VEGF DARPin®, or a VEGFR tyrosine kinase inhibitor; wherein optionally

(iv) the anti-VEGF antibody is ranibizumab (LUCENTIS®), RTH-258, or a bispecific anti-VEGF antibody; wherein optionally

(v) the bispecific anti-VEGF antibody is an anti-VEGF/anti-Ang2 antibody; and wherein optionally

(vi) the anti-VEGF/anti-Ang2 antibody is RG-7716.

20. The pharmaceutical composition of claim 19(iii), wherein (i) the soluble VEGF receptor fusion protein is aflibercept (EYLEA®); wherein optionally

(ii) the aptamer is pegaptanib (MACUGEN®); wherein optionally

(iii) the anti-VEGF DARPin® is abicipar pegol; and wherein optionally

(iv) the VEGFR tyrosine kinase inhibitor is selected from the group consisting of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474),

4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171), vatalanib (PTK787), semaxaminib (SU5416), and SUTENT® (sunitinib).

21. The pharmaceutical composition of claim 19(i), wherein the second biological molecule is selected from the group consisting of IL-1β; IL-6; IL-6R; IL-13; IL-13R; PDGF; angiopoietin; angiopoietin 2; Tie2; S1P; integrins αvβ3, αvβ5, and α5β1; betacellulin; apelin/APJ; erythropoietin; complement factor D; TNFα; HtrA1; a VEGF receptor; ST-2 receptor; and a protein genetically linked to AMD risk.

22. The pharmaceutical composition of claim 21, wherein (i) the VEGF receptor is VEGFR1, VEGFR2, VEGFR3, mbVEGFR, or sVEGFR; and wherein optionally

(ii) the protein genetically linked to AMD risk is selected from the group consisting of complement pathway components C2, factor B, factor H, CFHR3, C3b, C5, C5a, and C3a; HtrA1; ARMS2; TIMP3; HLA; IL-8; CX3CR1; TLR3; TLR4; CETP; LIPC, COL10A1; and TNFRSF10A.

23. The pharmaceutical composition of any one of claims 21(i) and 21-22, wherein (i) the compound that binds a second biological molecule is an antibody or antigen-binding fragment thereof; and wherein optionally

(ii) the antigen-binding antibody fragment is selected from the group consisting of Fab, Fab-C, Fab'-SH, Fv, scFv, and (Fab')2 fragments.

24. The pharmaceutical composition of any one of claims 18-23 for use as a medicament.

25. The pharmaceutical composition of any one of claims 18-23 for use in the manufacture of a medicament for treating a disorder associated with pathological angiogenesis in a subject.

26. The pharmaceutical composition of any one of claims 18-23 for use in reducing or inhibiting angiogenesis in a subject having a disorder associated with pathological angiogenesis.

27. The pharmaceutical composition of any one of claims 18-23 for use in treating a disorder associated with pathological angiogenesis in a subject.

28. The pharmaceutical composition for use of any one of claims 18-23, wherein (i) the disorder associated with pathological angiogenesis is an ocular disorder; wherein optionally

(ii) the ocular disorder is selected from the group consisting of age-related macular degeneration (AMD), macular degeneration, macular edema, diabetic macular edema (DME) (including focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic retinopathy (DR) (including proliferative DR (PDR), non-proliferative DR (NPDR), and high-altitude DR), other ischemia-related retinopathies, retinopathy of prematurity (ROP), retinal vein occlusion (RVO) (including central (CRVO) and branched (BRVO) forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, familial exudative vitreoretinopathy (FEVR), Coats' disease, Norrie Disease, Osteoporosis-Pseudoglioma Syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, cystoid macular edema (CME), vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease; and wherein optionally

(iii) the ocular disorder is AMD, DME, DR, or RVO.

29. The pharmaceutical composition for use of claim 28(ii) or 28(iii), wherein (i) the ocular disorder is AMD; and wherein optionally

(ii) the AMD is wet AMD.

30. Use of a solution of the composition of any one of claims 18-29 in the manufacture of a medicament for treating an ocular indication in a subject in need of such treatment.

31. The use of claim 30 wherein the administration is intraocular; or wherein

the composition is to be injected into the vitreum of the subject; wherein optionally

the composition is to be injected using a needle having a gauge of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, or 32.

32. The use of claim 30, wherein (i) the ocular indication is selected from age-related macular degeneration (AMD), macular degeneration, macular edema, diabetic macular edema (DME) (including focal, non-center DME and diffuse, center-involved DME), retinopathy, diabetic retinopathy (DR) (including proliferative DR (PDR), non-proliferative DR (NPDR), and high-altitude DR), other ischemia-related retinopathies, retinopathy of prematurity (ROP), retinal vein occlusion (RVO) (including central (CRVO) and branched (BRVO) forms), CNV (including myopic CNV), corneal neovascularization, a disease associated with corneal neovascularization, retinal neovascularization, a disease associated with retinal/choroidal neovascularization, pathologic myopia, von Hippel-Lindau disease, histoplasmosis of the eye, familial exudative vitreoretinopathy (FEVR), Coats' disease, Norrie Disease, Osteoporosis-Pseudoglioma Syndrome (OPPG), subconjunctival hemorrhage, rubeosis, ocular neovascular disease, neovascular glaucoma, retinitis pigmentosa (RP), hypertensive retinopathy, retinal angiomatous proliferation, macular telangiectasia, iris neovascularization, intraocular neovascularization, retinal degeneration, cystoid macular edema (CME), vasculitis, papilloedema, retinitis, conjunctivitis (including infectious conjunctivitis and non-infectious (e.g., allergic) conjunctivitis), Leber congenital amaurosis, uveitis (including infectious and non-infectious uveitis), choroiditis, ocular histoplasmosis, blepharitis, dry eye, traumatic eye injury, and Sjögren's disease; wherein optionally

(ii) the ocular indication is AMD, DME, DR, or RVO; wherein optionally

(iii) the ocular indication is AMD; and wherein optionally

(iv) the AMD is wet AMD.

33. A method for producing a hydrogel drug conjugate, the method comprising:

(a) providing a first hyaluronic acidor a derivative thereof, having at least three first reactive groups thereon;

(b) providing a second hyaluronic acid or a derivative thereof, having at least two second reactive group thereon, wherein the first and second reactive groups are capable reacting with each other to form a covalent bond;

(c) coupling at least one drug to one of the first reactive groups; and

(d) cross-linking the first and second hyaluronic acids by reaction of a first reactive group and a second reactive group to form a cross-linker and form the hydrogel conjugate;

wherein events (b) and (c) may optionally be interchanged.

34. The method of claim 33, wherein (i) the drug is coupled to the first hyaluronic acid via a reversible prodrug linker; wherein optionally

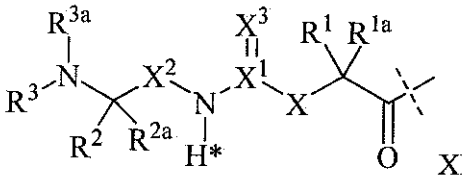
(ii) the drug is coupled to the reversible prodrug linker and purified to form a purified drug-reversible prodrug linker conjugate prior to coupling with the first hyaluronic acid, wherein the drug-reversible prodrug linker conjugate is purified by:

(a) tagging the drug-reversible prodrug linker conjugate with a purification tag to form a tagged drug-reversible prodrug linker conjugate mixture;

(b) purifying a tagged drug-reversible prodrug linker monoconjugate from the mixture by chromatographic separation; and

(c) removing the purification tag from the tagged drug-reversible prodrug linker monoconjugate to form the purified drug-reversible prodrug linker conjugate; or

wherein the reversible prodrug linker coupling the drug to the first hyaluronic acid is of formula XIIa



XIIa

wherein:

the dashed line indicates the attachment to a nitrogen of a drug compound (not shown) by forming an amide bond;

-X- is $-C(R^4R^{4a})$-; $-N(R^4)$-; -O-; $-C(R^4R^{4a})-C(R^5R^{5a})$-; $-C(R^5R^{5a})-C(R^4R^{4a})$-; $-C(R^4R^{4a})-N(R^6)$-; $-N(R^6)-C(R^4R^{4a})$-; $-C(R^4R^{4a})$-O-; $-O-C(R^4R^{4a})$-; or $-C(R^7R^{7a})$-;

X^1 is C; or S(O);

$-X^2-$ is $-C(R^8R^{8a})$-; or $-C(R^8R^{8a})-C(R^9R^{9a})$-;

$=X^3$ is =O; =S; or =N-CN;

$-R^1$, $-R^{1a}$, $-R^2$, $-R^{2a}$, $-R^4$, $-R^{4a}$, $-R^5$, $-R^{5a}$, $-R^6$, $-R^8$, $-R^{8a}$, $-R^9$, $-R^{9a}$ are independently selected from the group consisting of –H; and C_{1-6} alkyl;

$-R^3$, $-R^{3a}$ are independently selected from the group consisting of –H; and C_{1-6} alkyl, provided that in case one of $-R^3$, $-R^{3a}$ or both are other than –H they are connected to N to which they are attached through an SP^3-hybridized carbon atom;

$-R^7$ is $-N(R^{10}R^{10a})$; or $-NR^{10}-(C=O)-R^{11}$;

$-R^{7a}$, $-R^{10}$, $-R^{10a}$, $-R^{11}$ are independently of each other –H; or C_{1-10} alkyl;

optionally, one or more of the pairs $-R^{1a}/-R^{4a}$, $-R^{1a}/-R^{5a}$, $-R^{1a}/-R^{7a}$, $-R^{4a}/-R^{5a}$, $-R^{8a}/-R^{9a}$ form a chemical bond;

optionally, one or more of the pairs $-R^{1}/-R^{1a}$, $-R^{2}/-R^{2a}$, $-R^{4}/-R^{4a}$, $-R^{5}/-R^{5a}$, $-R^{8}/-R^{8a}$, $-R^{9}/-R^{9a}$ are joined together with the atom to which they are attached to form a C_{3-10} cycloalkyl; or 3- to 10-membered heterocyclyl;

optionally, one or more of the pairs $-R^{1}/-R^{4}$, $-R^{1}/-R^{5}$, $-R^{1}/-R^{6}$, $-R^{1}/-R^{7a}$, $-R^{4}/-R^{5}$, $-R^{4}/-R^{6}$, $-R^{8}/-R^{9}$, $-R^{2}/-R^{3}$ are joined together with the atoms to which they are attached to form a ring A;

optionally, R^3/R^{3a} are joined together with the nitrogen atom to which they are attached to form a 3- to 10-membered heterocycle;

Ring A is selected from the group consisting of phenyl; naphthyl; indenyl; indanyl; tetralinyl; C_{3-10} cycloalkyl; 3- to 10-membered heterocyclyl; and 8- to 11-membered heterobicyclyl; and

wherein the group of formula XIIa is substituted with $-L^4$ provided that the hydrogen marked with the asterisk in formula (XIIa) is not replaced by $-L^4$ or other substituent;

wherein $-L^4-$ is a single chemical bond or a spacer moiety as defined herein; wherein optionally

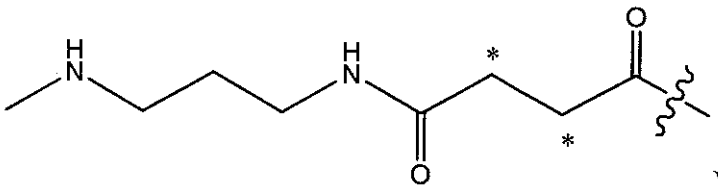
(iii) X^3 is =O;

$-R^1$ and $-R^{1a}$ are hydrogen;

$-R^3$ is methyl and R^{3a} is hydrogen; and

X is $-C(R^7R^{7a})-$; with R^7 being $NR^{10}-(C=O)-R^{11}$; and wherein optionally

(iv) the reversible prodrug linker is of the formula VIIa(i):

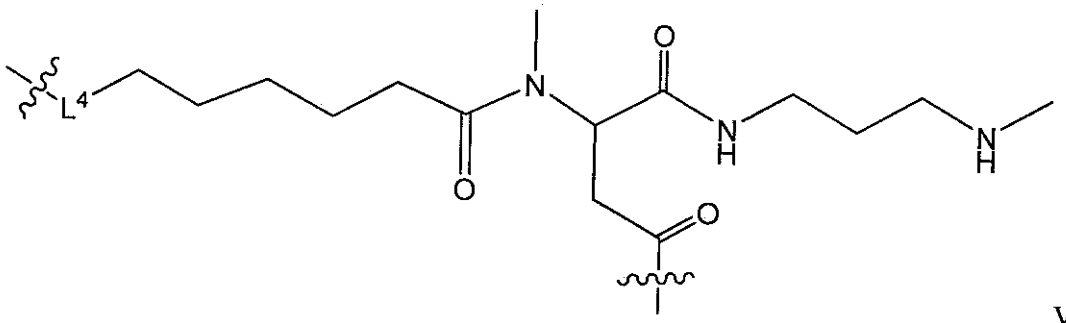


VIIa(i)

wherein:

each asterisk represents an independent site of attachment to the first hyaluronic acid by a spacer L^4 or

wherein the reversible prodrug linker together with an optionally spacer L_4, is of the formula VIIc:



VIIc

wherein:

the right-most wavy line represents the point of attachment to the nitrogen atom of a drug;

the left-most wavy line represents the point of attachment to the first hyaluronic acid; and

L^4 is an optionally biodegradable spacer.

35. The method of claim 33 wherein (i) the cross-linker comprises a biodegradable spacer moiety; and wherein optionally

(ii) the cross-linker comprises an azelaic acid ester moiety.

36. The method of claim 33, wherein (i) the drug is an antibody; wherein optionally

(ii) the antibody is a VEGF antagonist; wherein optionally

(iii) the antibody is an anti-VEGFantibody fragment; wherein optionally

(iv) the antibody fragment is a Fab antibody fragment; and wherein optionally

(v) the Fab antibody fragment is ranibizumab.

37. The method of claim 36(ii), wherein (i) the antibody comprises the following six hypervariable regions (HVRs):

(g) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(h) an HVR-H2 comprising the amino acid sequence of $GX_1TPX_2GGX_3X_4X_5YX_6DSVX_7X_8$ (SEQ ID NO: 2), wherein X_1 is Ile or His, X_2 is

Ala or Arg, X_3 is Tyr or Lys, X_4 is Thr or Glu, X_5 is Arg, Tyr, Gln, or Glu, X_6 is Ala or Glu, X_7 is Lys or Glu, and X_8 is Gly or Glu;

(i) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(j) an HVR-L1 comprising the amino acid sequence of RASQX_1VSTAVA (SEQ ID NO: 4), wherein X_1 is Asp or Arg;

(k) an HVR-L2 comprising the amino acid sequence of X_1ASFLYS (SEQ ID NO: 5), wherein X_1 is Ser or Met; and

(l) an HVR-L3 comprising the amino acid sequence of X_1QGYGX_2PFT (SEQ ID NO: 6), wherein X_1 is Gln, Asn, or Thr and X_2 is Ala, Asn, Gln, or Arg; wherein optionally

(ii) the antibody comprises the following six HVRs:

(g) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(h) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7), GITPAGGYEYYADSVKG (SEQ ID NO: 21), or GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(i) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(j) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(k) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(l) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10) or QQGYGNPFT (SEQ ID NO: 23); wherein optionally

(iii) the antibody comprises the following six HVRs:

(g) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(h) an HVR-H2 comprising the amino acid sequence of GITPAGGYTRYADSVKG (SEQ ID NO: 7);

(i) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(j) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(k) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(l) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10); wherein optionally

(iv) the antibody further comprises the following heavy chain variable (VH) domain framework regions (FRs):

(e) an FR-H1 comprising the amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTIS (SEQ ID NO: 13);

(f) an FR-H2 comprising the amino acid sequence of WVRQAPGKGLEWVA (SEQ ID NO: 14);

(g) an FR-H3 comprising the amino acid sequence of RFTISADTSKNTAYLQMRSLRAEDTAVYYCAR (SEQ ID NO: 15); and

(h) an FR-H4 comprising the amino acid sequence of WGQGTLVTVSS (SEQ ID NO: 16); and wherein optionally

(v) the antibody further comprises the following light chain variable (VL) domain FRs:

(e) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17);

(f) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18);

(g) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19); and

(h) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

38. The method of claim 37(i) or (ii), wherein (i) the antibody comprises the following six HVRs:

(g) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(h) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(i) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(j) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(k) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(l) an HVR-L3 comprising the amino acid sequence of QQGYGNPFT (SEQ ID NO: 23); wherein optionally

(ii) the antibody further comprises the following VL domain FRs:

(e) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17);

(f) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18);

(g) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO: 24); and

(h) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

39. The method of claim 37(i) or (ii), wherein (i) the antibody comprises the following six HVRs:

(g) an HVR-H1 comprising the amino acid sequence of DYWIH (SEQ ID NO: 1);

(h) an HVR-H2 comprising the amino acid sequence of GITPAGGYEYYADSVEG (SEQ ID NO: 22);

(i) an HVR-H3 comprising the amino acid sequence of FVFFLPYAMDY (SEQ ID NO: 3);

(j) an HVR-L1 comprising the amino acid sequence of RASQDVSTAVA (SEQ ID NO: 8);

(k) an HVR-L2 comprising the amino acid sequence of SASFLYS (SEQ ID NO: 9); and

(l) an HVR-L3 comprising the amino acid sequence of QQGYGAPFT (SEQ ID NO: 10); and wherein optionally

(ii) the antibody further comprises the following VL domain FRs:

(e) an FR-L1 comprising the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 17), DIQMTQSPESLSASVGDEVTITC (SEQ ID NO: 25), or DIQMTQSPSSLSASVGDEVTITC (SEQ ID NO: 26);

(f) an FR-L2 comprising the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO: 18) or WYQQKPGEAPKLLIY (SEQ ID NO: 27);

(g) an FR-L3 comprising the amino acid sequence of GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC (SEQ ID NO: 19) or GVPSRFSGSGSGTDFTLTIESLQPEDAATYYC (SEQ ID NO: 28); and

(h) an FR-L4 comprising the amino acid sequence of FGQGTKVEIK (SEQ ID NO: 20).

40. The method of any one of claims 38-39(i), wherein (i) the antibody further comprises the following VH domain FRs:

(e) an FR-H1 comprising the amino acid sequence of EEQLVEEGGGLVQPGESLELSCAASGFEIS (SEQ ID NO: 29) or EEQLVEEGGGLVQPGESLRLSCAASGFEIS (SEQ ID NO: 52);

(f) an FR-H2 comprising the amino acid sequence of WVRQEPGEGLEWVA (SEQ ID NO: 30);
(g) an FR-H3 comprising the amino acid sequence of RFTISADTSENTAYLQMNELRAEDTAVYYCAR (SEQ ID NO: 31); and
(h) an FR-H4 comprising the amino acid sequence of WGQGELVTVSS (SEQ ID NO: 32); wherein optionally

(ii) the antibody comprises (a) a VH domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11, 40, or 42; (b) a VL domain comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 12, 41, or 46; or (c) a VH domain as in (a) and a VL domain as in (b); wherein optionally

(iii) the antibody comprises (a) a VH domain comprising the amino acid sequence of SEQ ID NO:11, 40, or 42; (b) a VL domain comprising the amino acid sequence of SEQ ID NO: 12, 41, or 46; or (c) a VH domain as in (a) and a VL domain as in (b); wherein optionally

(iv) the antibody comprises a VH domain comprising the amino acid sequence of SEQ ID NO:11 and a VL domain comprising the amino acid sequence of SEQ ID NO:12; wherein optionally

(v) the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:48 and a light chain comprising the amino acid sequence of SEQ ID NO:50.

41. The method of claim 36(i), wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:49 and a light chain comprising the amino acid sequence of SEQ ID NO:50.