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(71) **Applicant**: **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(72) **Inventors**: **GUSAROVA, Viktoria**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **GROMADA, Jesper**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **MURPHY, Andrew J.**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **BUCKLER, David R.**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(74) **Agents**: **SWANSON & BRATSCHUN, L.L.C** et al.; 8210 Southpark Terrace, Littleton, Colorado 80120 (US).

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(54) **Title**: ANTI-ANGPTL8 ANTIBODIES AND USES THEREOF

(57) **Abstract**: The present invention provides antibodies that bind to ANGPTL8 and methods of using the same. According to certain embodiments, the antibodies of the invention bind human ANGPTL8 with high affinity. The antibodies of the invention may be fully human antibodies. The antibodies of the invention are useful for the treatment of various diseases or disorders characterized in part by elevated blood triglyceride levels.

ANTI-ANGPTL8 ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to antibodies, and antigen-binding fragments thereof, which specifically bind angiopoietin-like protein (ANGPTL) 8, compositions comprising these antibodies and methods of use thereof.

BACKGROUND

[0002] ANGPTL8 (alternatively called TD26, RIFL, Lipasin, C19orf80 and Betatrophin) is a newly recognized ANGPTL family member that has been implicated in both triglyceride (TG) and glucose metabolism. It is a circulating protein that is expressed primarily in liver and adipose tissue. Unlike ANGPTL3 and ANGPTL4, ANGPTL8 lacks a fibrinogen like domain at the C-terminus, but contains an N-terminal coiled-coil domain, much like other ANGPTL family members. Phylogenetic analysis reveals that ANGPTL8 shares common ancestors with ANGPTL3 and ANGPTL4 (Fu, Z. *et. al.*, (2013), Biochem. Biophys. Res. Commun. 430:1126-1131).

[0003] Hepatic overexpression of ANGPTL8 is associated with hypertriglyceridemia, whereas inactivation of *Angptl8* causes a reduction in plasma TG levels (Quagliarini, F. *et. al.* (2012), Proc. Natl. Acad. Sci. USA 109(48):19751-19756; Wang, Y. *et. al.* (2013), Proc. Natl. Acad. Sci. USA 110:16109-16114). Despite the consensus that ANGPTL8 is involved in lipid regulation, the mechanism responsible for this process is still under debate. One proposed mechanism is that ANGPTL8 inhibits lipoprotein lipase (LPL) activity, resulting in reduced triglyceride hydrolysis and clearance (Zhang, R. *et.al.,* (2012), Biochem. Biophys. Res. Commun. 424:786-792).

[0004] ANGPTL8 has also been reported to play a role in beta cell proliferation and beta cell mass in mice, where insulin resistance was induced by an insulin receptor antagonist, S961 (Yi, P. *et. al.* (2013), Cell 153:747-758). However, subsequent studies revealed that ANGPTL8 is not required for beta cell function, or the beta cell growth response to insulin resistance. Furthermore, overexpression of ANGPTL8 does not increase beta cell area or improve glycemic control (Gusarova, V. *et. al.* (2014) Cell 159:691-696).

[0005] Since hepatic overexpression of ANGPTL8 is associated with hypertriglyceridemia and since inactivation of *Angptl8* results in a reduction in plasma triglyceride levels, an inhibitor or antagonist of ANGPTL8 may prove effective in treating a disease characterized in part by elevated levels of triglycerides, such as, but not limited to, hypertriglyceridemia.

[0006] Zhang reported that a monoclonal antibody to lipasin, when injected intraperitoneally to wildtype mice, decreased serum triglyceride levels (Zhang, R. (2015), Endocrine Society's 97th Annual Meeting, Presentation No. OR13-6, March 5-8, San Diego, CA). However, no fully human antibodies specific for ANGPTL8 have been described to date that may be used in a

clinical setting to treat diseases, or conditions characterized by elevated levels of triglycerides, including hypertriglyceridemia.

[0007] Accordingly, there is a need in the art for novel antagonists of ANGPTL8, such as the antibodies described herein, for treating patients suffering from hypertriglyceridemia and other disorders or conditions associated with elevated triglyceride and lipid levels.

BRIEF SUMMARY OF THE INVENTION

[0008] The present invention provides antibodies and antigen-binding fragments thereof that bind to angiopoietin-like protein 8 (ANGPTL8). One aspect of the invention provides human antibodies and antigen-binding fragments thereof that bind to/interact with ANGPTL8, whereby such binding and/or interaction results in the lowering of triglyceride levels in a mammal.

[0009] Accordingly, in a first aspect, the invention provides fully human monoclonal antibodies (mAbs) and antigen-binding fragments thereof that specifically bind, neutralize, inhibit, block, abrogate, reduce, or interfere with, at least one activity of ANGTPL8, in particular, human ANGPTL8 (See amino acids 22-198 of GenBank accession number NP_061157.3 and amino acids 1-177 of SEQ ID NO:340). The activity of ANGPTL8 that can be neutralized, inhibited, blocked, abrogated, reduced or interfered with, by an antibody or antigen-binding fragment thereof of the invention, includes, but is not limited to, inhibition of LPL activity, or lowering of triglyceride levels *in vivo* and the like.

[0010] In one embodiment, the invention provides a monoclonal antibody or antigen-binding fragment thereof that specifically binds to ANGPTL8 and neutralizes, or inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment thereof exhibits one or more of the following characteristics:

a) is a fully human monoclonal antibody;

b) binds specifically to a linear epitope in the N-terminal region of human ANGPTL8 as defined by SEQ ID NO: 337;

c) does not bind to a linear epitope in the N-terminal region of human ANGPTL8 as defined by SEQ ID NO: 337;

d) does not bind to the N-terminal coiled-coil region of human ANGPTL3 peptide of SEQ ID NO: 338, or to the N-terminal coiled-coil region of human ANGPTL4 peptide of SEQ ID NO: 339;

e) binds human ANGPTL8 at 25℃ with a K_D of less than about 150pM and binds monkey ANGPTL8 at 25℃ with a K_D of less than about 90pM as measured by surface plasmon resonance;

f) lowers triglyceride levels in a mammal by about 68% (maximum) when administered subcutaneously at a dose of about 10 mg/kg;

g) lowers triglyceride levels in a mammal for a period ranging from about 7 days to 21 days,

when administered subcutaneously at doses ranging from about 5 mg/kg to about 25 mg/kg;

h) comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 266, 274, 282, 290, 298, 306, 314 and 330;

i) comprises a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, and 322; or

j) cross-competes with a reference antibody, wherein the reference antibody comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) amino acid sequence selected from the group consisting of any of the HCVR and LCVR amino acid sequences of Table 1.

[0011] In one embodiment, an antibody or antigen-binding fragment thereof of the present invention can neutralize, inhibit, block, abrogate, reduce or interfere with, an activity of hANGPTL8 by binding to an epitope of hANGPTL8 that is directly involved in the targeted activity of hANGPTL8 (*e.g.* the LPL inhibitory activity of ANGPTL8).

[0012] In another embodiment, an antibody or antigen-binding fragment thereof of the invention can neutralize, inhibit, block, abrogate, reduce or interfere with, an activity of hANGPTL8 by binding to an epitope of hANGPTL8 that is not directly involved in the targeted activity of hANGPTL8, but the antibody or fragment binding thereto may either by steric overlap or by allosteric effects at sites different from the antibody-antigen contact surface inhibit, block, abrogate, reduce or interfere with, the targeted activity of hANGPTL8.

[0013] In another embodiment, an antibody or fragment thereof of the invention binds to an epitope of hANGPTL8 that is not directly involved in the targeted activity (*e.g.*, inhibiting LPL activity, and the like) of hANGPTL8 (*i.e.*, a non-blocking antibody), but the antibody or fragment thereof results in lowering of triglyceride levels *in vivo*, compared to the lowering of triglyceride levels in the absence of the antibody or fragment thereof.

[0014] In one embodiment, the invention features an isolated anti-hANGPTL8 antibody or antigen-binding fragment thereof that binds to an epitope situated within the N-terminal region at residues 1-39 of SEQ ID NO: 340 (shown also as SEQ ID NO: 337).

[0015] In another embodiment, the invention provides an isolated antibody or antigen-binding fragment of an antibody that binds to an epitope situated within the N-terminal region of human ANGPTL8 at residues 1-39 of SEQ ID NO: 340 (shown also as SEQ ID NO: 337), but does not bind to the N-terminal coiled-coil region of hANGPTL3 (SEQ ID NO:338), or to the N-terminal coiled-coil region of hANGPTL4 (SEQ ID NO:339).

[0016] In one embodiment, the invention features an isolated anti-hANGPTL8 antibody or antigen-binding fragment thereof that binds to an epitope situated outside of the region of human ANGPTL8 defined by amino acid residues 1-39 of SEQ ID NO: 340 (shown also as SEQ

ID NO: 337), i.e. amino acid residues 40-177 of SEQ ID NO: 340), and neutralizes, inhibits, abrogates, reduces or interferes with, at least one activity of hANGPTL8.

[0017] In one embodiment, the invention features an isolated anti-hANGPTL8 antibody or antigen-binding fragment thereof that binds to human ANGPTL8 (amino acid residues 1-177 of SEQ ID NO: 340; See also amino acid residues 22-198 of GenBank accession number NP_061157.3), but does not cross react with a related protein, such as human ANGPTL3 (amino acid sequence of SEQ ID NO: 342, encoded by the nucleic acid sequence shown in SEQ ID NO: 343), or human ANGPTL4 (amino acid sequence of SEQ ID NO: 344, encoded by the nucleic acid sequence shown in SEQ ID NO:345).

[0018] The antibodies of the invention can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, e.g., to increase persistence in the host or to eliminate residual effector functions (Reddy et al., 2000, J. Immunol. 164:1925-1933). In certain embodiments, the antibodies may be bispecific.

[0019] Exemplary anti-ANGPTL8 antibodies of the present invention are listed in Tables 1 and 2 herein. Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of exemplary anti-ANGPTL8 antibodies. Table 2 sets forth the nucleic acid sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary anti-ANGPTL8 antibodies.

[0020] The present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0021] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0022] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1.

[0023] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42,

50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/250, 266/250, 274/250, 282/250, 290/250, 306/250, 314/322, and 330/322.

[0024] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 66/74, 162/170, 194/202 and 314/322.

[0025] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 162/170.

[0026] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that binds to and/or inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment comprises: (a) three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences as set forth in Table 1; and (b) three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences as set forth in Table 1.

[0027] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment comprises:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 268, 276, 284, 292, 300, 308, 316 and 332;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 270, 278, 286, 294, 302, 310, 318, and 334;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 272, 280, 288, 296, 304, 312, 320 and 336;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252 and 324;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, and 326; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256 and 328.

[0028] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0029] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0030] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0031] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0032] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0033] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0034] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-ANGPTL8 antibodies listed in Table 1. In certain embodiments, the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 72/80 (*e.g.*, H4H15321P), 168/176 (*e.g.*, H4H15341P), 200/208 (*e.g.,* H4H15345P), and 320/328 (*e.g.,* H4H15367P2). In one embodiment, the HCDR3/LCDR3 amino acid sequence pair is SEQ ID NO: 168/176 (*e.g.*,

H4H15341P).

[0035] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-ANGPTL8 antibodies listed in Table 1. In certain embodiments, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequence set is selected from the group consisting of SEQ ID NOs: 68-70-72-76-78-80 (*e.g.*, H4H15321P); 164-166-168-172-174-176 (*e.g.,* H4H15341P); 196-198-200-204-206-208 (*e.g.*, H4H15345P); 316-318-320-324-326-328 (*e.g.*, H4H15367P2). In one embodiment, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequence set is SEQ ID NOs: 164-166-168-172-174-176 (*e.g.,* H4H15341P).

[0036] In a related embodiment, the present invention provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-ANGPTL8 antibodies listed in Table 1. For example, the present invention includes antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 66/74 *(e.g.,* H4H15321P), 162/170 (*e.g.*, H4H15341P); 194/202 (*e.g.*, H4H15345P); 314/322 (*e.g.,* H415367P2). Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0037] The present invention includes anti-ANGPTL8 antibodies having a modified glycosylation pattern. In some embodiments, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield *et al.* (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0038] The present invention also provides for antibodies and antigen-binding fragments thereof that compete for specific binding to ANGPTL8 with a reference antibody or antigen-

binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[0039] In one embodiment, the invention provides an isolated monoclonal antibody or antigen-binding fragment thereof that competes for binding to ANGPTL8 with a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/250, 266/250, 274/250, 282/250, 290/250, 306/250, 314/322, and 330/322.

[0040] The present invention also provides antibodies and antigen-binding fragments thereof that bind the same epitope on ANGPTL8 as a reference antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[0041] In one embodiment, the invention provides an isolated monoclonal antibody or antigen-binding fragment thereof that binds to the same epitope on ANGPTL8 as a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/250, 266/250, 274/250, 282/250, 290/250, 306/250, 314/322, and 330/322.

[0042] In one embodiment, the isolated antibody that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, is a recombinantly produced human monoclonal antibody.

[0043] In one embodiment, the isolated antibody that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, is a recombinantly produced human monoclonal antibody having a HCVR and/or an LCVR sequence selected from the amino acid sequences found in Table 1.

[0044] In one embodiment, the isolated antibody that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, is a recombinantly produced human monoclonal antibody having a HCVR /LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/250, 266/250, 274/250, 282/250, 290/250, 306/250, 314/322, and 330/322.

[0045] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that neutralizes ANGPTL8 activity, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34,

50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 266, 274, 282, 290, 298, 306, 314 and 330; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, and 322; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 272, 280, 288, 296, 304, 312, 320 and 336, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256 and 328, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 268, 276, 284, 292, 300, 308, 316 and 332, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 270, 278, 286, 294, 302, 310, 318, and 334, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252 and 324, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, and 326, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) binds specifically to the N-terminal region of human ANGPTL8 defined by SEQ ID NO: 337; vi) does not bind specifically to the N-terminal region of human ANGPTL8 defined by SEQ ID NO: 337; vii) does not bind to the N-terminal coiled-coil region of human ANGPTL3 peptide of SEQ ID NO: 338, or to the N-terminal coiled-coil region of human ANGPTL4 peptide of SEQ ID NO: 339; viii) binds human ANGPTL8 at 25°C with a K_D of less than about 150pM and binds monkey ANGPTL8 at 25°C with a K_D of less than about 90pM as measured by surface plasmon resonance; ix) lowers triglyceride levels in a mammal by about 68% (maximum) when administered subcutaneously at a dose of about 10 mg/kg; x) lowers triglyceride levels in a mammal for a period ranging from about 7 days to 21 days, when administered subcutaneously at doses ranging from about 5 mg/kg to about 25 mg/kg; xi) cross-competes with a reference antibody, wherein the reference antibody comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) amino acid sequence selected from the group consisting of any of the HCVR and LCVR

amino acid sequences of Table 1.

[0046] In a second aspect, the present invention also provides nucleic acid molecules encoding anti-ANGPTL8 antibodies or portions thereof. For example, the present invention provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0047] The present invention also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 1. In certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0048] The present invention also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 1. In certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0049] The present invention also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 1. In certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0050] The present invention also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 1. In certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0051] The present invention also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 1. In certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0052] The present invention also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 1. In certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at

least 95%, at least 98% or at least 99% sequence identity thereto.

[0053] The present invention also provides nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 1. In certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0054] The present invention also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-ANGPTL8 antibodies listed in Table 1.

[0055] The present invention also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary anti-ANGPTL8 antibodies listed in Table 1.

[0056] The present invention also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 1, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 1. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In certain embodiments according to this aspect of the invention, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-ANGPTL8 antibody listed in Table 1.

[0057] The present invention also provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-ANGPTL8 antibody. For example, the present invention includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 1. Also included within the scope of the present invention are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[0058] In one embodiment, the isolated antibody that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, is a recombinantly produced human monoclonal

antibody having a HCVR and/or a LCVR encoded by a nucleic acid sequence selected from the nucleic acid sequences found in Table 2.

[0059] In one embodiment, the invention provides an isolated nucleic acid molecule encoding an antibody or fragment thereof that binds specifically to human ANGPTL8, wherein the antibody or an antigen binding fragment thereof comprises (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence as set forth in Table 1; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence as set forth in Table 1.

[0060] In one embodiment, the invention provides an isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof that binds specifically to human ANGPTL8, wherein the antibody or antigen-binding fragment comprises an HCVR selected from the group consisting of an amino acid sequence as set forth in Table 1 and a LCVR selected from the group consisting of an amino acid sequence as set forth in Table 1.

[0061] In a third aspect, the invention provides a pharmaceutical composition comprising a recombinant human monoclonal antibody or antigen-binding fragment thereof, which specifically binds ANGPTL8 and a pharmaceutically acceptable carrier.

[0062] In one embodiment, the invention provide a pharmaceutical composition comprising at least one antibody specific for human ANGPTL8 selected from an antibody or an antigen-binding fragment thereof of any of the anti-ANGPTL8 antibodies found in Table 1 and a pharmaceutically acceptable carrier or diluent.

[0063] In a related aspect, the invention features a composition, which is a combination of an anti-ANGPTL8 antibody and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-ANGPTL8 antibody.

[0064] In one embodiment, the second therapeutic agent may be an agent capable of lowering triglycerides or reducing at least one symptom in a patient suffering from a disease or condition characterized by high triglyceride levels, such as hypertriglyceridemia.

[0065] In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with the antibody or antigen-binding fragment of an antibody of the invention, if such side effect(s) should occur.

[0066] The second therapeutic agent may be a small molecule drug, a protein/polypeptide, an antibody, a nucleic acid molecule, such as an anti-sense molecule, or a siRNA. The second therapeutic agent may be synthetic or naturally derived.

[0067] It will also be appreciated that the antibodies and pharmaceutically acceptable compositions of the present invention can be employed in other combination therapies, that is, the antibodies and pharmaceutically acceptable compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures.

The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an antibody may be administered concurrently with another agent used to treat the same disorder), or they may achieve different effects (*e.g.,* control of any adverse effects). As used herein, additional therapeutic agents that are normally administered to treat or prevent a particular disease, or condition, are appropriate for the disease, or condition, being treated.

[0068] In a related embodiment, the invention features a composition, which is a combination of an antibody or antigen-binding fragment thereof of the invention, and a second therapeutic agent, such as (1) 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, such as cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, and the like; (2) inhibitors of cholesterol uptake and/or bile acid re-absorption; (3) niacin, which increases lipoprotein catabolism; (4) fibrates or amphipathic carboxylic acids, which reduce TG level, low-density lipoprotein (LDL) level and improve high-density lipoprotein (HDL) levels; and (5) activators of the LXR transcription factor that plays a role in cholesterol elimination such as 22-hydroxycholesterol, or fixed combinations such as ezetimibe plus simvastatin; a statin with a bile resin (*e.g.*, cholestyramine, colestipol, colesevelam), a fixed combination of niacin plus a statin (*e.g.*, niacin with lovastatin); or with other lipid lowering agents such as omega-3-fatty acid ethyl esters (for example, omacor).

[0069] Furthermore, the second therapeutic agent can be one or more other inhibitors of ANGPTL8 as well as inhibitors of other molecules, such as ANGPTL3, ANGPTL4, ANGPTL5, ANGPTL6, apolipoprotein C-III (APOC3) and proprotein convertase subtilisin/kexin type 9 (PCSK9), which are involved in lipid metabolism, in particular, cholesterol and/or triglyceride homeostasis. Inhibitors of these molecules include small molecules, antisense molecules and antibodies that specifically bind to these molecules and block their activity.

[0070] In one embodiment, if the anti-ANGPTL8 antibodies of the invention are used to treat a disease such as diabetes (*e.g.,* type 2 diabetes), then these antibodies may be used in combination with one or more of the following treatments for diabetes that are currently available. These include the following: insulin, an insulin analog (see below), a biguanide (metformin), a sulfonylurea (e.g. glyburide, glipizide), a PPAR gamma agonist (e.g. pioglitazone, rosiglitazone), an alpha glucosidase inhibitor (e.g. acarbose, voglibose), a glucagon-like peptide 1 (GLP-1) agonist (e.g., BYETTA® (exenatide), TRULICITY™(dulaglutide), VICTOZA® (liraglutide), Lyxumia® (lixisenatide), Tanzeum™ (albiglutide)), a dipeptidyl peptidase IV (DPP-4) inhibitor (e.g. saxagliptin (ONGLYZA®), sitaliptin (JANUVIA®), and vildagliptin (GALVUS®), a sodium-glucose co-transporter 2 (SGLT2) inhibitor (e.g., INVOKANA™ (canagliflozin), FORXIGA® (dapagliflozin), empagliflozin, ipragliflozin, tofogliflozin), SYMLIN® (pramlintide), a

glucagon receptor antagonist (as described in, for example, US8545847), and a glucagon antagonist.

[0071] In certain related embodiments, the composition may include a second agent selected from the group consisting of non-sulfonylurea secretagogues, insulin analogs, including fast acting (*e.g.,* Lispro, Aspart, Glulisine) and long acting (*e.g.* Detemir insulin, Degludec insulin, or Glargine insulin, exendin-4 polypeptides, beta 3 adrenoceptor agonists, inhibitors of cholesterol uptake and/or bile acid re-absorption, LDL-cholesterol antagonists, cholesteryl ester transfer protein antagonists (*e.g.* torcetrapib, anacetrapib, dalcetrapib, or evacetrapib), endothelin receptor antagonists, growth hormone antagonists, insulin sensitizers, amylin mimetics or agonists, cannabinoid receptor antagonists, glucagon-like peptide-1 receptor agonists, melanocortins, melanin-concentrating hormone receptor agonists, SNRIs, a fibroblast growth factor 21 (FGF21) mimetic (See, for example, US20110002845 and US20080261236), a fibroblast growth factor receptor 1c (FGFR1c) agonist (See, for example, US20110150901), an inhibitor of advanced glycation end product formation, such as, but not limited to, aminoguanidine, and protein tyrosine phosphatase inhibitors.

[0072] In related embodiments, the second therapeutic agent may be one or more other therapeutic agents, such as analgesics, anti-inflammatory agents, including non-steroidal anti-inflammatory drugs (NSAIDS), such as Cox-2 inhibitors, and the like, so as to ameliorate and/or reduce the symptoms accompanying the underlying condition, if needed.

[0073] In a fourth aspect, the invention provides a method for neutralizing, inhibiting, blocking, abrogating, reducing or interfering with, at least one activity associated with ANGPTL8 in a patient in need thereof, the method comprising administering any one or more of the antibodies of the invention, as found in Table 1, or a pharmaceutical composition comprising any one or more of these antibodies to a patient in need thereof, wherein at least one activity associated with ANGPTL8 is reduced or diminished.

[0074] In one embodiment, the invention provides a therapeutic method comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising one or more anti-hANGPTL8 antibodies or antigen-binding fragments thereof of the invention and, optionally one or more additional therapeutic agents as described above.

[0075] In a fifth aspect, the invention provides a method for treating a disease or condition associated in part with elevated expression and/or activity of ANGPTL8, the method comprising administering an ANGPTL8 inhibitor/antagonist, wherein the ANGPTL8 inhibitor/antagonist is an antibody or an antigen-binding fragment thereof specific for ANGPTL8. In one embodiment, the antibody or an antigen-binding fragment thereof specific for ANGPTL8 comprises an HCVR selected from the group consisting of an amino acid sequence from Table 1 and a LCVR selected from the group consisting of an amino acid sequence from Table 1.

[0076] In one embodiment, the disease or disorder treatable by the methods of the invention is any disease or condition which is improved, ameliorated, inhibited or prevented, or at least one symptom associated with the disease is reduced in severity or frequency of occurrence, compared to that without anti-hANGPTL8 antibody treatment (*e.g.*, ANGPTL8-mediated diseases or disorders), by removing, inhibiting, reducing, or otherwise interfering with, ANGPTL8 activity. Examples of diseases or disorders treatable by the methods of the invention include, but are not limited to, those involving lipid metabolism, such as hyperlipidemia, hyperlipoproteinemia and dyslipidemia, including atherogenic dyslipidemia, diabetic dyslipidemia, hypertriglyceridemia, including severe hypertriglyceridemia with TG > 1000 mg/dL and associated acute pancreatitis, hypercholesterolemia, chylomicronemia, mixed dyslipidemia (obesity, metabolic syndrome, diabetes, etc.), lipodystrophy, lipoatrophy, and the like, which are caused by, for example, decreased LPL activity and/or LPL deficiency, altered ApoC2, ApoE deficiency, increased ApoB, increased production and/or decreased elimination of very low-density lipoprotein (VLDL), certain drug treatment (*e.g.*, glucocorticoid treatment-induced dyslipidemia), any genetic predisposition, diet, life style, and the like.

[0077] The methods of the invention can also prevent or treat diseases or disorders associated with or resulting from triglyceridemia, hypertriglyceridemia, hyperlipidemia, hyper-lipoproteinemia, and/or dyslipidemia, including, but not limited to, cardiovascular diseases or disorders, such as atherosclerosis, aneurysm, hypertension, angina, stroke, cerebrovascular diseases, congestive heart failure, coronary artery diseases, myocardial infarction, peripheral vascular diseases, and the like; acute pancreatitis; nonalcoholic steatohepatitis (NASH); blood sugar disorders, such as diabetes; obesity, and the like.

[0078] In one embodiment, at least one antibody of the invention, or an antigen-binding fragment thereof, may be used to treat metabolic syndrome associated dyslipidemia, obesity, or for preventing weight gain, or for maintaining a normal weight.

[0079] In one embodiment, the invention provides a method for lowering blood triglyceride levels, or for treating a condition or disease associated with, or characterized in part by high blood triglyceride levels, or at least one symptom or complication associated with the condition or disease, the method comprising administering a pharmaceutical composition comprising one or more antibodies specific for human ANGPTL8 from Table 1, to a patient in need thereof, such that blood triglyceride levels are lowered or that the condition or disease is mediated, or at least one symptom or complication associated with the condition or disease is alleviated or reduced in severity.

[0080] In one embodiment, at least one antibody of the invention, or an antigen-binding fragment thereof, may be used alone or in combination with a second or third therapeutic agent to treat hypertriglyceridemia, or at least one symptom associated with hypertriglyceridemia, or may be used to treat a patient at risk for acquiring hypertriglyceridemia, for example, in a patient

who has a genetic predisposition for developing hypertriglyceridemia, *e.g.* familial hypertriglyceridemia or familial dysbetalipoproteinemia.

[0081] Other conditions may predispose a patient to high levels of triglycerides. For example, certain medications such as beta blockers, birth control pills, diuretics, steroids, or the use of tamoxifen may lead to elevated levels of triglycerides and as such, may increase a patient's likelihood of developing conditions, or complications associated with high levels of triglycerides, such as atherosclerosis, stroke, heart attack, and other cardiac conditions.

[0082] In addition, certain other conditions may lead to high levels of triglycerides, including obesity, poorly controlled diabetes, hypothyroidism, kidney disease, or alcohol consumption.

[0083] In one embodiment, the antibodies may be used to prevent the onset of a disease or disorder characterized in part by elevated blood triglyceride levels, or to prevent the likelihood of developing such disease or disorder, or to mitigate the severity of the disease or disorder, or at least one symptom associated with the disease or disorder. It is envisioned that the antibodies of the invention may be used alone, or as adjunct therapy with other agents or methods known to be standard care for treating patients suffering from diseases or conditions characterized in part by elevated blood triglyceride levels, such as, but not limited to, hypertriglyceridemia. Such standard therapy may include fluid administration, or administration of any other pharmaceutical agents useful for lowering blood triglycerides, or lipids, or for weight reduction.

[0084] In one embodiment, the use of the antibodies described herein, may be an effective means of achieving normal levels of triglycerides, thereby ameliorating, or preventing one or more symptoms of, or long term complications associated with a disease characterized by high triglyceride levels.

[0085] In one embodiment, the antibodies of the invention may be used in the preparation of a medicament for use in treating any disease or disorder characterized in part by elevated levels of triglycerides.

[0086] The antibodies of the invention may be used as short-term therapy in an acute setting, or they may be envisioned for long-term use as chronic therapy.

[0087] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0088] **Figure 1** shows the mean +/- SEM of serum triglyceride and total cholesterol concentration in humanized ANGPTL8 mice administered a single subcutaneous dose of H4H15341P. Doses administered were 1, 5, 10, or 25 mg/kg on day 0 of the study. Statistical comparison was done by 2-way ANOVA of differences from Control Ab, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

[0089] **Figure 2** shows the levels of circulating anti-human antibody after administration of one subcutaneous dose of H4H15341P at doses of 1, 5, 10, or 25 mg/kg.

[0090] **Figure 3** shows the effect of the H4H15341P mAb on serum lipoprotein lipase (LPL) and hepatic lipase in ANGPTL8 mice compared to control antibody. Statistics were done by unpaired student's t-test; **p<0.01

[0091] **Figure 4** shows the effect of the mAb H4H15341P in a lipid tolerance test in ANGPTL8 mice. Administration of the H4H15341P mAb was assessed for lowering of triglyceride levels after acute fat loading compared to control antibody. Statistics were done by 2-way ANOVA with Bonferroni post-test; ****p<0.0001

DETAILED DESCRIPTION

[0092] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0093] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0094] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All patents, applications and non-patent publications mentioned in this specification are incorporated herein by reference in their entireties.

Definitions

[0095] "Angiopoietin-like protein 8" or, "ANGPTL8," is a member of the angiopoietin family of proteins, and is sometimes referred to as TD26, RIFL, Lipasin, C19orf80 and Betatrophin. "ANGPTL8", as used herein, refers to human ANGPTL8 comprising the amino acid sequence as set forth in amino acid residues 1-177 of SEQ ID NO: 340. The full-length human ANGPTL8 amino acid sequence, including the signal sequence, can also be found in GenBank accession number NP_061157.3, while the full-length nucleic acid sequence encoding human ANGPTL8 can be found in GenBank accession number NM_018687.6. The N-terminal coiled-coil domain of human ANGPTL8 spans amino acid residues 1-39 of SEQ ID NO: 340 and is also depicted as SEQ ID NO: 337. All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, the expression "ANGPTL8" means human ANGPTL8 unless specified as being from a non-human species,

e.g., "mouse ANGPTL8," "monkey ANGPTL8," etc.

[0096] The term “human angiopoietin-like protein 3” or "hANGPTL3", as used herein, refers to ANGPTL3 having the nucleic acid sequence shown in SEQ ID NO:343 and the amino acid sequence of SEQ ID NO:342, or a biologically active fragment thereof. The N-terminal coiled-coil domain of human ANGPTL3 is depicted as SEQ ID NO: 338.

[0097] The term “human angiopoietin-like protein 4” or "hANGPTL4", as used herein, refers to ANGPTL4 having the nucleic acid sequence shown in SEQ ID NO:345 and the amino acid sequence of SEQ ID NO:344, or a biologically active fragment thereof.` The N-terminal coiled-coil domain of human ANGPTL4 is depicted as SEQ ID NO: 339.

[0098] The specific embodiments, antibody or antibody fragments of the invention may be conjugated to a therapeutic moiety (“immunoconjugate”), such as a second ANGPTL8 antagonist, or any other therapeutic moiety useful for treating a disease or condition caused in part by elevated triglyceride levels.

[0099] As used herein, the expression "anti-ANGPTL8 antibody" includes both monovalent antibodies with a single specificity, as well as bispecific antibodies comprising a first arm that binds ANGPTL8 and a second arm that binds a second (target) antigen, wherein the anti-ANGPTL8 arm comprises any of the HCVR/LCVR or CDR sequences as set forth in Table 1 herein.

[0100] The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, ANGPTL8). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-ANGPTL8 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0101] The term "antibody", as used herein, also includes antigen-binding fragments of full

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

[0102] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0103] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0104] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations

listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0105] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0106] The term "human antibody", as used herein, is intended to include non-naturally occurring human antibodies. The term includes antibodies that are recombinantly produced in a non-human mammal, or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject.

[0107] The antibodies of the invention may, in some embodiments, be recombinant human antibodies. The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. In certain embodiments, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0108] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a

molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[0109] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0110] The antibodies of the invention may be isolated antibodies. An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0111] The anti-ANGPTL8 antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to sequences available from, for example, public antibody sequence databases. Once obtained, antibodies and antigen-binding fragments that contain one or more mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0112] The present invention also includes anti-ANGPTL8 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-ANGPTL8 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences set forth in Table 1 herein.

[0113] A “blocking antibody” or a "neutralizing antibody", as used herein (or an "antibody that

neutralizes ANGPTL8 activity"), is intended to refer to an antibody whose binding to and/or interaction with ANGPTL8 results in inhibition of at least one biological activity of ANGPTL8. For example, an antibody of the invention may inhibit the lipoprotein lipase inhibitory activity of ANGPTL8, or it may lower plasma triglycerides through a mechanism other than through inhibition of the LPL inhibitory activity of ANGPTL8. This inhibition of the biological activity of ANGPTL8 can be assessed by measuring one or more indicators of ANGPTL8 biological activity by one or more of several standard *in vitro* or *in vivo* assays known in the art. An alternate activity is the triglyceride lowering activity associated with an antibody of the invention.

[0114] The term "surface plasmon resonance", or "SPR", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using a BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.), or a MASS-1 system (Sierra Sensors, Hamburg, Germany and Greenville, RI).

[0115] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0116] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[0117] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0118] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid

substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0119] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.,* GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.,* FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.,* Altschul *et al.* (1990) J. Mol. Biol. 215: 403 410 and (1997) Nucleic Acids Res. 25:3389 402, each of which is herein incorporated by reference.

[0120] By the phrase “therapeutically effective amount” is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for

example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[0121] The term “treating” or “treatment”, as used herein, refers to an approach for obtaining beneficial or desired clinical results. In one embodiment of the invention, a beneficial or desired clinical result includes, but is not limited to, an improvement in blood triglyceride levels, or an improvement in any one or more conditions, diseases, or symptoms associated with, or resulting from, elevated levels of triglycerides, including, but not limited to hypertriglyceridemia, etc.

pH-Dependent Binding

[0122] The present invention includes anti-ANGPTL8 antibodies with pH-dependent binding characteristics. For example, an anti-ANGPTL8 antibody of the present invention may exhibit reduced binding to ANGPTL8 at acidic pH as compared to neutral pH. Alternatively, anti-ANGPTL8 antibodies of the invention may exhibit enhanced binding to ANGPTL8 at acidic pH as compared to neutral pH. The expression "acidic pH" includes pH values less than about 6.2, *e.g.*, about 6.0, 5.95, 5,9, 5.85, 5.8, 5.75, 5.7, 5.65, 5.6, 5.55, 5.5, 5.45, 5.4, 5.35, 5.3, 5.25, 5.2, 5.15, 5.1, 5.05, 5.0, or less. As used herein, the expression "neutral pH" means a pH of about 7.0 to about 7.4. The expression "neutral pH" includes pH values of about 7.0, 7.05, 7.1, 7.15, 7.2, 7.25, 7.3, 7.35, and 7.4.

[0123] In certain instances, "reduced binding to ANGPTL8 at acidic pH as compared to neutral pH" is expressed in terms of a ratio of the K_D value of the antibody binding to ANGPTL8 at acidic pH to the K_D value of the antibody binding to ANGPTL8 at neutral pH (or vice versa). For example, an antibody or antigen-binding fragment thereof may be regarded as exhibiting "reduced binding to ANGPTL8 at acidic pH as compared to neutral pH" for purposes of the present invention if the antibody or antigen-binding fragment thereof exhibits an acidic/neutral K_D ratio of about 3.0 or greater. In certain exemplary embodiments, the acidic/neutral K_D ratio for an antibody or antigen-binding fragment of the present invention can be about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 20.0. 25.0, 30.0, 40.0, 50.0, 60.0, 70.0, 100.0 or greater.

[0124] Antibodies with pH-dependent binding characteristics may be obtained, *e.g.*, by screening a population of antibodies for reduced (or enhanced) binding to a particular antigen at acidic pH as compared to neutral pH. Additionally, modifications of the antigen-binding domain at the amino acid level may yield antibodies with pH-dependent characteristics. For example, by substituting one or more amino acids of an antigen-binding domain (*e.g.*, within a CDR) with a histidine residue, an antibody with reduced antigen-binding at acidic pH relative to neutral pH may be obtained.

Anti-ANGPTL8 Antibodies Comprising Fc Variants

[0125] According to certain embodiments of the present invention, anti-ANGPTL8 antibodies

are provided comprising an Fc domain comprising one or more mutations, which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes anti-ANGPTL8 antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (*e.g.,* A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P). In yet another embodiment, the modification comprises a 265A (*e.g.*, D265A) and/or a 297A (*e.g.*, N297A) modification.

[0126] For example, the present invention includes anti-ANGPTL8 antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); 257I and 311I (*e.g.*, P257I and Q311I); 257I and 434H (*e.g.*, P257I and N434H); 376V and 434H (*e.g.*, D376V and N434H); 307A, 380A and 434A (*e.g.*, T307A, E380A and N434A); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

[0127] The present invention also includes anti-ANGPTL8 antibodies comprising a chimeric heavy chain constant (C_H) region, wherein the chimeric C_H region comprises segments derived from the C_H regions of more than one immunoglobulin isotype. For example, the antibodies of the invention may comprise a chimeric C_H region comprising part or all of a C_H2 domain derived from a human IgG1, human IgG2 or human IgG4 molecule, combined with part or all of a C_H3 domain derived from a human IgG1, human IgG2 or human IgG4 molecule. According to certain embodiments, the antibodies of the invention comprise a chimeric C_H region having a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" amino acid sequence (amino acid residues from positions 216 to 227 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence (amino acid residues from positions 228 to 236 according to EU

numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. According to certain embodiments, the chimeric hinge region comprises amino acid residues derived from a human IgG1 or a human IgG4 upper hinge and amino acid residues derived from a human IgG2 lower hinge. An antibody comprising a chimeric C_H region as described herein may, in certain embodiments, exhibit modified Fc effector functions without adversely affecting the therapeutic or pharmacokinetic properties of the antibody. (*See*, *e.g.*, U.S. Provisional Appl. No. 61/759,578, filed February 1, 2013, the disclosure of which is hereby incorporated by reference in its entirety).

Biological Characteristics of the Antibodies

[0128] The present invention includes antibodies and antigen-binding fragments thereof that bind ANGPTL8 with high affinity. For example, the present invention includes anti-ANGPTL8 antibodies that bind human or monkey ANGPTL8 with a K_D of less than about 150 nM, as measured by surface plasmon resonance (SPR) at 25ºC, or at 37ºC, *e.g.*, using recombinant ANGPTL8 protein with a mouse IgG2a Fc C-terminal fusion, in an assay format as defined in Examples 3 and 4 herein, or a substantially similar assay. According to certain embodiments, anti-ANGPTL8 antibodies are provided that bind human or monkey ANGPTL8 at 25ºC or 37ºC with a K_D of less than about 90 nM, or less than about 50 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900 pM, less than about 500 pM, less than about 300 pM, less than about 150 pM, or less than about 90 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Examples 3 and 4 herein, or a substantially similar assay.

[0129] The present invention also includes antibodies and antigen-binding fragments thereof that bind the peptide of SEQ ID NO: 337 derived from the N-terminal region of human ANGPTL8 with a dissociative half-life (t½) of greater than about 100 minutes as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-ANGPTL8 antibodies are provided that bind peptides derived from the N-terminal region of human ANGPTL8 at 25ºC with a t½ of greater than or equal to about 110 minutes, greater than about 120 minutes, greater than about 130 minutes, greater than about 200 minutes, greater than about 300 minutes, greater than about 400 minutes, greater than about 500 minutes, or longer, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[0130] The present invention also includes antibodies and antigen-binding fragments thereof that lower triglycerides in a mammal by about 20%, or by about 30%, or by about 40%, or by about 50%, or by about 60%, or greater when administered subcutaneously at a dose of about

0.1mg/kg, or about 1mg/kg, or about 10mg/kg, or about 25mg/kg, or about 50mg/kg, or about 100mg/kg. The effect of an antibody of the invention on lowering plasma triglycerides may last from at least 7 days after administration to about 3 weeks, or 4 weeks after administration, or longer.

[0131] An antibody of the invention comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 266, 274, 282, 290, 298, 306, 314 and 330; and

a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, and 322; or may cross-compete with a reference antibody, wherein the reference antibody comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) amino acid sequence selected from the group consisting of any of the HCVR and LCVR amino acid sequences of Table 1.

[0132] An antibody of the invention may comprise a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/250, 266/250, 274/250, 282/250, 290/250, 306/250, 314/322, and 330/322.

[0133] An antibody of the invention may comprise:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 268, 276, 284, 292, 300, 308, 316 and 332;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 270, 278, 286, 294, 302, 310, 318, and 334;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 272, 280, 288, 296, 304, 312, 320 and 336;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252 and 324;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, and 326; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256 and 328.

[0134] The antibodies of the present invention may possess one or more of the aforementioned biological characteristics, or any combination thereof. The foregoing list of biological characteristics of the antibodies of the invention is not intended to be exhaustive. Other biological characteristics of the antibodies of the present invention will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Epitope Mapping and Related Technologies

[0135] The epitope to which the antibodies of the present invention bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids of an ANGPTL8 protein. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) of ANGPTL8.

[0136] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, *e.g.*, routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY), alanine scanning mutational analysis, peptide blots analysis (Reineke, 2004, Methods Mol Biol 248:443-463), and peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, 2000, Protein Science 9:487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water to allow hydrogen-deuterium exchange to occur at all residues except for the residues protected by the antibody (which remain deuterium-labeled). After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem. 73*:256A-265A.

[0137] The present invention further includes anti-ANGPTL8 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein). Likewise, the present invention also includes anti-ANGPTL8 antibodies that compete for binding to ANGPTL8 with any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein).

[0138] One can easily determine whether an antibody binds to the same epitope as, or

competes for binding with, a reference anti-ANGPTL8 antibody by using routine methods known in the art and exemplified herein. For example, to determine if a test antibody binds to the same epitope as a reference anti-ANGPTL8 antibody of the invention, the reference antibody is allowed to bind to an ANGPTL8 protein. Next, the ability of a test antibody to bind to the ANGPTL8 molecule is assessed. If the test antibody is able to bind to ANGPTL8 following saturation binding with the reference anti-ANGPTL8 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-ANGPTL8 antibody. On the other hand, if the test antibody is not able to bind to the ANGPTL8 molecule following saturation binding with the reference anti-ANGPTL8 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-ANGPTL8 antibody of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antibodies bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res. 1990:50:1495-1502). Alternatively, two antibodies are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0139] To determine if an antibody competes for binding (or cross-competes for binding) with a reference anti-ANGPTL8 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a ANGPTL8 protein under saturating conditions followed by assessment of binding of the test antibody to the ANGPTL8 molecule. In a second orientation, the test antibody is allowed to bind to an ANGPTL8 molecule under saturating conditions followed by assessment of binding of the reference antibody to the ANGPTL8 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the ANGPTL8 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to ANGPTL8. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the same epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Preparation of Human Antibodies

[0140] The anti-ANGPTL8 antibodies of the present invention can be fully human (non-naturally occurring) antibodies. Methods for generating monoclonal antibodies, including fully human monoclonal antibodies are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human ANGPTL8.

[0141] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to an allergen are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[0142] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0143] As described in the experimental section below, the high affinity chimeric antibodies, which are isolated having a human variable region and a mouse constant region, are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are then replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[0144] In general, the antibodies of the instant invention possess very high affinities, typically possessing K_D of from about 10^{-12} through about 10^{-9} M, when measured by binding to antigen either immobilized on solid phase or in solution phase.

Bioequivalents

[0145] The anti-ANGPTL8 antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies but that retain the ability to bind human ANGPTL8. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the anti-ANGPTL8 antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an anti-ANGPTL8 antibody or antibody fragment that is essentially bioequivalent to an anti-ANGPTL8 antibody or antibody fragment of the invention. Examples of such variant amino acid and DNA sequences are discussed above.

[0146] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single does or multiple dose. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[0147] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0148] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0149] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0150] Bioequivalence may be demonstrated by *in vivo* and *in vitro* methods. Bioequivalence measures include, e.g., (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other

mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[0151] Bioequivalent variants of anti-ANGPTL8 antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include anti-ANGPTL8 antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations which eliminate or remove glycosylation.

Multispecific Antibodies

[0152] The antibodies of the present invention may be monospecific or multispecific (*e.g.*, bispecific). Multispecific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al., 1991, J. Immunol. 147:60-69; Kufer *et al.*, 2004, Trends Biotechnol. 22:238-244. The anti-ANGPTL8 antibodies of the present invention can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multispecific antibody with a second binding specificity.

[0153] The present invention includes bispecific antibodies wherein one arm of an immunoglobulin binds human ANGPTL8, and the other arm of the immunoglobulin is specific for a second antigen. The ANGPTL8-binding arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 herein.

[0154] An exemplary bispecific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and

V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bispecific antibody format described above are contemplated within the scope of the present invention.

[0155] Other exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see*, *e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See*, *e.g.*, Kazane *et al.*, *J. Am. Chem. Soc.* [*Epub*: *Dec. 4, 2012*]).

Therapeutic Formulation and Administration

[0156] The invention provides pharmaceutical compositions comprising the anti-ANGPTL8 antibodies or antigen-binding fragments thereof of the present invention. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™, Life Technologies, Carlsbad, CA), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0157] The dose of antibody administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. In an adult patient, it may be advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering anti-ANGPTL8 antibodies may be determined empirically; for example, patient progress can be monitored by

periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti *et al.*, 1991, *Pharmaceut. Res. 8*:1351).

[0158] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al., 1987, J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0159] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0160] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (Sanofi-Aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (Sanofi-Aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand

Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0161] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0162] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0163] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Immunoconjugates

[0164] The invention encompasses a human anti-ANGPTL8 monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as an agent that is capable of reducing blood triglyceride or lipid levels. The type of therapeutic moiety that may be conjugated to the anti-ANGPTL8 antibody will take into account the condition to be treated and the desired therapeutic

effect to be achieved. For example, for treating hypertriglyceridemia, or any other condition whereby it is desirable to lower blood triglycerides, and/or to maintain normal blood triglyceride levels, an agent may be conjugated to the ANGPTL8 antibody. Alternatively, if the desired therapeutic effect is to treat the sequelae or symptoms associated with hypertriglyceridemia, or any other condition resulting from high, or uncontrolled blood triglyceride levels, it may be advantageous to conjugate an agent appropriate to treat the sequelae or symptoms of the condition. Examples of suitable agents for forming immunoconjugates are known in the art, see for example, WO 05/103081.

Therapeutic Uses of the Antibodies

[0165] The present antibodies are useful for lowering blood triglyceride levels, for example, in a patient suffering from hypertriglyceridemia, and also for the treatment of a wide range of conditions and disorders in which inhibiting the activity of ANGPTL8 is beneficial. Thus, the antibodies may find use for example to prevent, treat, or alleviate, diseases or conditions or associated symptoms or sequelae, of the endocrine system, the central nervous system, the peripheral nervous system, the cardiovascular system, the pulmonary system, and the gastrointestinal system, while reducing and or eliminating one or more of the unwanted side effects associated with the current treatments.

[0166] For example, the antibodies of the invention may be used to treat a disease or disorder including, but not limited to, those involving lipid metabolism, such as hyperlipidemia, hyperlipoproteinemia and dyslipidemia, including atherogenic dyslipidemia, diabetic dyslipidemia, hypertriglyceridemia, including severe hypertriglyceridemia with TG > 1000 mg/dL and associated acute pancreatitis, hypercholesterolemia, chylomicronemia, mixed dyslipidemia (obesity, metabolic syndrome, diabetes, etc.), lipodystrophy, lipoatrophy, and the like, which are caused by, for example, decreased LPL activity and/or LPL deficiency, altered ApoC2, ApoE deficiency, increased ApoB, increased production and/or decreased elimination of very low-density lipoprotein (VLDL), certain drug treatment (*e.g.*, glucocorticoid treatment-induced dyslipidemia), any genetic predisposition, diet, life style, and the like.

[0167] The methods of the invention can also prevent or treat diseases or disorders associated with or resulting from triglyceridemia, hypertriglyceridemia, hyperlipidemia, hyper-lipoproteinemia, and/or dyslipidemia, including, but not limited to, cardiovascular diseases or disorders, such as atherosclerosis, aneurysm, hypertension, angina, stroke, cerebrovascular diseases, congestive heart failure, coronary artery diseases, myocardial infarction, peripheral vascular diseases, and the like; acute pancreatitis; nonalcoholic steatohepatitis (NASH); blood sugar disorders, such as diabetes (e.g. Type II diabetes); obesity, and the like.

[0168] In one embodiment, at least one antibody of the invention, or an antigen-binding fragment thereof, may be used to treat metabolic syndrome associated dyslipidemia, obesity, or for preventing weight gain, or for maintaining a normal weight.

[0169] In one embodiment, the invention provides a method for lowering blood triglyceride levels, or for treating a condition or disease associated with, or characterized in part by high blood triglyceride levels, or at least one symptom or complication associated with the condition or disease, the method comprising administering a pharmaceutical composition comprising one or more antibodies specific for human ANGPTL8 from Table 1, to a patient in need thereof, such that blood triglyceride levels are lowered or that the condition or disease is mediated, or at least one symptom or complication associated with the condition or disease is alleviated or reduced in severity.

[0170] In one embodiment, at least one antibody of the invention, or an antigen-binding fragment thereof, may be used alone or in combination with a second or third therapeutic agent to treat hypertriglyceridemia, or at least one symptom associated with hypertriglyceridemia, or may be used to treat a patient at risk for acquiring hypertriglyceridemia, for example, in a patient who has a genetic predisposition for developing hypertriglyceridemia, *e.g.* familial hypertriglyceridemia or familial dysbetalipoproteinemia.

[0171] Other conditions may predispose a patient to high levels of triglycerides. For example, certain medications such as beta blockers, birth control pills, diuretics, steroids, or the use of tamoxifen may lead to elevated levels of triglycerides and as such, may increase a patient's likelihood of developing conditions, or complications associated with high levels of triglycerides, such as atherosclerosis, stroke, heart attack, and other cardiac conditions.

[0172] In addition, certain other conditions may lead to high levels of triglycerides, including obesity, poorly controlled diabetes, hypothyroidism, kidney disease, or alcohol consumption.

[0173] In one embodiment, the antibodies may be used to prevent the onset of a disease or disorder characterized in part by elevated blood triglyceride levels, or to prevent the likelihood of developing such disease or disorder, or to mitigate the severity of the disease or disorder, or at least one symptom associated with the disease or disorder. It is envisioned that the antibodies of the invention may be used alone, or as adjunct therapy with other agents or methods known to be standard care for treating patients suffering from diseases or conditions characterized in part by elevated blood triglyceride levels, such as, but not limited to, hypertriglyceridemia. Such standard therapy may include fluid administration, or administration of any other pharmaceutical agents useful for lowering blood triglycerides, or lipids, or for weight reduction.

[0174] In one embodiment, the use of the antibodies described herein, may be an effective means of achieving normal levels of triglycerides, thereby ameliorating, or preventing one or more symptoms of, or long term complications associated with a disease characterized by high triglyceride levels.

[0175] It is envisioned that the antibodies of the invention may be used in an acute setting (for short term use), or for longer term (chronic) use.

Combination Therapies

[0176] Combination therapies may include an anti-ANGPTL8 antibody of the invention and any additional therapeutic agent that may be advantageously combined with an antibody of the invention, or with a biologically active fragment of an antibody of the invention.

[0177] For example, when the antibodies of the invention are contemplated for use in treating a disease or condition characterized in part by elevated triglyceride levels, such as hypertriglyceridemia, a second therapeutic agent may be employed to aid in further lowering of triglyceride levels, or to reduce at least one symptom in a patient suffering from a disease or condition characterized by high blood triglyceride levels. Such a second agent may be selected from, for example, another ANGPTL8 antagonist (*e.g.* another different anti-ANGPTL8 antibody or small molecule inhibitor of ANGPTL8), or may include other therapeutic moieties useful for treating triglyceridemia, or other diseases or conditions associated with, or resulting from elevated blood triglyceride levels, or agents useful for treating any long term complications associated with elevated and/or uncontrolled blood triglyceride levels.

[0178] In related embodiments, the invention features a composition, which is a combination of an antibody or antigen-binding fragment thereof of the invention, and a second therapeutic agent, such as (1) 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, such as cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, and the like; (2) inhibitors of cholesterol uptake and/or bile acid re-absorption; (3) niacin, which increases lipoprotein catabolism; (4) fibrates or amphipathic carboxylic acids, which reduce low-density lipoprotein (LDL) level, improve high-density lipoprotein (HDL) and TG levels, and reduce the number of non-fatal heart attacks; and (5) activators of the LXR transcription factor that plays a role in cholesterol elimination such as 22-hydroxycholesterol, or fixed combinations such as ezetimibe plus simvastatin; a statin with a bile resin (*e.g.*, cholestyramine, colestipol, colesevelam), a fixed combination of niacin plus a statin (*e.g.*, niacin with lovastatin); or with other lipid lowering agents such as omega-3-fatty acid ethyl esters (for example, omacor).

[0179] Furthermore, the second therapeutic agent can be one or more other inhibitors/antagonists of glucagon or an inhibitor/antagonist of the glucagon receptor, as well as inhibitors of other molecules, such as other inhibitors of ANGPTL8, as well as inhibitors of other molecules, such as ANGPTL3, ANGPTL4, ANGPTL5, ANGPTL6, apolipoprotein C-III (also referred to as APOC3; see for example, inhibitors of APOC3 described in US8530439, US7750141, US7598227 and volanesorsen, also referred to as ISIS-APOCIIIRx) and proprotein convertase subtilisin/kexin type 9 (PCSK9), which are involved in lipid metabolism, in particular, cholesterol and/or triglyceride homeostasis. Inhibitors of these molecules include small molecules, antisense molecules and antibodies that specifically bind to these molecules and block their activity.

[0180] In one embodiment, if the anti-ANGPTL3 antibodies of the invention are used to treat a disease such as diabetes (*e.g.,* type 2 diabetes), then these antibodies may be used in combination with one or more of the following treatments for diabetes that are currently available. These include the following: insulin, an insulin analog (see below), a biguanide (metformin), a sulfonylurea (e.g. glyburide, glipizide), a PPAR gamma agonist (e.g. pioglitazone, rosiglitazone), an alpha glucosidase inhibitor (e.g. acarbose, voglibose), a glucagon-like peptide 1 (GLP-1) receptor agonist (e.g., BYETTA® (exenatide), TRULICITY™ (dulaglutide), VICTOZA® (liraglutide), LYXUMIA® (lixisenatide), TANZEUM™ (albiglutide), or an analogue of any of the foregoing), a dipeptidyl peptidase IV (DPP-4) inhibitor (e.g. saxagliptin (ONGLYZA®), sitaliptin (JANUVIA®), and vildagliptin (GALVUS®), a sodium-glucose co-transporter 2 (SGLT2) inhibitor (e.g., INVOKANA™ (canagliflozin), FORXIGA® (dapagliflozin), empagliflozin, ipragliflozin, tofogliflozin), (SYMLIN® (pramlintide), a glucagon receptor antagonist (as described in, for example, US8545847), and a glucagon antagonist.

[0181] In certain related embodiments, the composition may include a second agent selected from the group consisting of non-sulfonylurea secretagogues, insulin analogs, including fast acting (*e.g.,* Lispro, Aspart, Glulisine) and long acting (*e.g.* Detemir insulin, Degludec insulin, or Glargine insulin, exendin-4 polypeptides, beta 3 adrenoceptor agonists, inhibitors of cholesterol uptake and/or bile acid re-absorption, LDL-cholesterol antagonists, cholesteryl ester transfer protein antagonists (*e.g.* torcetrapib, anacetrapib, dalcetrapib, or evacetrapib), endothelin receptor antagonists, growth hormone antagonists, insulin sensitizers, amylin mimetics or agonists, cannabinoid receptor antagonists, glucagon-like peptide-1 receptor agonists, melanocortins, melanin-concentrating hormone receptor agonists, SNRIs, a fibroblast growth factor 21 (FGF21) mimetic (See, for example, US20110002845 and US20080261236), a fibroblast growth factor receptor 1c (FGFR1c) agonist (See, for example, US20110150901), an inhibitor of advanced glycation end product formation, such as, but not limited to, aminoguanidine, and protein tyrosine phosphatase inhibitors.

[0182] In related embodiments, the second therapeutic agent may be one or more other therapeutic agents, such as analgesics, anti-inflammatory agents, including non-steroidal anti-inflammatory drugs (NSAIDS), such as Cox-2 inhibitors, and the like, so as to ameliorate and/or reduce the symptoms accompanying the underlying condition, if needed.

[0183] The additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of the anti-ANGPTL8 antibody of the present invention. For purposes of the present disclosure, such administration regimens are considered the administration of an anti-ANGPTL8 antibody "in combination with" a second therapeutically active component.

Administration Regimens

[0184] According to certain embodiments of the present invention, multiple doses of an anti-ANGPTL8 antibody (or a pharmaceutical composition comprising a combination of an anti-ANGPTL8 antibody and any of the additional therapeutically active agents mentioned herein) may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an anti-ANGPTL8 antibody of the invention. As used herein, "sequentially administering" means that each dose of anti-ANGPTL8 antibody is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an anti-ANGPTL8 antibody, followed by one or more secondary doses of the anti-ANGPTL8 antibody, and optionally followed by one or more tertiary doses of the anti-ANGPTL8 antibody.

[0185] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the anti-ANGPTL8 antibody of the invention. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of anti-ANGPTL8 antibody, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of anti-ANGPTL8 antibody contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0186] In certain exemplary embodiments of the present invention, each secondary and/or tertiary dose is administered 1 to 26 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of anti-ANGPTL8 antibody, which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0187] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an anti-ANGPTL8 antibody. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are

administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient. The administration regimen may be carried out indefinitely over the lifetime of a particular subject, or until such treatment is no longer therapeutically needed or advantageous.

[0188] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks or 1 to 2 months after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 12 weeks after the immediately preceding dose. In certain embodiments of the invention, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

Diagnostic Uses of the Antibodies

[0189] The anti-ANGPTL8 antibodies of the present invention may also be used to detect and/or measure ANGPTL8 in a sample, *e.g.*, for diagnostic purposes. For example, an anti-ANGPTL8 antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of ANGPTL8. Exemplary diagnostic assays for ANGPTL8 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-ANGPTL8 antibody of the invention, wherein the anti-ANGPTL8 antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate ANGPTL8 protein from patient samples. Alternatively, an unlabeled anti-ANGPTL8 antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β-galactosidase, horseradish peroxidase, or luciferase.

[0190] Specific exemplary assays that can be used to detect or measure ANGPTL8 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0191] Samples that can be used in ANGPTL8 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of ANGPTL8 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of ANGPTL8 in a particular sample obtained from a healthy patient (*e.g.*, a

patient not afflicted with a disease or condition associated with abnormal ANGPTL8 levels or activity) will be measured to initially establish a baseline, or standard, level of ANGPTL8. This baseline level of ANGPTL8 can then be compared against the levels of ANGPTL8 measured in samples obtained from individuals suspected of having a ANGPTL8 related disease or condition, or symptoms associated with such disease or condition.

EXAMPLES

[0192] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[0193] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0194] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Example 1. Generation of Anti-ANGPTL8 Antibodies

[0195] Anti-ANGPTL8 antibodies were obtained by immunizing a VELOCIMMUNE® mouse (*i.e.*, an engineered mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions) with an immunogen comprising a recombinant human ANGPTL8 expressed with a C-terminal mouse IgG2a tag (See SEQ ID NO: 340). The antibody immune response was monitored by an ANGPTL8-specific immunoassay. When a desired immune response was achieved, several fully human anti-ANGPTL8 antibodies were generated from antigen-positive B cells as described in US 2007/0280945A1, incorporated by reference herein in its entirety.

[0196] Certain biological properties of the exemplary anti-ANGPTL8 antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid and Nucleic Acid Sequences

[0197] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-ANGPTL8 antibodies of the invention. The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 1: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H4H15314P2	2	4	6	8	10	12	14	16
H4H15316P	18	20	22	24	26	28	30	32
H4H15318P	34	36	38	40	42	44	46	48
H4H15319P	50	52	54	56	58	60	62	64
H4H15321P	66	68	70	72	74	76	78	80
H4H15323P	82	84	86	88	90	92	94	96
H4H15330P	98	100	102	104	106	108	110	112
H4H15331P	114	116	118	120	122	124	126	128
H4H15334P	130	132	134	136	138	140	142	144
H4H15335P	146	148	150	152	154	156	158	160
H4H15341P	162	164	166	168	170	172	174	176
H4H15343P	178	180	182	184	186	188	190	192
H4H15345P	194	196	198	200	202	204	206	208
H4H15346P	210	212	214	216	218	220	222	224
H4H15347P	226	228	230	232	234	236	238	240
H4H15350P2	242	244	246	248	250	252	254	256
H4H15353P2	258	260	262	264	250	252	254	256
H4H15354P2	266	268	270	272	250	252	254	256
H4H15355P2	274	276	278	280	250	252	254	256
H4H15357P2	282	284	286	288	250	252	254	256
H4H15361P2	290	292	294	296	250	252	254	256
H4H15362P2	298	300	302	304	250	252	254	256
H4H15363P2	306	308	310	312	250	252	254	256
H4H15367P2	314	316	318	320	322	324	326	328
H4H15369P2	330	332	334	336	322	324	326	328

Table 2: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H4H15314P2	1	3	5	7	9	11	13	15
H4H15316P	17	19	21	23	25	27	29	31
H4H15318P	33	35	37	39	41	43	45	47
H4H15319P	49	51	53	55	57	59	61	63
H4H15321P	65	67	69	71	73	75	77	79
H4H15323P	81	83	85	87	89	91	93	95
H4H15330P	97	99	101	103	105	107	109	111
H4H15331P	113	115	117	119	121	123	125	127

H4H15334P	129	131	133	135	137	139	141	143
H4H15335P	145	147	149	151	153	155	157	159
H4H15341P	161	163	165	167	169	171	173	175
H4H15343P	177	179	181	183	185	187	189	191
H4H15345P	193	195	197	199	201	203	205	207
H4H15346P	209	211	213	215	217	219	221	223
H4H15347P	225	227	229	231	233	235	237	239
H4H15350P2	241	243	245	247	249	251	253	255
H4H15353P2	257	259	261	263	249	251	253	255
H4H15354P2	265	267	269	271	249	251	253	255
H4H15355P2	273	275	277	279	249	251	253	255
H4H15357P2	281	283	285	287	249	251	253	255
H4H15361P2	289	291	293	295	249	251	253	255
H4H15362P2	297	299	301	303	249	251	253	255
H4H15363P2	305	307	309	311	249	251	253	255
H4H15367P2	313	315	317	319	321	323	325	327
H4H15369P2	329	331	333	335	321	323	325	327

[0198] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H1H," "H1M," "H2M", "H4H", etc.), followed by a numerical identifier (*e.g.* "15321," "15341," "15350," etc.), followed by a "P" or "N" suffix, as shown in Tables 1 and 2. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H4H15321P", etc. The H4H prefix on the antibody designations used herein indicate the particular Fc region isotype of the antibody. For example, an "H4H" antibody has a human IgG4 Fc, an "H1M" antibody has a mouse IgG1 Fc, and an "H2M" antibody has a mouse IgG2 Fc, (all variable regions are fully human as denoted by the first 'H' in the antibody designation). As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Tables 1 and 2 – will remain the same, and the binding properties are expected to be identical or substantially similar regardless of the nature of the Fc domain.

Example 3: Surface plasmon resonance (SPR) determination of dissociation rate constants (k_d) for ANGPTL8 antibodies binding to ANGPTL8, ANGPTL3, and ANGPTL4 peptides

[0199] It was previously demonstrated that antibodies binding to the N-terminal coiled-coil region of ANGPTL3 [WO 2012/174178 A1; Lee et al. (2009) JBC, 284:13735-13745] and ANGPTL4 [Desai et al. (2007) PNAS, 104:11766-11771] blocked the LPL inhibitory activity of the ANGPTL proteins. In this experiment, antibodies against ANGPTL8 were tested for binding to a peptide from the N-terminal region of ANGPTL8.

[0200] Dissociation rate constants for ANGPTL8 antibodies binding to human ANGPTL8 peptide (hANGPTL8 peptide, SEQ ID NO: 337) were determined using a real-time surface

plasmon resonance based MASS-1 biosensor platform. The assay utilized a format where ANGPTL8 antibodies were captured on the sensor surface and peptides were injected over the antibody surface. Peptides from the N-terminal coiled-coil region of human ANGPTL3 (hANGPTL3 peptide, SEQ ID NO: 338) and human ANGPTL4 (hANGPTL4 peptide, SEQ ID NO: 339) were also included as controls. Also included was a control antibody (H4H268P from US2011/0159015A1) that binds to the ANGPTL4 peptide and a negative isotype control antibody. All binding studies were performed in 10mM HEPES pH 7.4, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20 (HBS-ET running buffer) at 25°C. The HCA sensor surface was derivatized via amine coupling to a monoclonal mouse anti-human Fc antibody (GE, # BR-1008-39), and to this surface was captured approximately 1000RU of each ANGPTL8 antibody or control antibody. Peptide stock solutions were diluted in HBS-ET running buffer to 500nM and injected over the antibody-captured surfaces for 4 minutes at a flow rate of 30µL/minute followed by the dissociation of bound peptide in HBS-ET running buffer for 10 minutes.

[0201] The association phase of peptides binding to captured ANGPTL8 antibodies could not be fit to a 1:1 binding model; therefore, only the dissociation rate constant (k_d) values were calculated by fitting the real-time binding sensorgrams using Scrubber 2.0c curve-fitting software. Dissociative half-lives (t½) were calculated from k_d as:

$$t½\ (min) = \frac{\ln(2)}{60 \times kd}$$

[0202] Binding parameters for the ANGPTL8, ANGPTL3, and ANGPTL4 N-terminal region peptides binding to captured ANGPTL8 antibodies, the control ANGPTL4 antibody, and the isotype control antibody are shown in Tables 3-5.

Results:

[0203] Under these experimental conditions, the maximum non-specific binding signal exhibited by 500nM of hANGPTL8, hANGPTL3, or hANGPTL4 peptides to blank anti-hFc surface was 3 RUs. Hence, binding interactions with signals that were three-fold above the 3 RU non-specific background (i.e., ≥ 9RU) were considered specific binding interactions. Based on this criterion, antibody-peptide binding signals less than 9 RUs were considered non-binding (NB in Table 1).

[0204] From this binding study it was shown that ANGPTL8 antibodies H4H15321P, H4H15367P2, and H4H15345P bind specifically to the N-terminal region ANGPTL8 peptide (SEQ ID NO: 337). None of the ANGPTL8 antibodies bound to the hANGPTL3 (SEQ ID NO: 338) or hANGPTL4 (SEQ ID NO: 339) N-terminal region peptides.

Table 3: Binding of anti-ANGPTL8 monoclonal antibody to hANGPTL8 peptide at 25ºC

mAb Captured	mAb Capture Level (RU)*	500nM hANGPTL8 peptide Bound (RU)	kd (1/s)	t½ (min)
H4H15321P	1101 ± 6.1	61	8.29E-05	139
H4H15367P2	1116 ± 17.4	43	9.82E-05	118
H4H15345P	1096 ± 3.6	37	2.03E-05	570
H4H15361P2	1394 ± 12.3	4	NB	NB
H4H15347P	1554 ± 54.6	0	NB	NB
H4H15318P	1087 ± 31.5	0	NB	NB
H4H15350P2	1298 ± 30.7	0	NB	NB
H4H15363P2	1281 ± 13.7	0	NB	NB
H4H15346P	1277 ± 26.3	0	NB	NB
H4H15334P	1256 ± 5.3	0	NB	NB
H4H15335P	1625 ± 31	0	NB	NB
H4H15343P	1129 ± 19.8	0	NB	NB
H4H15357P2	1159 ± 13.1	0	NB	NB
H4H15353P2	1296 ± 8.5	0	NB	NB
H4H15341P	1023 ± 30.1	0	NB	NB
H4H15369P2	1196 ± 54.2	0	NB	NB
H4H15330P	1168 ± 20.1	0	NB	NB
H4H15362P2	1131 ± 15.5	0	NB	NB
H4H15319P	974 ± 3.5	0	NB	NB
H4H15316P	1107 ± 24.7	0	NB	NB
H4H15323P	1068 ± 16.4	0	NB	NB
H4H15354P2	1297 ± 8.5	0	NB	NB
H4H15355P2	1323 ± 25.4	0	NB	NB
H4H15314P2	1011 ± 3.4	0	NB	NB
H4H15331P	1264 ± 16.8	-1	NB	NB
(α-AngPTL4 Ab)	1281 ± 50.2	0	NB	NB
Negative isotype control Ab	1092 ± 41.5	0	NB	NB
Blank α-hFc Surface	5 ± 0.3	3	NB	NB

* This column displays the average and standard deviation of antibody surface densities used for binding to ANGPTL8.

Table 4: Binding of anti-ANGPTL8 monoclonal antibody to hANGPTL3 shift peptide at 25ºC

mAb Captured	mAb Capture Level (RU)*	500nM hANGPTL3 peptide Bound (RU)	kd (1/s)	t½ (min)
H4H15321P	1101 ± 6.1	0	NB	NB
H4H15367P2	1116 ± 17.4	0	NB	NB

H4H15345P	1096 ± 3.6	0	NB	NB
H4H15361P2	1394 ± 12.3	0	NB	NB
H4H15347P	1554 ± 54.6	-1	NB	NB
H4H15318P	1087 ± 31.5	0	NB	NB
H4H15350P2	1298 ± 30.7	0	NB	NB
H4H15363P2	1281 ± 13.7	0	NB	NB
H4H15346P	1277 ± 26.3	0	NB	NB
H4H15334P	1256 ± 5.3	0	NB	NB
H4H15335P	1625 ± 31	-1	NB	NB
H4H15343P	1129 ± 19.8	0	NB	NB
H4H15357P2	1159 ± 13.1	0	NB	NB
H4H15353P2	1296 ± 8.5	0	NB	NB
H4H15341P	1023 ± 30.1	0	NB	NB
H4H15369P2	1196 ± 54.2	0	NB	NB
H4H15330P	1168 ± 20.1	-1	NB	NB
H4H15362P2	1131 ± 15.5	0	NB	NB
H4H15319P	974 ± 3.5	0	NB	NB
H4H15316P	1107 ± 24.7	0	NB	NB
H4H15323P	1068 ± 16.4	0	NB	NB
H4H15354P2	1297 ± 8.5	0	NB	NB
H4H15355P2	1323 ± 25.4	0	NB	NB
H4H15314P2	1011 ± 3.4	0	NB	NB
H4H15331P	1264 ± 16.8	0	NB	NB
(α-AngPTL4 Ab)	1281 ± 50.2	0	NB	NB
Negative isotype control Ab	1092 ± 41.5	0	NB	NB
Blank α-hFc Surface	5 ± 0.3	0	NB	NB

* This column displays the average and standard deviation of antibody surface densities used for binding to ANGPTL3 peptide.

Table 5: Binding of anti-ANGPTL8 monoclonal antibody to hAngPTL4 peptide at 25ºC

mAb Captured	mAb Capture Level (RU)*	500nM hAngPTL4 peptide Bound (RU)	kd (1/s)	t½ (min)
H4H15321P	1101 ± 6.1	0	NB	NB
H4H15367P2	1116 ± 17.4	0	NB	NB
H4H15345P	1096 ± 3.6	0	NB	NB
H4H15361P2	1394 ± 12.3	0	NB	NB
H4H15347P	1554 ± 54.6	0	NB	NB
H4H15318P	1087 ± 31.5	0	NB	NB
H4H15350P2	1298 ± 30.7	0	NB	NB

H4H15363P2	1281 ± 13.7	0	NB	NB
H4H15346P	1277 ± 26.3	0	NB	NB
H4H15334P	1256 ± 5.3	1	NB	NB
H4H15335P	1625 ± 31	1	NB	NB
H4H15343P	1129 ± 19.8	0	NB	NB
H4H15357P2	1159 ± 13.1	0	NB	NB
H4H15353P2	1296 ± 8.5	0	NB	NB
H4H15341P	1023 ± 30.1	0	NB	NB
H4H15369P2	1196 ± 54.2	0	NB	NB
H4H15330P	1168 ± 20.1	0	NB	NB
H4H15362P2	1131 ± 15.5	0	NB	NB
H4H15319P	974 ± 3.5	0	NB	NB
H4H15316P	1107 ± 24.7	0	NB	NB
H4H15323P	1068 ± 16.4	0	NB	NB
H4H15354P2	1297 ± 8.5	0	NB	NB
H4H15355P2	1323 ± 25.4	0	NB	NB
H4H15314P2	1011 ± 3.4	1	NB	NB
H4H15331P	1264 ± 16.8	0	NB	NB
(α-AngPTL4 Ab)	1281 ± 50.2	23	1.02E-03	11
Negative isotype control Ab	1092 ± 41.5	0	NB	NB
Blank α-hFc Surface	5 ± 0.3	0	NB	NB

* This column displays the average and standard deviation of antibody surface densities used for binding to ANGPTL4 peptide.

Example 4: Determination of kinetic binding parameters for H4H15341P binding to full-length human and monkey ANGPTL8 proteins by surface plasmon resonance (SPR)

[0205] The equilibrium dissociation constant (K_D) for ANGPTL8 antibody H4H15341P binding to full-length human and cynomolgus monkey ANGPTL8 proteins was determined using a real-time surface plasmon resonance-based MASS-1 biosensor platform. For the assay H4H15341P was injected over sensor surfaces onto which human or monkey ANGPTL8 proteins were immobilized. All binding studies were performed in 10mM HEPES pH 7.4, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20 (HBS-ET running buffer) at 25°C. The HCA sensor surface was first derivatized by amine coupling goat anti-mouse IgG2a polyclonal antibody (Southern Biotech, # 1080-01) onto which was then captured approximately 30 RU (binding units) of human ANGPTL8 expressed with C-terminal mouse IgG2a Fc tag (hANGPTL8-mFc; SEQ ID NO: 340) or monkey ANGPTL8 expressed with C-terminal mouse IgG2a Fc tag (MfANGPTL8-mFc; SEQ ID NO: 341). Different concentrations of ANGPTL8 mAb were first prepared in HBS-ET running buffer (300nM – 1.23nM; 3-fold serial dilution) and then injected

over the ANGPTL8-mFc captured surfaces for 4 minutes at a flow rate of 30μL/minute followed by the dissociation of bound mAb in HBS-ET running buffer for 10 minutes.

[0206] Kinetic association (k_a) and dissociation (k_d) rate constants were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using Scrubber 2.0c curve-fitting software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives (t½) were calculated from the kinetic rate constants as:

$$K_D\ (M) = \frac{kd}{ka}, \quad \text{and} \quad t½\ (min) = \frac{\ln(2)}{60 \times kd}$$

[0207] Binding kinetic parameters for anti-ANGPTL8 mAb binding to hANGPTL8-mFc and MfANGPTL8-mFc at 25℃ is shown in Table 6.

Results:

[0208] Antibody H4H15341 bound to both human and monkey ANGPTL8 proteins immobilized on the sensor surface and did not exhibit measureable dissociation during the recorded dissociation phase. To obtain an estimate of the binding affinity the dissociation rate constant, k_d, was fixed at the upper detection limit under the experimental conditions, 1.0E-05 1/s. The equilibrium dissociation constant (K_D) values of H4H15341P binding to hANGPTL8-mFc and MfANGPTL8-mFc were estimated to be 117pM and 86pM or lower, respectively.

Table 6: Binding kinetics parameters of H4H15341P binding to hANGPTL8-mFc and MfANGPTL8-mFc at 25℃.

Capture Surface	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
hANGPTL8-mFc	8.50E+04	1.00E-05*	≤ 1.17E-10	≥ 1155
MfANGPTL8-mFc	1.16E+05	1.00E-05*	≤ 8.60E-11	≥ 1155

*No dissociation of anti-ANGPTL8 mAb was observed under the experimental conditions; therefore, the value of k_d was fixed at the upper detection limit of 1.00E-05s^{-1}.

Example 5: Determination of human and monkey ANGPTL8 binding specificity by Bio-Layer Interferometry (BLI)

[0209] Binding of ANGPTL8 antibodies to human and monkey ANGPTL8 proteins was investigated using Bio-layer Interferometry with an Octet HTX biosensor platform (ForteBio, A Division of Pall Life Sciences). All experiments were performed at 25℃ in 10mM HEPES pH 7.4, 150mM NaCl, 0.05% v/v Surfactant Tween-20, and 1mg/ml BSA with the reaction multiwell plate agitated at 1000rpm. Approximately 1.6nm of human ANGPTL8 produced with a C-terminal mouse IgG2a Fc tag (hANGPTL8-mFc; SEQ ID NO: 340) or cynomolgus monkey

ANGPTL8 produced with a C-terminal mouse IgG2a Fc tag (MfANGPTL8-mFc; SEQ ID NO: 341) was captured onto anti-mFc (AMC) Octet biosensors by submerging the sensors into wells containing 10µg/mL of each protein for 4 minutes. Under the same conditions a negative control protein with the same mFc tag (hLDLR-mFc) was also coupled to the AMC sensor. All four sensors, three protein-coupled and one blank, were then submerged into wells containing 100nM of different ANGPTL8 monoclonal antibodies or an isotype control for 4 minutes. Binding signals observed after the 4 minute binding step are tabulated in Table 7.

Results:

[0210] Among 25 ANGPTL8 mAbs tested in this study, 24 antibodies displayed binding signals higher than the maximum binding signals on the irrelevant control sensor tips (0.03 nm; this value was used to calculate binding signals as fold above background). Among the 24 human ANGPTL8 binders, 20 displayed positive binding on the monkey ANGPTL8 protein. The 4 antibodies that did not bind to monkey ANGPTL8 protein also displayed low binding signal on the human ANGPTL8 protein with values between 1-2 fold above the background binding signal. For the 24 antibodies binding to human ANGPTL8 protein, 4 antibodies (H4H15362P2, H4H15321P, H4H15330P, H4H15367P2) showed binding signals of 10-fold above background. Another group of 12 antibodies displayed binding signals between 5-10-fold above background. The remaining antibodies bound the human ANGPTL8 protein with binding signals that were between 1-5-fold above the background level.

Table 7: Binding specificity of 100 nM ANGPTL8 monoclonal antibodies to human and monkey ANGPTL8-mFc captured on Octet biosensors

	mAb Binding Response (nm)			
mAb PID#	**hANGPTL8.mFc Captured Surface**	**MfANGPTL8.mFc Captured Surface**	**Irrelevant control (hLDLR.mFc) Captured Surface**	**Blank AMC Sensor**
H4H15362P2	0.39	0.36	0.03	0.01
H4H15321P	0.36	0.51	0.01	0.00
H4H15330P	0.34	0.39	0.02	0.01
H4H15367P2	0.32	0.33	0.01	0.00
H4H15363P2	0.25	0.26	0.01	0.02
H4H15347P	0.25	0.29	0.01	0.03
H4H15345P	0.25	0.31	0.00	0.01
H4H15319P	0.22	0.26	-0.01	0.00
H4H15361P2	0.20	0.21	0.01	0.02
H4H15318P	0.19	0.20	0.01	0.01

H4H15323P	0.18	0.15	0.00	0.00
H4H15350P2	0.17	0.20	0.00	-0.01
H4H15343P	0.17	0.20	-0.01	0.01
H4H15331P	0.16	0.21	0.01	0.01
H4H15355P2	0.15	0.13	0.02	0.03
H4H15353P2	0.15	0.11	0.02	0.02
H4H15369P2	0.14	0.17	0.00	0.01
H4H15357P2	0.13	0.08	0.01	0.02
H4H15341P	0.12	0.10	0.02	0.03
H4H15346P	0.07	0.01	0.00	-0.01
H4H15335P	0.06	0.04	0.01	0.01
H4H15354P2	0.05	0.03	0.01	0.02
H4H15334P	0.05	0.01	0.00	0.03
H4H15314P2	0.04	0.01	0.01	0.00
H4H15316P	0.03	0.02	0.02	0.02
Negative Isotype control Ab	0.01	0.00	0.01	0.01

Example 6: *In Vivo* Effect of IgG4 Anti-hANGPTL8 Antibodies on circulating tryglyceride levels in humanized ANGPTL8 mice

[0211] The effect of anti-hANGPTL8 antibodies on serum triglyceride (TG) levels was determined in humanized ANGPTL8 mice. Mice were pre-bled 7 days before the experiment and put into groups of five mice each for each antibody tested. Antibodies were administered at 10mg/kg dose (anti-hANGPTL8 and isotype-matched (hIgG4) control with irrelevant specificity) by subcutaneous injection on Day 0 of the study. Mice were bled (nonfasted) at consecutive days after antibody injections and TG levels were determined in the serum by ADVIA® 1800 Serum Chemistry Analyzer (Siemens). Averages were calculated for each of the time points for all tested antibodies. Results, expressed as (mean ± SEM) of serum TG concentration, are shown in Tables 8-13.

Levels of circulating anti-hANGPTL8 (Serum Ab) were also determined using a standard ELISA assay. Briefly, plates were coated with a goat anti-human Fc antibody (Sigma-Aldrich) to capture Serum Ab. Serum was then added to the plates and captured antibodies were detected by chemiluminescence using a horseradish peroxidase (HRP) conjugated goat anti-human IgG antibody (Sigma-Aldrich). Results, expressed as (mean ± SEM) of are shown in Tables 14-19.

Control: Mice that received an isotype-matched Control Ab

Results:

[0212] The effect of 25 mAbs to hANGPTL8 on circulating TG levels were tested in humanized ANGPTL8 mice. Antibody H4H15341P led to significant reduction in circulating TG (up to 68% average) after administration (compared to control mAb).

Table 8. Study 1, serum triglycerides (mg/dL)

Days after injection	Antibody									
	Control		H4H15321P		H4H15331P		H4H15343P		H4H15367P2	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
- 7	205.4	14.20	203.8	19.68	206.6	16.20	205.2	12.13	203.6	14.21
1	233.6	16.93	239.4	28.61	259.8	35.52	196.8	16.05	222.0	27.41
4	210.4	12.79	233.2	26.19	244.4	33.83	175.2	10.32	234.8	27.28
7	261.0	19.66	235.6	33.82	241.8	55.74	201.8	23.50	203.2	27.79

Table 9. Study 2, serum triglycerides (mg/dL)

Days after injection	Antibody							
	Control		H4H15341P		H4H15319P		H4H15318P	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
- 7	214.8	20.08	211.4	21.67	213.6	20.50	212.8	20.00
1	255.4	25.18	82.0	3.35	217.4	26.92	235.2	24.62
4	228.6	33.43	93.6	7.69	195.0	29.93	270.6	34.28
7	197.0	21.22	90.8	7.68	235.4	35.70	209.6	31.88
14	223.0	14.98	126.4	21.75	185.2	29.94	166.0	24.58

Table 9 (continued)

Days after injection	Antibody			
	H4H15355P2		H4H15345P	
	Mean	SEM	Mean	SEM
- 7	214.4	19.18	213.0	20.34
1	248.2	45.93	228.8	37.97
4	221.2	30.30	195.80	23.87
7	254.2	37.93	252.60	25.24
14	219.4	36.69	190.60	13.13

Table 10. Study 3, serum triglycerides (mg/dL)

Days after injection	Antibody									
	Control		H4H15350P2		H4H15314P2		H4H15330P		H4H15361P2	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
- 7	247.8	23.88	242.8	21.60	244.0	26.34	243.2	22.29	242.4	25.29
1	214.6	20.37	206.6	21.60	228.2	35.33	206.6	25.44	215.4	20.20
4	222.4	13.78	198.2	22.61	192.4	17.25	216.6	15.84	200.0	15.89
7	288.8	35.41	274.6	45.48	238.6	21.21	244.4	14.61	247.4	37.93

Table 11. Study 4, serum triglycerides (mg/dL)

Days after injection	Antibody									
	Control		H4H15357P2		H4H15363P2		H4H15347P		H4H15369P	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
- 7	197.0	18.29	201.6	25.18	201.6	26.15	200.4	24.71	198.8	22.43
1	227.6	46.41	221.6	37.35	189.4	5.963	194.6	28.33	217.0	39.68
6	194.0	18.06	211.2	35.96	190.6	20.21	248.2	16.12	223.0	25.61

Table 12. Study 5, serum triglycerides (mg/dL)

Days after injection	Antibody							
	Control		H4H15353P2		H4H15323P		H4H15362P2	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
- 7	199.2	26.68	197.4	27.02	199.8	30.33	200.8	27.55
2	217.2	16.09	184.4	28.67	179.8	35.99	166.6	26.76
8	161.8	18.58	185.4	24.78	187.0	38.76	180.2	18.22
14	227.2	33.70	216.4	11.74	212.4	31.29	173.2	17.75

Table 12 (continued)

Days after injection	Antibody			
	H4H15334P		H4H15354P2	
	Mean	SEM	Mean	SEM
- 7	199.8	26.25	200.0	26.33
2	183.0	16.93	169.8	23.14
8	160.0	16.56	162.6	20.50
14	167.6	18.73	197.4	34.20

Table 13. Study 6, serum triglycerides (mg/dL)

Days after injection	Antibody							
	Control		H4H15316P		H4H15335P		H4H15346P	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
- 7	232.0	24.94	232.0	28.26	232.4	23.88	232.8	30.30
2	211.0	23.19	248.2	35.35	203.2	6.785	197.2	20.42
7	256.8	32.02	249.6	35.72	248.0	17.28	234.8	66.74

Table 14. Study 1, Serum Ab (µg/mL)

Days after injection	Antibody									
	Control		H4H15321P		H4H15331P		H4H15343P		H4H15367P2	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	64.1	9.0	76.4	8.6	9.8	2.0	74.4	8.5	113.0	9.6
4	55.8	6.3	66.5	4.6	3.3	0.7	68.3	4.4	101.4	11.3

Table 15. Study 2, Serum Ab (µg/mL)

Days after injection	Antibody							
	Control		H4H15341P		H4H15319P		H4H15318P	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	50.8	3.9	104.0	18.7	81.8	8.2	74.4	8.5
4	51.2	9.5	70.6	23.6	59.1	9.6	68.3	4.4
7	40.9	5.4	50.7	13.3	46.8	8.9	68.3	4.4
14	32.2	3.1	8.2	4.6	24.1	8.7	68.3	4.4

Table 15 (continued)

Days after injection	Antibody			
	H4H15355P2		H4H15345P	
	Mean	SEM	Mean	SEM
1	68.4	3.8	59.3	3.6
4	58.4	3.0	46.3	16.2
7	35.7	6.6	50.1	3.9
14	3.1	0.8	35.9	4.6

Table 16. Study 3, Serum Ab (µg/mL)

Days after injection	Antibody									
	Control		H4H15350P2		H4H15314P2		H4H15330P		H4H15361P2	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	47.3	7.0	57.2	23.4	89.9	13.0	38.3	14.7	50.0	13.6
4	50.6	13.4	66.1	22.6	69.9	12.9	35.4	0.9	57.4	10.1
7	38.8	9.2	39.9	14.7	48.6	17.3	30.0	5.1	38.7	11.1

Table 17. Study 4, Serum Ab (µg/mL)

Days after injection	Antibody									
	Control		H4H15357P2		H4H15363P2		H4H15347P		H4H15369P	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	100.9	100.9	78.4	26.7	93.2	10.1	53.6	7.5	99.7	15.6
6	84.0	84.0	56.9	14.8	62.0	7.6	9.5	2.9	68.0	12.0

Table 18. Study 5, Serum Ab (µg/mL)

Days after injection	Antibody							
	Control		H4H15353P2		H4H15323P		H4H15362P2	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
2	93.7	14.4	63.5	17.6	99.9	34.5	91.0	24.6

8	79.8	8.7	42.8	11.1	50.3	9.7	50.8	10.3
14	55.1	11.4	18.2	14.0	32.3	10.0	29.5	20.9

Table 18 (continued)

Days after injection	Antibody			
	H4H15334P		H4H15354P2	
	Mean	SEM	Mean	SEM
2	64.4	15.0	71.3	7.2
8	38.7	7.1	46.0	15.4
14	8.6	4.6	30.1	26.6

Table 19. Study 6, Serum Ab (µg/mL)

Days after injection	Antibody							
	Control		H4H15316P		H4H15335P		H4H15346P	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
2	87.4	9.2	79.3	18.5	66.9	17.5	61.1	22.8
7	97.4	23.0	77.9	12.8	78.6	16.2	56.7	23.7

Example 7: Dose Response of hANGPTL8 Antibody H4H15341P in humanized ANGPTL8 mice

[0213] The effects of different doses of hANGPTL8 mAb, H4H15341P, on serum triglycerides (TG) were evaluated in humanized ANGPTL8 mice. Mice were pre-bled 7 days before the experiment and put into groups of five mice each for each dose tested. H4H15341P was administered at 1, 5, 10 and 25 mg/kg and isotype-matched (hIgG4) control with irrelevant specificity at 10mg/kg by single-dose subcutaneous injection on Day 0 of the study. Mice were bled (nonfasted) at days 2, 7, 14 and 21 after antibody injection and TG levels were determined in the serum by ADVIA® 1800 Chemistry System (Siemens). Averages were calculated for each time point. Results, expressed as (mean ± SEM) of serum TG concentration, are shown in Figure 1.

[0214] Levels of circulating anti-human antibodies (Serum Ab) were determined using a standard ELISA assay. Briefly, plates were coated with a goat anti-human Fc antibody (Sigma-Aldrich) to capture Serum Ab. Serum was then added to the plates and captured antibodies were detected by chemiluminescence using a horseradish peroxidase (HRP) conjugated goat anti-human IgG antibody (Sigma-Aldrich). Results, expressed as (mean ± SEM) are shown in Figure 2.

Control Ab refers to mice that received an isotype-matched control Ab.

Results:

[0215] The effect of 4 different doses of H4H15341P (anti-hANGPTL8) on circulating TG and cholesterol levels were tested in humanized ANGPTL8 mice. H4H15341P led to dose-dependent sustained significant reduction in serum TG (up to 66% average, compared to control mAb) with 5mg/kg being the lowest efficacious dose. No effect was observed on total cholesterol levels.

Example 8. Evaluation of lipoprotein lipase (LPL) activity after hANGPTL8 mAb treatment in humanized ANGPTL8 mice

[0216] The effect of hANGPTL8 mAb (H4H15341P) administration on LPL activity was evaluated in humanized ANGPTL8 mice. Mice were pre-bled 7 days before the experiment and put into groups of five mice each for each mAb tested. H4H15341P and Control Ab were administered at 10 mg/kg by single-dose subcutaneous injection on Day 0 of the study. On day 4 of the study, mice were dosed with heparin by intravenous injection via tail vein at 250U/kg that releases LPL from vascular endothelial surfaces. Five minutes later mice were bled from the retro-orbital sinus and post-heparin plasma collected and fractionated to separate LPL from hepatic lipase using heparin-Sepharose chromatography. Post-heparin plasma was loaded onto 1.0-ml heparin-Sepharose HiTrap columns (GE Healthcare) controlled by the GE Akta Prime, equilibrated with 0.25 M NaCl, 20% glycerol, 1% BSA, 10 mM sodium phosphate, pH 6.5. The column was washed with 10 ml of the equilibration buffer and eluted with a 30 ml NaCl gradient (0.25–1.5 M in 20% glycerol, 1% BSA, 10 mM sodium phosphate, pH 6.5). Resulting fractions were pooled by hepatic lipase and LPL peaks and the lipase activities were assayed using Invitrogen Enzchek Lipase substrate (cat#E33955). The kinetic reaction was read on Molecular Devices SpectraMax i3 plate reader at 482nm excitation / 518nm emission. Results, expressed as relative fluorescence units (RFU) (mean ± SEM) are shown in Figure 3. Control Ab refers to mice that received an isotype-matched negative control Ab.

Results

[0217] The results showed that administration of H4H15341P (anti-hANGPTL8) to humanized ANGPTL8 mice leads to a significant increase in LPL activity and has no effect on hepatic lipase activity.

Example 9. Lipid Tolerance Test in Humanized ANGPTL8 Mice Treated with hANGPTL8 mAb H4H15341P

[0218] The effect of ANGPTL8 inhibition with the mAb H4H15341P on triglyceride clearance was evaluated by acute fat loading. Humanized ANGPTL8 mice were pre-bled 8 days before the experiment and put into groups of 6 mice each for each mAb tested. H4H15341P and isotype-matched control Ab were administered at 10 mg/kg by single-dose subcutaneous injection on Day 0 of the study. On day 4 of the study mice were fasted for 4 hours following intravenous administration of 20% intralipid (Baxter Healthcare, IL) at 2.5 µl/g body weight. TG

level was evaluated in blood collected from the tail vein at subsequent time points. Results, expressed as (mean ± SEM) of TG concentration are shown in Figure 4. Control Ab refers to mice that received an isotype-matched negative control Ab.

Results

[0219] Administration of H4H15341P (anti-hANGPTL8) to humanized ANGPTL8 mice leads to a significantly lower TG level after acute fat load compared to control antibody. These data suggest that H4H15341P, by blocking ANGPTL8, promotes accelerated TG clearance from the circulation.

Example 10. HiSense Linear Epitope Mapping for Angiopoietin-Like Protein 8

[0220] Pepscan analysis using HiSense linear peptides was employed to establish linear epitopes for antibodies H4H15341P and H4H15367P2. The study was conducted at Pepscan Presto BV, (Zuidersluisweg 2, 8243RC Lelystad, The Netherlands). All Pepscan data is stored in the software package Peplab™, a proprietary database application developed in-house and built on a PostgreSQL storage back-end.

SYNTHESIS OF PEPTIDES

[0221] To reconstruct epitopes of the target molecule, a library of peptides was synthesized. An amino functionalized polypropylene support was obtained by grafting with a proprietary hydrophilic polymer formulation, followed by reaction with t-butyloxycarbonyl-hexamethylenediamine (BocHMDA) using dicyclohexylcarbodiimide (DCC) with Nhydroxybenzotriazole (HOBt) and subsequent cleavage of the Boc-groups using trifluoroacetic acid (TFA). Standard Fmocpeptide synthesis was used to synthesize peptides on the amino-functionalized solid support by custom modified JANUS liquid handling stations (Perkin Elmer).

COUPLING OF ANGIOPOIETIN-LIKE PROTEIN 8 ONTO THE ARRAY

[0222] The target protein was coupled on the mini-card as a positive control. To couple Angiopoietin-like protein 8 (hANGPTL8-mFc) onto the arrays, two cross-linking agents were used - m-maleimidobenzoyl-Nhydroxysuccinimide ester (MBS) and glutaraldehyde (GDA). For MBS 40 µl of hANGPTL8-mFc were mixed with 1 µl of MBS (2 mg/ml), incubated for 45 min at room temperature, and then applied onto the array at positions containing the linker motif CGGCGG (SEQ ID NO:346). For the GDA linking, 0.05% GDA in phosphate buffer (pH 5.0) was applied onto the array, incubated at room temperature for 4 hours, then hANGPTL8-mFc at concentration 5 or 20 µg/ml in phosphate buffer pH 8.0 was added onto the array on positions containing Gly only to allow coupling to the free N terminus.

ELISA SCREENING

[0223] The binding of antibody to each of the synthesized peptides was tested in a

PEPSCAN-based ELISA. The peptide arrays were incubated with primary antibody solution (overnight at 4℃). After washing, the peptide arrays were incubated with a 1/1000 dilution of an appropriate antibody peroxidase conjugate (goat anti-human HRP conjugate, Southern Biotech, catalog no. 2010-05) for one hour at 25℃. After washing, the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 20 µl/ml of 3 percent H_2O_2 were added. After one hour, the color development was measured. The color development was quantified with a charge coupled device (CCD) - camera and an image processing system.

SCREENING DETAILS

[0224] Antibody binding depends on a combination of factors, including concentration of the antibody and the amounts and nature of competing proteins in the ELISA buffer. Also, the pre-coat conditions (the specific treatment of the peptide arrays prior to incubation with the experimental sample) affect binding. These details are summed up as follows:

Label	Dilution	Sample buffer	Pre-conditioning
H4H15341P	1 µg/ml	100% SQ	100% SQ
H4H15367P2	1 µg/ml	100% SQ	100% SQ
Negative isotype control	1 µg/ml	100% SQ	100% SQ

For the Pepscan Buffer and Preconditioning (SQ), the numbers indicate the relative amount of competing protein (a combination of horse serum and ovalbumin).

DATA PROCESSING

[0225] The values obtained from the CCD camera range from 0 to 3000 mAU, similar to a standard 96-well plate ELISA-reader. The results are quantified and stored into the Peplab database. Occasionally, a well contains an air-bubble resulting in a false-positive value, the cards are manually inspected and any values caused by an air-bubble are scored as 0.

SYNTHESIS QUALITY CONTROL

[0226] To verify the quality of the synthesized peptides, a separate set of positive and negative control peptides was synthesized in parallel. These were screened with antibody 57.9 (Posthumus, *et al.* 1990 *J Virol* 64:3304-3309).

Results

DESIGN OF PEPTIDES

[0227] The following sets of peptides were synthesized on the target sequence:

[0228] Human ANGPTL8, mature sequence, amino acids 22-198 from NP_061157.3

1 APMGGPELAQ HEELTLLFHG TLQLGQALNG VYRTTEGRLT KARNSLGLYG 50

51 RTIELLGQEV SRGRDAAQEL RASLLETQME EDILQLQAEA TAEVLGEVAQ 100

101 AQKVLRDSVQ RLEVQLRSAW LGPAYREFEV LKAHADKQSH ILWALTGHVQ 150

151 RQRREMVAQQ HRLRQIQERL HTAALPA 177 (SEQ ID NO:347)

[0229] The antibodies were tested for binding a series of 15-mer peptides covering the full sequence of ANGPTL8, each peptide offset by one amino acid from the next. Also included were double alanine ("AA") substitutions within the series of tested peptides for finer epitope analysis.

SET 1. Mimic: linear. Type: LIN

Description Peptides of length 15 derived from the target sequence of Angiopoietin-like protein 8 with an offset of one residue.

Sequences (first 10)

APMGGPELAQHEELT (SEQ ID NO: 348)

PMGGPELAQHEELTL (SEQ ID NO: 349)

MGGPELAQHEELTLL (SEQ ID NO: 350)

GGPELAQHEELTLLF (SEQ ID NO: 351)

GPELAQHEELTLLFH (SEQ ID NO: 352)

PELAQHEELTLLFHG (SEQ ID NO: 353)

ELAQHEELTLLFHGT (SEQ ID NO: 354)

LAQHEELTLLFHGTL (SEQ ID NO: 355)

AQHEELTLLFHGTLQ (SEQ ID NO: 356)

QHEELTLLFHGTLQL (SEQ ID NO: 357)

SET 2. Mimic: linear. Type: LIN.AA

Description Peptides of set 1, but with residues on positions 10 and 11 replaced by Ala. When a native Ala would occur on either position, it is replaced by Gly. The order of peptides in this set was randomized. The actual order on the array is shown.

Sequences (first 10)

TAEVLGEVAAGQKVL (SEQ ID NO: 358)

VYRTTEGRLAAARNS (SEQ ID NO: 359)

GVYRTTEGRAAKARN (SEQ ID NO: 360)

VQRLEVQLRAGWLGP (SEQ ID NO: 361)

LTGHVQRQRAAMVAQ (SEQ ID NO: 362)

VLKAHADKQAAILWA (SEQ ID NO: 363)

LRDSVQRLEAALRSA (SEQ ID NO: 364)

RREMVAQQHAARQIQ (SEQ ID NO: 365)

VSRGRDAAQAARASL (SEQ ID NO: 366)

AYREFEVLKGAADKQ (SEQ ID NO: 367)

[0230] The raw ELISA results of the screening were provided and plotted (box plot, data not shown) to depict each dataset and indicate the average ELISA signal, the distribution, and the outliers within each dataset. Depending on experiment conditions (amount of antibody, blocking strength, etc.), different distributions of ELISA data were obtained.

ANTIBODY H4H15367P2

[0231] When tested under high stringency conditions, antibody H4H15367P2 avidly bound only one linear peptide comprised of sequence 1APMGGPELAQHEELT15 (SEQ ID NO: 348). This sample was tested twice under the same conditions and repeatedly yielded the same result. Antibody H4H15367P2 also strongly bound Angiopoietin-like protein 8, which was coupled onto the array as a positive control. Interestingly, somewhat weaker binding was obtained with the target protein coupled using MBS when compared to GDA coupling.

ANTIBODY H4H15341P

[0232] When tested under high stringency conditions, antibody H4H15341P avidly bound a series of linear peptides, which contain common sequence $_{150}$QRQRREMVAQ$_{159}$ (SEQ ID NO: 368). Comparison of intensity profiles recorded on set 1 (native linear epitope mimics) and set 2 (double Ala mutants) indicates that residues R154, E155, and Q159 are essential for antibody binding. Antibody H4H15341P also strongly bound Angiopoietin-like protein 8, which was coupled onto the array as a positive control, regardless of the immobilization.

NEGATIVE ISOTYPE CONTROL

[0233] Negative isotype control did not bind any peptide present on the array. Furthermore, no detectable binding was recorded with Angiopoietin-like protein 8, which was coupled onto the array as a positive control. Negative isotype control was additionally tested with goat anti-human secondary conjugate used in Pepscan ELISA. The antibody can be recognized by this secondary.

Conclusion

[0234] Three antibodies provided for this study were tested on HiSense peptide arrays. It was possible to establish tentative linear epitopes for two antibodies. Despite repeated incubations, antibody Negative isotype control did not bind to the array. Core tentative epitopes identified in this study are listed as follows:

Antibody	Core epitope
H4H15341P	$_{150}$QRQRREMVAQ$_{159}$ (SEQ ID NO: 368)
H4H15367P2	$_{1}$APMGGPELAQHEELT$_{15}$ (SEQ ID NO: 348)
Negative isotype control	-.

[0235] Thus, Antibodies H4H15341P and H4H15367P2 recognize distinct linear sequences within C- and N-termini respectively. The fact that signal obtained for antibody H4H15367P2 with MBS coupling was less than with GDA coupling, together with its localization on the extreme N terminus indicate that the N terminal amine itself may be part of the epitope. Additionally, for antibody H4H15341P double alanine mutants served to pinpoint residues that are critical for binding (residues shaded in light grey, above).

[0236] Antibody H4H15341P targets a C-terminal region of Angiopoietin-like protein 8, while H4H15367P2 targets the very N-terminus. Antibody Negative isotype control did not bind to the array.

What is claimed is:

1. An antibody or antigen-binding fragment thereof that binds to the same epitope on ANGPTL8 as a reference antibody, and/or competes for binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises HCDR1 / HCDR2 / HCDR3 / LCDR1 / LCDR2 / LCDR3 domains having amino acid sequences of SEQ ID NO: 164 / 166 / 168 / 172 / 174 / 176 or 316 / 318 / 320 / 324 / 326 / 328, respectively.

2. The antibody of claim 1, wherein the reference antibody:

a) comprises HCDR1 / HCDR2 / HCDR3 / LCDR1 / LCDR2 / LCDR3 domains having amino acid sequences of SEQ ID NO: 164 / 166 / 168 / 172 / 174 / 176 and binds specifically to the epitope as defined by SEQ ID NO: 368; or

b) comprises HCDR1 / HCDR2 / HCDR3 / LCDR1 / LCDR2 / LCDR3 domains having amino acid sequences of SEQ ID NO: 316 / 318 / 320 / 324 / 326 / 328 and binds specifically to a linear epitope in the N-terminal region of human ANGPTL8 as defined by SEQ ID NO: 348.

3. The antibody of claim 1, wherein the reference antibody:

a) is a fully human monoclonal antibody;

b) does not bind to the N-terminal coiled-coil region of human ANGPTL3 peptide of SEQ ID NO: 338, or to the N-terminal coiled-coil region of human ANGPTL4 peptide of SEQ ID NO: 339;

c) binds human ANGPTL8 at 25℃ with a K_D of less than about 150pM and binds monkey ANGPTL3 at 25℃ with a K_D of less than about 90pM as measured by surface plasmon resonance;

d) lowers triglyceride levels in a mammal by about 68% when administered subcutaneously at a dose of about 10 mg/kg;

e) lowers triglyceride levels in a mammal for a period ranging from about 7 days to 21 days, when administered subcutaneously at doses ranging from about 5 mg/kg to about 25 mg/kg;

f) comprises a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 162 or 314;

g) comprises a light chain variable region (LCVR) having an amino acid sequence of SEQ ID NO: 170 or 322; or

h) cross-competes with a reference antibody, wherein the reference antibody comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) amino acid sequence selected from the group consisting of any of the HCVR and LCVR amino acid sequences of Table 1.

4. The antibody of any of claims 1-3, wherein the antibody is a recombinantly produced human antibody.

5. An isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof that binds specifically to human ANGPTL8, wherein the antibody or antigen-binding fragment thereof comprises (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence as set forth in SEQ ID NO:162 or 314; or (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence as set forth in SEQ ID NO:170 or 322.

6. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1 to 4 and a pharmaceutically acceptable carrier or diluent.

7. A method for inhibiting the activity of ANGPTL8 in a patient in need thereof, the method comprising administering any one or more of the antibodies or antigen-binding fragments thereof of any one of claims 1-4, or a pharmaceutical composition comprising any one or more of the antibodies or antigen-binding fragments thereof of any one of claims 1-4 to the patient, wherein at least one activity of ANGPTL8 is reduced or diminished.

8. The method of claim 7, wherein the administering results in a reduction in blood triglyceride levels in the patient.

9. A method for treating a disease or condition associated in part with elevated levels of ANGPTL8 activity, the method comprising administering an ANGPTL8 inhibitor/antagonist, wherein the ANGPTL8 inhibitor/antagonist is an antibody or antigen-binding fragment thereof specific for ANGPTL8.

10. The method of claim 9, wherein the antibody or antigen-binding fragment thereof

specific for ANGPTL8 comprises HCVR/LCVR sequences as set forth in SEQ ID NOs:162/170 or 314/322.

11. A method for treating a condition or disease associated with, or characterized in part by high blood triglyceride levels, or at least one symptom or complication associated with the condition or disease, the method comprising administering the antibody or antigen-binding fragment thereof of any one of claims 1-4, or the pharmaceutical composition of claim 6, to a patient in need thereof, such that blood triglyceride levels are lowered or that the condition or disease is mediated, or the at least one symptom or complication associated with the condition or disease is alleviated or reduced in frequency or severity.

12. The method of claim 11, wherein the condition or disease is selected from the group consisting of hyperlipidemia, hyperlipoproteinemia, dyslipidemia (atherogenic dyslipidemia, diabetic dyslipidemia, mixed dyslipidemia), hypertriglyceridemia, severe hypertriglyceridemia with TG > 1000 mg/dL and associated acute pancreatitis, hypercholesterolemia, chylomicronemia, obesity, metabolic syndrome, diabetes, lipodystrophy, lipoatrophy resulting from, or caused by altered ApoC2, ApoE deficiency, increased ApoB, increased production and/or decreased elimination of very low-density lipoprotein (VLDL), nonalcoholic steatohepatitis (NASH), certain drug treatments (*e.g.*, glucocorticoid treatment-induced dyslipidemia), and any genetic predisposition, diet, or life style that results in elevated triglycerides or lipids.

13. The method of claim 11, wherein the condition or disease is a cardiovascular disease or disorder selected from the group consisting of atherosclerosis, aneurysm, hypertension, angina, stroke, cerebrovascular diseases, congestive heart failure, coronary artery diseases, myocardial infarction, and peripheral vascular diseases.

14. The method of claim 11, wherein the pharmaceutical composition is administered to the patient in combination with a second therapeutic agent.

15. The method of claim 14, wherein the second therapeutic agent is selected from the group consisting of: 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors; apolipoprotein C-III inhibitors; inhibitors of cholesterol uptake and/or bile acid re-absorption; niacin; fibrates or amphipathic carboxylic acids; activators of the LXR transcription factor (*e.g.* 22-hydroxycholesterol); or fixed combinations (*e.g.* ezetimibe plus simvastatin); a statin with a

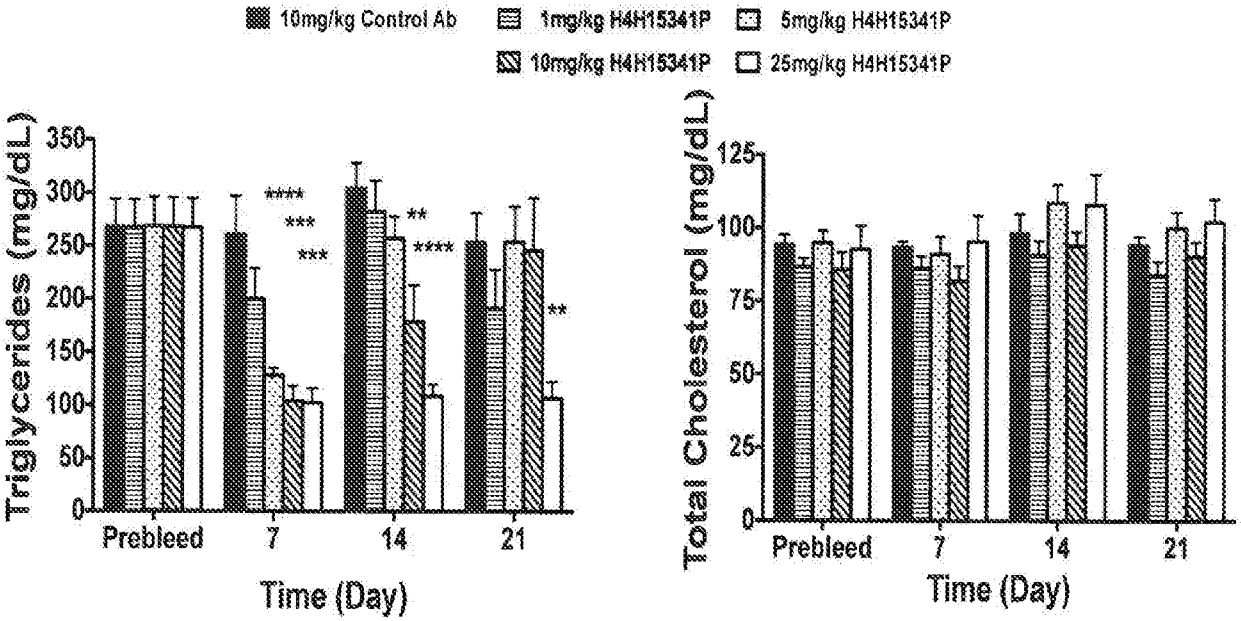
bile resin (*e.g.*, cholestyramine, colestipol, colesevelam), a fixed combination of niacin plus a statin (*e.g.*, niacin with lovastatin); and other lipid lowering agents (*e.g.* omega-3-fatty acid ethyl esters.

16. The method of claim 15, wherein the HMG-CoA reductase inhibitor is selected from the group consisting of cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin, and pravastatin.

17. The method of claim 14, wherein the second therapeutic agent is selected from the group consisting of an isolated antibody, or an antigen-binding fragment thereof, that specifically binds to angiopoietin-like protein 3 (ANGPTL3), angiopoietin-like protein 4 (ANGPTL4), angiopoietin-like protein 5 (ANGPTL5), angiopoietin-like protein 6 (ANGPTL6) and human proprotein convertase subtilisin/kexin type 9 (PCSK9).

18. The method of claim 14, wherein the second therapeutic agent is selected from the group consisting of insulin, an insulin analog, a biguanide (metformin), a sulfonylurea (e.g. glyburide, glipizide), a PPAR gamma agonist (e.g. pioglitazone, rosiglitazone), an alpha glucosidase inhibitor (e.g. acarbose, voglibose), a glucagon-like peptide 1 (GLP-1) agonist (e.g., BYETTA® (exenatide), TRULICITY™ (dulaglutide), VICTOZA® (liraglutide), LYXUMIA® (lixisenatide, TANZEUM™ (albiglutide), or an analogue of any of the foregoing, a dipeptidyl peptidase IV (DPP-4) inhibitor (e.g. saxagliptin (ONGLYZA®), sitaliptin (JANUVIA®), and vildagliptin (GALVUS®), a sodium-glucose co-transporter 2 (SGLT2) inhibitor (e.g., INVOKANA™ (canagliflozin), FORXIGA® (dapagliflozin), empagliflozin, ipragliflozin, tofogliflozin), SYMLIN® (pramlintide), a glucagon receptor antagonist, a non-sulfonylurea secretagogue, an insulin analog (*e.g.,* fast acting Lispro, Aspart, Glulisine and long acting Detemir insulin, Degludec insulin, or Glargine insulin), exendin-4 polypeptides, beta 3 adrenoceptor agonists, inhibitors of cholesterol uptake and/or bile acid re-absorption, LDL-cholesterol antagonists, cholesteryl ester transfer protein antagonists (*e.g.* torcetrapib, anacetrapib, dalcetrapib, or evacetrapib), endothelin receptor antagonists, growth hormone antagonists, insulin sensitizers, amylin mimetics or agonists, cannabinoid receptor antagonists, melanocortins, melanin-concentrating hormone receptor agonists, SNRIs, a fibroblast growth factor 21 (FGF21) mimetic, a fibroblast growth factor receptor 1c (FGFR1c) agonist, an inhibitor of advanced glycation endproduct formation (*e.g.* aminoguanidine), and protein tyrosine phosphatase inhibitors.

19. The method of claim 14, wherein the second therapeutic agent is an analgesic or an anti-inflammatory agent.



*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Figure 1

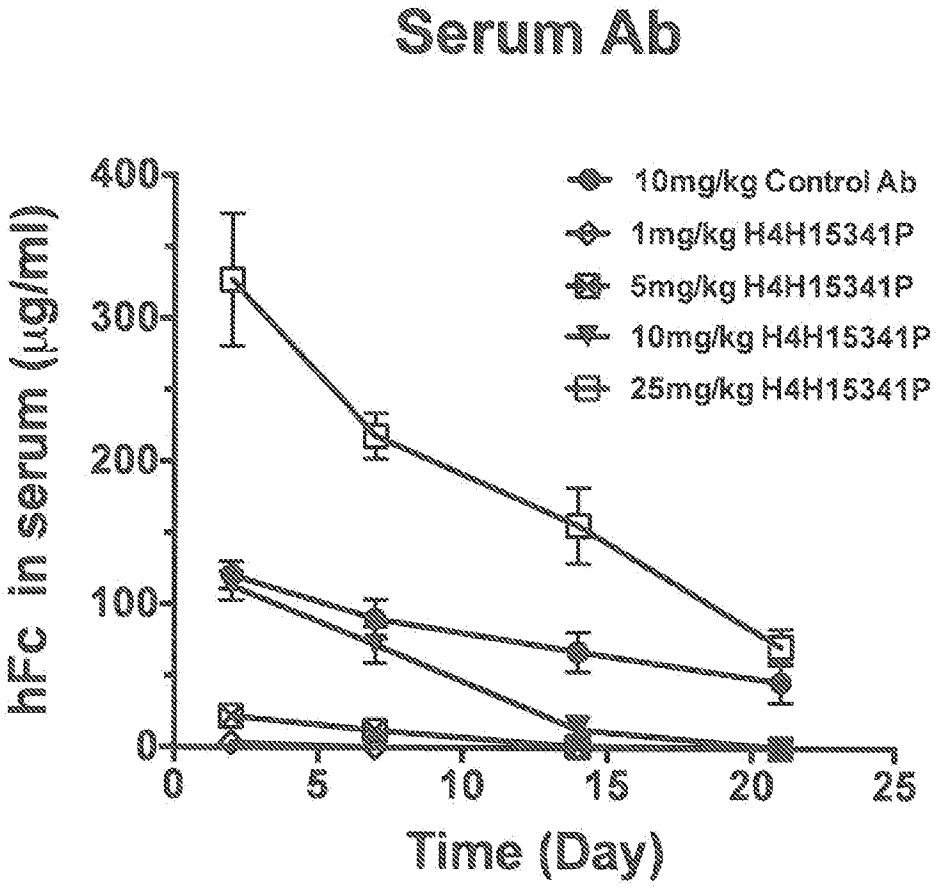
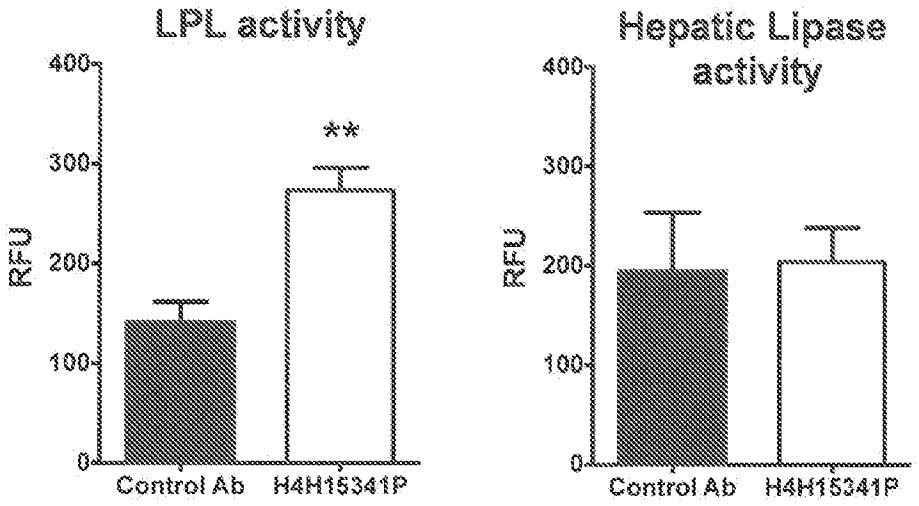

Serum Ab
hFc in serum (µg/ml)
400
300
200
100
0
0
5
10
15
20
25
Time (Day)
10mg/kg Control Ab
1mg/kg H4H15341P
5mg/kg H4H15341P
10mg/kg H4H15341P
25mg/kg H4H15341P

Figure 2



**p<0.01

Figure 3

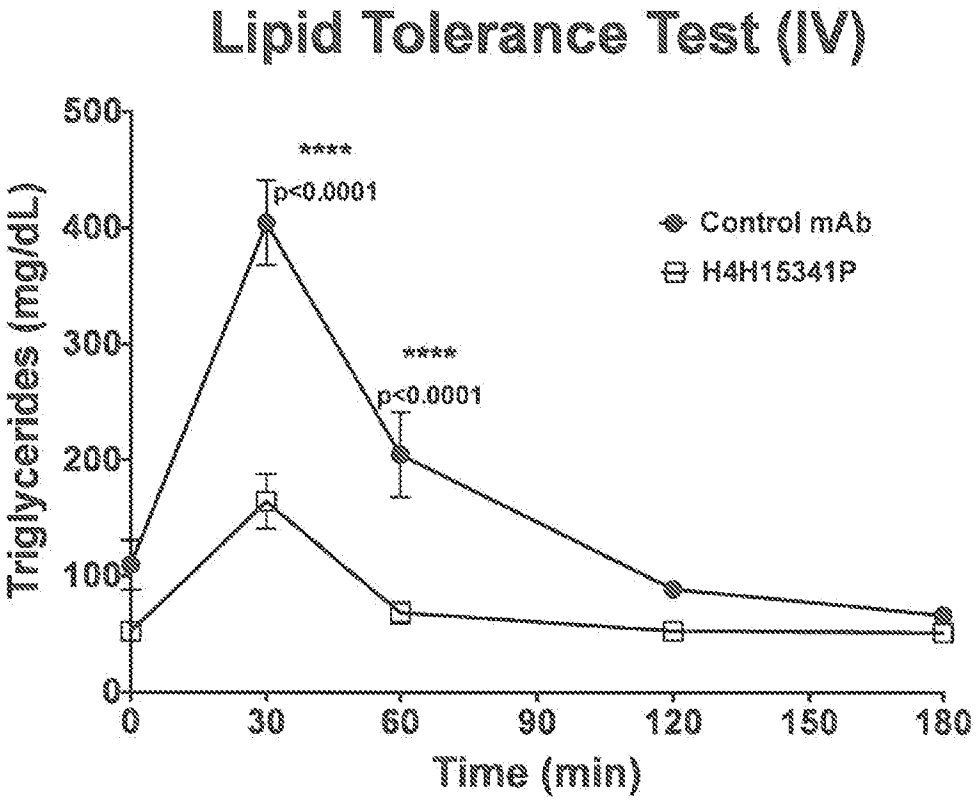

Lipid Tolerance Test (IV)
Triglycerides (mg/dL)
500
400
300
200
100
0
****
p<0.0001
****
p<0.0001
Control mAb
H4H15341P
0
30
60
90
120
150
180
Time (min)

Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/045535

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/00 C07K16/18 C07K16/22
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Zhang Ren: "OR13-6: A Monoclonal Neutralizing Antibody Against Lipasin (Angptl8), a Novel Lipid Regulator, Reduces Serum Triglycerides in Mice By Enhancing Lipoprotein Lipase-Mediated Triglyceride Clearance", ENDOCRINE SOCIETY'S 97TH ANNUAL MEETING AND EXPO, March 5-8, 2015 - SAN DIEGO CV Risk - Drugs, Sex, and Rock and Roll, 6 March 2015 (2015-03-06), XP002763964, DOI: 10.1210/endo-meetings.2015.DGM.3.OR13-6 Retrieved from the Internet: URL:http://press.endocrine.org/doi/10.1210/endo-meetings.2015.DGM.3.OR13-6 [retrieved on 2016-11-08] cited in the application	1-4,6-19
Y	the whole document	5

-/--

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 November 2016	20/12/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Scheffzyk, Irmgard

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/045535

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Anonymous: "Product Data Sheet - Purified anti-Betatrophin (ANGPTL8)", BioLegend , 25 July 2014 (2014-07-25), XP002763965, Retrieved from the Internet: URL:http://www.biolegend.com/pop_pdf.php?id=10113 [retrieved on 2016-11-08]	1-4,6-19
Y	the whole document	5

4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/045535

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: