HUMAN ANTIBODIES TO HUMAN ANGIOPOIETIN-LIKE PROTEIN 4

Field of the invention

[0001] The present invention is related to human antibodies and antigen-binding fragments of human antibodies that specifically bind human angiopoietin-like protein 4 (hANGPTL4), and therapeutic methods of using those antibodies.

Statement of Related Art

[0002] Lipoprotein lipase (LPL) has a central role in lipoprotein metabolism to maintain normal lipoprotein levels in blood and, through tissue specific regulation of its activity, to determine when and in what tissues triglycerides (TG) are unloaded. It has been reported that ANGPTL4 inhibits LPL and retards lipoprotein catabolism, in humans and rodents. ANGPTL4 null mice exhibit a significant decrease in serum TG. Conversely, ANGPTL4 injection into mice produces a rapid increase in circulating lipids and this is at a higher rate than the injection of angiopoietin-like protein 3 (ANGPTL3) (Yoshida *et al.*, 2002, *J Lipid Res* 43:1770-1772). The N-terminal coiled-coil region, not the C-terminal fibrinogen-like domain, of ANGPTL4 is known to be important in the inhibition of LPL activity and, therefore, for the hypertriglyceridemia indication. These observations indicate that inhibition of ANGPTL4 could be beneficial in treating diseases characterized by elevated lipid levels, including primary dyslipidemia and hypertriglyceridemia associated with obesity, metabolic syndrome, type II diabetes, and the like. ANGPTL4 has also been implicated as having a role in angiogenesis and cancer (Galaup *et al.*, 2006, *PNAS* 103(49):18721-18726; Kim *et al.*, 2000, *Biochem J* 346:603-610; and Ito *et al.*, 2003, *Cancer Res* 63(20):6651-6657).

[0003] The nucleic acid and the amino acid sequences of human ANGPTL4 are shown in SEQ ID NOS: 475 and 476, respectively. Antibodies to ANGPTL4 are disclosed in, for example, WO 2006/074228 and WO 2007/109307.

BRIEF SUMMARY OF THE INVENTION

[0004] In a first aspect, the invention provides fully human monoclonal antibodies (mAbs) and antigen-binding fragments thereof that specifically bind and neutralize human ANGPTL4 (hANGPTL4) activity.

[0005] The antibodies (Abs) 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 eliminate residual effector functions (Reddy *et al.*, 2000, *J. Immunol.* 164:1925-1933).

[0006] In one embodiment, the invention comprises an antibody or antigen-binding fragment of an antibody comprising a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NO:2, 18, 22, 26, 42, 46, 50, 66, 70, 74, 90, 94, 98, 114, 118, 122, 138, 142, 146, 162, 166, 170, 186, 190, 194, 210, 214, 218, 234, 238, 242, 258, 262, 266, 282, 286,

290, 306, 310, 314, 330, 334, 338, 354, 358, 362, 378, 382, 386, 402, 406, 410, 426, 430, 434, 450, 454, 458, 466, 468 and 487, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In another embodiment, the antibody or an antigen-binding fragment thereof comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO:26, 42, 46, 487, 74, 90, 94, 122, 138, 142, 146, 162 and 166. In yet another embodiment, the antibody or fragment thereof comprises a HCVR comprising SEQ ID NO:42, 487, 90, 138 or 162.

[0007] In one embodiment, an antibody or antigen-binding fragment of an antibody comprises a light chain variable region (LCVR) selected from the group consisting of SEQ ID NO:10, 20, 24, 34, 44, 48, 58, 68, 72, 82, 92, 96, 106, 116, 120, 130, 140, 144, 154, 164, 168, 178, 188, 192, 202, 212, 216, 226, 236, 240, 250, 260, 264, 274, 284, 288, 298, 308, 312, 322, 332, 336, 346, 356, 360, 370, 380, 384, 394, 404, 408, 418, 428, 432, 442, 452 and 456, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In another embodiment, the antibody or antigen-binding portion of an antibody comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 44, 48, 82, 92, 96, 130, 140, 144, 154, 164 and 168. In yet another embodiment, the antibody or fragment thereof comprises a LCVR comprising SEQ ID NO:44, 92, 140 or 164.

[0008] In further embodiments, the antibody or fragment thereof comprises a HCVR and LCVR (HCVR/LCVR) sequence pair selected from the group consisting of SEQ ID NO:2/10, 18/20, 22/24, 26/34, 42/44, 487/44, 46/48, 50/58, 66/68, 70/72, 74/82, 90/92, 94/96, 98/106, 114/116, 118/120, 122/130, 138/140, 142/144, 146/154, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/394, 466/404 and 468/408. In one embodiment, the antibody or fragment thereof comprises a HCVR and LCVR selected from the amino acid sequence pairs of SEQ ID NO:26/34, 42/44, 487/44, 46/48, 74/82, 90/92, 94/96, 122/130, 138/140, 142/144, 146/154, 162/164 and 166/168. In another embodiment, the antibody or fragment thereof comprises a HCVR/LCVR pair comprising SEQ ID NO:42/44, 487/44, 90/92, 138/140 or 162/164.

[0009] In a second aspect, the invention features an antibody or antigen-binding fragment of an antibody comprising a heavy chain complementarity determining region 3 (HCDR3) amino acid sequence selected from the group consisting of SEQ ID NO:8, 32, 56, 80, 104, 128, 152, 176, 200, 224, 248, 272, 296, 320, 344, 368, 392, 416, 440 and 464, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) amino acid sequence selected from the group consisting of SEQ ID NO:16, 40, 64, 88, 112, 136, 160, 184, 208, 232, 256, 280, 304, 328, 352,

376, 400, 424 and 448, or substantially similar sequences thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In one embodiment, the antibody or fragment thereof comprises a HCDR3/LCDR3 amino acid sequence pair comprising SEQ ID NO:32/40, 80/88, 128/136 or 152/160. In another embodiment, the antibody or fragment thereof comprises a HCDR3/LCDR3 amino acid sequence pair comprising SEQ ID NO:32/40 or 80/88.

[0010] In a further embodiment, the antibody or fragment thereof further comprises a heavy chain CDR1 (HCDR1) amino acid sequence selected from the group consisting of SEQ ID NO:4, 28, 52, 76, 100, 124, 148, 172, 196, 220, 244, 268, 292, 316, 340, 364, 388, 412, 436 and 460, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a heavy chain CDR2 (HCDR2) amino acid sequence selected from the group consisting of SEQ ID NO:6, 30, 54, 78, 102, 126, 150, 174, 198, 222, 246, 270, 294, 318, 342, 366, 390, 414, 438 and 462, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and optionally further comprises a light chain CDR1 (LCDR1) amino acid sequence selected from the group consisting of SEQ ID NO:12, 36, 60, 84, 108, 132, 156, 180, 204, 228, 252, 276, 300, 324, 348, 372, 396, 420 and 444, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and/or a light chain CDR2 (LCDR2) amino acid sequence selected from the group consisting of SEQ ID NO:14, 38, 62, 86, 110, 134, 158, 182, 206, 230, 254, 278, 302, 326, 350, 374, 398, 422 and 446, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0011] Alternatively, the invention features an antibody or antigen-binding fragment of an antibody comprising a HCDR1/HCDR2/HCDR3 combination selected from the group consisting of SEQ ID NO:4/6/8, 28/30/32, 52/54/56, 76/78/80, 100/102/104, 124/126/128, 148/150/152, 172/174/176, 196/198/200, 220/222/224, 244/246/248, 268/270/272, 292/294/296, 316/318/320, 340/342/344, 364/366/368, 388/390/392, 412/414/416, 436/438/440 and 460/462/464; and/or a LCDR1/LCDR2/LCDR3 combination selected from the group consisting of SEQ ID NO:12/14/16, 36/38/40, 60/62/64, 84/86/88, 108/110/112, 132/134/136, 156/158/160, 180/182/184, 204/206/208, 228/230/232, 252/254/256, 276/278/280, 300/302/304, 324/326/328, 348/350/352, 372/374/376,396/398/400, 420/422/424 and 444/446/448.

[0012] In one embodiment, the HCDR1, HCDR2 and HCDR3 are selected from the group consisting of SEQ ID NO:28/30/32, 76/78/80, 124/126/128, and 148/150/152; and/or the LCDR1, LCDR2 and LCDR3 are selected from the group consisting of SEQ ID NO:36/38/40, 84/86/88, 132/134/136, and 156/158/160. In yet another embodiment, the heavy and light chain CDR amino acid sequences comprise a CDR sequence combination selected from the group consisting of SEQ ID NO:28/30/32/36/38/40, 76/78/80/84/86/88, 124/126/128/132/134/136, and 148/150/152/156/158/160. In yet another embodiment, the antibody or antigen-binding fragment thereof comprises heavy and light chain CDR sequences of SEQ ID NO: 28/30/32/36/38/40, or

76/78/80/84/86/88.

[0013] In a related embodiment, the invention comprises an antibody or antigen-binding fragment of an antibody which specifically binds hANGPTL4, wherein the antibody or fragment thereof comprises heavy and light chain CDR domains contained within a HCVR/LCVR pair selected from the group consisting of SEQ ID NO: 2/10, 18/20, 22/24, 26/34, 42/44, 487/44, 46/48, 50/58, 66/68, 70/72, 74/82, 90/92, 94/96, 98/106, 114/116, 118/120, 122/130, 138/140, 142/144, 146/154, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/394, 466/404 and 468/408. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are known in the art and can be applied to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Conventional definitions that can be applied to identify the boundaries of CDRs include 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. In one embodiment, the antibody or fragment thereof comprises CDR sequences contained within a HCVR and LCVR pair selected from the group consisting of the amino acid sequence pairs of SEQ ID NO: 26/34, 42/44, 487/44, 46/48, 74/82, 90/92, 94/96, 122/130, 138/140, 142/144, 146/154, 162/164 and 166/168. In another embodiment, the antibody or fragment thereof comprises CDR sequences contained within the HCVR and LCVR sequence pair of SEQ ID NO: 42/44, 487/44, 90/92, 138/140 or 162/164.

[0014] In another related embodiment, the invention provides an antibody or antigen-binding fragment thereof that competes for specific binding to hANGPTL4 with an antibody or antigen-binding fragment comprising heavy and light chain CDR sequences of SEQ ID NO: 28/30/32/36/38/40, 76/78/80/84/86/88, 124/126/128/132/134/136, or 148/150/152/156/158/160. In one embodiment, the antibody or antigen-binding fragment of the invention competes for specific binding to hANGPTL4 with an antibody comprising a HCVR/LCVR sequence pair of SEQ ID NO:42/44, 487/44, 90/92, 138/140, or 162/164.

[0015] In another related embodiment, the invention provides an antibody or antigen-binding fragment thereof that binds the same epitope on hANGPTL4 that is recognized by an antibody or fragment thereof comprising heavy and light chain CDR sequences of SEQ ID NO: 28/30/32/36/38/40, 76/78/80/84/86/88, 124/126/128/132/134/136, or 148/150/152/156/158/160. In one embodiment, the antibody or antigen-binding fragment of the invention recognizes the

epitope on hANGPTL4 that is recognized by an antibody comprising a HCVR/LCVR sequence pair of SEQ ID NO:42/44, 487/44, 90/92, 138/140, or 162/164.

[0016] In a third aspect, the invention provides nucleic acid molecules encoding anti-ANGPTL4 antibodies or fragments thereof, in particular, those described above. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells, *e.g.*, bacterial cells, such as *E. coli*, or mammalian cells, such as CHO cells, into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0017] In one embodiment, the invention provides an antibody or fragment thereof comprising a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 17, 21, 25, 41, 45, 49, 65, 69, 73, 89, 93, 97, 113, 117, 121, 137, 141, 145, 161, 165, 169, 185, 189, 193, 209, 213, 217, 233, 237, 241, 257, 261, 265, 281, 285, 289, 305, 309, 313, 329, 333, 337, 353, 357, 361, 377, 381, 385, 401, 405, 409, 425, 429, 433, 449, 453, 457, 465, 467 and 486, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In another embodiment, the antibody or fragment thereof comprises a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 25, 41, 45, 73, 89, 93, 121, 137, 141, 145, 161, 165 and 486. In yet another embodiment, the antibody or fragment thereof comprises a HCVR encoded by the nucleic acid sequence of SEQ ID NO: 41, 89, 137, 161 or 486.

[0018] In one embodiment, an antibody or antigen-binding fragment thereof comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, 19, 23, 33, 43, 47, 57, 67, 71, 81, 91, 95, 105, 115, 119, 129, 139, 143, 153, 163, 167, 177, 187, 191, 201, 211, 215, 225, 235, 239, 249, 259, 263, 273, 283, 287, 297, 307, 311, 321, 331, 335, 345, 355, 359, 369, 379, 383, 393, 403, 407, 417, 427, 431, 441, 451 and 455, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In another embodiment, the antibody or fragment thereof comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 33, 43, 47, 81, 91, 95, 129, 139, 143, 153, 163 and 167. In yet another embodiment, the antibody or fragment thereof comprises a LCVR encoded by the nucleic acid sequence of SEQ ID NO: 43, 91, 139 or 163.

[0019] In further embodiments, the antibody or fragment thereof comprises a HCVR and LCVR (HCVR/LCVR) sequence pair encoded by a nucleic acid sequence pair selected from the group consisting of SEQ ID NO:1/9, 17/19, 21/23, 25/33, 41/43, 486/43, 45/47, 49/57, 65/67, 69/71, 73/81, 89/91, 93/95, 97/105, 113/115, 117/119, 121/129, 137/139, 141/143, 145/153, 161/163, 165/167, 169/177, 185/187, 189/191, 193/201, 209/211, 213/215, 217/225, 233/235, 237/239, 241/249, 257/259, 261/263, 265/273, 281/283, 285/287, 289/297, 305/307, 309/311, 313/321, 329/331, 333/335, 337/345, 353/355, 357/359, 361/369, 377/379, 381/383, 385/393, 401/403,

405/407, 409/417, 425/427, 429/431, 433/441, 449/451, 453/455, 457/393, 465/403 and 467/407. In one embodiment, the antibody or fragment thereof comprises a HCVR/LCVR sequence pair encoded by a nucleic acid sequence pair selected from the group consisting of SEQ ID NO: 25/33, 41/43, 486/43, 45/47, 73/81, 89/91, 93/95, 121/129, 137/139, 141/143, 145/153, 161/163 and 165/167. In yet another embodiment, the antibody or fragment thereof comprises a HCVR/LCVR pair encoded by a nucleic acid sequence pair of SEQ ID NO:41/43, 486/43, 89/91, 137/139 or 161/163.

[0020] In one embodiment, the invention features an antibody or antigen-binding fragment of an antibody comprising a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:7, 31, 55, 79, 103, 127, 151, 175, 199, 223, 247, 271, 295, 319, 343, 367, 391, 415, 439 and 463, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 15, 39, 63, 87, 111, 135, 159, 183, 207, 231, 255, 279, 303, 327, 351, 375, 399, 423 and 447, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In another embodiment, the antibody or fragment thereof comprises a HCDR3 and LCDR3 sequence pair encoded by the nucleic acid sequence pair of SEQ ID NO: 31/39, 79/87, 127/135 or 151/159. In yet another embodiment, the antibody or fragment thereof comprises a HCDR3 and LCDR3 sequence pair encoded by the nucleic acid sequence pair of SEQ ID NO:31/39 or 79/87.

[0021] In a further embodiment, the antibody or fragment thereof further comprises a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 3, 27, 51, 75, 99, 123, 147, 171, 195, 219, 243, 267, 291, 315, 339, 363, 387, 411, 435 and 459, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:5, 29, 53, 77, 101, 125, 149, 173, 197, 221, 245, 269, 293, 317, 341, 365, 389, 413, 437 and 461, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and optionally further comprises a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 11, 35, 59, 83, 107, 131, 155, 179, 203, 227, 251, 275, 299, 323, 347, 371, 395, 419 and 443, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and/or a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 13, 37, 61, 85, 109, 133, 157, 181, 205, 229, 253, 277, 301, 325, 349, 373, 397, 421 and 445, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0022] Alternatively, the invention features an antibody or antigen-binding fragment of an antibody comprising a HCDR1/HCDR2/HCDR3 combination encoded by a nucleotide sequence combination selected from the group consisting of SEQ ID NO:3/5/7, 27/29/31, 51/53/55,

75/77/79, 99/101/103, 123/125/127, 147/149/151, 171/173/175, 195/197/199, 219/221/223, 243/245/247, 267/269/271, 291/293/295, 315/317/319, 339/341/343, 363/365/367, 387/389/391, 411/413/415, 435/437/439 and 459/461/463; and/or a LCDR1/LCDR2/LCDR3 combination encoded by a nucleotide sequence combination selected from the group consisting of SEQ ID NO:11/13/15, 35/37/39, 59/61/63, 83/85/87, 107/109/111, 131/133/135, 155/157/159, 179/181/183, 203/205/207, 227/229/231, 251/253/255, 275/277/279, 299/301/303, 323/325/327, 347/349/351, 371/373/375, 395/397/399, 419/421/423 and 443/445/447.

[0023] In one embodiment, the HCDR1, HCDR2 and HCDR3 are encoded by a nucleotide sequence combination selected from the group consisting of SEQ ID NO:27/29/31, 75/77/79, 123/125/127, and 147/149/151; and/or the LCDR1, LCDR2 and LCDR3 are encoded by a nucleotide sequence combination selected from the group consisting of SEQ ID NO:35/37/39, 83/85/87, 131/133/135, and 155/157/159. In yet another embodiment, the antibody or fragment thereof comprises heavy and light chain CDR sequences encoded by a nucleotide sequence combination selected from the group consisting of SEQ ID NO: 27/29/31/35/37/39; 75/77/79/83/85/87; 123/125/127/131/133/135; and 147/149/151/155/157/159. In another embodiment, the antibody or antigen-binding portion thereof comprises heavy and light chain CDR sequences encoded by the nucleotide sequence combination of SEQ ID NO: 27/29/31/35/37/39; or 75/77/79/83/85/87.

[0024] In a fourth aspect, the invention features an isolated antibody or antigen-binding fragment of an antibody that specifically binds hANGPTL4, comprising a HCDR3 and a LCDR3, wherein the HCDR3 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18} - X^{19} - X^{20} (SEQ ID NO:471) wherein X^1 is Ala, X^2 is Arg or Lys, X^3 is Gly or Glu, X^4 is Gly, Asp or absent, X^5 is Asp or absent, X^6 is Leu, Arg or absent, X^7 is Arg or Ser, X^8 is Phe, Gly or Arg, X^9 is Leu, His or Asn, X^{10} is Asp, Pro or Tyr, X^{11} is Trp, Tyr or Phe, X^{12} is Leu, Phe, Val or Asp, X^{13} is Ser, Tyr or Gly, X^{14} is Ser, Tyr or Asp, X^{15} is Tyr, X^{16} is Phe or Gly, X^{17} is Leu or absent, X^{18} is Asp, X^{19} is Tyr, Val or Phe, and X^{20} is Trp; and the LCDR3 comprises an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} (SEQ ID NO:474) wherein X^1 is Gln, X^2 is Asn or Gln, X^3 is Tyr or Leu, X^4 is Asn, His, Ser or Asp, X^5 is Thr or Ser, X^6 is Ala or Tyr, X^7 is Pro, Ser or Phe, X^8 is Leu or Arg, X^9 is Thr, and X^{10} is Phe.

[0025] In a further embodiment, the antibody or fragment thereof further comprises a HCDR1 sequence comprising an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:469), wherein X^1 is Gly, X^2 is Gly or Phe, X^3 is Ser or Thr, X^4 is Phe, X^5 is Ser, X^6 is Ile, Ser or Thr, X^7 is His or Tyr, and X^8 is His, Gly or Asp; a HCDR2 sequence comprising an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:470), wherein X^1 is Ile, X^2 is Asn, Ser or Gly, X^3 is His, Phe, Ser or Val, X^4 is Arg, Asp or Ala, X^5 is Gly, X^6 is Gly or absent, X^7 is Ser, Asn or Asp, and X^8 is Thr or Lys; a LCDR1 sequence comprising an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 (SEQ ID NO:472) wherein X^1 is

Gln, X^2 is Gly or Ser, X^3 is Ile, X^4 is Ser or Asn, X^5 is Asp, Ser or Arg, and X^6 is Tyr or Trp; and a LCDR2 sequence comprising an amino acid sequence of the formula X^1 - X^2 - X^3 (SEQ ID NO:473) wherein X^1 is Ala or Lys, X^2 is Ala, and X^3 is Ser. The sequence alignments of H1H268P, H1H284P, H1H291P and H1H292P monoclonal antibodies are shown in Fig. 1 (HCVR) and Fig. 2 (LCVR).

[0026] In a fifth aspect, the invention features a human anti-ANGPTL4 antibody or antigen-binding fragment thereof comprising a heavy chain variable region (HCVR) encoded by nucleotide sequence segments derived from V_H, D_H and J_H germline sequences, and a light chain variable region (LCVR) encoded by nucleotide sequence segments derived from V_K and J_K germline sequences, wherein the HCVR and the LCVR are encoded by nucleotide sequence segments derived from a germline gene combination selected from the group consisting of: (i) V_H3-30, D_H5-12, J_H6, V_K1-9 and J_K4; (ii) V_H4-34, D_H3-3, J_H4, V_K1-27 and J_K4; and (iii) V_H3-13, D_H1-26, J_H4, V_K1-5 and J_K1.

[0027] In a sixth aspect, the invention features an antibody or antigen-binding fragment thereof that specifically binds to hANGPTL4 with an equilibrium dissociation constant (K_D) of about 1 nM or less, as measured by surface plasmon resonance assay (for example, BIACORE™). In certain embodiments, the antibody of the invention exhibits a K_D of about 500 pM or less; about 400 pM or less; about 300 pM or less; about 200 pM or less; about 150 pM or less; about 100 pM or less; or about 50 pM or less.

[0028] In a seventh aspect, the present invention provides an anti-hANGPTL4 antibody or antigen-binding fragment thereof that binds hANGPTL4 protein of SEQ ID NO:476, but does not cross-react with a related protein, such as a human angiopoietin-like protein 3 (hANGPTL3; SEQ ID NO:485), as determined by, for example, ELISA, surface plasmon resonance assay, or LUMINEX® XMAP® Technology, as described herein. ANGPTL3 is another secreted protein that is known to reduce LPL activity and has an N-terminal coiled-coil region and a C-terminal fibrinogen-like domain (Ono *et al.*, 2003, *J Biol Chem* 43:41804-41809). In related embodiments, the invention provides an anti-hANGPTL4 antibody or antigen binding fragment thereof that binds a hANGPTL4 protein and cross-reacts with a hANGPTL3 protein. In certain embodiments, the binding affinity of the hANGPTL4 antibody or fragment thereof to hANGPTL3 protein is about 75% or less, or about 50% or less, of the binding affinity of the antibody or fragment to the hANGPTL4 protein. In another related embodiment, the invention provides an anti-hANGPTL4 antibody or antigen binding fragment thereof that does not cross-react with mouse ANGPTL4 (mANGPTL4: SEQ ID NO:478) but does cross-react with cynomolgus monkey (*Macaca fascicularis*; the amino acid sequence of the N-terminal 1-148 residues and the encoding DNA sequences are shown as SEQ ID NOS:490 and 489, respectively) and/or rhesus monkey (*Macaca mulatta*; the amino acid sequence of the N-terminal 1-148 residues and the encoding DNA sequences are shown as SEQ ID NOS:492 and 491, respectively) ANGPTL4.

[0029] The invention encompasses anti-hANGPTL4 antibodies having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or *e.g.*, removal of a fucose moiety to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield *et al.* (2002) JBC 277:26733). In other applications, removal of N-glycosylation site may reduce undesirable immune reactions against the therapeutic antibodies, or increase affinities of the antibodies. In yet other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0030] In an eighth aspect, the invention features a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds hANGPTL4 and a pharmaceutically acceptable carrier. In one 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. The second therapeutic agent may be one or more of any 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). Furthermore, the second therapeutic agent can be one or more other inhibitors of ANGPTL4 as well as inhibitors of other molecules, such as ANGPTL3, ANGPTL5, ANGPTL6 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 and antibodies that specifically bind to these molecules and block their activity.

[0031] In related embodiments, the second therapeutic agent may be one or more anti-cancer agents, such as chemotherapeutic agents, anti-angiogenic agents, growth inhibitory agents, cytotoxic agents, apoptotic agents, and other agents well known in the art to treat cancer or other proliferative diseases or disorders, as well as 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 cancer/tumor.

[0032] In a ninth aspect, the invention features methods for inhibiting hANGPTL4 activity using the anti-hANGPTL4 antibody or antigen-binding portion of the antibody of the invention, wherein the therapeutic methods comprise administering a therapeutically effective amount of a

pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody of the invention and, optionally one or more additional therapeutic agents described above. The disease or disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented, or its occurrence rate reduced compared to that without anti-hANGPTL4 antibody treatment (*e.g.*, ANGPTL4-mediated diseases or disorders), by removal, inhibition or reduction of ANGPTL4 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, 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, decreased LDL receptor activity and/or LDL receptor 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. The methods of the invention can also prevent or treat diseases or disorders associated with or resulting from hyperlipidemia, hyperlipoproteinemia, 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.

[0033] Other examples of diseases or disorders treatable by the methods of the invention include cancer/tumor as well as non-neoplastic angiogenesis-associated diseases or disorders, including ocular angiogenic diseases or disorders, such as age-related macular degeneration, central retinal vein occlusion or branch retinal vein occlusion, diabetic retinopathy, retinopathy of prematurity, and the like, inflammatory diseases or disorders, such as arthritis, rheumatoid arthritis (RA), psoriasis, and the like.

[0034] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURE

[0035] Figure 1 shows a sequence alignment of heavy chain variable regions (HCVR) of antibodies H1H268P, H1H284P, H1H291P AND H1H292P.

[0036] Figure 2 shows a sequence alignment of light chain variable regions (LCVR) of antibodies H1H268P, H1H284P, H1H291P AND H1H292P.

[0037] Figure 3A and 3B show the pharmacokinetic clearance of anti-ANGPTL4 antibodies in

wild-type mice (Fig. 3A) and in transgenic mice expressing human ANGPTL4 [hAngptl4(+/+) mice; or "humanized ANGPTL4 mice"] (Fig. 3B). H4H268P2 (□); H4H284P (▲); and hIgG4 control (●).

[0038] Figure 4 shows the effect of anti-ANGPTL4 antibody, H4H268P2, on serum triglyceride (TG) levels in humanized ANGPTL4 mice crossed to ApoE null mice. Percent (%) changes of serum TG levels by H4H268P2, compared to control antibody with irrelevant specificity, are shown. Control Ab (○); and H4H268P2 (■).

[0039] Figure 5 shows the effects of anti-ANGPTL4 antibody H4H268P2 and TG-reducing drug fenofibrate, each alone or in combination, on serum TG levels in humanized ANGPTL4 mice.

[0040] Figure 6 shows the results of phase I pilot study on effects of anti-ANGPTL4 antibodies on fasting serum TG levels, among other lipids, in obese rhesus monkeys (*Macaca mulatta*). All monkeys received a vehicle (10 mM histidine, pH 6) intravenous (IV) infusion on Day -5 and either H4H268P2 (n=3) (●) or H4H284P (n=3) (□) at 10 mg/kg IV on Day 0. Serum samples were collected from the baseline period through Day 35 post-dosing. The average baseline for each animal was determined based on the samples taken on Days -7, -5 and 0, and percent (%) changes of serum TG levels from the baseline determined and averaged for each Ab group.

[0041] Figure 7 shows the results of phase II pilot study on effects of anti-ANGPTL4 antibody H4H268P2 on fasting serum TG levels in obese monkeys, as described for Figure 6, except that the step of vehicle infusion was omitted. The average baseline was obtained for each monkey based on the samples taken on Days -7, -3 and 0. Monkeys were divided into Groups based on their baselines: A. TG<150 mg/dL (n=3; □); B. 150 mg/dL<TG<500 mg/dL (n=4; ●); and C. TG>1000 mg/dL (n=1; ▽). Percent (%) changes of fasting TG levels from the baseline were determined for each monkey and averaged for each Group.

[0042] Error bars in all graphs indicate mean ± SEM.

DETAILED DESCRIPTION

[0043] Before the present invention is described in detail, 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.

[0044] 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. 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.

Definitions

[0045] The term “human angiopoietin-like protein 4” or "hANGPTL4", as used herein, refers to hANGPTL4 having the nucleic acid sequence shown in SEQ ID NO:475 and the amino acid sequence of SEQ ID NO:476, or a biologically active fragment thereof.

[0046] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (HCVR) and a heavy chain constant region (C_H; comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region (LCVR) and a light chain constant region (C_L). The HCVR and LCVR can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each HCVR and LCVR 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, and FR4.

[0047] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 J Mol Biol 320:415-428).

[0048] CDR residues not contacting antigen can be identified based on previous studies (for example, residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[0049] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (*e.g.*, mouse), have been grafted onto human FR sequences.

[0050] The fully-human anti-hANGPTL4 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 as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residues(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline back-mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residues of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline 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.

[0051] The present invention also includes anti-ANGPTL4 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-ANGPTL4 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, 2 or 1, conservative amino acid substitution(s) relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein. In one embodiment, a HCVR comprises the amino acid sequence of SEQ ID NO:487 with 10 or fewer conservative

amino acid substitutions therein. In another embodiment, a HCVR comprises the amino acid sequence of SEQ ID NO:487 with 8 or fewer conservative amino acid substitutions therein. In another embodiment, a HCVR comprises the amino acid sequence of SEQ ID NO:487 with 6 or fewer conservative amino acid substitutions therein. In another embodiment, a HCVR comprises the amino acid sequence of SEQ ID NO:487 with 4 or fewer conservative amino acid substitutions therein. In yet another embodiment, a HCVR comprises the amino acid sequence of SEQ ID NO:487 with 2 or 1 conservative amino acid substitution(s) therein. In one embodiment, a LCVR comprises the amino acid sequence of SEQ ID NO:44 with 10 or fewer conservative amino acid substitutions therein. In another embodiment, a LCVR comprises the amino acid sequence of SEQ ID NO:44 with 8 or fewer conservative amino acid substitutions therein. In another embodiment, a LCVR comprises the amino acid sequence of SEQ ID NO:44 with 6 or fewer conservative amino acid substitutions therein. In another embodiment, a LCVR comprises the amino acid sequence of SEQ ID NO:44 with 4 or fewer conservative amino acid substitutions therein. In yet another embodiment, a LCVR comprises the amino acid sequence of SEQ ID NO:44 with 2 or 1 conservative amino acid substitution(s) therein.

[0052] Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. 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-display 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.

[0053] 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)). Other engineered molecules, such as diabodies, triabodies, tetrabodies and minibodies, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0054] 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.

[0055] 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)).

[0056] 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.

[0057] In certain embodiments, antibody or antibody fragments of the invention may be conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope.

[0058] The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiological conditions. Specific binding can be characterized by an equilibrium dissociation constant (K_D) of about $1x10^{-6}$ M or less (*i.e.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. An isolated antibody that

specifically binds hANGPTL4 may, however, exhibit cross-reactivity to other antigens, such as ANGPTL4 molecules from other species, for example, cynomolgus monkey ANGPTL4, and/or hANGPTL3 having the amino acid sequence of SEQ ID NO:485. Moreover, multi-specific antibodies (*e.g.*, bispecifics) that bind to hANGPTL4 and one or more additional antigens are nonetheless considered antibodies that "specifically bind" hANGPTL4, as used herein.

[0059] The term "high affinity" antibody refers to those antibodies having a binding affinity to hANGPTL4, expressed as K_D, of about $1x10^{-9}$ M or less, about $0.5x10^{-9}$ M or less, about $0.25x10^{-9}$ M or less, about $1x10^{-10}$ M or less, or about $0.5x10^{-10}$ M or less, as measured by surface plasmon resonance, *e.g.*, BIACORE™ or solution-affinity ELISA.

[0060] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0061] By the term "slow off rate", "Koff" or "k_d" is meant an antibody that dissociates from hANGPTL4 with a rate constant of 1×10^{-3} s^{-1} or less, preferably 1×10^{-4} s^{-1} or less, as determined by surface plasmon resonance, e.g., BIACORE™.

[0062] By the term "intrinsic affinity constant" or "k_a" is meant an antibody that associates with hANGPTL4 at a rate constant of about 1×10^{3} $M^{-1}s^{-1}$ or higher, as determined by surface plasmon resonance, e.g., BIACORE™.

[0063] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other mAbs having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds hANGPTL4 is substantially free of mAbs that specifically bind antigens other than hANGPTL4). An isolated antibody that specifically binds hANGPTL4 may, however, have cross-reactivity to other antigens, such as ANGPTL4 molecules from other species, such as cynomolgus monkey, and/or other related proteins, such as human ANGPTL3.

[0064] A "neutralizing antibody", as used herein (or an "antibody that neutralizes ANGPTL4 activity"), is intended to refer to an antibody whose binding to ANGPTL4 results in inhibition of at least one biological activity of ANGPTL4. This inhibition of the biological activity of ANGPTL4 can be assessed by measuring one or more indicators of ANGPTL4 biological activity by one or more of several standard *in vitro* or *in vivo* assays known in the art (also see examples below).

[0065] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[0066] The term "epitope" is a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl

groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[0067] 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 90%, and more preferably at least about 95%, 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.

[0068] 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 90% sequence identity, even more preferably at least 95%, 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 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. 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: 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 45. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0069] 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.

[0070] 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, the age and the size of a subject treated, the route of administration, and the like, 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).

Preparation of Human Antibodies

[0071] Methods for generating human antibodies in transgenic mice 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 ANGPTL4.

[0072] Using VELOCIMMUNE™ technology or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to ANGPTL4 are initially isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, and the like.

[0073] In general, the antibodies of the instant invention possess very high affinities, typically possessing K_D of from about 10^{-12} M through about 10^{-9} M, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions, for example, wild-type IgG1 (SEQ ID NO:481) or IgG4 (SEQ ID NO:482), or modified IgG1 or IgG4 (for example, SEQ ID NO:483), to generate the fully human antibodies of the invention. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics of the antibodies reside in the variable region.

Epitope Mapping and Related Technologies

[0074] To screen for antibodies that bind to a particular epitope, a routine cross-blocking assay such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY) can be performed. Other methods include alanine scanning mutants, peptide blots (Reineke (2004) Methods Mol Biol 248:443-63), or 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).

[0075] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0076] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (US 2004/0101920). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical mAbs, such that characterization can be focused on genetically distinct mAbs. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the anti-ANGPTL4 mAbs of the invention into groups of mAbs binding different epitopes.

[0077] ANGPTL4 contains an amino-terminal coiled-coil domain and a carboxyl-terminal fibrinogen like domain and the full-length ANGPTL4 protein forms an oligomer held by intermolecular disulfide bonds (Ge *et al.*, 2004, *J Bio Chem* 279(3):2038-2045). It has been reported that the N-terminal coiled-coil domain mediates ANGPTL4's oligomerization (Ge *et al.*, *supra*) and is also important in the inhibition of LPL activity (Ge *et al.*, 2005, *J Lipid Res* 46:1484-1490; and Ono *et al.*, 2003, *J Biol Chem* 278:41804-41809). Thus, in certain embodiments, the anti-hANGPTL4 antibody or antigen-binding fragment of an antibody binds an epitope within the N-terminal coiled-coil domain (residues 1-123) of hANGPTL4 (SEQ ID NO:476). In certain embodiments, anti-hANGPTL4 antibody or fragment thereof binds an epitope within the region from about residue 1 to about residue 25, from about residue 25 to about residue 50, from about residue 50 to about residue 75, from about residue 75 to about residue 100, from about residue 100 to about residue 125, from about residue 125 to about residue 150, of hANGPTL4 (SEQ ID NO:476). In some embodiments, the antibody or antibody fragment binds an epitope which includes more than one of the enumerated epitopes within the N-terminal coiled-coil domain of hANGPTL4. In other embodiments, hANGPTL4 antibody or fragment thereof binds one or more fragments of hANGPTL4, for example, a fragment from residues 26 to 406, from residues 26 to 148, from residues 34 to 66, and/or residues 165 to 406, of SEQ ID NO:476.

[0078] The present invention includes hANGPTL4 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein. Likewise, the present invention also includes anti-hANGPTL4 antibodies that compete for binding to hANGPTL4 or a hANGPTL4 fragment with any of the specific exemplary antibodies described herein.

[0079] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-hANGPTL4 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-hANGPTL4 antibody of the invention, the reference antibody is allowed to bind to a hANGPTL4 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the hANGPTL4 molecule is assessed. If the test antibody is able to bind to hANGPTL4 following saturation binding with the reference anti-hANGPTL4 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-hANGPTL4 antibody. On the other hand, if the test antibody is not able to bind to the hANGPTL4 molecule following saturation binding with the reference anti-hANGPTL4 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-hANGPTL4 antibody of the invention.

[0080] To determine if an antibody competes for binding with a reference anti-hANGPTL4 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a hANGPTL4 molecule under saturating conditions followed by assessment of binding of the test antibody to the hANGPTL4 molecule. In a second orientation, the test antibody is allowed to bind to a hANGPTL4 molecule under saturating conditions followed by assessment of binding of the reference antibody to the ANGPTL4 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the ANGPTL4 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to hANGPTL4. 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 identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0081] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, 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 have 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 have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0082] 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, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0083] The invention encompasses a human anti-ANGPTL4 monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope. Cytotoxin agents include any agent that is detrimental to cells. Examples of suitable cytotoxin agents and chemotherapeutic agents for forming immunoconjugates are known in the art, see for example, WO 05/103081.

Bispecifics

[0084] The antibodies of the present invention may be monospecific, bispecific, or multispecific. Multispecific mAbs 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. The human anti-hANGPTL4 mAbs 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 bispecific or a multispecific antibody with a second binding specificity.

[0085] An exemplary bi-specific 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 bi-specific antibody format described above are contemplated within the scope of the present invention.

Bioequivalents

[0086] The anti-hANGPTL4 antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described mAbs, but that retain the ability to bind human ANGPTL4. Such variant mAbs 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 mAbs. Likewise, the hANGPTL4 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-hANGPTL4 antibody or antibody fragment that is essentially bioequivalent to an anti-hANGPTL4 antibody or antibody fragment of the invention. Examples of such variant amino acid and DNA sequences are discussed above.

[0087] 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. In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0088] 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.

[0089] 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.

[0090] 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.

[0091] Bioequivalent variants of anti-hANGPTL4 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.

Therapeutic Administration and Formulations

[0092] The invention provides therapeutic compositions comprising the anti-hANGPTL4 antibodies or antigen-binding fragments thereof of the present invention and the therapeutic methods using the same. The administration of therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to 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™), 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.

[0093] The dose may vary depending upon the age and the size of a subject to be administered, target disease, the purpose of the treatment, conditions, route of administration, and the like. When the antibody of the present invention is used for treating various conditions and diseases directly or indirectly associated with ANGPTL4, including hypercholesterolemia, disorders associated with LDL and apolipoprotein B, and lipid metabolism disorders, and the like, in an adult patient, it is advantageous to intravenously or subcutaneously administer the antibody of the present invention 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. In certain embodiments, the antibody or antigen-binding fragment thereof of the invention can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0094] 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.

[0095] The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see Langer (1990) Science 249:1527-1533; Treat *et al.* (1989) in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez Berestein and Fidler (eds.), Liss, New York, pp. 353-365; Lopez-Berestein, ibid., pp. 317-327; see generally ibid.).

[0096] 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.), CRC Pres., Boca Raton, Florida (1974). 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, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138, 1984).

[0097] 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. 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.

[0098] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, 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 certainly are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly).

[0099] 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 0.1 to about 800 mg per dosage form in a unit dose; especially in the form of injection, the aforesaid antibody is contained in about 1 to about 500 mg, in about 5 to 300 mg, in about 8 to 200 mg, and in about 10 to about 100 mg for the other dosage forms.

Combination Therapies

[0100] The invention further provides therapeutic methods for treating diseases or disorders, which is directly or indirectly associated with hANGPTL4, by administering the hANGPTL4 antibody or fragment thereof of the invention in combination with one or more additional therapeutic agents. The additional therapeutic agent may be one or more of any agent that is advantageously combined with the antibody or fragment thereof of the invention, including HMG-CoA reductase inhibitors, such as cerovastatin, atorvastatin, simvastatin, pitavastin, ros uvastatin, fluvastatin, lovastatin, pravastatin, and the like; niacin; various fibrates, such as fenofibrate, bezafibrate, ciprofibrate, clofibrate, gemfibrozil, and the like; LXR transcription factor activators, and the like. Furthermore, the hANGPTL4 antibody or fragment thereof of the invention can be co-administered with other ANGPTL4 inhibitors as well as inhibitors of other molecules, such as ANGPTL3, ANGPTL5, ANGPTL6 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 and antibodies that specifically bind to these molecules and block their activity (see, for example, anti-PCSK9

antibodies disclosed in U.S. 2010/0166768 A1).

[0101] Furthermore, the additional therapeutic agent may be one or more anti-cancer agents, such as chemotherapeutic agents, anti-angiogenic agents, growth inhibitory agents, cytotoxic agents, apoptotic agents, and other agents well known in the art to treat cancer or other proliferative diseases or disorders. Examples of anti-cancer agents include, but are not limited to, an anti-mitotic agent, such as docetaxel, paclitaxel, and the like; a platinum-based chemotherapeutic compound, such as cisplatin, carboplatin, iproplatin, oxaliplatin, and the like; or other conventional cytotoxic agent, such as 5-fluorouracil, capecitabine, irinotecan, leucovorin, gemcitabine, and the like, and anti-angiogenic agents, including vascular endothelial growth factor (VEGF) antagonists, such as anti-VEGF antibodies, *e.g.*, bevacizumab (AVASTIN®, Genentech) and a receptor-based blocker of VEGF, *e.g.*, "VEGF trap" described in US Patent No. 7,070,959, delta-like ligand 4 (Dll4) antagonists, such as anti-Dll4 antibodies as described in U.S. Patent Application Publication No. 2008/0181899, and a fusion protein containing the extracellular domain of Dll4, *e.g.*, Dll4-Fc as described in U.S. Patent Application Publication No. 2008/0107648; inhibitors of receptor tyrosine kinases and/or angiogenesis, including sorafenib (NEXAVAR® by Bayer Pharmaceuticals Corp.), sunitinib (SUTENT® by Pfizer), pazopanib (VOTRIENT™ by GlaxoSmithKline), toceranib (PALLADIA™ by Pfizer), vandetanib (ZACTIMA™ by AstraZeneca), cediranib (RECENTIN® by AstraZeneca), regorafenib (BAY 73-4506 by Bayer), axitinib (AG013736 by Pfizer), lestaurtinib (CEP-701 by Cephalon), erlotinib (TARCEVA® by Genentech), gefitinib (IRESSA™ by AstraZeneca), BIBW 2992 (TOVOK™ by Boehringer Ingelheim), lapatinib (TYKERB® by GlaxoSmithKline), neratinib (HKI-272 by Wyeth/Pfizer), and the like, and pharmaceutically acceptable salts, acids or derivatives of any of the above. In addition, other therapeutic agents, such as analgesics, anti-inflammatory agents, including non-steroidal anti-inflammatory drugs (NSAIDS), such as Cox-2 inhibitors, and the like, may be also co-administered with the hANGPTL4 antibody or fragment thereof of the invention so as to ameliorate and/or reduce the symptoms accompanying the underlying cancer/tumor.

[0102] The hANGPTL4 antibody or fragment thereof of the invention and the additional therapeutic agent(s) can be co-administered together or separately. Where separate dosage formulations are used, the antibody or fragment thereof of the invention and the additional agents can be administered concurrently, or separately at staggered times, *i.e.*, sequentially, in appropriate orders.

Diagnostic Uses of the Antibodies

[0103] The anti-ANGPTL4 antibodies of the present invention can be also used to detect and/or measure ANGPTL4 in a sample, *e.g.*, for diagnostic purposes. For example, an anti-ANGPTL4 Ab or fragment thereof, can be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of

ANGPTL4. Exemplary diagnostic assays for ANGPTL4 may comprise, *e.g.*, contacting a sample obtained from a patient, with an anti-ANGPTL4 Ab of the invention, wherein the anti-ANGPTL4 antibody is labeled with a detectable label or reporter molecule or used to selectively capture and isolate ANGPTL4 protein from patient samples. Alternatively, an unlabeled anti-ANGPTL4 Ab 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, ^{131}I 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. Assays that can be used to detect or measure ANGPTL4 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), fluorescence-activated cell sorting (FACS), and the like.

EXAMPLES

[0104] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used but some experimental errors and deviations should be accounted for. Unless indicated otherwise, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Generation of Human Antibodies to Human ANGPTL4

[0105] VELOCIMMUNE™ mice were immunized with human ANGPTL4, and the antibody immune response monitored by antigen-specific immunoassay using serum obtained from these mice. Anti-hANGPTL4 expressing B cells were harvested from the spleens of immunized mice shown to have elevated anti-hANGPTL4 antibody titers and were fused with mouse myeloma cells to form hybridomas. The hybridomas were screened and selected to identify cell lines expressing hANGPTL4-specific antibodies using assays as described below. The assays identified several cell lines that produced chimeric anti-hANGPTL4 antibodies designated as H1M222, H1M223, H1M224, H1M225, H1M234 and H1M236.

[0106] Human ANGPTL4-specific antibodies were also isolated directly from antigen-immunized B cells without fusion to myeloma cells, as described in U.S. 2007/0280945 A1. Heavy and light chain variable regions were cloned to generate fully human anti-hANGPTL4 antibodies designated as H1H257, H1H268, H1H283, H1H284, H1H285, H1H291, H1H292, H1H295, H1H624, H1H637, H1H638, H1H644 and H1H653. Stable recombinant antibody-expressing CHO cell lines were established.

Example 2. Variable Gene Utilization Analysis

[0107] To analyze the structure of antibodies produced, the nucleic acids encoding antibody

variable regions were cloned and sequenced. From the nucleic acid sequence and predicted amino acid sequence of the antibodies, gene usage was identified for each Heavy Chain Variable Region (HCVR) and Light Chain Variable Region (LCVR). Table 1 shows the gene usage for selected antibodies in accordance with the invention.

Table 1

Antibody	HCVR			LCVR	
	V_H	D_H	J_H	V_K	J_K
H1M225	3-9	1-7	1	1-5	2
H1M236	3-9	6-6	5	3-15	5
H1H283	3-13	1-26	4	1-5	1
H1H285	3-13	1-26	4	1-5	1
H1H291	3-13	1-26	4	1-5	1
H1H292	3-13	1-26	4	1-5	1
H1H295	3-13	1-26	4	1-5	1
H1H637	3-13	1-26	4	1-5	1
H1H638	3-13	1-26	4	1-5	1
H1H644	3-13	1-26	4	1-5	1
H1H257	3-13	1-26	4	1-5	1
H1M224	3-13	3-3	4	1-16	3
H1M223	3-15	3-3	4	1-12	3
H1M234	3-15	3-3	4	1-12	3
H1H624	3-23	5-5	6	1-9	3
H1H284	3-30	5-12	6	1-9	4
H1M222	3-33	3-9	5	3-20	4
H1H653	3-33	2-8	6	1-17	2
H1H268	4-34	3-3	4	1-27	4

[0108] Table 2 shows the heavy and light chain variable region amino acid sequence pairs of selected anti-hANGPTL4 antibodies and their corresponding antibody identifiers. The N, P and L designations refer to antibodies having heavy and light chains with identical CDR sequences but with sequence variations in regions that fall outside of the CDR sequences (*i.e.*, in the framework regions). Thus, N, P and L variants of a particular antibody have identical CDR sequences within their heavy and light chain variable regions but contain modifications within the framework regions.

Table 2

Name	HCVR/LCVR SEQ ID NOs	Name	HCVR/LCVR SEQ ID NOs	Name	HCVR/LCVR SEQ ID NOs
H1M222N	314/322	H1M222P	330/332	H1M222L	334/336
H1M223N	410/418	H1M223P	426/428	H1M223L	430/432
H1M224N	338/346	H1M224P	354/356	H1M224L	358/360
H1M225N	362/370	H1M225P	378/380	H1M225L	382/384
H1M234N	434/442	H1M234P	450/452	H1M234L	454/456
H1M236N	386/394	H1M236P	402/404	H1M236L	406/408
H1H236N2	458/394	H1H236P2	466/404	H1H236L2	468/408
H1H257N	2/10	H1H257P	18/20	H1H257L	22/24
H1H268N	26/34	H1H268P	42/44	H1H268L	46/48
-	-	H4H268P2	487/44	-	-
H1H283N	50/58	H1H283P	66/68	H1H283L	70/72
H1H284N	74/82	H1H284P	90/92	H1H284L	94/96
H1H285N	98/106	H1H285P	114/116	H1H285L	118/120
H1H291N	122/130	H1H291P	138/140	H1H291L	142/144
H1H292N	146/154	H1H292P	162/164	H1H292L	166/168
H1H295N	170/178	H1H295P	186/188	H1H295L	190/192
H1H624N	194/202	H1H624P	210/212	H1H624L	214/216
H1H637N	218/226	H1H637P	234/236	H1H637L	238/240
H1H638N	242/250	H1H638P	258/260	H1H638L	262/264
H1H644N	266/274	H1H644P	282/284	H1H644L	286/288
H1H653N	290/298	H1H653P	306/308	H1H653L	310/312

Example 3. hANGPTL4 Binding Affinity Determination.

[0109] Equilibrium dissociation constants (K_D values) for antigen binding to selected antibodies that bind amino acid residues 26-148 of human ANGPTL4 fused in-line to mouse IgG2a (hANGPTL4-mFc; SEQ ID NO:480) were determined by surface kinetics using a real-time biosensor surface plasmon resonance assay (BIACORE™ T100). hANGPTL4-mFc was captured with goat anti-mouse IgG polyclonal antibody (GE Healthcare) that was chemically coupled to a BIACORE™ chip through free amino groups. Varying concentrations (ranging from 12.5 nM to 50 nM) of anti-ANGPTL4 antibodies were injected over the captured antigen surface for 90 seconds. Antigen-antibody binding and dissociation were monitored in real time at 25°C and 37°C. Kinetic analysis was performed to calculate K_D and half-life of antigen/antibody complex dissociation. Results are shown in Table 3. A human anti-EGFR antibody was used as a negative control, which showed no binding to the captured hANGPTL4-mFc.

Table 3

Antibody	25°C		37°C	
	K_D (pM)	$t_{1/2}$ (min)	K_D (pM)	$t_{1/2}$ (min)
H1H257P	201	91	238	63
H1H268P	275	80	389	57
H1H283P	130	119	1360	12
H1H284P	168	162	349	81
H1H285P	92.5	156	194	71
H1H291P	87.6	303	178	122
H1H292P	136	112	167	88
H1H295P	30.7	874	2620	10
H1H624P	1190	7	3710	3
H1H638P	193	85	299	48
H1H644P	111	144	3000	6
H1H653P	411	43	2130	6

[0110] For H1H268P and H1H284P, Fab fragments were prepared by papain digestion and purified by standard purification methods, and their binding affinities to hANGPTL4 were measured at 25°C at pH 7.2 and pH 5.75 using the BIACORE™ system, essentially according to the method described above. Briefly, various concentrations (3.125 nM-100 nM) of anti-hANGPTL4 antibodies (*i.e.*, H1H268 Fab, entire H1H268 mAb, H1H284 Fab, and entire H1H284 mAb) were injected over a low density anti-mFc captured hANGPTL4(26-148)-mFc (~68 ± 4 RU) surface, or the surface of amino-coupled hANGPTL4(26-406)-His (R&D Systems) (450 RU) or amino-coupled cynomolgus monkey N-terminal region (amino acid residues 1-130 of SEQ ID NO:490) expressed with an C-terminal hexa-histidine tag (MfANGPTL4(1-130)-His) (1,028 RU). Kinetic analysis was performed to measure k_a and k_d, and K_D values and half-life of antigen/antibody complex dissociation were calculated. The results are shown in Table 4 (H1H268P) and Table 5 (H1H284P).

Table 4

Antigen	Antibody H1H268P	pH	Antigen captured (RU)	50 mM mAb or Fab bound	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)	$t_{1/2}$ (min)
hANGPTL4 (26-148)-mFc	Full mAb	7.2	35 ± 1.9	40	1.53×10^5	9.59×10^{-5}	629	120
		5.75	27 ± 0.6	77	6.28×10^5	1.38×10^{-4}	220	84
	Fab	7.2	35 ± 1.9	10	3.00×10^5	6.01×10^{-4}	2,000	19
		5.75	27 ± 0.6	20	1.74×10^5	3.04×10^{-3}	17.5 (nM)	4
hANGPTL4 (26-406)-His	Full mAb	7.2	Amino-coupled 450 RU	38	4.89×10^5	2.00×10^{-4}	408	58
		5.75		45	9.23×10^5	4.46×10^{-4}	483	26
	Fab	7.2		13	7.26×10^5	1.18×10^{-2}	16.3 (nM)	1
		5.75		10	4.44×10^5	6.57×10^{-3}	14.8 (nM)	2
MfANGPTL4 (1-130)-His	Full mAb	7.2	Amino-coupled 1,028 RU	279	3.92×10^5	4.76×10^{-5}	122	243
		5.75		583	1.07×10^5	8.24×10^{-5}	77.2	140
	Fab	7.2		167	2.67×10^5	1.71×10^{-3}	6,420	7
		5.75		178	3.12×10^5	4.32×10^{-3}	13.8 (nM)	3

Table 5

Antigen	Antibody H1H284P	pH	Antigen captured (RU)	50 mM mAb or Fab bound	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)	$t_{1/2}$ (min)
hANGPTL4 (26-148)-mFc	Full mAb	7.2	35 ± 1.9	99	2.74×10^5	5.36×10^{-5}	196	216
		5.75	27 ± 0.6	171	1.09×10^6	8.91×10^{-5}	81.9	130
	Fab	7.2	35 ± 1.9	29	2.45×10^5	2.02×10^{-4}	823	57
		5.75	27 ± 0.6	56	4.72×10^5	1.60×10^{-3}	3,400	7
hANGPTL4 (26-406)-His	Full mAb	7.2	Amino-coupled 450 RU	77	8.50×10^5	8.85×10^{-5}	105	130
		5.75		101	1.93×10^6	2.72×10^{-4}	141	42
	Fab	7.2		32	1.11×10^6	3.44×10^{-4}	310	34
		5.75		33	1.21×10^6	1.73×10^{-3}	1,440	7
MfANGPTL4 (1-130)-His	Full mAb	7.2	Amino-coupled 1,028 RU	414	4.67×10^5	5.83×10^{-5}	125	198
		5.75		804	1.55×10^6	8.42×10^{-5}	54.3	137
	Fab	7.2		214	3.10×10^5	3.13×10^{-3}	10.1 (nM)	4
		5.75		255	7.24×10^5	6.19×10^{-3}	8,540	2

[0111] Both Fab fragments were capable of binding to all forms of ANGPTL4, albeit with lower affinities than the whole antibody molecules.

Example 4. Anti-hANGPTL4 Antibody Cross-Reactivity Determination

[0112] Possible cross-reactivity of the anti-hANGPTL4 antibodies to related proteins, *i.e.*, hANGPTL3, human angiopoietin-like protein 5 (hANGPTL5) and mouse ANGPTL4 (mANGPTL4), was tested for the selected antibodies, *i.e.*, H1H268P and H1H284P, using the BIACORE™ system. Briefly, anti-hANGPTL4 antibodies as well as negative controls, *i.e.*, two monoclonal antibodies (Control a and Control b) that are non-binders to any ANGPTL proteins, were injected at 3.125 µg/mL-50 µg/mL over amine coupled chip surfaces of hANGPTL3-His (R&D Systems, cat # 3829-AN) at 5228 RU, hANGPTL4-His (R&D Systems, cat # 4487-AN) at 6247 RU, hANGPTL5-His (Abnova Corp., cat # H00253935-P01) at 5265 RU, and mANGPTL4-His [R26-S410 of mANGPTL4 (SEQ ID NO: 478) fused with an AS linker to a C-terminal 6-histidine tag] at 5233 RU, respectively. A polyclonal antibody specific for hANGPTL3 (R&D System, cat # BAF3485) was also tested. The binding of each antibody, expressed as a specific RU value, was determined and the results are shown in Table 6.

Table 6

mAb injected	Specific RU			
	hANGPTL3-His	hANGPTL4-His	hANGPTL5-His	mANGPTL4-His
Buffer	-17	-23	-18	-7
H1H268P	-17	768	-16	-7
H1H284P	-6	1351	-13	18
Control a	-16	-23	-18	-6
Control b	-17	-23	-18	-6
Anti-hANGPTL3	680	-1	-1	2319

[0113] H1H268P and H1H284P only bound specifically to hANGPTL4-His and did not bind any of the other related ANGPTL proteins.

[0114] Further, the binding affinities of H1H268P and H1H284P for various ANGPTL3 and ANGPTL4 peptides were also determined by BIACORE™ system. Briefly, H1H268P (1348 ± 11 RU) and H1H284P (868 ± 13 RU) were captured over anti-human Fc surface and various concentrations (62.5 nM-500 nM) of the hANGPTL3 and hANGPTL4 peptides were injected. The peptides tested were hANGPTL4 (R34-L66 of SEQ ID NO:476), N-terminally biotinylated hANGPTL4 (R34-L66 of SEQ ID NO:476), hANGPTL3 (R36-I68 of SEQ ID NO:485), and N-terminally biotinylated hANGPTL3 (R36-I68 of SEQ ID NO:485). Kinetic analysis was performed to measure k_a and k_d, and K_D values and half-life of antigen/antibody complex dissociation were calculated. The results are shown in Table 7. NB: No binding under the experimental conditions described.

Table 7

Anti-hANGPTL4 Antibody	Peptide	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)	$t_{1/2}$ (min)
H1H268P	hANGPTL3-Nterm biotin	NB	-	-	-
	hANGPTL4-Nterm biotin	4.53×10^3	2.94×10^{-4}	64.8	39
	hANGPTL3	NB	-	-	-
	hANGPTL4	6.49×10^3	3.65×10^{-4}	56.3	32

[0115] Neither of the antibodies bound to any of the hANGPTL3 peptides. In addition, H1H284P did not bind to any of the hANGPTL4 peptides even at the highest peptide concentration tested (500 nM), while H1H268P was able to bind to both hANGPTL4 peptides. This suggests that H1H268P recognizes a linear epitope within the 34-66 region. In contrast, H1H284P either binds outside this region or does not recognize a linear epitope in this region.

Example 5. Inhibition of hANGPTL4 by Anti-ANGPTL4 Antibodies

[0116] Lipoprotein Lipase (LPL) plays a critical role in lipid metabolism in humans. LPL catalyzes hydrolysis of triglycerides and releases fatty acids to be metabolized. ANGPTL4 inhibits LPL activity leading to increased level of lipids (Oike *et al.*, 2005, *Trends in Molecular Medicine* 11(10):473-479). The N-terminal coiled-coil region of ANGPTL4 undergoes homo-multimerization, both in isolation and when joined to the C-terminal fibrinogen-like region. The N-terminal region also inhibits LPL when expressed without the C-terminal fibrinogen region. A cell-free bioassay was developed to determine the ability of selected anti-hANGPTL4 antibodies to inhibit ANGPTL4-induced decrease in LPL activity.

[0117] Inhibition of hANGPTL4 activity by selected anti-hANGPTL4 antibodies was determined using the CONFLUOLIP™ Continuous Fluorometric Lipase Test (Progen, Germany) using two hANGPTL4 proteins: full-length hANGPTL4 (*i.e.*, amino acid residues 26-406 of SEQ ID NO:476) with a C-terminal hexa-histidine tag (hANGPTL4-His; R&D Systems, MN) and hANGPTL4-mFc (SEQ ID NO:480) containing the N-terminal coiled-coil region.

[0118] Briefly, 2 nM bovine LPL, 0.25 µM human ApoCII (a cofactor of LPL), 2 mg/mL BSA and 1.6mM $CaCl_2$, were premixed in a 96-well assay plate. Either hANGPTL4-His or hANGPTL4-mFc protein was added to the Apo/LPL mixture to a final concentration of 10 nM and 2 nM, respectively. The Apo/LPL/ANGPTL4 protein mixtures were then added together with serially diluted anti-hANGPTL4 antibodies with a starting concentration of 300 nM (for inhibition of hANGPTL4-His) or 100 nM (for inhibition of hANGPTL4-mFc) and incubated at room temperature for 30 minutes (final volume 50 µl). Following the incubation, 200 µl of reconstituted lipase substrate, 1-trinitrophenyl-amino-dodecanoyl-2-pyrendecanoyl-3-0-hexadecyl-*sn*-glycerol (LS-A, Progen), was added to the antibody mixture and incubated at 37°C for two hours. Fluorescence was then measured at 342 nm/400 nm (excitation/emission) using

a FlexStation® 3 Microplate Reader (Molecular Devices, CA). Fluorescence is directly proportional to LPL activity. Results are shown in Table 8. Control I: a rabbit polyclonal antibody specific for hANGPTL4 (BioVendor). Control II: an irrelevant human antibody that does not bind hANGPTL4. NT: not tested. The total inhibition (*i.e.*, 100% inhibition) was determined from the relative fluorescence unit (RFU) of the assay with 2 nM bovine LPL, 0.25 μM human ApoCII, 2 mg/mL BSA and 1.6mM $CaCl_2$, in the absence of anti-ANGPTL4 antibodies and ANGPTL4 proteins.

Table 8

Antibody	% Inhibition of hANGPTL4 Activity	
	hANGPTL4-mFc (2 nM)	hANGPTL4-His (10 nM)
H1H236N2	13	19
H1H257P	16	NT
H1H268P	76	80
H1H284P	82	90
H1H285P	28	47
H1H292P	20	53
H1H624P	62	48
H1H653P	55	48
Control I	29	66
Control II	No inhibition	No inhibition

[0119] Antibodies H1H284P and H1H268P exhibited the highest inhibition of ANGPTL4's inhibitory activity against LPL among the antibodies tested, including the polyclonal hANGPTL4 antibody control. For antibodies H1H284P and H1H268P, the antibody concentrations required for 50% maximum inhibition (IC50) of 2 nM hANGPTL4-mFc were determined to be 0.8 nM and 1.2 nM, respectively. In addition, the antibody concentrations required for 50% maximum inhibition of 10 nM hANGPTL4-His were determined to be 0.5 nM and 0.2 nM, respectively.

[0120] Similarly, H1H284P and H1H268P were tested in the LPL bioassay for their ability to inhibit cross-species orthologs: the cynomolgus monkey N-terminal region (amino acid residues 26-148) expressed with an N-terminal hexa-histidine tag (His-MfANGPTL4; SEQ ID NO:488) and the mouse full-length ortholog (amino acid residues 26-410 of SEQ ID NO:478) with a C-terminal hexa-histidine tag (mANGPTL4-His). A full dose-response using the ANGPTL4 protein in the LPL assay was first performed to determine the ANGPTL4 EC50 for each experiment and IC50 determinations for each antibody were then performed using constant concentrations of ANGPTL4 protein, as shown in Table 9. Antibody concentrations ranged from 0 to 100 nM. NB: Not blocking.

Table 9

		hANGPTL4(26-406)-His	hANGPTL4(26-148)-mFc	His-MfANGPTL4 (26-148)	mANGPTL4(26-410)-His
EC50 (nM)		6.00	0.50	3.89	0.63
Constant ANGPTL4 (nM)		10	2	10	1
IC50 (nM)	H1H268P	0.46	0.47	0.42	NB
	H1H284P	0.31	0.51	0.42	NB
	IgG1 control	NB	NB	NB	NB

[0121] Both antibodies inhibited both human ANGPTL4 (full-length and N-terminal) and monkey ANGPTL4 (N-terminal) protein activity with IC50s of about 0.3-0.5 nM; but neither antibody inhibited mouse ANGPTL4 (full-length) up to the highest antibody concentrations tested (*i.e.*, 100 nM).

Example 6. In Vivo Effect of ANGPTL4 on Plasma Lipid Levels

[0122] hANGPTL4 was administered intravenously to C57BL/6 mice to determine the biological effect of hANGPTL4 on plasma lipid levels. Briefly, C57BL/6 mice were put into four groups of five animals and each group was administered with different amount of hANGPTL4-mFc protein (SEQ ID NO:480): 25 µg, 50 µg, 100 µg and 300 µg, per mouse. A control group received injections of PBS. hANGPTL4-mFc protein and PBS were administered by intravenous injection (i.v.) via tail vein. Mice were bled at 15min, 30min, 60min and 120min after delivery of hANGPTL4-mFc or PBS and plasma lipid levels were determined by ADVIA® 1650 Chemistry System (Siemens). Measurements of triglycerides, total cholesterol, low density lipoprotein (LDL), nonesterified fatty acids (NEFA-C) and high density lipoprotein (HDL) were determined for each dose group. Measurements of total cholesterol, LDL, NEFA-C and HDL were not significantly different across each dose group for each time point post-injection. Injection of 25 µg/mouse of hANGPTL4-mFc increased circulating triglycerides greater than two-fold as compared to control mice (PBS) 30 minutes post injection. Thus, the 25 µg dose of hANGPTL4-mFc was selected as a possible minimum dosage for analysis of inhibition of ANGPTL4-induced increase in serum triglyceride levels by selected anti-hANGPTL4 antibodies as described below.

Example 7. In Vivo Inhibition of ANGPTL4 by Anti-ANGPTL4 Antibodies

[0123] In another set of experiments, selected anti-hANGPTL4 antibodies were tested for their ability to inhibit hANGPTL4-induced increase of triglyceride levels. Measurements of total cholesterol (Total-C), LDL, NEFA-C and HDL were also made. Briefly, C57BL/6 mice were put into groups of five mice each for each antibody tested. Antibodies were administered at 5 mg/kg dose by subcutaneous injection. Control group I, *i.e.*, mice that received neither anti-hANGPTL4 antibodies nor hANGPTL4, were administered with PBS. Twenty-four hours post-injection of antibody, hANGPTL4-mFc (SEQ ID NO:480) was administered (i.v.) at a dose of 25 µg/mouse

to each antibody group. Mice were bled at 30 min after hANGPTL4-mFc injection and lipid levels were determined by ADVIA® 1650 Chemistry System (Siemens). Averages were calculated for each of the measurements of triglycerides, total cholesterol, LDL, NEFA-C and HDL for each antibody or control group. Levels of circulating anti-hANGPTL4 antibodies (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 anti-hANGPTL4 antibodies were detected by chemiluminescence using a horseradish peroxidase (HRP) conjugated goat anti-human IgG antibody (Sigma-Aldrich). Results, expressed as (mean ± SEM) of serum lipid concentration, are shown in Tables 10-12. Control I: Mice that received PBS, but neither anti-hANGPTL4 antibodies nor hANGPTL4-mFc. Control II: Mice that received a human antibody specific for CD20 (*i.e.*, the mAb having the sequence of 2F2 clone disclosed in US 2008/0260641) and hANGPTL4-mFc.

Table 10

Antibody	Triglycerides (mg/dL)	Total-C (mg/dL)	LDL (mg/dL)	NEFA-C (mg/dL)	HDL (mg/dL)	Serum Ab (µg/mL)
Control I	98.20 ± 5.49	89.80 ± 4.28	5.60 ± 0.66	1.01 ± 0.04	44.18 ± 2.43	-
Control II	211.60 ± 58.29	93.40 ± 5.52	6.30 ± 0.22	1.33 ± 0.17	44.62 ± 3.14	12.76 ± 0.52
H1H284P	99.20 ± 9.52	80.80 ± 6.40	4.98 ± 0.87	0.99 ± 0.11	39.60 ± 3.46	7.96 ± 0.55
H1H257P	115.80 ± 6.43	84.40 ± 3.53	5.30 ± 0.36	0.97 ± 0.03	41.38 ± 3.24	8.43 ± 0.86

Table 11

Antibody	Triglycerides (mg/dL)	Total-C (mg/dL)	LDL (mg/dL)	NEFA-C (mg/dL)	HDL (mg/dL)	Serum Ab (µg/mL)
Control I	66.60 ± 7.94	70.00 ± 2.3	3.88 ± 0.36	0.76 ± 0.08	35.26 ± 1.09	-
Control II	161.00 ± 17.83	73.60 ± 0.93	4.12 ± 0.17	1.18 ± 0.09	35.10 ± 0.6	11.05 ± 2.28
H1H236N2	151.80 ± 9.26	72.40 ± 1.81	4.26 ± 0.25	1.11 ± 0.18	33.78 ± 1.13	9.20 ± 0.63
H1H624P	81.20 ± 9.26	72.80 ± 5.49	4.36 ± 0.92	0.86 ± 0.07	35.40 ± 2.68	11.76 ± 0.89
H1H268P	92.60 ± 11.44	76.00 ± 2.14	4.94 ± 0.51	0.82 ± 0.04	35.94 ± 1.64	8.05 ± 1.06

Table 12

Antibody	Triglycerides (mg/dL)	Total-C (mg/dL)	LDL (mg/dL)	NEFA-C (mg/dL)	HDL (mg/dL)	Serum Ab (µg/mL)
Control I	94.20 ± 10.91	75.00 ± 5.32	3.98 ± 0.25	1.01 ± 0.05	40.46 ± 3.25	-
Control II	179.80 ± 28.06	76.80 ± 3.46	4.38 ± 0.09	1.30 ± 0.13	39.06 ± 2.94	11.59 ± 1.2
H1H291P	111.00 ± 7.51	71.20 ± 3.26	3.84 ± 0.12	1.11 ± 0.04	38.24 ± 2.14	9.46 ± 0.73
H1H283P	113.60 ± 8.74	75.80 ± 1.53	4.62 ± 0.39	1.13 ± 0.05	40.30 ± 0.72	7.85 ± 1.00
H1H295P	104.80 ± 9.44	74.60 ± 4.82	4.04 ± 0.40	1.12 ± 0.04	39.70 ± 3.13	12.61 ± 0.83
H1H653P	88.00 ± 13.52	74.20 ± 4.49	3.84 ± 0.32	1.04 ± 0.1	40.10 ± 2.72	8.77 ± 1.06
H1H285P	91.40 ± 11.99	76.40 ± 1.75	3.72 ± 0.44	0.97 ± 0.06	42.20 ± 0.91	10.49 ± 0.67
H1H292P	85.80 ± 7.00	74.40 ± 2.11	3.96 ± 0.15	1.06 ± 0.06	39.44 ± 1.68	12.61 ± 0.55
H1H638P	102.80 ± 10.75	73.80 ± 2.78	4.14 ± 0.18	1.06 ± 0.05	39.54 ± 1.65	12.20 ± 0.80

[0124] After injection of hANGPTL4-mFc (25 µg), most of the anti-hANGPTL4 antibodies tested (shown in Tables 10-12) exhibited significantly reduced levels of serum triglycerides compared to mice treated with irrelevant antibody (control II).

Example 8. Preparation of Anti-ANGPTL4 Antibodies with hIgG4 Isotype.

[0125] The antibodies H1H268P and H1H284P with hIgG1 isotype were converted to hIgG4 isotype by replacing the respective constant regions with the hIgG4 amino acid sequence of SEQ ID NO:483, which contains a S108P mutation in the hinge region. Furthermore, a single amino acid substitution was introduced in the framework region 1 of H1H268P (SEQ ID NO:42) to form H4H268P2 (SEQ ID NO:487) in the IgG4 version. K_D (pM) and $t_{1/2}$ values of the IgG4 antibodies, designated as H4H268P2 and H4H284P, respectively, for hANGPTL4-mFc (SEQ ID NO:480) binding were obtained by Biacore at pH7.4 and 25°C, according to the protocol as described in Example 3 above. The results are shown in Table 13 below.

Table 13

Antibody	K_D (pM)	$t_{1/2}$ (min)
H4H268P2	146	195
H4H284P	143	205

[0126] H4H268P2 and H4H284P together with the corresponding IgG1 versions, H1H268P and H1H284P, respectively, were tested in the LPL inhibition assay, as described in Example 5 above, to determine IC50 values. The results are shown in Table 14. NB: Not blocking.

Table 14

		hAngPTL4(26-406)-His	hANGPTL4(26-148)-mFc
EC50 (nM)		4.54	0.29
Constant ANGPTL4 (nM)		10	2
IC50 (nM)	**H1H268P**	0.20	0.67
	H1H284P	0.42	0.33
	IgG1 control	NB	NB
	H4H268P2	1.61	1.85
	H4H284P	1.19	1.69
	IgG4 control	NB	NB

[0127] In this assay, H1H268P and H1H284P showed IC50s ranging from about 0.2-0.7 nM for the full-length and the N-terminal hANGPTL4 proteins, while H4H268P2 and H4H284P showed IC50s ranging from about 1.0-2.0 nM.

Example 9. Pharmacokinetic Study of Anti-ANGPTL4 Antibodies

[0128] Pharmacokinetic clearance rates of anti-hANGPTL4 antibodies H4H268P2 and H4H284P were determined in wild-type mice and in transgenic mice expressing human ANGPTL4 [hANGPTL4(+/+) mice]. The strain background for both wild-type and transgenic mice was C57BL6 (75%) and 129Sv (25%). Separate cohorts consisting of 5 mice each of either wild-type or hANGPTL4(+/+) mice received subcutaneously (s.c.) 1 mg/kg of H4H268P2, H4H284P, or an isotype-matched (hIgG4) control with irrelevant specificity. Blood samples were collected at 0 hour, 6 hours, 1 day, 2 days, 3 days, 4 days, 7 days, 10 days, and 15 days, 22 days, and 30 days after the injection. Serum levels of human antibodies were determined by a sandwich ELISA. Briefly, a goat polyclonal anti-human IgG (Fc-specific) capture antibody (Jackson ImmunoResearch, PA) was coated in 96-well plates at a concentration of 1 µg/mL and incubated overnight at 4°C. After the plates were blocked with BSA, serum samples in a six-point serial dilution and reference standards of the respective antibody in a twelve-point serial dilution were added to the plate and incubated for one hour at room temperature. After washing with a suitable washing buffer, captured human antibodies were detected using the same goat polyclonal anti-human IgG (Fc-specific) antibody conjugated with horse radish peroxidase (HRP) (Jackson ImmunoResearch, PA) and developed by standard colorimetric response using tetramethylbenzidine (TMB) substrate, measuring absorbance at 450 nm in a plate reader. Concentrations of human antibodies in serum were determined using the reference standard curve generated for the same sample plate. The results are shown in Table 15 and Figs. 3A and 3B.

Table 15

Antibody	Mouse Genotype	Cmax (μg/mL)	AUC (hr*μg/mL)
H4H268P2	Wild-type	18.4	318
H4H284P	Wild-type	15.7	200
hIgG4 control	Wild-type	14.2	199
H4H268P2	hANGPTL4(+/+)	13.3	37.0
H4H284P	hANGPTL4(+/+)	5.86	7.60
hIgG4 control	hANGPTL4(+/+)	11.6	168

[0129] As shown in Table 15, both anti-hANGPTL4 antibodies showed similar clearance rates as the isotype-matched control antibody in wild-type mice, as reflected in the area under the curve (AUC) calculated over the 30-day period of about 318, 200, and 199 (hr*μg/mL), respectively, for H4H268P2, H4H284P, and hIgG4 control (*also see* Fig. 3A). In the transgenic mice expressing only human ANGPTL4 [hANGPTL4(+/+)], the clearance rates as reflected in AUCs were faster for both H4H268P2 (37.0 hr*μg/mL) and H4H284P (7.60 hr*μg/mL) compared to clearance rates in wild-type mice (318 and 200 hr*μg/mL, respectively) and compared to the clearance rate of the isotype-matched control antibody in either the hANGPTL4(+/+) mice (168 hr*μg/mL) or the wild-type mice (199 hr*μg/mL) (*also see* Fig. 3B). In the hANGPTL4(+/+) mice, the 30-day AUC for H4H284P (7.60 hr*μg/mL) was about 5-fold less than that for H4H268P2 (37.0 hr*μg/mL). Together these results suggest that both anti-hANGPTL4 antibodies exhibit target-mediated clearance in mice expressing human ANGPTL4, and H4H284P exhibits a substantially faster clearance rate than H4H268P2.

Example 10. In Vivo Effect of IgG1 Anti-hANGPTL4 Antibodies on Circulating TG Levels in Humanized ANGPTL4 Mice

[0130] The effect of anti-hANGPTL4 antibodies H1H268P and H1H284P on serum TG levels was determined in mice expressing the ANGPTL4 protein containing the human N-terminal coil-coil region ("humanized ANGPTL4 mice"). The humanized ANGPTL4 mouse was made by replacing first three exons of the mouse *Angptl4* gene (the N terminal coil-coil region) with the corresponding human N-terminal coil-coil ANGPLT4 sequence in C57BL6/129 (F1H4) embryonic stem cells. After germ line transmission was established, heterozygous mice (ANGPTL4hum/+) were bred together to generate homozygous mice [ANGPTL4hum/hum or hANGPTL4(+/+)] on a C57BL6 background. Humanized ANGPTL4 mice were pre-bled 7 days before (day -7) the experiment and put into groups of six mice each for each antibody tested. Antibodies (H1H268P, H1H284P and isotype-matched (hIgG1) control with no known cross-reactivity to mouse antigens) were administered at 10 mg/kg dose by subcutaneous injection.

Mice were bled after 4 hours of fasting at days 1, 4, 7 and 11 after antibody injection; and serum TG levels were determined by ADVIA® 1800 Chemistry System (Siemens). Average TG levels were calculated for each time point for each antibody. Results, expressed as (mean ± SEM) of serum TG concentration, are shown in Table 16.

Table 16

Days after injection	Serum TG (mg/dL)		
	hIgG1 Control	H1H268P	H1H284P
- 7	117 ± 18	112 ± 9.3	113 ± 11
1	138 ± 21	129.8 ± 5.6	125 ± 18
4	102 ± 14	73.3 ± 8.9	67 ± 9.8
7	112 ± 10	83 ± 14	91 ± 7.2
11	110 ± 15	76 ± 5.5	109 ± 11

[0131] Levels of circulating anti-hANGPTL4 antibodies ("serum human 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 human Ab. Serum was then added to the plates and captured anti-hANGPTL4 antibodies were detected by chemiluminescence using a horseradish peroxidase (HRP)-conjugated goat anti-human IgG antibody (Sigma-Aldrich). Results, expressed as (mean ± SEM) of serum human Ab, are shown in Table 17.

Table 17

Days after injection	Serum Human Antibody (µg/mL)		
	hIgG1 Control	H1H268P	H1H284P
- 7	2.71 ± 2.18	2.92 ± 2.92	3.02 ± 2.38
1	24384 ± 911	24130 ± 1788	16459 ± 1455
4	22553 ± 1811	16557 ± 1369	9103 ± 767
7	13833 ± 467	12586 ± 1176	2428 ± 525
11	13145 ± 1598	6106 ± 1111	135 ± 38

[0132] Administration of H1H268P to humanized ANGPTL4 mice led to ~25-30% reduction in circulating TG 4-11 days after the antibody administration, compared to mice dosed with an isotype-matched control antibody. TG reduction resulting from H1H284P administration was most effective at day 4 after injection of the antibody (~34% in TG reduction), but by day 11 TG levels were increased back to control level, probably due to fast clearance rate of the antibody.

Example 11. In Vivo Effect of IgG4 Anti-hANGPTL4 Antibodies on Circulating TG Levels in Humanized ANGPTL4 Mice

[0133] The effect of anti-hANGPTL4 antibodies, H4H268P2 and H4H284P, on serum TG levels

was determined in humanized ANGPTL4 mice. Humanized ANGPTL4 mice were pre-bled 7 days before the experiment and put into groups of six mice each for each antibody tested. Antibodies (H4H268P2, H4H284P and isotype-matched (hIgG4) control with no known cross-reactivity to mouse antigens) were administered at 10 mg/kg dose by subcutaneous injection. Mice were bled after 4 hours of fasting at days 1, 4, 7 and 11 after antibody injection; and TG levels were determined in the serum by ADVIA® 1800 Chemistry System (Siemens). Average TG levels were calculated for each time point for each antibody. Results, expressed as (mean ± SEM) of serum TG concentration, are shown in Table 18.

Table 18

Days after injection	Serum TG (mg/dL)		
	hIgG4 Control	H4H268P2	H4H284P
- 7	103 ± 9.5	101 ± 9.3	103 ± 8.0
1	118 ± 13	81 ± 6.8	86 ± 8.6
4	115 ± 9.8	67 ± 5.3	69 ± 6.4
7	81 ± 9.7	56 ± 7.1	71 ± 11
11	109 ± 10	87 ± 8.7	83 ± 7.3

[0134] Administration of H4H268P2 and H4H284P to humanized ANGPTL4 mice led to a significant reduction in circulating TG on day 1 (H4H268P2) and day 4 (H4H268P2 and H4H284P) after the antibodies administration, compared to mice dosed with isotype-matched control antibody.

[0135] The effect of H4H268P2 on circulating TG levels was further studied in humanized ANGPTL4 mice crossed to ApoE null mice. The ApoE null mouse model is known as a highly atherogenic and hyperlipidemic model with the majority of cholesterol and TG circulating in VLDL particles due to impaired VLDL remnant clearance. Humanized ANGPTL4 x ApoE null mice were pre-bled 7 days before the experiment and put into 2 groups of six mice each. Antibodies H4H268P2 and Control Ab were administered at 10 mg/kg by subcutaneous injection. Mice were bled after 4 hours of fasting on days 1, 4, 7, 11 and 17 after antibody injection and TG levels were determined in the serum by ADVIA® 1800 Chemistry System (Siemens). TG reductions shown in Fig. 4 are expressed as a percent of TG levels compared to Control Ab.

[0136] TG levels were significantly reduced for all 17 days (more than 42%) with the greatest reduction at day 7 (~50%) after administration of H4H268P2, compared to mice dosed with Control Ab.

Example 12. In Vivo Effect of Anti-hANGPTL4 Antibodies in Combination with Fenofibrate on Serum TG Levels

[0137] The effects of the anti-ANGPTL4 antibody H4H268P2 and TG-reducing drug fenofibrate,

each alone or in combination, on serum TG levels were evaluated in humanized ANGPTL4 mice. The mice were pre-bled 7 days before the experiment after 4 hours of fasting and put into 4 groups of six mice each. Groups 2 and 4 were administered with H4H268P2 at 10 mg/kg by subcutaneous injection on day 0 and groups 1 (control group) and 2 were placed on regular chow diet. Groups 3 and 4 received chow diet supplemented with 0.05% (w/w) of fenofibrate (the dosage level was determined experimentally in a pilot study). Serum was collected from a terminal bleed on day 7 after H4H268P2 and/or fenofibrate administration (after 4 hours of fasting) and analyzed by ADVIA® 1800 Chemistry System (Siemens). The results are shown in Fig. 5.

[0138] Administration of H4H268P2 alone and in combination with fenofibrate led to significant reduction in circulating TG levels 7 days after administration. TG levels were reduced by ~40% (mean) 7 days after H4H268P2 administration alone, ~25% after fenofibrate treatment alone, and ~50% after combination treatment of H4H268P2 and fenofibrate, compared to control group treated with chow diet. H4H268P2 showed more efficacy than fenofibrate in reducing circulating TG levels in mouse models. Combination treatment showed a synergistic effect of H4H268P2 and fenofibrate on TG levels. Remarkably, livers collected from mice consumed fenofibrate (groups 3 and 4) for 7 days were significantly enlarged (1.8 times, liver weight/body weight), compared to mice consumed control chow diet (groups 1 and 2) (data not shown).

Example 13. Pilot Study on Pharmacokinetics/Pharmacodynamics of Anti-ANGPTL4 Antibodies in Obese Rhesus Monkeys

[0139] Phase I: In a pilot non-GLP pharmacokinetics/pharmacodynamic (PK/PD) study, H4H268P2 and H4H284P were administered as a single bolus intravenous (IV) injection, to obese rhesus monkeys (*Macaca mulatta*). Rhesus monkeys were selected because this species is closely related, both phylogenically and physiologically, to humans and is a species commonly used for nonclinical toxicity evaluations. Obese monkeys that had been on a high fat diet for greater than 6 months were selected because they typically display moderately elevated TG levels (*i.e.*, mean >100 mg/dL; hyper-TG). Eight confirmed healthy, male monkeys were acclimated and assigned to the study for baseline assessment for 7 days prior to dosing. All animals received a vehicle (10 mM histidine, pH 6) IV infusion on Day -5, after which four of them received H4H268P2 and another four H4H284P at 10mg/kg IV on day 0. No injection site reactions or other adverse effects were observed at any time point after infusion.

[0140] Serum samples were collected from the baseline period through day 35 post-dosing and assessed for serum lipid levels by ADVIA® 1800 Chemistry System (Siemens). The average baseline for each animal was determined based on the samples taken on Days -7, -5 and 0. Preliminary analysis of samples taken after vehicle administration revealed that 3 obese animals from each group displayed elevated fasted TG levels (*i.e.*, TG > 100mg/dL) while one animal from each group had an average TG level well within the normal range (*i.e.*, mean fasting TG

level of 42 mg/dL and 84 mg/dL). Thus the data analysis was done with 3 animals in each group. Percent (%) changes of serum TG levels from the baseline were determined for each animal and averaged for each Ab group. The results are shown in Fig. 6.

[0141] Administration of H4H268P2 to the three mildly hyper-TG animals showed a maximal reduction of 57% at day 4 post-dosing. The mean serum TG levels for these animals after H4H268P2 treatment remained at or below 100 mg/dL until approximately Day 25. Moderate effects were observed on additional lipids, such as LDL-cholesterol (LDL-C) and HDL-C; however total-C was unchanged (data not shown). Administration of H4H268P2 to the single obese animal with low TG levels did not yield any significant effect to lower fasting TG's or any other lipid parameters (data not shown), suggesting a lower limit for the reduction of TG levels by the antibody.

[0142] Phase II: In the second non-GLP pharmacokinetics/pharmacodynamic (PK/PD) study only H4H268P2 was administered as a single bolus intravenous (IV) injection to eight obese rhesus monkeys (*Macaca mulatta*). The step of pre-dosing vehicle injection was omitted in this study. Three baseline assessments were taken at days -7, -3 and 0 of the study and all eight monkeys received H4H268P2 at 10 mg/kg by IV on day 0. Serum samples were collected from the baseline period through day 35 post-dosing and assessed for serum lipid levels by ADVIA® 1800 Chemistry System (Siemens). Animals were grouped for data analysis according to average baseline TG levels so as to prospectively predict the effect: A. TG < 150 mg/dL (n=3); B. 150 mg/dL < TG < 500 mg/dL (n=4); and C. TG > 1000 mg/dL (n=1).

[0143] Fig. 7 shows the TG levels of the three groups expressed as percent changes of TG levels from the average of 3 baseline TG values. As expected on the basis of preclinical data, the greater reduction in fasting TG levels was seen in the animals with higher basal serum TG levels. An especially dramatic and rapid drop in TG level was observed in an individual animal whose baseline TG level was > 1000 mg/dL. A robust decrease in TG levels (50-68%) was observed for animals with baseline 150 mg/dL < TG < 500 mg/dL. In this group of obese monkeys administration of H4H268P2 increased HDL-C, but had no effect on LDL-C or Total-C (data not shown). Animals with baseline TG < 150 mg/dL (normal TG levels) were largely unresponsive to H4H268P2.

We claim:

1. An isolated human antibody or antigen-binding fragment thereof that specifically binds human angiopoietin-like protein 4 (hANGPTL4), comprising a heavy chain complementarity determining region 1 (HCDR1)/HCDR2/HCDR3 combination selected from SEQ ID NO: 28/30/32, 76/78/80, 4/6/8, 52/54/56, 100/102/104, 124/126/128, 148/150/152, 172/174/176, 196/198/200, 220/222/224, 244/246/248, 268/270/272, 292/294/296, 316/318/320, 340/342/344, 364/366/368, 388/390/392, 412/414/416, 436/438/440 and 460/462/464.

2. An isolated human antibody or antigen-binding fragment thereof that specifically binds human angiopoietin-like protein 4 (hANGPTL4), comprising a light chain complementarity determining region 1 (LCDR1)/LCDR2/LCDR3 combination selected from SEQ ID NO:36/38/40, 84/86/88, 12/14/16, 60/62/64, 108/110/112, 132/134/136, 156/158/160, 180/182/184, 204/206/208, 228/230/232, 252/254/256, 276/278/280, 300/302/304, 324/326/328, 348/350/352, 372/374/376,396/398/400, 420/422/424 and 444/446/448.

3. The antibody or antigen-binding fragment according to claim 1 or 2, comprising heavy and light chain CDR sequences HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 comprising a CDR sequence combination selected from SEQ ID NO:28/30/32/36/38/40, 76/78/80/84/86/88, 4/6/8/12/14/16, 52/54/56/60/62/64, 100/102/104/108/110/112, 124/126/128/132/134/136, 148/150/152/156/158/160, 172/174/176/180/182/184, 196/198/200/204/206/208, 220/222/224/228/230/232, 244/246/248/252/254/256, 268/270/272/276/278/280, 292/294/296/300/302/304, 316/318/320/324/326/328, 340/342/344/348/350/352, 364/366/368/372/374/376, 388/390/392/396/398/400, 412/414/416/420/422/424, 436/438/440/444/446/448 and 460/462/464/396/398/400.

4. An isolated human antibody or antigen-binding fragment of an antibody that specifically binds human angiopoietin-like protein 4 (hANGPTL4), comprising a heavy chain variable region (HCVR) selected from SEQ ID NO:487, 90, 2, 18, 22, 26, 42, 46, 50, 66, 70, 74, 94, 98, 114, 118, 122, 138, 142, 146, 162, 166, 170, 186, 190, 194, 210, 214, 218, 234, 238, 242, 258, 262, 266, 282, 286, 290, 306, 310, 314, 330, 334, 338, 354, 358, 362, 378, 382, 386, 402, 406, 410, 426, 430, 434, 450, 454, 458, 466 and 468.

5. The antibody or antigen-binding fragment according to claim 4, further comprising a light chain variable region (LCVR) selected from SEQ ID NO:44, 92, 10, 20, 24, 34, 48, 58, 68, 72, 82, 96, 106, 116, 120, 130, 140, 144, 154, 164, 168, 178, 188, 192, 202, 212, 216, 226, 236, 240, 250, 260, 264, 274, 284, 288, 298, 308, 312, 322, 332, 336, 346, 356, 360, 370, 380, 384, 394, 404, 408, 418, 428, 432, 442, 452 and 456.

6. The antibody or antigen-binding fragment according to any one of claims 1 to 5, comprising a HCVR/LCVR sequence pair selected from SEQ ID NO:487/44, 90/92, 2/10, 18/20, 22/24, 26/34, 42/44, 46/48, 50/58, 66/68, 70/72, 74/82, 94/96, 98/106, 114/116, 118/120, 122/130, 138/140,

142/144, 146/154, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/394, 466/404 and 468/408.

7. The antibody or antigen-binding fragment according to any one of claims 1 to 6, comprising a HCVR/LCVR sequence pair SEQ ID NO:487/44 or 90/92.

8. An isolated human antibody or antigen-binding fragment thereof that specifically binds hANGPTL4, comprising heavy and light chain CDR sequences contained within the HCVR/LCVR sequence pair of the antibody or antigen-binding fragment according to claim 6 or 7.

9. An antibody or antigen-binding fragment thereof which competes for binding to hANGPTL4 with the antibody or antigen-binding fragment according to any one of claims 3, 6 and 7.

10. An antibody or antigen-binding fragment thereof which binds the same epitope as the antibody or antigen-binding fragment according to any one of claims 3, 6, 7 and 9.

11. The antibody or antigen-binding fragment according to any one of claims 3, 6 and 7, wherein the antibody or antigen-binding fragment cross-reacts with cynomolgus monkey ANGPTL4 and rhesus monkey ANGPTL4.

12. An isolated antibody or antigen-binding fragment thereof that specifically binds human angiopoietin-like protein 4 (hANGPTL4), comprising:

(a) a heavy chain complementarity determining region 3 (HCDR3) comprising an amino cid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18} - X^{19} - X^{20} (SEQ ID NO:471), wherein X^1 is Ala, X^2 is Arg or Lys, X^3 is Gly or Glu, X^4 is Gly, Asp or absent, X^5 is Asp or absent, X^6 is Leu, Arg or absent, X^7 is Arg or Ser, X^8 is Phe, Gly or Arg, X^9 is Leu, His or Asn, X^{10} is Asp, Pro or Tyr, X^{11} is Trp, Tyr or Phe, X^{12} is Leu, Phe, Val or Asp, X^{13} is Ser, Tyr or Gly, X^{14} is Ser, Tyr or Asp, X^{15} is Tyr, X^{16} is Phe or Gly, X^{17} is Leu or absent, X^{18} is Asp, X^{19} is Tyr, Val or Phe, and X^{20} is Trp; and

(b) a light chain complementarity determining region 3 (LCDR3) comprising an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} (SEQ ID NO:474), wherein X^1 is Gln, X^2 is Asn or Gln, X^3 is Tyr or Leu, X^4 is Asn, His, Ser or Asp, X^5 is Thr or Ser, X^6 is Ala or Tyr, X^7 is Pro, Ser or Phe, X^8 is Leu or Arg, X^9 is Thr, and X^{10} is Phe.

13. The antibody or antigen-binding fragment of claim 12, further comprising:

(c) a HCDR1 comprising an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 -

X^6 - X^7 - X^8 (SEQ ID NO:469), wherein X^1 is Gly, X^2 is Gly or Phe, X^3 is Ser or Thr, X^4 is Phe, X^5 is Ser, X^6 is Ile, Ser or Thr, X^7 is His or Tyr, and X^8 is His, Gly or Asp;

(d) a HCDR2 comprising an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO:470), wherein X^1 is Ile, X^2 is Asn, Ser or Gly, X^3 is His, Phe, Ser or Val, X^4 is Arg, Asp or Ala, X^5 is Gly, X^6 is Gly or absent, X^7 is Ser, Asn or Asp, and X^8 is Thr or Lys;

(e) a LCDR1 comprising an amino acid sequence of the formula X^1 - X^2 - X^3 - X^4 - X^5 - X^6 (SEQ ID NO:472), wherein X^1 is Gln, X^2 is Gly or Ser, X^3 is Ile, X^4 is Ser or Asn, X^5 is Asp, Ser or Arg, and X^6 is Tyr or Trp; and

(f) a LCDR2 comprising an amino acid sequence of the formula X^1 - X^2 - X^3 (SEQ ID NO:473), wherein X^1 is Ala or Lys, X^2 is Ala, and X^3 is Ser.

14. An isolated human antibody or antigen-binding fragment thereof that specifically binds human angiopoietin-like protein 4 (hANGPTL4), comprising a heavy chain variable region (HCVR) encoded by nucleotide sequence segments derived from V_H, D_H and J_H germline gene segments, and a light chain variable region (LCVR) encoded by nucleotide sequence segments derived from V_K and J_K germline gene segments, wherein the HCVR and the LCVR are encoded by nucleotide sequence segments derived from a combination of (i) V_H4-34, D_H3-3, J_H4, V_K1-27 and J_K4; or (ii) V_H3-30, D_H5-12, J_H6, V_K1-9 and J_K4.

15. An isolated nucleic acid molecule encoding the antibody or antigen-binding fragment according to any one of the preceding claims.

16. An expression vector comprising the nucleic acid molecule of claim 15.

17. A host cell comprising the expression vector of claim 16.

18. A method of producing an anti-hANGPTL4 antibody or antigen-binding fragment thereof, comprising growing the host cell of claim 17 under conditions permitting production of the antibody or fragment thereof, and recovering the antibody or fragment thereof so produced.

19. A pharmaceutical composition comprising the antibody or antigen-binding fragment according to any one of claims 1 to 14, and a pharmaceutically acceptable carrier.

20. The pharmaceutical composition of claim 19, further comprising one or more additional therapeutic agents selected from an inhibitor of HMG-CoA reductase; an inhibitor of cholesterol uptake or bile acid re-absorption, or both; an agent which increases lipoprotein catabolism; an agent which reduces the number of non-fatal heart attacks; and an activator of the LXR transcription factor.

21. The pharmaceutical composition of claim 19, further comprising one or more additional therapeutic agents selected from statin, niacin, fibrate, and anti-PCSK9 antibody.

22. A method for preventing or treating a disease or disorder which is prevented, ameliorated, improved or inhibited by reduction or inhibition of ANGPTL4 activity, comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition according to any one of claims 19 to 21.

23. The method of claim 22, wherein the disease or disorder is selected from hypertriglyceridemia, hypercholesterolemia, chylomicronemia, atherogenic dyslipidemia, a cardiovascular disease or disorder, acute pancreatitis, nonalcoholic steatohepatitis (NASH), diabetes and obesity.

24. An antibody or antigen-binding fragment according to any one of claims 1 to 14 for use to improve, ameliorate, inhibit or prevent an ANGPTL4-mediated disease or disorder.

25. The antibody or antigen-binding fragment according to claim 24, wherein the ANGPTL4-mediated disease or disorder is selected from hypertriglyceridemia, hypercholesterolemia, chylomicronemia, atherogenic dyslipidemia, a cardiovascular disease or disorder, acute pancreatitis, nonalcoholic steatohepatitis (NASH), diabetes and obesity.

26. Use of an antibody or antigen-binding fragment thereof according to any one of claims 1 to 14 in the manufacture of a medicament for use to improve, ameliorate, inhibit or prevent an ANGPTL4-mediated disease or disorder.

27. The use according to claim 26, wherein the ANGPTL4-mediated disease or disorder is selected from hypertriglyceridemia, hypercholesterolemia, chylomicronemia, atherogenic dyslipidemia, a cardiovascular disease or disorder, acute pancreatitis, nonalcoholic steatohepatitis (NASH), diabetes and obesity.