Patent Right	Date	January 26, 2023	Court	Intellectual Property High Court, Fourth Division
	Case number	2021 (Gyo-Ke) 10094		

- With regard to an invention having an invention-specifying matter of "which can neutralize binding between PCSK9 and LDLR protein" and which "competes" "for binding to PCSK9" "with an antibody which comprises: a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence of SEQ ID NO: 67; and a light chain comprising a light chain variable region consisting of an amino acid sequence of SEQ ID NO: 12" (hereinafter referred to as a "reference antibody"), a technical significance of the present invention should be deemed to exist in that it has been identified that an antibody which competes with an reference antibody for binding to PCSK9 has a functional property as a binding neutralizing antibody. However, it cannot be deemed that this point was disclosed in the present description. Therefore, the present invention does not comply with the support requirement.
- Even if it is interpreted that the present invention is directed to only an antibody which "can neutralize binding between PCSK9 and LDLR protein" among antibodies which compete for binding to PCSK9 with a reference antibody, the present invention also comprises an antibody which competes in a manner that binds to a position for steric hindrance to the binding between PCSK9 and LDLR protein to occur. However, the present description does not state anything about a mechanism, etc. by which these antibodies neutralize the binding. From this point as well, the present invention does not comply with the support requirement.

Case type: Rescission of Trial Decision

Result: Appeal granted (Granted)

References: Article 36, paragraph (6), item (i) of the Patent Act

Related rights, etc.: Invalidation Trial No. 2020-800012, Patent No. 5906333

Summary of the Judgment

No. 1 Background

1. The Defendant filed a patent application (hereinafter referred to as "the present application") with regard to an invention titled "ANTIGEN BINDING PROTEIN TO PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 (PCSK9)" on February 23, 2015 by dividing a part of a patent application (Patent Application No. 2010-522084) of which the international filing date is August 22, 2008 (Priority date: August 23, 2007, December 21 of the same year, January 9, 2008, and August 4 of the same year (hereinafter referred to as "the priority date of the present case"), Priority country: United States of America). The Defendant obtained a registration of establishment of a patent right (Patent No. 5906333, Number of claims: 5, this patent is hereinafter referred to as "the present patent") on March 25, 2016.

For the present patent, in invalidation trial procedures of a trial for invalidation (Invalidation Trial No. 2016-800066) by Sanofi (a French corporation), among a

group of claims consisting of Claims 1, 2, and 5 in the scope of claims, the following corrections were made: Claims 1 and 5 were corrected; Claim 2 was deleted; and a group of claims consisting of Claims 3 and 4 were deleted (hereinafter referred to as "the correction of the present case") (Note that the JPO made a trial decision dismissing the request for a trial for invalidation of the present patent with regard to corrected Claims 1 and 5, then Sanofi instituted a suit against the above trial decision made by the JPO, but Sanofi's claim was dismissed in the suit, and the judgment became final).

2. The Plaintiff filed a request for a trial for invalidation of the present patent with regard to a portion according to Claims 1 and 5 (Invalidation Trial No. 2020-800012) on February 12, 2020, but received a decision that "The request for a trial is dismissed." (hereinafter referred to as "the trial decision of the present case") on April 7, 2021. Accordingly, the Plaintiff instituted a suit of the present case to seek rescission of the trial decision of the present case.

3. The recitation of Claims 1 and 5 after the correction of the present case is as follows (hereinafter, an invention according to Claim 1 is referred to as "Present Invention 1", an invention according to Claim 5 is referred to as "Present Invention 5", and Present Invention 1 and Present Invention 5 are collectively referred to as "the present invention," and in addition, "an antibody which comprises: a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence of SEQ ID NO: 67; and a light chain comprising a light chain variable region consisting of an amino acid sequence of SEQ ID NO: 12" is referred to as "31H4 antibody" and is also referred to as a "reference antibody.").

[Claim 1] An isolated monoclonal antibody, which can neutralize binding between PCSK9 and LDLR protein and which competes for binding to PCSK9 with an antibody which comprises: a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence of SEQ ID NO: 67; and a light chain comprising a light chain variable region consisting of an amino acid sequence of SEQ ID NO: 12."

[Claim 5] A pharmaceutical composition, comprising the isolated monoclonal antibody as claimed in Claim 1.

No. 2 Summary of the Court decision

1. The term "neutralize" in the present invention includes an embodiment of altering a binding ability of PCSK9 to LDLR protein through indirect means (such as structural or energetic alterations in a ligand) in addition to interfering with, blocking, reducing, or modulating an interaction between PCSK9 and LDLR protein by directly

blocking a protein binding site. However, a reference antibody itself can be acknowledged as a neutralizing antibody which sterically interferes with binding between PCSK9 and LDLR protein and which strongly blocks the binding at a position which partially overlaps with a position of EGFa domain of LDLR (this EGFa domain binds to a catalytic domain of PCSK9, and an antibody which interacts with or blocks any of PCSK9 residues present within the region can be useful as an antibody which inhibits the interaction between PCSK9 and LDLR) in the crystal structure. Based on this fact, the invention-specifying matter of "which competes for binding to PCSK9 with 31H4 antibody" in the present invention also has a technical significance in that it is revealed that an antibody which competes with the 31H4 antibody interferes with, blocks, reduces, or modulates the interaction between PCSK9 and LDLR protein by directly blocking a binding site of LDLR protein (specifically, by the antibody binding to PCSK9 at a position which overlaps with a position of EGFa domain of LDLR in the crystal structure) by a mechanism similar to that of the 31H4 antibody.

2. Regarding an antibody stated as having a neutralizing activity among antibodies identified as antibodies which compete with 31H4 antibody, the present description does not specifically state a position where the above antibody stated as having a neutralizing activity binds on PCSK9. In this regard, it can be deemed to be highly probable that a group of antibodies having an amino acid sequence with high identity to the 31H4 antibody bind to PCSK9 in a position similar to the 31H4 antibody. However, regarding several groups of antibodies having an amino acid sequence other than the above, a knowledge that a position where such an antibody binds on PCSK9 is revealed by the fact that the antibody was evaluated as competing in an assay such as epitope binning cannot be acknowledged to be common general technical knowledge. Thus, the position where the above antibody binds to PCSK9 cannot be deemed to be apparent.

Further, it is obvious that an antibody having a property "which competes for binding to PCSK9 with a reference antibody" of the present invention encompasses a very wide variety of antibodies in addition to several groups of antibodies specifically stated in the Detailed Description of the Invention in the present description. Furthermore, the antibody of the present invention encompasses not only an antibody which prevents or inhibits (e.g., reduces) specific binding of the reference antibody by binding to a site which overlaps with a site on PCSK9 where 31H4 antibody binds to PCSK9, but also an antibody which prevents or inhibits (e.g., reduces) specific binding of the reference antibody to PCSK9 in various degrees by binding to PCSK9

in a manner that sterically interferes with the binding between the reference antibody and PCSK9. Then, the antibodies mentioned above can include, for example, an antibody which prevents or inhibits (e.g., reduces) specific binding of the 31H4 antibody to PCSK9 by binding to a site which differs from a site where the 31H4 antibody binds to PCSK9 and which differs from a position of EGFa domain of LDLR in the crystal structure and bringing minor steric hindrance to the 31H4 antibody. However, a site where such an antibody binds to PCSK9 is not a position where the antibody overlaps with a position of EGFa domain of LDLR in the crystal structure. Thus, such an antibody cannot be deemed to interfere with, block, reduce, or modulate the interaction between PCSK9 and LDLR protein by directly blocking a binding site of LDLR protein.

As mentioned above, it cannot be deemed that the "antibody which competes for binding to PCSK9 with 31H4 antibody" interferes with, blocks, reduces, or modulates the interaction between PCSK9 and LDLR protein by directly blocking a binding site of LDLR protein (specifically, by the antibody binding to PCSK9 at a position which overlaps with a position of EGFa domain of LDLR in the crystal structure) in a manner similar to 31H4 antibody. Thus, it also cannot be acknowledged that the "antibody which competes for binding to PCSK9 with 31H4 antibody" has a functional property as a binding-neutralizing antibody.

3. The Defendant asserts that there is no reason why the present invention violates the support requirement, on the grounds that even if there exists an antibody which competes with 31H4 antibody (a reference antibody) but which cannot neutralize the binding between PCSK9 and LDLR protein, such an antibody is literally excluded from the technical scope of Present Invention 1.

However, a technical significance of the present invention should be deemed to exist in that it has been identified that an antibody which competes with 31H4 antibody has a functional property as an antibody which neutralizes the binding between PCSK9 and LDLR protein by the mechanism similar to that of 31H4 antibody. If an antibody which competes with 31H4 antibody includes one which does not have a binding neutralizing activity, it is apparent that the assumption of its technical significance will collapse.

Note that even if it is interpreted that the scope of claims of Present Invention 1 is, as asserted by the Defendant, directed to only an antibody which "can neutralize binding between PCSK9 and LDLR protein" among antibodies which compete for binding to PCSK9 with a reference antibody, the invention-specifying matter of that which competes for binding to PCSK9 with a reference antibody according to the

present invention is not limited to an antibody which binds to a position that is the same as or overlaps with a position where the reference antibody binds as asserted by the Defendant, but also includes an antibody which competes in a manner that binds to a position for steric hindrance to the binding between PCSK9 and LDLR protein to occur. Thus, it must be supported that such an antibody is also a binding neutralizing antibody. In this regard, unlike the case of an antibody which binds to a position that is the same as or overlaps with a position where a reference antibody binds, the present description does not state anything about a mechanism by which an antibody neutralizes the binding between PCSK9 and LDLR protein in which the antibody competes in a manner that binds to a position for steric hindrance to the binding to occur. In addition, binding neutralizing antibodies based on experimental results by binning are all likely to be antibodies which bind to a position that is the same as or overlaps with a position where a reference antibody binds, of which the mechanism pertaining to binding and neutralizing is disclosed. Even if this point is excluded, at least, the present description does not state anything to suggest that these are sterically interfering antibodies.

Thus, it must be deemed that the Detailed Description of the Invention in the present description does not disclose anything about the fact that among antibodies which compete with a reference antibody, when an antibody competes in a manner that binds to a position for steric hindrance to the binding between PCSK9 and LDLR protein to occur, the antibody has a binding neutralizing activity. From this point as well, the present invention does not comply with the support requirement.

Judgment rendered on January 26, 2023
2021 (Gyo-Ke) 10094 Case of seeking rescission of trial decision
Date of Conclusion of Oral Argument: November 7, 2022
Judgment

Plaintiff: Regeneron Pharmaceuticals Incorporated

Defendant: Amgen Incorporated

Main text
1. The court shall rescind the decision made by the Japan Patent Office (JPO) on April 7, 2021 with regard to the case of Invalidation Trial No. 2020-800012.
2. The court costs shall be borne by the Defendant.
3. The additional period for filing a final appeal and a petition for acceptance of final appeal against this judgment shall be 30 days.
Facts and reasons
No. 1 Claim
The same gist as the main text, the first paragraph.
No. 2 Outline of the case
1. History, etc. of Procedures at the JPO
(1) The Defendant filed a patent application (hereinafter referred to as "the Present Application") with regard to an invention titled "ANTIGEN BINDING PROTEIN TO PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 (PCSK9)" on February 23, 2015 by dividing a part of a patent application (Patent Application No. 2010-522084) whose international filing date is August 22, 2008 (Priority date: August 23, 2007, December 21 of the same year, January 9, 2008, and August 4 of the same year (hereinafter referred to as "the priority date of the present case"), Priority country: United States of America). The Defendant obtained a registration of establishment of a patent right (Patent No. 5906333, Number of claims: 5, this patent is hereinafter referred to as "the Present Patent") on March 25, 2016.
(2) Sanofi (a French corporation) filed a request for a patent invalidation trial with regard to the present patent on May 31, 2016 (the case of Invalidation Trial No. 2016-800066, hereinafter referred to as "the invalidation trial of the separate case").

The Defendant received an advance notice of a trial decision dated March 9, 2017 and thus, on May 8 of the same year, filed a request for correction to the effect that in the scope of claims, Claims 1 and 5 be corrected and Claim 2 be deleted among

a group of claims consisting of Claims 1, 2 and 5 and that a group of claims consisting of Claims 3 and 4 be deleted (hereinafter referred to as "the correction of the present case").

Thereafter, on August 2, 2017, the Japan Patent Office allowed the correction of the present case and rendered a trial decision that "The request for the trial with regard to the inventions according to Claims 1 and 5 of the present patent is groundless. The request for the trial with regard to the inventions according to Claims 2 to 4 shall be dismissed" (hereinafter referred to as "the trial decision of the separate case").

Sanofi instituted a suit for seeking rescission of the portion pertaining to Claims 1 and 5 of the Present Patent in the trial decision of the separate case (hereinafter referred to as "the suit against the trial decision of the separate case") on December 8, 2017 (Intellectual Property High Court, 2017 (Gyo-Ke) 10226). However, the Intellectual Property High Court dismissed Sanofi's claim on December 27, 2018 (the Intellectual Property High Court denied the presence of all grounds for rescission (error in the determination of an inventive step, error in the determination of the support requirement, and error in the determination of the enablement requirement) asserted by Sanofi). Sanofi filed a petition for acceptance of a final appeal against the same judgment. However, the Supreme Court ruled not to accept the final appeal on April 24, 2020, and the same judgment became final and binding.

(3) The Plaintiff filed a request for a patent invalidation trial with regard to the portion pertaining to Claims 1 and 5 of the Present Patent on February 12, 2020 (the case of Invalidation Trial No. 2020-800012).

However, the Japan Patent Office rendered a trial decision that "The request for the trial of the present case is groundless." (hereinafter referred to as "the trial decision of the present case") on April 7, 2021, a certified copy of which was served on the Plaintiff on the 16th of the same month (additional time frame: 90 days).

(4) The Plaintiff instituted a suit of the present case for seeking rescission of the trial decision of the present case on August 13, 2021.

2. Statement of the Scope of Claims

The statement of Claims 1 and 5 after the correction of the present case is as follows (Hereinafter, an invention according to Claim 1 is referred to as "Present Invention 1," an invention according to Claim 5 is referred to as "Present Invention 5," and Present Invention 1 and Present Invention 5 are collectively referred to as "the present invention." In addition, "an antibody which comprises: a heavy chain comprising a heavy-chain variable region consisting of an amino acid sequence of

SEQ ID NO: 67; and a light chain comprising a light-chain variable region consisting of an amino acid sequence of SEQ ID NO: 12" is referred to as "31H4 antibody" and is also referred to as a "reference antibody.").

[Claim 1] An isolated monoclonal antibody, which can neutralize binding between PCSK9 and LDLR protein and which competes for binding to PCSK9 with an antibody which comprises: a heavy chain comprising a heavy-chain variable region consisting of an amino acid sequence of SEQ ID NO: 67; and a light chain comprising a light-chain variable region consisting of an amino acid sequence of SEQ ID NO: 12."

[Claim 5] A pharmaceutical composition, comprising the isolated monoclonal antibody as claimed in Claim 1.

3. Summary of the Trial Decision of the Present Case

(1) Reason 1 for Invalidation (Violation of the Support Requirement)

The present invention is "an isolated monoclonal antibody" that has both of a property "which can neutralize binding between PCSK9 and LDLR protein" and a property "which competes for binding to PCSK9 with 31H4 antibody" and is "a pharmaceutical composition comprising" this. In this regard, according to each statement of [0002], [0003], [0066], [0071], [0155], [0270], [0271], and [0276] of the description attached to the written application of the present application (hereinafter referred to as "the present description," including the Drawings), it can be understood that a problem of the present invention is to provide such a novel antibody and to produce a pharmaceutical composition comprising this, which neutralizes binding between PCSK9 and LDLR and increases the amount of LDLR, thereby achieving an effect of causing a decrease in serum cholesterol in a subject, treating or preventing diseases related to elevated cholesterol levels such as hypercholesterolemia, and reducing the risk of such diseases.

In addition, the present description specifically states a method of producing an anti-PCSK9 monoclonal antibody (preparation of immunized mice and production of hybridomas (antibody-producing cells) using immunized mice), a method of screening for an antibody which neutralizes binding between PCSK9 and LDLR, and a method of screening for an antibody which competes with 31H4 antibody ([0138], [0312], [0313], [0320], [0322] to [0328], [0332] to [0334], [0336], [0377], [0378], and Table 1 to Table 3). Further, the working examples of the present description show results of two independent experiments in which hybridomas that produced antibodies which strongly neutralized binding between PCSK9 and LDLR were selected from hybridomas obtained by injecting human PCSK9 antigen into two groups of mice

containing human immunoglobulin genes, and epitope binning of these antibodies was performed (Example 10, Example 37), which specifically demonstrates that a number of antibodies of the present invention can be repeatedly identified with sufficiently high probability by performing two screenings of anti-PCSK9 monoclonal antibodies; that is, one screening is to select antibodies "which can neutralize binding between PCSK9 and LDLR" and the other screening is to select antibodies "which compete with 31H4 antibody." Furthermore, the present description states a mechanism of action in which the binding between PCSK9 and LDLR is neutralized thereby to increase the amount of LDLR, causing a decrease in serum cholesterol in a subject ([0066], [0155], [0270], [0271], [0276]). Thus, it can be reasonably acknowledged that an antibody of the present invention having a property "which can neutralize binding between PCSK9 and LDLR protein" achieves an effect of causing a decrease in serum cholesterol in a subject and can solve the problem of treating or preventing diseases related to elevated cholesterol levels such as hypercholesterolemia, and reducing the risk of such diseases.

Hence, a person ordinarily skilled in the art can acknowledge from the statement of the present description that the antibody of the present invention can solve the above problem, and the present invention can be deemed to be stated in the description. Therefore, the present patent complies with the support requirement.

(2) Reason 2 for Invalidation (Violation of the Enablement Requirement)

As mentioned in (1) above, the present description specifically states a method of producing an antibody of the present invention, such as a method of producing an anti-PCSK9 monoclonal antibody (preparation of immunized mice and production of hybridomas using immunized mice), a method of screening for an antibody which neutralizes binding between PCSK9 and LDLR, and a method of screening for an antibody which competes with 31H4 antibody. In addition, the present description shows through specific data that a number of antibodies of the present invention can be repeatedly identified with sufficiently high probability by performing two screenings of anti-PCSK9 monoclonal antibodies; that is, the screening for being able to neutralize binding between PCSK9 and LDLR and the screening for competing with 31H4 antibody. Thus, it can be deemed that a person ordinarily skilled in the art can produce an antibody of the present invention with sufficiently high probability on the basis of these specific statements of the present description.

Further, a structure (amino acid sequence) of a binding region in an antibody is a resulting product from reconstituting antibody genes in immune cells of an immunized animal. Thus, it can be acknowledged that a person ordinarily skilled in

the art will reasonably understand that a wide variety of antibodies other than the working examples encompassed in the present invention can be produced countlessly by changing the types of animals to be immunized and the immunization program using the statement of the present description as a hint.

Hence, the present description can be deemed to be stated to the extent that a person ordinarily skilled in the art can obtain an antibody of the present invention without undue burden. Therefore, the present patent complies with the enablement requirement.

(3) Reason 3 for Invalidation (Lack of Inventive Step)

A. Invention Disclosed in Nature Structural & Molecular Biology, vol. 14(5), pp. 413-419 (2007) (Exhibit Ko 1, hereinafter referred to as "Exhibit Ko 1 document," see Attachment 2) as Found by the Trial Decision of the Present Case (hereinafter referred to as "Exhibit Ko 1 invention")

"PCSK9 which binds to LDLR, i.e., gain-of-function PCSK9 mutants F216L, S127R, D374Y, or wild-type PCSK9."

B. Common Feature and Difference as Found by the Trial Decision of the Present Case

Both of the "monoclonal antibody" of Present Invention 1 and "PCSK9" of the Exhibit Ko 1 invention are proteins. Thus, the two are common in that they are proteins and differ in the following point.

(Difference)

Present Invention 1 is "an isolated monoclonal antibody, which can neutralize binding between PCSK9 and LDLR protein and which competes for binding to PCSK9 with 31H4 antibody." In contrast, the Exhibit Ko 1 invention is "PCSK9 which binds to LDLR; i.e., gain-of-function PCSK9 mutants F216L, S127R, D374Y, or wild-type PCSK9."

C. Determination on Difference

From the disclosures of Exhibit Ko 1-11 in Attachment 2 (hereinafter referred to simply as "Exhibit Ko 1-11" or the like) and Exhibit Ko 1-12, by obtaining anti-PCSK9 antibodies using full-length PCSK9 as an antigen through a method of obtaining monoclonal antibodies, such as an animal immunization method and a phage display method, and then screening the obtained anti-PCSK9 antibodies for neutralizing binding between PCSK9 and LDLR, it is possible to acknowledge up to the extent that there is possibility of obtaining some anti-PCSK9 monoclonal antibodies which neutralize binding between PCSK9 and LDLR. However, when anti-PCSK9 monoclonal antibodies which neutralize binding between PCSK9 and

LDLR are obtained by obtaining anti-PCSK9 monoclonal antibodies using full-length PCSK9 as an antigen and then screening the obtained anti-PCSK9 monoclonal antibodies through a binding-neutralizing assay, it can be considered that such anti-PCSK9 monoclonal antibodies encompass a considerable variety of monoclonal antibodies which bind to various epitopes present on or around binding surfaces. Thus, in order to obtain an anti-PCSK9 monoclonal antibody which can compete with 21B12 antibody among anti-PCSK9 monoclonal antibodies which can neutralize binding between PCSK9 and LDLR, it is essential to select anti-PCSK9 monoclonal antibodies by performing a competition assay with 31H4 antibody, and in order to do so, it is a prerequisite that 31H4 antibody has been obtained.

However, the Exhibit Ko 1 document neither discloses nor suggests 31H4 antibody and does not disclose any information that would provide a hint to obtaining 31H4 antibody among anti-PCSK9 monoclonal antibodies which can inhibit binding between PCSK9 and LDLR antibody and to obtain a monoclonal antibody which competes for binding to PCSK9 with 31H4 antibody. Further, it cannot be acknowledged that 31H4 antibody had been widely known prior to the priority date of the present case.

Thus, even a person ordinarily skilled in the art cannot arrive at obtaining a monoclonal antibody which competes with 31H4 antibody. Therefore, it cannot be deemed that Present Invention 1 that comprises the invention-specifying matter of "which competes with 31H4 antibody" could have easily been made by a person ordinarily skilled in the art on the basis of the Exhibit Ko 1 invention and well-known art. The same applies to Present Invention 5 that is a pharmaceutical composition comprising the antibody of Present Invention 1.

(4) Reason 4 for Invalidation (Violation of the Clarity Requirement)

In the present invention, it should be interpreted that the term "compete" means a common-general knowledge degree of competing to a person ordinarily skilled in the art. In the present description as well, as in [0140], the term "compete" is used in the meaning of common general knowledge, testing methods therefor are stated together with specific documents, and it is stated that the term "compete" means a common-general knowledge degree of inhibiting binding. Therefore, it cannot be deemed that the outer limit of "which competes with 31H4 antibody" is unclear.

(5) Reason 5 for Invalidation (Violation of the Requirement that the Invention Should Fall within Its Definition)

Both Present Inventions 1 and 5 provide specific means for solving their problem and can be deemed to be creation of technical ideas utilizing the laws of

nature. Therefore, both Present Inventions 1 and 9 fall within the definition of an "invention" under the Patent Act.

4. Ground for Rescission

(1) Error in Determination on Inventive Step Based on Exhibit Ko 1 Invention (Ground 1 for Rescission)

A. Error in Finding Common Feature and Difference Between Present Invention 1 and the Exhibit Ko 1 Invention (Ground 1-1 for Rescission)

B. Error in Determination on Whether Present Invention could have been Easily Conceived of (Ground 1-2 for Rescission)

(2) Error in Determination on Violation of the Support Requirement (Ground 2 for Rescission)

(3) Error in Determination on Violation of the Enablement Requirement (Ground 3 for Rescission)

(4) Error in Determination on Violation of the Clarity Requirement (Ground 4 for Rescission)

(5) Error in Determination on Violation of the Requirement that the Invention should Fall within Its Definition (Ground 5 for Rescission)

(omitted)

No. 4 Judgment of this court

1. Statements of the Present Description (Exhibit Ko 201), etc.

A. In the [Detailed Description of the Invention] of the present description, there are statements as shown in Attachment 1. According to these statements, the following matters are disclosed with regard to the present invention.

(A) PCSK9 (proprotein convertase subtilisin kexin type 9) is a serine protease involved in regulating the levels of low-density lipoprotein receptor (LDLR) protein, directly interacts with LDLR protein, and is endocytosed into liver cells along with LDLR, decreasing the levels of LDLR protein in the liver and further decreasing the amount of LDLR protein available to bind to LDL on cell surfaces (extracellularly), thereby increasing the amount of LDL in a subject ([0002], [0003], [0071]).

(B) The antibody which comprises: a heavy chain comprising a heavy-chain variable region consisting of an amino acid sequence of SEQ ID NO: 67 and a light chain comprising a light-chain variable region consisting of an amino acid sequence of SEQ ID NO: 12 ("31H4 antibody") ("reference antibody") is a neutralizing antibody which strongly blocks binding between PCSK9 and LDLR protein (Example

11, [0377] to [0379], [0138], Table 2). The reference antibody partially overlaps with a position of EGFa domain of LDLR in the crystal structure and sterically interferes with its binding to PCSK9 (Example 31, [0444], Figure 20A).

The EGFa domain of LDLR binds to the catalytic domain of PCSK9. PCSK9 amino acid residues that are present within 5 angstroms of the EGFa domain in the crystal structure are specific core PCSK9 amino acid residues of the interaction interface with the EGFa domain of LDLR (core residues). PCSK9 residues that are present at 5 angstroms to 8 angstroms of the EGFa domain are boundary PCSK9 amino acid residues of the interaction interface with the EGFa domain of LDLR. An antibody which interacts with or blocks any of these amino acid residues can be useful as an antibody which inhibits the interaction between PCSK9 and the EGFa domain of LDLR (and/or LDLR in general) (Example 28, [0428] to [0432]).

(C) The monoclonal antibody which "competes" with the reference antibody is an antibody which prevents or inhibits (e.g., reduces) binding of the reference antibody to PCSK9 ([0138], [0140], [0262], [0269]).

(D) A neutralizing ABP (antibody) to PCSK9 neutralizes binding between PCSK9 and LDLR to increase the amount of LDLR, thereby achieving an effect of causing a decrease in the amount of LDL in a subject and a decrease in serum cholesterol in the subject. In addition, owing to this effect, diseases related to elevated cholesterol levels, such as hypercholesterolemia, can be treated or prevented, and the risk of such diseases can be reduced. Thus, the neutralizing ABP to PCSK9 can be therapeutically useful ([0155], [0270], [0271], [0276]).

B. According to the disclosures of the present description as summarily excerpted in A above, the present invention increases the amount of LDLR protein, thereby achieving an effect of causing a decrease in the amount of LDL in a subject and a decrease in serum cholesterol in the subject, and owing to this effect, diseases related to elevated cholesterol levels, such as hypercholesterolemia, can be treated or prevented, and the risk of such diseases can be reduced. For this purpose, the present invention made its problem to provide an antibody which neutralizes binding between PCSK9 and LDLR protein, in which the PCSK9 binds to the LDLR protein to thereby decrease the amount of LDLR protein in a subject and increase the amount of LDL in the subject, or to provide a pharmaceutical composition comprising this. On this basis, the present invention reveals that PCSK9 binds to the EGFa domain of LDLR, and that the reference antibody is a neutralizing antibody which sterically interferes with binding between PCSK9 and LDLR protein at a position that partially overlaps with a position of the EGFa domain of the LDLR in the crystal structure and

which strongly blocks the binding, and that the monoclonal antibody which "competes" with the reference antibody is an antibody which prevents or inhibits (e.g., reduces) binding of the reference antibody to PCSK9.

(2) Statements of Exhibit Ko 1 Document

A. In the Exhibit Ko 1 document that is a publication distributed prior to the priority date of the present case, there are statements as shown in Attachment 2 (note that the statements are shown as translations). According to these statements, the Exhibit Ko 1 document can be found to disclose the following matters.

(A) Familial hypercholesterolemia results from elevated LDL cholesterol levels in plasma or the like. Proprotein convertase subtilisin kexin type 9 (PCSK9) reduces the abundance of low-density lipoprotein receptor (LDLR) present on cell surfaces, and gain-of-function mutations of PCSK9 result in more severe decrease in the number of LDLR with consequent hypercholesterolemia. (The disclosure up to here is from Exhibit Ko 1-2.)

Secreted PCSK9 interacts with human liver cell surfaces and can be co-immunoprecipitated with LDLR, and study results have shown that PCSK9 binds to LDLR with more greatly increased affinity by as much as 170-fold at the endosomal pH (Exhibit Ko 1-5).

(B) Gain-of-function mutations in human PCSK9 are associated with familial hypercholesterolemia. In particular, the D374Y mutant is about 10 times more active than wild-type PCSK9 in lowering the number of LDLR. The increased effect of the D374Y mutant in lowering LDLR is likely to result from enhanced binding to cell-surface LDLR (Exhibits Ko 1-6, 1-9).

(C) Genetic evidence suggests that PCSK9 is an attractive target for the treatment of cardiovascular disease. Because binding to LDLR protein in plasma and receptor-dependent intracellular uptake are mostly the rate-determining step for PCSK9 function, antibodies or small molecules which bind to PCSK9 in plasma and inhibit its binding to LDLR can be effective inhibitors of PCSK9 function. In particular, the structure of PCSK9-LDLR complex is useful for designing novel therapies (Exhibit Ko 1-12).

B. According to each statement as summarily excerpted in the above, it can be found that the Exhibit Ko 1 document discloses the following matters: familial hypercholesterolemia results from elevated LDL cholesterol levels in plasma; in this regard, proprotein convertase subtilisin kexin type 9 (PCSK9) reduces the abundance of low-density lipoprotein receptor (LDLR) present on cell surfaces; thus, PCSK9 is an attractive target for the treatment, and antibodies or the like which bind to PCSK9

in plasma and inhibit its binding to LDLR can be effective inhibitors.

(3) Results of the Demonstration Experiment and Its Evaluation as Stated in Affidavit (1) by Dr. [A] (Exhibit Ko 2-1)

A. (A) According to the abridged translation of Affidavit (1) by Dr. [A] (Exhibit Ko 2-1), experimental results as shown in Attachment 3 are stated.

(B) According to the abridged translation of Affidavit (1) by Dr. [B] (Exhibit Ko 2-2), there are the following statements.

a. "4. I have also been provided with details of experiments performed by Yumab GmbH (hereinafter "Yumab") on a panel of antibodies to hPCSK9 (a group of antibodies) and with a copy of the "Affidavit (1) by Dr. [A]([A])" (hereinafter "D1") from Yumab. The Affidavit shows the overall explanation of these experiments. I have been requested to review and comment on these results, ..." (Page 2, lines 19 to 24 (The relevant part refers to the original text. The same applies hereinafter.))

b. "C. Analysis of Competition and Neutralization

14. Data on the Regeneron antibodies are summarized in Table 1, using the criterion for competition; i.e., "a reduction of greater than or equal to 50% in the binding of 21B12 or 31H4 antibody."

Table 1: Neutralizing Property of Regeneron Antibody which Competes with 21B12/31H4 Using 50% Threshold Value for Competition

	Competing with 21B12	Competing with 31H4
Neutralizing	3	6
Non-neutralizing	10	28
Total	13	34
Non-neutralizing (%)	76.90%	82.40%

15. As Table 1 shows, about 80% of Regeneron antibodies classified as competing with 21B12 antibody cannot neutralize the hPCSK9-LDLR interaction. Further, as Table 1 shows, more than 80% of Regeneron antibodies classified as competing with 31H4 antibody cannot neutralize the hPCSK9-LDLR interaction.

16. ....Similarly, in summary, these results demonstrate that most of the antibodies which compete for binding with 31H4 cannot neutralize binding between hPCSK9 and LDLR.... According to the present patent, this is because the binding site of 31H4 antibody only partially overlaps with the binding site of LDLR on hPCSK9. Thus, a binding site of another antibody can overlap with the 31H4 binding site without overlapping with the binding site of LDLR, and in this manner, another antibody can

compete with 31H4 without neutralizing the hPCSK9-LDLR interaction. Further, two antibodies can compete with each other without necessarily binding to the same site (e.g., in a case where one antibody sterically prevents another antibody from binding to a close binding site). Therefore, in order for competition to be seen, overlap of binding sites is not required." (Page 5, lines 8 to page 6, line 11)

c. "D. Conclusion

19. ....Similarly, taking these results into consideration, it is scientifically erroneous to state that an antibody which competes with 31H4 antibody would "neutralize binding to LDLR." ... The fact that antibodies which compete with 31H4 antibody do not have the effect of strongly neutralizing binding of hPCSK9-LDLR obviously indicates that such antibodies do not have any effects on this interaction.

20. I also disagree that an antibody which competes with 21B12 antibody would necessarily have affinity and/or a binding site similar to that of the 21B12 antibody....

21. Therefore, the idea that an antibody which competes with 21B12 must have an activity similar to said specific 21B12 antibody cannot be accepted as my scientific opinion. Therefore, the idea that an antibody which competes with 31H4 must similarly have an activity similar to that of said specific 31H4 antibody cannot be accepted as my scientific opinion. It is certainly erroneous to state that all of such antibodies would have an effect of neutralizing binding. The fact that so many of such antibodies cannot actually inhibit binding of hPCSK9-LDLR demonstrates that this idea is unrealistic. Whatever the case may be, any biological effect of these non-neutralizing antibodies can be produced through a mechanism different from those of 21B12 and 31H4 and does not fall within the definition of neutralization in the claims of the present patent (i.e., inhibition by preventing hPCSK9 and LDLR from interacting with each other as verified in an in vitro competition assay)." (Page 6, line 19 to page 7, line 15)

B. (A) As mentioned in No. 3, 2(2)C(A) above, with regard to the demonstration experiment stated in Affidavit (1) by Dr. [A] (Exhibit Ko 2-1), the Defendant relies on the Expert Opinion by Professor [C] (Exhibit Otsu 24) and points out the following matters: [i] In Example 10, the "premix" method is used, but Dr. [A] did not use this method, and in the method used by Dr. [A] in which PCSK9 was first added to a tested antibody on a plate to allow to bind and the reference antibody was then added, the binding site of the reference antibody on PCSK9 was occluded depending on the direction in which the tested antibody was immobilized on the plate, which caused the reference antibody to be unable to bind to the PCSK9 + tested antibody complex, showing the result as if they had been apparently competing; [ii] PCSK9 at a much

higher concentration than in Example 10 and the tested antibody at a higher concentration than in Example 10 were used, and nevertheless a blocking buffer at a slightly lower concentration than in Example 10 was used, which caused PCSK9 to nonspecifically bind to the tested antibody in such a manner as to occlude the binding site of the reference antibody, making it impossible to prevent the phenomenon such that the reference antibody became unable to bind to PCSK9, leading to the results as if they had been apparently competing; [iii] Experiments were performed using a strong blocking buffer, which showed that 081006A, 081006B, and 190515-41 antibodies, which Dr. [A] concluded to be "competing," did not compete with the reference antibody. On the above basis, the Defendant asserts that the results of the demonstration experiment by Dr. [A] contain false positives and are thus erroneous.

However, the above assertion compares the above demonstration experiment by Dr. [A] with the experimental conditions in Example 10 of the present description. For example, Example 37 of the present description does not use the premix method, but the blocking buffer is phosphate buffered saline plus 1% BSA that has an even lower concentration than 2% BSA (containing 0.05% Tween 20) used in the above demonstration experiment. Thus, on the basis of the comparison of the experimental conditions with Example 10, it cannot be deemed that the conditions of the above demonstration experiment are inappropriate. In addition, needless to say, the present invention does not define the measurement method and measurement conditions for competition and neutralization of monoclonal antibodies for neutralizing binding between PCSK9 and LDLR.

Further, in the above demonstration experiment, among 63 Regeneron antibodies, it has been confirmed that five antibodies (081211B, 190515-35, 190515-7, 190515-8, 190515-9) do not bind to PCSK9 (see Material B1 of Attachment 3). From this point as well, it cannot be deemed that the above demonstration experiment was performed under conditions in which nonspecific binding of PCSK9 produced false-positive results. In addition, the above demonstration experiment is intended to verify binding specificity, compatibility, and neutralizing property using the antibodies disclosed in the present description (9C9, 3B6, and 27B2) as controls. As shown in Material B1 of Attachment 3, it has been confirmed that 9C9 and 3B6 bind to PCSK9, compete with 21B12 antibody, and have neutralizing property. It has been confirmed that the results of this demonstration experiment are the same as the results disclosed in the present description (see [0374], [0493]).

Thus, it cannot be deemed that the above results of the demonstration experiment are inappropriate experimental results, (If the results of the experiment

were inappropriate, the Defendant should have presented experiment results performed under the same conditions as in Example 10, as pointed out by the Plaintiff. However, the Defendant failed to do so and instead entirely focused on impeaching the above results of the experiment.) Further, it also cannot be deemed that Affidavit (1) by Dr. [B] (Exhibit Ko 2-2) based on this demonstration experiment is an inappropriate opinion.

(B) Incidentally, after the date for preparatory proceedings (both parties gave technical explanations concerning the present invention under the involvement of three technical advisors) had concluded, it was agreed that both parties may submit any written arguments to the extent that they would supplement their explanations given on the same date (see the record of the first preparatory proceedings). Nevertheless, immediately before the date for the first oral argument, the Defendant newly submitted the Expert Opinion by Professor [C] (Exhibit Otsu 48). Then, the Defendant points out the following matters about the demonstration experiment by Dr. [A]: [i] 25 of the 63 Regeneron antibodies used are disclosed in the Plaintiff's U.S. provisional application (No. 61/122,482), among which 081211B and 190515-35 are identical to antibodies H1H314P and H1H317P disclosed in the Plaintiff's provisional application, respectively, and the Plaintiff's provisional application states that both of these antibodies bind to PCSK9, whereas the above demonstration experiment concludes that these antibodies do not bind to PCSK9 at all; [ii] 190515-36 and 190515-37 are identical to antibodies H1H320P and H1H321P disclosed in the Plaintiff's provisional application (Exhibit Otsu 48), respectively, and in the provisional application, the blocking activity of binding between PCSK9 and LDLR is confirmed, whereas in the results of the above demonstration experiment, the activity is described as non-neutralizing; [iii] 081008B and 190515-43 have the same amino acid sequence, and thus competition test results should be the same, and in spite of this, the above results of the demonstration experiment conclude that 190515-43 competes with 31H4 antibody, whereas 081008B does not compete with 31H4 antibody. On the above basis, the Defendant asserts that these points diminish the reliability of the results of the above demonstration experiment.

The above assertion goes beyond the limit of supplementing the Defendant's explanation concerning the reliability of the results of the above demonstration experiment on the date of preparatory proceedings, and falls under delayed allegation and evidence, the dismissal of which is inescapable. However, since the Plaintiff submitted the ninth brief as a counter-assertion against this on the date of conclusion of the oral argument, the following can be pointed out as additional remarks just to

make sure. Regarding point [i], it is apparent that both H1H314P and H1H317P disclosed in the provisional application have lower binding activity than other antibodies (see Table 4 in Exhibit Ko 222). Thus, the fact that these are evaluated as not binding in the experimental assay different from that in the provisional application does not necessarily impair the reliability of the results of the above demonstration experiment. Regarding point [ii], the provisional application (Exhibit Ko 222) discloses in paragraph [0076] that H1H320P (190515-36) and H1H321P (190515-37) block binding between hPCSK9 and hLDLR-EGF-A domain. However, H1H320P and H1H321P are not stated in Table 6 prepared as examples of antibodies having clear binding blocking property to PCSK9 (paragraph [0077]). Further, similarly to point [i], the fact that different evaluations are made in the experimental assay different from that in the provisional application does not necessarily impair the overall reliability of the results of the above demonstration experiment. Regarding point [iii], in the Plaintiff's provisional application, it can be found from the sequence listings in Exhibit Ko 222 and Exhibit Otsu 10 that 190515-43 (H1M505 (SEQ ID NOs: 266/274) in the provisional application) and 081008B (H1HM504 (SEQ ID NOs: 242/250) in the provisional application) are stated as different antibodies, and Table 7 differently states the binding specificity to the chimeric protein and D374Y mutant of PCSK9. Thus, the fact that different evaluations are made in the demonstration experiment by Dr. [A] does not necessarily affect the reliability of the results of the same demonstration experiment.

(C) According to the foregoing, the results of the demonstration experiment stated in Affidavit (1) by Dr. [A] (Exhibit Ko 2-1) should be deemed to have reliability.

2. Ground 2 for Rescission (Error in Determination on Violation of the Support Requirement)

In accordance with the case, first, a determination will be made on Ground 2 for Rescission (error in determination on violation of the support requirement).

(1) The Defendant asserts, as mentioned in No. 3, 3(2)A above, that it violates Article 167 of the Patent Act that the Plaintiff asserts Ground 2 for Rescission on the premise that the Plaintiff and Sanofi substantially share common interests and on the grounds that the trial decision of the separate case has become final and binding with regard to the invalidation trial of the separate case which was requested for reasons that the present patent violates the support requirement, etc.

However, Article 167 of the Patent Act provides that "Once the trial decision in a trial for patent invalidation ... has become final and binding, neither the parties nor intervenors may file a request for either such kind of trial on the basis of the same

facts or evidence." In this regard, although the Plaintiff and Sanofi or Sanofi K.K. have a relationship in which they jointly commercialize a formulation, etc. concerning the dispute on the present patent, the Plaintiff is a corporation separate from Sanofi, and it cannot be acknowledged that there are special circumstances in which the Plaintiff and Sanofi or Sanofi K.K. should be regarded as the same party from a practical point of view, such as a relationship between parent company and subsidiary company, or a relationship between a Japanese corporation and a foreign corporation (needless to say, the Plaintiff is not also an intervenor in the invalidation trial of the separate case). Therefore, in the first place, it should be deemed that the same Article is not applied.

(2) Article 36, paragraph (6), item (i) of the Patent Act provides that the statement of the scope of claims shall not be made beyond the scope of the invention stated in the Detailed Description of the Invention. The purpose of this provision can be interpreted as follows: if an invention that is not stated in the Detailed Description of the Invention were to be claimed in the scope of claims, it would be improper to claim a monopolistic and exclusive right for the unpublished invention; and thus, the purpose of this provision is to prevent this.

Then, it is reasonable to interpret that whether or not the statement of the scope of claims complies with the requirement provided in the same item (support requirement) should be determined by comparing between the statement of the scope of claims and the statement of the Detailed Description of the Invention, then examining: whether or not the invention stated in the scope of claims is the invention stated in the Detailed Description of the Invention, and falls within the scope where a person ordinarily skilled in the art can recognize that the problem of the invention can be solved by the statement of the Detailed Description of the Invention; or whether or not, even if it is neither stated nor suggested in the Detailed Description of the Invention, the invention stated in the scope of claims falls within the scope where a person ordinarily skilled in the art can recognize that the problem of the invention can be solved in light of the common general technical knowledge at the time of filing the application.

(3) Under the circumstances, the statement of the scope of claims according to the present invention will be examined. It can be interpreted that Claim 1 of the present invention has the invention-specifying matters: [i] "which can neutralize binding between PCSK9 and LDLR protein"; [ii] "which competes" for binding to PCSK9 with "an antibody which comprises: a heavy chain comprising a heavy-chain variable region consisting of an amino acid sequence of SEQ ID NO: 67; and a light chain

comprising a light-chain variable region consisting of an amino acid sequence of SEQ ID NO: 12" (31H4 antibody) (reference antibody); and [iii] "An isolated monoclonal antibody," and that the invention-specifying matters [i] and [ii] determine properties of [iii] the isolated monoclonal antibody.

A. When the statement of the present description is examined in order to interpret the technical significance of "neutralize" in the invention-specifying matter [i] "which can neutralize binding between PCSK9 and LDLR protein," there are the following statements: "The term 'neutralizing antigen binding protein' or 'neutralizing antibody' represents an antigen binding protein or antibody, respectively, which binds to a ligand and prevents or reduces a biological effect of that ligand. This can be done, for example, by directly blocking a binding site on the ligand or by binding to the ligand to alter the binding ability of the ligand through indirect means (such as structural or energetic alterations in the ligand)." ([0138]); and "the antigen binding protein provided in the present description can interfere with, block, reduce, or modulate the interaction between PCSK9 and LDLR. Such an antigen binding protein is denoted as 'neutralizing'.... the neutralizing ABP binds to PCSK9 in a position and/or manner that prevents PCSK9 from binding to LDLR. Such an ABP can be specifically described as a 'competitively neutralizing' ABP." ([0155]). According to these statements, the term "neutralize" in the present invention means interfering with, blocking, reducing, or modulating the interaction between PCSK9 and LDLR protein and includes not only an aspect of directly blocking a binding site between PCSK9 and LDLR protein, but also an aspect of altering the binding ability of PCSK9 to LDLR protein through indirect means (such as structural or energetic alterations in the ligand).

B. Next, when the statement of the present description is examined in order to interpret the technical significance of the invention-specifying matter [ii] "which 'competes' for binding to PCSK9 with a reference antibody," there are the following statements in the present description: "Antigen binding proteins identified by the competition assay (competing antigen binding proteins) include an antigen binding protein which binds to the same epitope as a reference antigen binding protein and an antigen binding protein which binds to an adjacent epitope sufficiently close to an epitope bound by the reference antigen binding protein for steric interference to occur...." ([0140]); "Competing Antigen Binding Proteins ... for specific binding to PCSK9, there are provided antigen binding proteins which compete with one of the exemplified antibodies or functional fragments binding to the epitope described in the present description. Such antigen binding proteins can also bind to the epitope that

is the same as or overlaps with one of the antigen binding proteins exemplified in the present description" ([0269]); "The term 'compete' when used in the context of an antigen binding protein (e.g., a ... neutralizing antibody) which competes for the same epitope means competition between antigen binding proteins as measured by an assay in which the antigen binding protein (e.g., an antibody or immunologically functional fragment thereof) being tested prevents or inhibits (e.g., reduces) specific binding of a reference antigen binding protein (e.g., a ligand or reference antibody) to a common antigen (e.g., PCSK9 or a fragment thereof)." ([0140]). According to these statements, it can be interpreted that "competing" with a reference antibody in the present invention means preventing or inhibiting (e.g., reducing) specific binding of the reference antibody by binding to a site on PCSK9 that is the same as or overlaps with a site where the reference antibody binds to PCSK9 and also means preventing or inhibiting (e.g., reducing) specific binding of the reference antibody by sterically interfering with binding between the reference antibody and PCSK9, which is evaluated as "competing" between antibodies if the fact that an antibody prevents or inhibits (e.g., reduces) specific binding of the reference antibody to PCSK9 is measured by an assay. In the present invention, the degree of "competing" is not specified.

Thus, it can be acknowledged that the monoclonal antibody of the present invention, which competes with the reference antibody, prevents or inhibits (e.g., reduces) specific binding of the reference antibody in various degrees and is not necessarily limited to a monoclonal antibody having a property of preventing or inhibiting (e.g., reducing) specific binding of the reference antibody by binding to the same site on PCSK9 as a site where the reference antibody binds to PCSK9, but also includes a monoclonal antibody having a property of preventing or inhibiting (e.g., reducing) specific binding of the reference antibody by binding to a site that overlaps with a site on PCSK9 where the reference antibody binds to PCSK9, and also includes a monoclonal antibody having a property of preventing or inhibiting (e.g., reducing) specific binding of the reference antibody to PCSK9 by binding to PCSK9 in a manner that sterically interferes with binding between the reference antibody and PCSK9.

(4) A. Next, when the statement of the present description is further specifically examined, there are the following statements in addition to the statements mentioned in 1(1)A above:

(A) In order to produce antibodies to PCSK9, two groups of XenoMouse (R) mice were used to prepare immunized mice according to the schedule of the

immunization program in Table 3. Then, mice (10 mice) that produced antibodies specific to PCSK9 were selected, and splenocytes and lymphocytes were isolated from the spleens and lymph nodes (Example 1, [0312] to [0314], [0320], [0321]).

(B) B cells were dissociated from the lymphoid tissue of the mice selected in (A) and mixed with non-secretory myeloma P3X63Ag8.653 cells. Then, through procedures such as centrifugal sedimentation of the fused cells, hybridomas that produce antigen binding proteins to PCSK9 were produced (Example 2, [0322] to [0324]).

(C) The primary screening by ELISA using biotinylated-PCSK9 without a V5 tag bound to a NeutrAvidin-coated plate as a capture sample was performed, thereby obtaining a total of 3104 antigen (wild-type PCSK9) specific hybridomas (Example 3, [0325] to [0328]).

In order to confirm that stable hybridomas were established, a total of 3000 positives mentioned above were rescreened for binding to wild-type PCSK9, and a total of 2441 positives were repeated in the second screening (confirmatory screening). Then, in order to confirm that the antibodies can bind to both human and mouse, "mouse cross-reaction screening" was performed to confirm that 579 antibodies cross-reacted with mouse PCSK9 ([0329], [0330]).

(D) The hybridoma exhaust supernatant and biotinylated D374Y PCSK9 mutant having a high binding affinity to LDLR were transferred to a plate bound to LDLR as a capture sample. Screening to detect the LDLR-bound biotinylated D374Y mutant using streptavidin HRP (large-scale receptor ligand blocking screening) was performed to identify 384 antibodies which blocked the interaction between PCSK9 and the LDLR wells, among which it was confirmed that 100 antibodies inhibited the binding interaction between PCSK9 and LDLR by more than 90% ([0332]).

Then, the receptor ligand assay was repeated using the D374Y mutant on 384 neutralizing substances identified in the first large-scale receptor ligand inhibition assay, which identified 85 antibodies that blocked the interaction between the D374Y mutant and LDLR by more than 90% ([0333], [0334]).

(E) 31H4 antibody (reference antibody) produced from the hybridoma that produces an antibody having the desired interaction with PCSK9 as identified on the basis of these assays (screenings) is a neutralizing antibody which strongly blocks binding between PCSK9 and LDLR (Example 11, [0377] to [0379], [0138], Table 2).

(F) The EGFa domain of LDLR binds to the catalytic domain of PCSK9. PCSK9 amino acid residues that are present within 5 angstroms of the EGFa domain in the crystal structure are specific core PCSK9 amino acid residues of the interaction

interface with the EGFa domain of LDLR (core residues). PCSK9 residues that are present at 5 angstroms to 8 angstroms of the EGFa domain are boundary PCSK9 amino acid residues of the interaction interface with the EGFa domain of LDLR. An antibody which interacts with or blocks any of these amino acid residues can be useful as an antibody which inhibits the interaction between PCSK9 and the EGFa domain of LDLR (and/or LDLR in general) (Example 28, [0428] to [0432], Figure 17).

(G) As illustrated in Figure 19B and Figure 19A, 31H4 antibody binds to the catalytic domain of PCSK9, has a binding site different from that of 21B12 antibody, and binds to PCSK9 simultaneously with 21B12 antibody. PCSK9 amino acid residues that are present within 5 angstroms of 31H4 antibody in the crystal structure are specific core PCSK9 amino acid residues of the interaction interface with 31H4 antibody (core residues). An antibody which interacts with or blocks any residue of the specific core PCSK9 amino acid residues of the interaction interface with the EGFa domain of LDLR (core residues) or boundary PCSK9 amino acid residues of the interaction interface with the EGFa domain of LDLR can be useful for inhibition of the PCSK9/LDLR interaction (Example 29, [0433], [0434], [0437], Figure 18B, Example 30, [0438] to [0440], [0443], Figure 19A).

The result by superimposing the structure of the ternary complex (PCSK9/31H4/21B12) (Figure 19A) obtained from Example 30 on the PCSK9/EGFa domain structure (Example 28, Figure 17A) is Figure 20A, in which both 21B12 antibody and 31H4 antibody partially overlap with the position of the EGFa domain of LDLR and sterically interfere with its binding to PCSK9 (Example 31, [0444], Figure 20A). Fifteen specific core PCSK9 amino acid residues of the interaction interface with the EGFa domain of LDLR (core residues) and 32 specific core PCSK9 amino acid residues of the interaction interface with 31H4 antibody (core residues) have three amino acid residues in common. Further, the residues that are involved in binding to the EGFa domain and are close to the regions to which the antigen binding protein (21B12 antibody or 31H4 antibody) binds can be useful for manipulating the binding of PCSK9 to LDLR ([0446], [0447], Table 12).

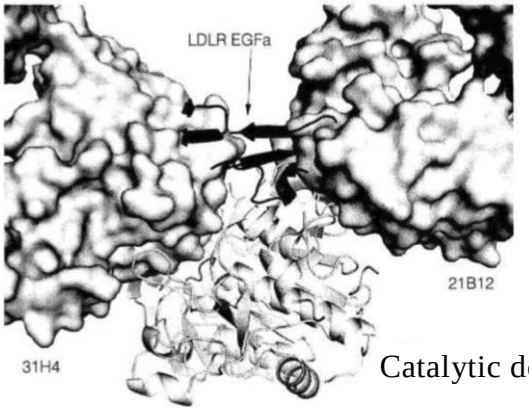


FIG. 20A

(H) An ELISA plate was coated with antibodies containing a first antibody (each 2 μg/mL) and blocked with 3% bovine serum albumin (BSA), and biotinylated human PCSK9 (30 ng/mL) together with a second antibody was applied on the ELISA plate, which was washed. Then, antibodies having the same binding property were grouped into the same epitope bin. The results are shown in Table 8.3, in which 21B12 antibody was grouped into bin 1 and 31H4 antibody was grouped into bin 3 (Example 10, [0373], [0374], Table 8.3).

Then, bead codes coated with human IgG capture antibody were washed three times with phosphate buffered saline plus 1% BSA (PBSA), and 2 μg/mL anti-PCSK9 antibody was added, which was washed three times with PBSA. Then, 2 μg/mL PCSK9 was added and 2 μg/mL anti-PCSK9 antibody was further added to perform binning and identify competing antibodies, the results of which are as shown in Table 37.1. Bin 1 (competing with 21B12 antibody) and bin 3 (competing with 31H4 antibody) are exclusive of each other, bin 2 competes with bin 1 and bin 3, and bin 4 does not compete with bin 1 and bin 3. The antibodies in each of the bins are representative of different types of epitope positions on PCSK9, some of which overlap with each other (Example 37, [0489] to [0495], Table 37.1, Figures 23A to D).

Table 37.1

Bin 1	Bin 2	Bin 3	Bin 4	Bin 5
01A12.2	27B2.1	16F12.1	11G1.5	30A4.1
03B6.1	27B2.5	22E2.1	03C4.1	13B5.1
09C9.1	12H11.1	27A6.1		13H1.1
17C2.1		28B12.1		31A4.1
21B12.2		28D6.1		31B12.1
23G1.1		31G11.1		
25G4.1		31H4.1		
26E10.1		08A1.2		
11H4.1		08A3.1		
11H8.1		11F1.1		
19H9.2				
26H5.1				
27E7.1				
27H5.1				
30B9.1				
02B5.1				
23B5.1				
27B2.6				
09H6.1				

(I) Figure 27D illustrates the 12H11 epitope hits mapped onto the crystal structure of PCSK9 with 31H4 and 21B12 antibodies. 12H11 competes with 21B12 antibody and 31H4 antibody in the binning assay ([0523], [0526], Figure 27D).

(J) Single bolus injections of either 10 mg/kg or 30 mg/kg of 21B12 antibody and 31H4 antibody were performed to gene recombinant mice that express human PCSK9. As a result, both 31H4 and 21B12 showed significant LDL-cholesterol lowering up to 48 hours (including 48 hours) after administration, as compared to control mice (Example 26, [0422], [0423], Figure 14A, Figure 14B).

B. (A) According to the disclosures of the present description as summarily excerpted in A above, the following matters are disclosed: [i] 3104 hybridomas that produce antibodies specific to PCSK9 were obtained, and the assay (screening) of antibodies produced by these hybridomas was performed for the binding interaction between PCSK9 and LDLR protein, which identified antibodies having activity to neutralize binding between PCSK9 and LDLR protein; [ii] From the identified hybridomas, 31H4 antibody was produced, and this antibody is a neutralizing antibody which strongly blocks binding between PCSK9 and LDLR protein; [iii] The binding region of the 21B12 antibody on PCSK9 was identified in the crystal structure, and a mechanism has been solved in which the 31H4 antibody partially overlaps the position of the EGFa domain of LDLR to sterically interfere with its binding to PCSK9; [iv] Amino acid residues that are involved in binding to the EGFa domain and that are present at a position on PCSK9 close to the regions to which the antigen binding protein (21B12 antibody or 31H4 antibody) binds can be useful for

manipulating the binding of PCSK9 to LDLR protein; [v] In the working examples, epitope binning was performed on antibodies confirmed to have high binding-neutralizing activity, and antibodies that belong to the group which competes with 31H4 antibody were grouped.

According to these disclosures, it can be deemed that a person ordinarily skilled in the art can understand that among antibodies having high neutralizing binding for binding between PCSK9 and LDLR protein, antibodies which compete with 31H4 antibody (reference antibody) are selected, and that 31H4 antibody partially overlaps with a position of EGFa domain of LDLR in the crystal structure to sterically interfere with its binding to PCSK9.

(B) Next, Table 37.1 of the present description is a table grouped on the basis of the data of Figures 23A to D. (It can be found that some of the clones stated in Table 8.3 are contained, but there are no statements of the original data for grouping in Table 8.3 in the Detailed Description of the Invention and the Drawings in the present description. A few unclear points on the relationship between the two can be found; that is, in relation to 27B2 designated as ND in Table 8.3, Table 37.1 shows that 27B2.6 is included in bin 1, and 27B2.1 and 27B2.5 are included in bin 2; in relation to 30A4 included in bin 1 in Table 8.3, Table 37.1 shows that 30A4.1 is included in bin 5; and other points.). Regarding 19 antibodies other than 31H4 antibody included in bin 3 of Table 37.1 that is stated as competing with 31H4 antibody, and for three antibodies in bin 2 that can be deemed to include an antibody which competes with 31H4 antibody in view of their competing with bin 3, the following matters are confirmed.

That is, each of 16F12.1, 22E2.1, 27A6.1, 28B12.1, 28D6.1, and 31G1.1 antibodies has high identity of amino acid sequence, but does not have high identity of amino acid sequence with 31H4 antibody. Each of 08A1.2, 08A.3.1, and 11F1.1 antibodies has high identity of amino acid sequence, but it is not stated that these antibodies are neutralizing antibodies. Regarding 12A11.1 antibody as well, it is not stated that this is a neutralizing antibody. Further, each of 27B2.1 and 27B2.5 antibodies is a non-neutralizing antibody ([0138]).

On the basis of these disclosures, as an antibody which competes with 31H4 antibody and which neutralizes binding between PCSK9 and LDLR protein, the Detailed Description of the Invention in the present description can be found to disclose a group of antibodies that differ from 31H4 antibody in the amino acid sequence and have high identity of amino acid sequences with each other.

(5) A. Examination based on the premises above will be conducted. As explained in

(2) above, it is reasonable to interpret that whether or not the statement of the scope of claims complies with the support requirement should be determined by comparing between the statement of the scope of claims and the statement of the Detailed Description of the Invention, then examining: whether or not the invention stated in the scope of claims is the invention stated in the Detailed Description of the Invention, and falls within the scope where a person ordinarily skilled in the art can recognize that the problem of the invention can be solved by the statement of the Detailed Description of the Invention; or whether or not, even if it is neither stated nor suggested in the Detailed Description of the Invention, the invention stated in the scope of claims falls within the scope where a person ordinarily skilled in the art can recognize that the problem of the invention can be solved in light of the common general technical knowledge at the time of filing the application. In this regard, as shown in 1(1) above, the present invention can be understood as follows: the present invention increases the amount of LDLR protein, thereby achieving an effect of causing a decrease in the amount of LDL in a subject and a decrease in serum cholesterol in the subject, and owing to this effect, diseases related to elevated cholesterol levels, such as hypercholesterolemia, can be treated or prevented, and the risk of such diseases can be reduced; and for this purpose, the present invention made its problem to provide an antibody which neutralizes binding between PCSK9 and LDLR protein, in which the PCSK9 binds to the LDLR protein to thereby decrease the amount of LDLR protein in a subject and increase the amount of LDL in the subject, or to provide a pharmaceutical composition comprising this; and the present invention reveals that an antibody which competes with the reference antibody that is a neutralizing antibody which strongly blocks binding between PCSK9 and LDLR protein is an isolated monoclonal antibody which prevents or inhibits binding of the reference antibody to PCSK9.

In addition, according to (3) above, the term "neutralize" in the present invention includes an aspect of altering a binding ability of PCSK9 to LDLR protein through indirect means (such as structural or energetic alterations in the ligand) in addition to interfering with, blocking, reducing, or modulating the interaction between PCSK9 and LDLR protein by directly blocking the protein binding site. However, as mentioned in 1(1) above, the reference antibody itself can be acknowledged as a neutralizing antibody which sterically interferes with binding between PCSK9 and LDLR protein and which strongly blocks the binding at a position which partially overlaps with a position of the EGFa domain of LDLR (this EGFa domain binds to a catalytic domain of PCSK9, and an antibody which interacts with or blocks any of

PCSK9 residues present within the region can be useful as an antibody which inhibits the interaction between PCSK9 and LDLR) in the crystal structure. On this basis, it should be deemed that the invention-specifying matter of "which competes for binding to PCSK9 with 31H4 antibody" in the present invention also has a technical significance in that it is revealed that an antibody which competes with the 31H4 antibody interferes with, blocks, reduces, or modulates the interaction between PCSK9 and LDLR protein by directly blocking the binding site of LDLR protein (specifically, by the antibody binding to PCSK9 at a position which overlaps with a position of the EGFa domain of LDLR in the crystal structure) by a mechanism similar to that of the 31H4 antibody. Conversely, it can also be deemed that precisely because an antibody which competes with the reference antibody binds at such a position, it makes neutralization possible. This point is also supported by the fact that the Defendant itself asserts in No. 3, 3(2)C above that according to the Detailed Description of the Invention in the present description, in light of the common general technical knowledge at the time of filing the application, it can be deemed that a person ordinarily skilled in the art understands that an antibody which binds to a specific position within a specific region among multiple binding surfaces on PCSK9 (a position that overlaps with the site which binds to the EGFa domain of LDLR (or a similar position)) by competing with the reference antibody can neutralize binding between PCSK9 and LDLR protein, and the person has been able to recognize that the problem of the invention can be solved throughout the entire technical scope of the invention.

In addition, according to the disclosures of the Exhibit Ko 1 document as found in 1(2) above, familial hypercholesterolemia results from elevated LDL cholesterol levels in plasma. In this regard, since PCSK9 reduces the abundance of LDLR protein present on cell surfaces, it can be found to have already been shown that PCSK9 is an attractive target for the treatment and that antibodies or the like which bind to PCSK9 in plasma and which inhibit its binding to LDLR protein can be effective inhibitors. Thus, from these points of view as well, the technical significance of the present invention can also be deemed to lie in the point that it has been identified that an antibody which competes with 31H4 antibody has a functional property as an antibody which inhibits the binding to LDLR protein as mentioned above by a mechanism similar to that of the 31H4 antibody; i.e., a binding-neutralizing antibody. Originally, as found in 1(1)B above, the problem of the present invention is to provide an antibody which neutralizes binding between PCSK9 and LDLR protein, in which the PCSK9 binds to the LDLR protein to thereby

decrease the amount of LDLR protein in a subject and increase the amount of LDL in the subject, or to provide a pharmaceutical composition comprising this. In a relationship between such a problem and its solution, competing itself with the reference antibody cannot be found to have any unique meaning. Thus, from these points of view as well, as mentioned above, the technical significance of the present invention should be deemed to lie in the point that it has been identified that an antibody which competes with 31H4 antibody has a functional property as a binding-neutralizing antibody by the mechanism similar to that of the 31H4 antibody.

B. Further examination will be made. As mentioned in (4)B(B) above, the Detailed Description of the Invention in the present description discloses that epitope binning was performed and as a result, a group of antibodies with amino acid sequences that cannot be deemed to have high identity with 31H4 antibody was identified as competing with the 31H4 antibody. Regarding an antibody stated as having a neutralizing activity among antibodies identified as an antibody which competes as mentioned above, the present description does not specifically state a position where the above antibody stated as having a neutralizing activity binds on PCSK9. In addition, regarding a group of antibodies that differs from 31H4 antibody in the amino acid sequence, knowledge that a position where such an antibody binds on PCSK9 is revealed by the fact that the antibody was evaluated as competing in an assay such as epitope binning cannot be acknowledged to be common general technical knowledge. Thus, the position where the above antibody binds on PCSK9 cannot be deemed to be apparent.

Further, it is obvious that an antibody having a property "which competes for binding to PCSK9 with a reference antibody" of the present invention encompasses a very wide variety of antibodies in addition to several groups of antibodies specifically stated in the Detailed Description of the Invention in the present description mentioned above. Furthermore, as mentioned in 2(3)B above, the antibody of the present invention encompasses not only an antibody which prevents or inhibits (e.g., reduces) specific binding of the reference antibody by binding to a site which overlaps with a site on PCSK9 where 31H4 antibody binds to PCSK9, but also an antibody which prevents or inhibits (e.g., reduces) specific binding of the reference antibody to PCSK9 in various degrees by binding to PCSK9 in a manner that sterically interferes with binding between the reference antibody and PCSK9, as the Defendant asserts. Then, the antibodies mentioned above can include, for example, an antibody which prevents or inhibits (e.g., reduces) specific binding of the 31H4 antibody to PCSK9 by binding to a site which differs from a site where the 31H4 antibody binds to PCSK9

and which differs from a position of EGFa domain of LDLR in the crystal structure and bringing minor steric hindrance to the 31H4 antibody. However, a site where such an antibody binds to PCSK9 is not a position where the antibody overlaps with a position of EGFa domain of LDLR in the crystal structure. Thus, such an antibody cannot be deemed to interfere with, block, reduce, or modulate the interaction between PCSK9 and LDLR protein by directly blocking the binding site of LDLR protein.

Incidentally, the present description states that "An antigen binding protein and fragment which compete with or bind to the same epitope as the exemplified antigen binding proteins are expected to show similar functional properties." ([0269]) However, as mentioned above, since the matter "which competes for binding to PCSK9 with 31H4 antibody" does not specify binding to PCSK9 at the same position as the 31H4 antibody, it cannot be deemed that an antibody which competes with 31H4 antibody is an antigen binding protein (antibody) which competes with or binds to the same epitope as the 31H4 antibody. Further, no specific explanations can be found on a mechanism supporting that such antibodies in general show functional properties similar to those of the 31H4 antibody. Therefore, it cannot be deemed that the "antibody which competes for binding to PCSK9 with 31H4 antibody" of the present invention "shows functional properties similar" to the 31H4 antibody.

As mentioned above, the technical significance of the present invention should be deemed to lie in the point that the present invention identifies that an antibody which competes with 31H4 antibody has a property as an antibody which neutralizes binding between PCSK9 and LDLR protein by the mechanism similar to that of the 31H4 antibody. In this regard, as mentioned above, it cannot be deemed that an antibody which competes with 31H4 antibody would not directly block the binding site between PCSK9 and LDLR protein by binding to the site where the antibody interacts with the EGFa domain of LDLR (from the statement of the present description, it can be understood as specific core PCSK9 amino acid residues of the interaction interface with the EGFa domain of LDLR (core residues), which are defined as PCSK9 residues present within 5 angstroms of the EGFa domain, and boundary PCSK9 amino acid residues of the interaction interface with the EGFa domain of LDLR, which are defined as PCSK9 residues present at 5 angstroms to 8 angstroms of the EGFa domain). Other than the above, there is no disclosure on the mechanism by which any antibody which competes with 31H4 antibody will be an antibody which inhibits the interaction (binding) between PCSK9 and the EGFa domain of LDLR (and/or LDLR in general). Therefore, it can only be deemed to be

difficult for a person ordinarily skilled in the art to arrive at the understanding that an antibody which competes with 31H4 antibody is a binding-neutralizing antibody.

C. As mentioned in the foregoing, it cannot be deemed that an "antibody which competes for binding to PCSK9 with 31H4 antibody" interferes with, blocks, reduces, or modulates the interaction between PCSK9 and LDLR protein by directly blocking the binding site of LDLR protein (specifically, by the antibody binding to PCSK9 at a position which overlaps with a position of EGFa domain of LDLR in the crystal structure) in a manner similar to 31H4 antibody. Thus, it also cannot be acknowledged that the "antibody which competes for binding to PCSK9 with 31H4 antibody" has a functional property as a binding-neutralizing antibody. Incidentally, as mentioned in (3)A above, the term "neutralize" in the present invention includes not only an aspect of directly blocking the binding site between PCSK9 and LDLR protein, but also an aspect of altering the binding ability of PCSK9 to LDLR protein through indirect means (such as structural or energetic alterations in the ligand). However, it cannot be deemed that it was the common general technical knowledge at the time of filing the present application that an "antibody which competes for binding to PCSK9 with 31H4 antibody" will be an antibody which alters the binding ability of PCSK9 to LDLR protein through the above indirect means, nor can it be deemed that it was disclosed in the Detailed Description of the Invention in the present description.

D. These points are supported by the results of the demonstration experiment by Dr. [A], the reliability of which has been acknowledged in 1(3) above, and Affidavit (1) by Dr. [B] based on the same demonstration experiment. That is, in this demonstration experiment, 63 antibodies of Regeneron were tested for competition with the reference antibody and their binding-neutralizing activity. As a result of using a threshold value of 50% for competition, it was confirmed that 34 antibodies competed with the reference antibody, among which 28 antibodies (more than 80%) had no binding-neutralizing activity (Material B1 of Attachment 3 and 1(3)A(B)b above). Thus, the specific experimental result demonstrates that it cannot be deemed that an antibody which competes with the reference antibody has a binding-neutralizing activity. Further, in addition to this experimental result, Dr. [B] states that "According to the present patent, this is because the binding site of 31H4 antibody only partially overlaps with the binding site of LDLR on hPCSK9 ... a binding site of another antibody can overlap with the 31H4 binding site without overlapping with the binding site of LDLR, and in this manner, another antibody can overlap with the 31H4 binding site without overlapping with the hPCSK9-LDLR binding site" (1(3)A(B)b above) and provides an opinion that "it is certainly erroneous

to state that 'all of ... antibodies which compete with 31H4 antibody would have an effect of neutralizing binding.'" (1(3)A(B)c above).

E. The Defendant asserts in No. 3, 3(2)C above that there is no reason why the present invention violates the support requirement, on the grounds that even if there exists an antibody which competes with 31H4 antibody (a reference antibody) but which cannot neutralize binding between PCSK9 and LDLR protein, such an antibody is literally excluded from the technical scope of Present Invention 1. However, as already explained, a technical significance of the present invention should be deemed to lie in the point that it has been identified that an antibody which competes with 31H4 antibody has a functional property as an antibody which neutralizes binding between PCSK9 and LDLR protein by a mechanism similar to that of the 31H4 antibody. If an antibody which competes with 31H4 antibody includes one which does not have a binding-neutralizing activity, it is apparent that the assumption of its technical significance will collapse. (In an instance like the present case, if it were interpreted to be sufficient to literally exclude an antibody that does not have a binding-neutralizing activity, it would be allowed to make a very broad definition of a position where the antibody binds to PCSK9, such as the most part of PCSK9, which would allow the scope of claims to be made broad without a justifiable basis. Therefore, such an interpretation is not reasonable.) In addition, even if it is interpreted that the scope of claims of Present Invention 1 is, as asserted by the Defendant, directed to only an antibody which "can neutralize binding between PCSK9 and LDLR protein" among antibodies which compete for binding to PCSK9 with a reference antibody, the invention-specifying matter of that which competes for binding to PCSK9 with a reference antibody according to the present invention is not limited to an antibody which binds to a position that is the same as or overlaps with a position where the reference antibody binds as asserted by the Defendant, but also includes an antibody which competes in a manner that binds to a position for steric interference with the binding between PCSK9 and LDLR protein to occur, as explained above. Thus, it must be supported that such an antibody is also a binding-neutralizing antibody. In this regard, unlike the case of an antibody which binds to a position that is the same as or overlaps with a position where the reference antibody binds, the present description does not state anything about a mechanism by which an antibody neutralizes the binding between PCSK9 and LDLR protein in which the antibody competes in a manner that binds to a position for steric interference with the binding to occur. In addition, binding-neutralizing antibodies based on experimental results by binning ((4)B(B) above) are all likely to be antibodies which bind to a

position that is the same as or overlaps with a position where the reference antibody binds, whose mechanism on binding-neutralizing is disclosed. Even if this point is excluded, at least, the present description does not state anything to suggest that these are sterically interfering antibodies. Thus, it must be deemed that the Detailed Description of the Invention in the present description does not disclose anything about the fact that among antibodies which compete with a reference antibody, when an antibody competes in a manner that binds to a position for steric interference with the binding between PCSK9 and LDLR protein to occur, the antibody has a binding-neutralizing activity. From this point as well, the present invention does not comply with the support requirement.

Further, as mentioned in No. 2, 3(1) above, the trial decision of the present case determines that the present description specifically demonstrates that a number of antibodies of the present invention are repeatedly identified with sufficiently high probability by performing the preparation and selection of immunized mice according to the procedure and schedule of the immunization program as stated in the present description, the production of hybridomas using the selected immunized mice, and the screening and epitope binning assay for identifying an antibody which strongly blocks the binding interaction between PCSK9 and LDLR as stated in the present description from the beginning, repeatedly. However, as the second Expert Opinion by Professor [F]([F]) (referred to as Professor [F]) (Exhibit Ko 230) states that "It is impossible to generate and screen all possible candidate antibodies, because whether a particular mouse generates a particular antibody is controlled by luck," even if the production process of antibodies stated in the present description has been undergone, it is "controlled by luck" what position on PCSK9 an antibody obtained in an immunized mouse will bind to. Also, it cannot be deemed that a method of producing an antibody which binds to an antigen protein in a manner that sterically interferes with binding of the antibody to the antigen protein was common general technical knowledge at the time of filing the present application. Therefore, on the basis of the statement on a method of producing an antibody as stated in the present description, it cannot be deemed that various antibodies encompassed in the present invention were stated in the Detailed Description of the Invention in the present description.

F. Furthermore, Present Invention 5, which is an invention relating to a pharmaceutical composition comprising the monoclonal antibody of Present Invention 1, also does not comply with the support requirement, for the same reasons as mentioned above.

(6) According to the foregoing, since neither of Present Inventions 1 and 5 can be acknowledged to comply with the support requirement, the determination of the trial decision of the present case which differs from this is erroneous. (Note that among the Plaintiff's assertions, it is considered that the point concerning "EGFa mimic antibodies" mentioned in No. 3, 3(1)B(C) above contains something worth affirming and raises doubts about the Defendant's assertion that the support requirement is complied with. However, without making a determination on this point, neither of Present Inventions 1 and 5 can be acknowledged to comply with the support requirement, as mentioned above. Therefore, the court shall refrain from adding further determinations.)

(7) Hereinafter, additional remarks will be made just to be sure.

A. With regard to the international situation surrounding the present invention, the Plaintiff asserts that in Europe, the corresponding European patent, which is substantially the same as the present invention, was judged to be invalid for lack of an inventive step in 2020 in the court of appeal against opposition, and that in the U.S., the corresponding U.S. patent, which is more limited than the present invention, was judged to be invalid for lack of the enablement requirement on February 11, 2021 in the Court of Appeals for the Federal Circuit of the United States, and that Japan is currently the only country in the world where the validity of the present patent has been maintained by the court. On the other hand, with regard to the above judgment by the Court of Appeals for the Federal Circuit, the Defendant asserts that since the Federal Supreme Court granted the petition for acceptance of discretionary appeal on November 4, 2022, the above judgment is extremely likely to be overturned. However, needless to say, it is apparent that the judgments in other countries do not immediately affect the judgment in the present case (Note that with regard to the U.S., even if the judgment of invalidation by the Court of Appeals for the Federal Circuit is overturned, the corresponding U.S. patent is not directly relevant to the judgment on the present invention in either case, because it can be found that the matter "competition" with the reference antibody is not defined as an invention-specifying matter (For example, Claim 1 of the U.S. Patent No. 8829165 recites the invention-specifying matter that is "An isolated monoclonal antibody, which, when binding to PCSK9, binds to at least one of the following residues: S153, I154, P155, R194, D238, A239, I369, S372, D374, C375, T377, C378, F379, V380, or S381 of SEQ ID NO: 3, and inhibits PCSK9 from binding to LDLR" (Exhibit Ko 19).).).

B. In the suit against the trial decision of the separate case concerning the present invention, as mentioned in No. 2, 1(2) above, Sanofi's assertion on violation of the

support requirement has been rejected. However, this can also be understood to be due to the fact that, in view of the circumstances of the assertions and proof at the time, it was naturally premised that an antibody which competes with 31H4 antibody would bind to almost the same position on PCSK9 as the 31H4 antibody and have a function similar to that of the 31H4 antibody. In contrast thereto, in the present suit, although doubts about the above premise have been raised by new assertions based on new evidence such as the structural analysis according to each of the Affidavits by Dr. [A] and Dr. [B], the Expert Opinion by Professor [F], etc. (Exhibits Ko 18, 230), and the relevant documentary evidence concerning "EGFa mimic antibodies" (Exhibits Ko 4-1 and 4-2), no information for determination to support this premise can be found. Therefore, there should be a reasonable reason why the conclusion of the judgment in the separate case differs from the determination in the present case.

3. Conclusion

According to the foregoing, Ground 2 for Rescission asserted by the Plaintiff is well founded. Therefore, without going so far as to determine other grounds for rescission, the trial decision of the present case should be rescinded.

For the foregoing reasons, the judgment is rendered as mentioned in the main text.

Intellectual Property High Court, Fourth Division

Presiding Judge	KANNO Masayuki
Judge	NAKAMURA Kyo
Judge	OKAYAMA Tadahiro

(Attachment 1)

[Detailed Description of the Invention]

[Technical Field]

[0002]

Field of the Invention

The present invention relates to an antigen binding protein which binds to proprotein convertase subtilisin kexin type 9 (PCSK9) and to a method of using and producing said antigen binding protein.

[Background Art]

[0003]

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a serine protease involved in regulating the levels of low-density lipoprotein receptor (LDLR) protein (Horton et al., 2007; Seidah and Prat, 2007). In vitro experiments have shown that adding PCSK9 to HepG2 cells lowers the levels of cell-surface LDLR (Benjannet et al., 2004; Lagace et al., 2006; Maxwell et al., 2005; Park et al., 2004). Experiments with mice have shown that increasing PCSK9 protein levels lowers the levels of the LDLR protein in the liver (Benjannet et al., 2004; Lagace et al., 2006; Maxwell et al., 2005; Park et al., 2004), while PCSK9 knockout mice have increased levels of LDLR in the liver (Rashid et al., 2005). Additionally, various human PCSK9 mutations that result in either increased or decreased levels of plasma LDL have been identified (Kotowski et al., 2006; Zhao et al., 2006). PCSK9 has been shown to directly interact with the LDLR protein, be endocytosed into cells along with the LDLR, and emit immunofluorescence simultaneously with the LDLR throughout the endosomal pathway (Lagace et al., 2006). Degradation of LDLR by PCSK9 has not been observed, and the mechanism through which extracellular LDLR protein levels are lowered is uncertain.

[Description of Embodiments]

[0066]

As will be appreciated by a person ordinarily skilled in the art, in light of the present disclosure, altering the interaction between PCSK9 and LDLR increases the amount of LDLR available for binding to LDL, which in turn decreases the amount of serum LDL in a subject, resulting in a reduction in the subject's serum cholesterol level. Hence, antigen binding proteins to PCSK9 can be used in various methods and compositions for treating a subject with elevated serum cholesterol levels, a subject at risk for elevated serum cholesterol levels, or a subject that could benefit from a reduction in serum cholesterol levels. Thus, various methods and techniques

for lowering, maintaining, or preventing an increase in serum cholesterol are also described in the present description. In some embodiments, the antigen binding protein allows for binding between PCSK9 and LDLR, but the antigen binding protein prevents or reduces the adverse activity of PCSK9 on LDLR. In some embodiments, the antigen binding protein prevents or reduces the binding of PCSK9 to LDLR.
[0071]

The term "PCSK9 activity" includes any biological effect of PCSK9. In certain embodiments, PCSK9 activity includes the ability of PCSK9 to interact with a substrate or receptor or to bind to a substrate or receptor. In some embodiments, PCSK9 activity is represented by the ability of PCSK9 to bind to an LDL receptor (LDLR). In some embodiments, PCSK9 binds to and catalyzes a reaction including LDLR. In some embodiments, PCSK9 activity includes the ability of PCSK9 to alter (e.g., reduce) the availability of LDLR. In some embodiments, PCSK9 activity includes the ability of PCSK9 to increase the amount of LDL in a subject. In some embodiments, PCSK9 activity includes the ability of PCSK9 to decrease the amount of LDLR available for binding to LDL. In some embodiments, "PCSK9 activity" includes any biological activity resulting from PCSK9 signaling. Exemplary activities include, but are not limited to, binding of PCSK9 to LDLR, PCSK9 enzyme activity which cleaves LDLR or other proteins....
[0109]

An "antigen binding protein" ("ABP") as used in the present description means any protein which binds a specified target antigen. In the present application, the specified target antigen is the PCSK9 protein or a fragment thereof. The "antigen binding protein" includes, but is not limited to, an antibody and a binding part thereof (such as an immunologically functional fragment). A peptibody is another example of antigen binding proteins. The term "immunologically functional fragment" (or simply "fragment") of an antibody or immunoglobulin chain (heavy chain or light chain) antigen binding protein as used in the present description is a species of antigen binding protein comprising a portion (regardless of how said portion is obtained or synthesized) of an antibody that lacks at least some of the amino acids present in a full-length chain but which can still bind specifically to the antigen. Such a fragment is biologically active, in that the fragment binds to the target antigen and can compete with other antigen binding proteins, including intact antibodies, for binding to a certain epitope. In some embodiments, the fragment is a neutralizing fragment. In some embodiments, the fragment can block or reduce the possibility of the interaction between LDLR and PCSK9. In one aspect, such a fragment retains at

least one CDR present in the full-length light chain or heavy chain and, in some embodiments, comprises a single heavy chain and/or light chain or a portion thereof....
[0123]

An "antigen binding region" means a protein or a portion of a protein which specifically binds to a particular antigen (e.g., a paratope). For example, that portion of an antigen binding protein comprising amino acid residues which interact with an antigen and give the antigen binding protein its specificity and affinity for the antigen is referred to as the "antigen binding region." The antigen binding region generally includes one or more "complementary binding regions" ("CDRs"). A certain antigen binding region also includes one or more "framework" regions. The "CDR" is an amino acid sequence that contributes to antigen binding specificity and affinity. The "framework" region can aid in maintaining the proper conformation of the CDR to promote binding between an antigen binding region and an antigen. Structurally, the framework region can be located between CDRs in an antibody. Examples of framework and CDR regions are shown in Figures 2A to 3D, 3CCC to JJJ, and 15A to 15D....
[0127]

The variable region typically exhibits the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions (also referred to as complementarity determining regions or CDRs). The CDR obtained from the two chains of each pair is generally aligned by the framework regions, which can enable binding to a specific epitope. From N-terminal to C-terminal, both light-chain and heavy-chain variable regions generally include domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The assignment of amino acids to each domain is generally in accordance with the definition of Kabat sequences of proteins of immunological interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or "Chothia & Lesk, J. Mol. Biol., 196: 901-917 (1987); Chothia et al., Nature, 342: 878-883 (1989)".
[0132]

The term "light chain" includes a full-length light chain and a fragment thereof having a variable region sequence sufficient to give binding specificity. The full-length light chain includes a variable region domain V_L and a constant region domain C_L. The variable region domain of the light chain is located at the amino-terminus of a polypeptide. The light chain includes a κ chain and a λ chain.
[0133]

The term "heavy chain" includes a full-length heavy chain and a fragment

thereof having a variable region sequence sufficient to give binding specificity. The full-length heavy chain includes a variable region domain V_H and three constant region domains C_H1, C_H2, and C_H3. The V_H domain is located at the amino-terminus of a polypeptide, and the C_H domains are located at the carboxyl-terminus, in which the C_H3 is closest to the carboxy-terminus of the polypeptide. The heavy chain can be any isotype, such as IgG (including IgG1, IgG2, IgG3, and IgG4 subtypes), IgA (including IgA1 and IgA2 subtypes), IgM, and IgE.

[0138]

The term "neutralizing antigen binding protein" or "neutralizing antibody" represents an antigen binding protein or antibody, respectively, which binds to a ligand and prevents or reduces a biological effect of that ligand. This can be done, for example, by directly blocking a binding site on the ligand or by binding to the ligand to alter the binding ability of the ligand through indirect means (such as structural or energetic alterations in the ligand). In some embodiments, this term can also represent an antigen binding protein that prevents a protein to which it binds from exerting a biological function. In assessing the binding and/or specificity of an antigen binding protein (e.g., an antibody or immunologically functional fragment thereof), when an excess of antibody reduces the amount of binding partner bound to a ligand by at least about 1 to 20%, 20 to 30%, 30 to 40%, 40 to 50%, 50 to 60%, 60 to 70%, 70 to 80%, 80 to 85%, 85 to 90%, 90 to 95%, 95 to 97%, 97 to 98%, 98 to 99%, or more (when used in an in vitro competitive binding assay), an antibody or fragment can substantially inhibit binding of the ligand to its binding partner.... In some embodiments, in the case of PCSK9 antigen binding protein, such neutralizing molecules can reduce the ability of PCSK9 to bind LDLR. In some embodiments, the neutralizing ability is characterized and/or described via a competition assay.... In some embodiments, ABP27B2, 13H1, 13B5, and 3C4 are non-neutralizing ABPs, 3B6, 9C9, and 31A4 are weak neutralizing substances, and the remaining ABPs in Table 2 are strong neutralizing substances. In some embodiments, the antibody or antigen binding protein neutralizes by binding to PCSK9 and preventing PCSK9 from binding to LDLR (or reducing the ability of PCSK9 to bind to LDLR). In some embodiments, the antibody or ABP neutralizes by binding to PCSK9 and while allowing the PCSK9 to bind to LDLR, preventing or reducing PCSK9-mediated degradation of the LDLR....

[0140]

The term "compete" when used in the context of an antigen binding protein (e.g., a neutralizing antigen binding protein or neutralizing antibody) which competes

for the same epitope means competition between antigen binding proteins as measured by an assay in which the antigen binding protein (e.g., an antibody or immunologically functional fragment thereof) being tested prevents or inhibits (e.g., reduces) specific binding of a reference antigen binding protein (e.g., a ligand or reference antibody) to a common antigen (e.g., PCSK9 or a fragment thereof). In order to determine if one antigen binding protein competes with another binding protein, there can be used numerous types of competitive binding assays, e.g., solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see, e.g., Stahli et al., 1983, Methods in Enzymology 9: 242-253); solid phase direct biotin-avidin EIA (see, e.g., Kirkland et al., 1986, J. Immunol. 137: 3614-3619) ... solid phase direct biotin-avidin EIA (see, e.g., Cheung, et al., 1990, Virology 176: 546-552). Typically, such an assay includes using a purified antigen bound to a solid surface or cell having any of these, an unlabeled test antigen binding protein, and a labeled reference antigen binding protein. Competitive inhibition is determined by measuring the amount of label bound to the solid surface or cell in the presence of the test antigen binding protein. Usually, the test antigen binding protein is present in excess. Antigen binding proteins identified by the competition assay (competing antigen binding proteins) include an antigen binding protein which binds to the same epitope as a reference antigen binding protein, and an antigen binding protein which binds to an adjacent epitope sufficiently close to an epitope bound by the reference antigen binding protein for steric interference to occur....

[0142]

The term "epitope" includes any determinant which can be bound by an antigen binding protein such as an antibody or T-cell receptor. An epitope is a region of an antigen bound by an antigen binding protein which targets the antigen, and when the antigen is a protein, the epitope includes a particular amino acid that directly contacts the antigen binding protein. Most often, the epitope is present on a protein. However, in some instances, the epitope can be present on other kinds of molecules such as nucleic acids. Epitope determinants can include chemically active surface groups of molecules such as amino acids, sugar side chains, a phosphoryl or sulfonyl group, and can have specific three-dimensional structural characteristics and/or specific charge characteristics. Generally, an antibody specific to a particular target antigen will preferentially recognize an epitope on a target antigen in a complex mixture of proteins and/or macromolecules.

[0154]

Antigen Binding Proteins to PCSK9

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a serine protease involved in regulating the levels of low-density lipoprotein receptor (LDLR) protein (Horton et al., 2007; Seidah and Prat, 2007). PCSK9 is a prohormone-proprotein convertase in the subtilisin (S8) family of serine proteases (Seidah et al., 2003).... The structure of the PCSK9 protein has recently been solved by two groups.... PCSK9 comprises a signal sequence, an N-terminal prodomain, a subtilisin-like catalytic domain, and a C-terminal domain.

[0155]

Antigen binding proteins (ABPs) which bind PCSK9, including human PCSK9, are described in the present description. In some embodiments, the antigen binding protein provided is a polypeptide comprising one or more complementary determining regions (CDRs), as described in the present description. In the same antigen binding protein, the CDR is embedded into a "framework" region that orients the CDR such that the proper antigen binding property of the CDR is achieved. In some embodiments, the antigen binding protein provided in the present description can interfere with, block, reduce, or modulate the interaction between PCSK9 and LDLR. Such an antigen binding protein is denoted as "neutralizing." In some embodiments, binding between PCSK9 and LDLR can still occur even though the antigen binding protein is neutralizing and bound to PCSK9. For example, in some embodiments, the ABP prevents or reduces the adverse influence of PCSK9 on LDLR without blocking the LDLR binding site on PCSK9. Thus, in some embodiments, the ABP modulates or alters the ability of PCSK9 to cause degradation of LDLR without having to prevent the binding interaction between PCSK9 and LDLR. Such an ABP can be specifically denoted as a "non-competitively neutralizing" ABP. In some embodiments, the neutralizing ABP binds to PCSK9 in a position and/or manner that prevents PCSK9 from binding to LDLR. Such an ABP can be specifically described as a "competitively neutralizing" ABP. Both of the above neutralizing substances can result in a greater amount of free LDLR present in a subject, which results in more LDLR bound to LDL (thereby reducing the amount of LDL in a subject). In turn, this results in a reduction in the amount of serum cholesterol present in a subject.

[0170]

Some specific examples of the light-chain and heavy-chain variable regions of the antibodies provided and their corresponding amino acid sequences are summarized in Table 2.

[0171]

[Table 2]

Table 2
Exemplary Heavy-Chain and Light-Chain Variable Regions

Antibody	Light/Heavy SEQ ID NO
30A4	5/74
3C4	7/85
23B5	9/71
25G4	10/72
31H4	12/67
27B2	13/87
25A7	15/58
27H5	16/52
26H5	17/51
31D1	18/53
20D10	19/48
27E7	20/54
30B9	21/55
19H9	22/56
26E10	23/49
21B12	23/49
17C2	24/57
23G1	26/50
13H1	28/91
9C9	30/64
9H6	31/62
31A4	32/89
1A12	33/65
16F12	35/79
22E2	36/80
27A6	37/76
28B12	38/77
28D6	39/78
31G11	40/83
13B5	42/69
31B12	44/81
3B6	46/60

[0172]

Likewise, each of the exemplary variable heavy chains listed in Table 2 can be combined with any of the exemplary variable light chains shown in Table 2 to form an antibody. Table 2 shows exemplary pairs of light chains and heavy chains found in some of the antibodies disclosed in the present description....

[0262]

... In some embodiments, the ABP competes with ABP 31H4.

[0268]

In some embodiments, ABP 21B12 binds to an epitope comprising residues 162 to 167 (e.g., residues D162 to E167 of SEQ ID NO: 1)....

[0269]

Competing Antigen Binding Proteins

In another aspect, for specific binding to PCSK9, there are provided antigen binding proteins which compete with one of the exemplified antibodies or functional fragments binding to the epitope described in the present description. Such antigen binding proteins can also bind to the epitope that is the same as or overlaps with one

of the antigen binding proteins exemplified in the present description. An antigen binding protein and fragment which competes with or binds to the same epitope as the exemplified antigen binding proteins are expected to show similar functional properties. The exemplified antigen binding proteins and fragments include those described above, such as those having heavy-chain and light-chain variable region domains, and CDRs included in Table 2 and/or Figures 2 to 3 and 15. Thus, as specific examples, the antigen binding proteins provided include those which compete with an antibody or antigen binding protein having:

(a) all 6 of the CDRs listed for antibodies listed in Figures 2 to 3 and 15;

(b) VH and VL listed for antibodies listed in Table 2; or

(c) two light chains and two heavy chains as specified for antibodies listed in Table 2.

[0270]

Certain Therapeutic Uses and Pharmaceutical Compositions

In certain instances, PCSK9 activity correlates with a number of human disease conditions. For example, in certain instances, too high or too low PCSK9 activity correlates with certain conditions such as hypercholesterolemia. Thus, in certain instances, modulating PCSK9 activity can be therapeutically useful. In certain embodiments, a neutralizing antigen binding protein to PCSK9 is used to modulate at least one PCSK9 activity (e.g., binding to LDLR). Such a method can treat and/or prevent diseases that relate to elevated serum cholesterol levels or to which elevated cholesterol levels relate, and/or reduce the risk of these diseases.

[0271]

As will be appreciated by a person ordinarily skilled in the art, in light of the present disclosure, diseases that relate to varied cholesterol, LDL, or LDLR levels, involve varied cholesterol, LDL, or LDLR levels, or can be influenced by varied cholesterol, LDL, or LDLR levels can be addressed by various embodiments of the antigen binding proteins. In some embodiments, "cholesterol related diseases" (including "serum cholesterol related diseases") include any one or more of those that can be manifested, for example, by elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated VLDL, and/or low HDL, as follows: hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular disease, Alzheimer's disease, and dyslipidemia in general....

[0276]

In some embodiments, an antigen binding protein to PCSK9 is used to decrease the amount of PCSK9 activity from an abnormally high level or a normal level. In

some embodiments, an antigen binding protein to PCSK9 is used to treat or prevent hypercholesterolemia and/or in the preparation of medicaments for hypercholesterolemia and/or for other cholesterol related diseases (such as those described in the present description). In certain embodiments, an antigen binding protein to PCSK9 is used to treat or prevent conditions such as hypercholesterolemia in which PCSK9 activity is normal. In such conditions, for example, a reduction in PCSK9 activity to below normal can provide a therapeutic effect.

[0312]

(Example 1)

Immunization and Titer Measurement

Production of Anti-PCSK9 Antibodies and Hybridomas

Antibodies to the mature form of PCSK9 (illustrated as the sequence in Figure 1A, where the prodomain is underlined) were produced in XenoMouse(R) mice (Abgenix, Fremont, CA) that are mice containing human immunoglobulin genes. Two groups of XenoMouse(R) mice (Groups 1 and 2) were used to produce antibodies to PCSK9. Group 1 included mice of XenoMouse(R) strain XMG2-KL that produce fully human $IgG2_\kappa$ and $IgG2_\lambda$ antibodies. Group 1 mice were immunized with human PCSK9. PCSK9 was prepared by use of standard recombinant techniques using the GenBank sequence as reference (NM_174936). Group 2 included mice of XenoMouse(R) strain XMG4-KL that produce fully human $IgG4_\kappa$ and $IgG4_\lambda$ antibodies. Group 2 mice were also immunized with human PCSK9.

[0313]

The mice of both groups were injected with the antigen 11 times according to the schedule in Table 3. In the first immunization, each mouse was injected with a total of 10 μg of the antigen delivered intraperitoneally into the abdomen. Subsequent boost immunizations were doses of 5 μg, and the injection method was staggered between intraperitoneal injections into the abdomen and subcutaneous injections at the base of the tail. For intraperitoneal injections, the antigen was prepared as an emulsion with TiterMax(R) Gold (Sigma, Cat #T2684) added. For subcutaneous injections, the antigen was mixed with Alum (aluminum phosphate) and CpG oligos. In injections 2 to 8 and 10, each mouse was injected with a total of 5 μg of the antigen in the adjuvant alum gel. A final injection of 5 μg of the antigen per mouse was delivered in phosphonate-buffered saline and delivered into two sites (50% intraperitoneal into the abdomen and 50% subcutaneous at the base of the tail). The immunization program is summarized in Table 3 shown below.

[0314]

[Table 3]

Table 3

Mouse strain	XMG2/kl	XMG4/kl
Number of animals	10	10
Immunogen	PCSK9-V5/His	PCSK9-V5/His
First boost immunization	Intraperitoneal injection 10 μg each	Intraperitoneal injection 10 μg each
	Titermax Gold	Titermax Gold
Second boost immunization	Tail injection 5 μg each	Tail injection 5 μg each
	Alum/CpG ODN	Alum/CpG ODN
Third boost immunization	Intraperitoneal injection 5 μg each	Intraperitoneal injection 5 μg each
	Titermax Gold	Titermax Gold
Fourth boost immunization	Tail injection 5 μg each	Tail injection 5 μg each
	Alum/CpG ODN	Alum/CpG ODN
Fifth boost immunization	Intraperitoneal injection 5 μg each	Intraperitoneal injection 5 μg each
	Titermax Gold	Titermax Gold
Sixth boost immunization	Tail injection 5 μg each	Tail injection 5 μg each
	Alum/CpG ODN	Alum/CpG ODN
Seventh boost immunization	Intraperitoneal injection 5 μg each	Intraperitoneal injection 5 μg each
	Titermax Gold	Titermax Gold
Eighth boost immunization	Tail injection 5 μg each	Tail injection 5 μg each
	Alum/CpG ODN	Alum/CpG ODN
Blood collection		
Ninth boost immunization	Intraperitoneal injection 5 μg each	Intraperitoneal injection 5 μg each
	Titermax Gold	Titermax Gold
10th boost immunization	Tail injection 5 μg each	Tail injection 5 μg each
	Alum/CpG ODN	Alum/CpG ODN
11th boost immunization	BIP 5 μg each	BIP 5 μg each
	PBS	PBS
Harvest		

[0320]

Titers of antibodies to human PCSK9 were tested by ELISA assay for mice

immunized with the soluble antigen as described. Table 4 summarizes the ELISA data and indicates that there were some mice that appeared to be specific to PCSK9. See, for example, Table 4. Therefore, at the end of the immunization program, 10 mice (in bold type in Table 4) were selected for harvest, and splenocytes and lymphocytes were isolated from the spleens and lymph nodes, respectively, as described in the present description.

[0321]

[Table 4]

Table 4

Summary of ELISA results

	Animal ID	Titer b-hu PCSK9 (V5His) @ 2 μg/ml	Titer b-hu PCSK9 @ 2μg/ml
Group 1 IgG2k/l	P175807	>72900 @ OD 2.2	68359
	P175808	>72900 @ OD 2.3	>72900 @ OD 2.5
	P175818	>72900 @ OD 3.2	>72900 @ OD 3.0
	P175819	>72900 @ OD 3.4	>72900 @ OD 3.2
	P175820	>72900 @ OD 2.4	>72900 @ OD 2.5
	P175821	>72900 @ OD 3.4	>72900 @ OD 3.0
	P175830	>72900 @ OD 2.6	>72900 @ OD 2.5
	P175831	>72900 @ OD 3.1	>72900 @ OD 3.1
	P175832	>72900 @ OD 3.8	>72900 @ OD 3.6
	P175833	>72900 @ OD 2.6	>72900 @ OD 2.3
Group 2 IgG4k/l	P174501	19369	17109
	P174503	31616	23548
	P174508	48472	30996
	P174509	23380	21628
	P174510	15120	9673
	P175773	19407	15973
	P175774	54580	44424
	P175775	60713	55667
	P175776	30871	22899
	P175777	16068	12532
	Naive G2	<100 @ OD 0.54	<100 @ OD 0.48
	Naive G4	<100 @ OD 1.57	<100 @ OD 1.32

[0322]

(Example 2)

Recovery of Lymphocytes, Isolation of B-cells, Fusions, and Production of Hybridomas

This example outlines how the immune cells were recovered and how

hybridomas were produced. Selected immunized mice were sacrificed by cervical dislocation. From each cohort, draining lymph nodes were harvested and pooled. In order to release cells from the tissue, B cells were dissociated from the lymphoid tissue by grinding in DMEM, and the cells were suspended in DMEM. The cells were counted. In order to resuspend the cells gently but completely, 0.9 mL of DMEM per 100 million lymphocytes was added to the cell sediment.

[0323]

Lymphocytes were mixed with non-secretory myeloma P3X63Ag8.653 cells purchased from ATCC, cat. #CR11580 (Kearney et al., (1979) J. Immunol. 123, 1548-1550) at a ratio of 1:4. The cell mixture was gently sedimented by centrifugation at 400 × g for 4 minutes. After removing the supernatant by tipping the container, the cells were gently mixed using a 1 mL pipette. Preheated PEG/DMSO solution obtained from Sigma (cat #P7306) (1 mL per million of B-cells) was slowly added with gentle agitation over 1 minute and then mixed for 1 minute. Subsequently, preheated IDMEM (2 mL per million of B cells) (DMEM without glutamine, L-glutamine, penicillin/streptomycin, MEM non-essential amino acids) (all obtained from Invitrogen) was added over 2 minutes with gentle agitation. Finally, preheated IDMEM (8 mL per 10^6 B-cells) was added over 3 minutes.

[0324]

The fused cells were spun down at 400 × g for 6 minutes and resuspended in 20 mL of selection medium (DMEM (Invitrogen), 15% FBS (Hyclone), supplemented with L-glutamine, penicillin/streptomycin, MEM non-essential amino acids, sodium pyruvate, 2-mercaptoethanol (all obtained from Invitrogen), HA-azaserine hypoxanthine and OPI (oxaloacetate, pyruvate, bovine insulin) (both obtained from Sigma) and IL-6 (Boeringer Mannheim)) per million B cells. The cells were incubated at 37°C for 20 to 30 minutes, and then resuspended in 200 mL of selection medium and cultured in T175 flasks for 3 to 4 days prior to seeding into a 96-well. Thus, hybridomas that produce antigen binding proteins to PCSK9 were produced.

[0325]

(Example 3)

Selection of PCSK9 Antibodies

The present example outlines how the various PCSK9 antigen binding proteins were characterized and selected. The binding of secreted antibodies (produced from the hybridomas produced in Examples 1 and 2) to PCSK9 was assessed. Selection of antibodies was based on binding data and inhibition of binding of PCSK9 to LDLR and affinity. As described below, binding to soluble PCSK9 was analyzed by ELISA.

BIAcore(R) (surface plasmon resonance) was used to quantify binding affinity.

[0326]

Primary Screening

Primary screening for antibodies which bind to wild-type PCSK9 was performed. The primary screening was performed on two harvests. The primary screening comprised an ELISA assay and was performed using the following protocol.

[0327]

Costar 37-2 medium-binding 384-well plates (Corning Life Sciences) were used. The plates were coated with NeutrAvidin at a concentration of 4 μg/mL in 1×PBS/0.05% azide at a volume of 40 μL/well. The plates were incubated at 4°C overnight. Then, the plates were washed using a Titertek plate washer (Titertek, Huntsville, AL). Three cycles of washing were performed. The plates were blocked with 90 μL of 1×PBS/1% milk and incubated at room temperature for about 30 minutes. Then, the plates were washed. Again, three cycles of washing were performed. The capture sample was biotinylated-PCSK9 without a V5 tag and added at 0.9 μg/mL in 1×PBS/1% milk/10 mM Ca^{2+} at a volume of 40 μL/well. Then, the plates were incubated at room temperature for 1 hour. Next, the plates were washed using the Titertek plate washer operated using three-cycle washing. Ten microliters of the supernatant was transferred into 40 μL of 1×PBS/1% milk/10 mM Ca^{2+} and incubated at room temperature for 1.5 hours. Again, the plates were washed using the Titertek plate washer operated using three-cycle washing. Forty microliters/well of goat anti-human IgG Fc POD at a concentration of 100 ng/mL (1:4000) in 1×PBS/1% milk/10 mM Ca^{2+} was added to the plates and incubated at room temperature for 1 hour. The plates were washed once again using three-cycle washing. Finally, 40 μL/well of One-step TMB (Neogen, Lexington, Kentucky) was added to the plates, and quenching was performed with 40 μL/well of 1N hydrochloric acid at room temperature after 30 minutes. OD was read immediately at 450 nm using a Titertek plate reader.

[0328]

The primary screening resulted in a total of 3104 antigen specific hybridomas identified from the two harvests. Based on the highest ELISA OD, 1500 hybridomas per harvest were used for further manipulation for a total of 3000 positives.

[0329]

Confirmatory Screening

Then, in order to confirm that stable hybridomas were established, 3000 positives were rescreened for binding to wild-type PCSK9.... A total of 2441

positives were repeated in the second screening. Then, these antibodies were used in the subsequent screenings.

[0330]

Mouse Cross-Reaction Screening

In order to confirm that the antibodies can bind to both human and mouse PCSK9, the panel of hybridomas was then screened for cross-reactivity to mouse PCSK9.... It was observed that 579 antibodies cross-reacted with mouse PCSK9. Then, these antibodies were used in the subsequent screenings.

[0331]

D374Y Mutant Binding Screening

The D374Y mutation in PCSK9 in human populations has been stated in documents (e.g., Timms KM et al., "A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in a Utah pedigree", Hum. Genet. 114: 349-353, 2004). In order to determine if the antibodies were specific to the wild type or also bound to the D374Y form of PCSK9, the samples were then screened for binding to the mutant PCSK9 sequence comprising the mutation D374Y.... More than or equal to 96% of the positive hits on the wild-type PCSK9 also bound the mutant PCSK9.

[0332]

Large-Scale Receptor Ligand Blocking Screening

An assay was developed using the D374Y PCSK9 mutant in order to screen for antibodies which block PCSK9 binding to LDLR. The mutant was used for this assay because the mutant has a higher binding affinity to LDLR, enabling the development of a more sensitive receptor ligand blocking assay. In the receptor ligand blocking screening, the following protocol was used. In the screening, Costar 3702 medium-binding 384-well plates (Corning Life Sciences) were used. The plates were coated with 2 μg/mL goat anti-LDLR (R&D Cat #AF2148) in 1×PBS/0.05% azide at a volume of 40 μL/well. The plates were incubated at 4°C overnight. Then, the plates were washed using a Titertek plate washer (Titertek, Huntsville, AL). Three cycles of washing were performed. The plates were blocked with 90 μL of 1×PBS/1% milk and incubated at room temperature for about 30 minutes. Then, the plates were washed using the Titertek plate washer. Three cycles of washing were performed. The capture sample was LDLR (R&D, Cat #2148LD/CF), and was added at 0.4 μg/mL in 1×PBS/1% milk/10 mM Ca^{2+} at a volume of 40 μL/well. Then, the plates were incubated at room temperature for 1 hour and 10 minutes. Simultaneously, 20 ng/mL of biotinylated human D374Y PCSK9 was incubated together with 15 μL of hybridoma exhaust supernatant in Nunc

polypropylene plates and the exhaust supernatant concentration was diluted at 1:5. Then, the plates were preincubated at room temperature for about 1 hour and 30 minutes. Next, the plates were washed using the Titertek plate washer operated using three-cycle washing. Fifty microliters/well of the preincubated mixture was transferred onto the LDLR-coated ELISA plates and incubated at room temperature for 1 hour. To detect LDLR-bound b-PCSK9, 40 μL/well of 500 ng/mL streptavidin HRP in assay diluent was added to the plates. The plates were incubated at room temperature for 1 hour. Again, the plates were washed using the Titertek plate washer. Three cycles of washing were performed. Finally, 40 μL/well of One-step TMB (Neogen, Lexington, Kentucky) was added to the plates, and quenching was performed with 40 μL/well of 1N hydrochloric acid at room temperature after 30 minutes. OD was read immediately at 450 nm using a Titertek plate reader. The screening identified 384 antibodies which blocked the interaction between PCSK9 and the LDLR wells, among which 100 antibodies blocked the interaction strongly (OD < 0.3). These antibodies inhibited the binding interaction between PCSK9 and LDLR by more than 90% (more than 90% inhibition).

[0333]

Receptor Ligand Binding Assay on Subset of Blocking Substances

The receptor ligand assay was then repeated using the mutant enzyme on the subset of 384 neutralizing substances identified in the first large-scale receptor ligand inhibition assay. The screening of the 384 blocking substance subset assays used the same protocol as that performed in the large-scale receptor ligand blocking screening. This repeated screening confirmed the first screening data.

[0334]

This screening of the 384-member subset identified 85 antibodies which blocked the interaction between the PCSK9 mutant enzyme and LDLR by more than 90%.

[0335]

Receptor Ligand Binding Assay of Blocking Substances Which Bind Wild-Type PCSK9 but Does Not Bind the D374Y Mutant In the initial panel of 3000 supernatants, there were 86 antibodies shown to specifically bind to the wild-type PCSK9 and not to the huPCSK9 (D374Y) mutant. These 86 supernatants were tested for the ability to block the wild-type PCSK9 from binding to the LDLR receptor....

[0336]

Screening Results

Based on the results of the assays described, several hybridoma lines were

identified as producing antibodies having the desired interaction with PCSK9. Limiting dilution was used to isolate a manageable number of clones from each line. The clones were designated by hybridoma line number (e.g., 21B12) and clone number (e.g., 21B12.1). Generally, differences among different clones of a particular line were detected by the functional assays described in the present description. In a few instances, clones were identified from a particular line that behaved differently in the functional assays. For example, it was found that 25A7.1 does not block PCSK9/LDLR, but 25A7.3 (referred to as 25A7 in the present description) has a neutralizing property. Each of the isolated clones was allowed to grow in 50 to 100 mL of hybridoma solvent until exhausted (i.e., cell viability of less than about 10%). The concentration and potency of the antibodies to PCSK9 in the supernatants of these cultures were measured by ELISA and by in vitro functional testing, as described in the present description. As a result of the screening described in the present description, the hybridoma having the highest titer of antibodies to PCSK9 was identified. The selected hybridomas are shown in Figures 2A to 3D and Table 2.

[0373]

(Example 10)

Epitope Binning

Competition ELISA was used for anti-PCSK9 antibody binning. In summary, in order to determine if two antibodies belong to the same epitope bin, one of the antibodies (mAb1) was first coated onto an ELISA plate (NUNC) at 2 μg/mL by overnight incubation. Then, the plate was washed and blocked with 3% BSA. On the other hand, 30 ng/mL of biotinylated hPCSK9 was incubated with the second antibody (mAb2) at room temperature for 2 hours. The mixture was applied to coated mAb1 and incubated at room temperature for 1 hour. Then, the ELISA plate was washed and incubated with Neutravidin-HRP (Pierce) at a dilution of 1:5000 for 1 hour. After further washing, the plate was incubated with TMB substrate and the signal was detected at 650 nm using a Titertek plate reader. Antibodies having the same binding property were grouped into the same epitope bin. The results of the antibody binning studies are shown in Table 8.3.

[0374]

[Table 11]

Table 8.3

Clone	Bin
21B12.2	1
31H4	3
20D10	1
25A7.1	2
25A7.3	1
23G1	1
26H5	1
31D1	1
16F12	3
28D6	3
27A6	3
31G11	3
27B2	ND
28B12	3
22E2	3
1A12.2	1
3B6	1
3C4	4
9C9	1
9H6	1
13B5	6
13H1	7
17C2	1
19H9.2	1
23B5	1
25G4	1
26E10	1
27E7	1
27H5	1
30A4	1
30B9	1

Clone	Bin
31A4	5
31B12	5

[0377]

(Example 11)

Effects of 31H4 and 21B12 on Blocking D374Y PCSK9/LDLR Binding

The present example provides IC50 values for two of the antibodies in blocking the ability of PCSK9 D374Y to bind to LDLR. Clear 384-well plates (Costar) were coated with 2 μg/mL of goat anti-LDL receptor antibody (R&D Systems) diluted in buffer A (100 mM sodium cacodylate, pH 7.4). The plates were washed thoroughly with buffer A and then blocked for 2 hours with buffer B (1% milk in buffer A). After washing, the plates were incubated with 0.4 μg/mL of LDL receptor (R&D Systems) diluted in buffer C (buffer B supplemented with 10 mM $CaCl_2$) for 1.5 hours. Simultaneously with this incubation, 20 ng/mL of biotinylated D374Y PCSK9 was incubated with various concentrations of the 31H4 IgG2, 31H4 IgG4, 21B12 IgG2, or 21B12 IgG4 antibody that was diluted in buffer A, or buffer A alone (control). The

LDL receptor-containing plates were washed, and the biotinylated D374Y PCSK9/antibody mixture was transferred to the plates and incubated at room temperature for 1 hour. Binding of the biotinylated D374Y to the LDL receptor was detected by incubation with 500 ng/mL of streptavidin-HRP (Biosource) in buffer C, followed by incubation with TMB substrate (KPL). The signal was quenched with 1N HCl and the absorbance was read at 450 nm.

[0378]

The results of this binding study are shown in Figures 6A to 6D. In summary, IC_{50} values were measured for each antibody and found to be 199 pM for 31H4 IgG2 (Figure 6A), 156 pM for 31H4 IgG4 (Figure 6B), 170 pM for 21B12 IgG2 (Figure 6C), and 169 pM for 21B12 IgG4 (Figure 6D).

[0379]

The antibodies also blocked the binding of wild-type PCSK9 to LDLR in this assay.

[0380]

(Example 12)

Cell LDL Uptake Assay

The present example demonstrates that various antigen binding proteins can reduce LDL uptake by cells....

[0381]

The results of the cell uptake assay are shown in Figures 7A to 7D. In summary, IC_{50} values were measured for each antibody and found to be 16.7 nM for 31H4 IgG2 (Figure 7A), 13.3 nM for 31H4 IgG4 (Figure 7B), 13.3 nM for 21B12 IgG2 (Figure 7C), and 18 nM for 21B12 IgG4 (Figure 7D). These results demonstrate that the applied antigen binding proteins can reduce the effect of PCSK9 (D374Y) to block LDL uptake by cells. The antibodies also blocked the effect of wild-type PCSK9 in this assay.

[0382]

(Example 13)

Serum Cholesterol Lowering Effect of 31H4 Antibody in a 6-Day Study

In order to assess total serum cholesterol (TC) lowering in wild type (WT) mice via antibody therapy against PCSK9 protein, the following procedure was performed.

[0383]

Male WT mice (C57BL/6 strain, 9 to 10 weeks old, 17 to 27 g) obtained from Jackson Laboratory (Bar Harbor, ME) were fed a normal diet (Harland-Teklad, Diet

2918) throughout the duration of the experiment. Mice were administered either anti-PCSK9 antibody 31H4 (2 mg/mL in PBS) or control IgG (2 mg/mL in PBS) at a level of 10 mg/kg through the mouse's tail vein at t = 0. Naive mice were also set aside as a naive control group. Dosing groups and time of sacrifice are shown in Table 9.

[0384]

[Table 12]

Table 9

Group	Treatment	Time point after dosing	Number
1	IgG	8 hours	7
2	31H4	8 hours	7
3	IgG	24 hours	7
4	31H4	24 hours	7
5	IgG	72 hours	7
6	31H4	72 hours	7
7	IgG	144 hours	7
8	31H4	144 hours	7
9	Naive	n/a	7

[0385]

Mice were sacrificed with CO_2 asphyxiation at the predetermined time points shown in Table 9. Blood was collected via vena cava into Eppendorf tubes and was allowed to clot at room temperature for 30 minutes. Then, in order to separate the serum, the samples were spun down in a table-top centrifuge at 12,000 × g for 10 minutes. Serum total cholesterol and HDL-C were measured using Hitachi 912 clinical analyzer and Roche/Hitachi TC and HDL-C kits.

[0386]

The results of the experiment are shown in Figures 8A to 8D. In summary, mice to which antibody 31H4 was administered showed decreased serum cholesterol levels over the duration of the experiment (Figure 8A and Figure 8B). In addition, it is noted that the mice also showed decreased HDL levels (Figure 8C and Figure 8D). For Figure 8A and Figure 8C, the change in % is relative to the control IgG at the same time point (*P < 0.01, #P < 0.05). For Figure 8B and Figure 8D, the change in % is relative to total serum cholesterol and HDL levels measured in naive animals at t = 0 hours (*P < 0.01, #P < 0.05).

[0387]

For the lowered HDL levels, it is noted that it will be appreciated by a person

ordinarily skilled in the art that the decrease in HDL in mice does not suggest that an HDL decrease will occur in humans, and merely further reflects that the serum cholesterol in this organism decreased. It is noted that mice transport most of serum cholesterol into high-density lipoprotein (HDL) particles, which differs from humans having most serum cholesterol on LDL particles. In mice, the measurement of total serum cholesterol most closely resembles the serum HDL-C levels. Mouse HDL contains apolipoprotein E (apoE) that is a ligand for the LDL receptor (LDLR) and allows HDL to be cleared by the LDLR. Thus, examining HDL is an appropriate indicator for the present example in mice (it is appreciated that a decrease in HDL is not expected for humans). In contrast thereto, for example, human HDL does not contain apoE and is not a ligand for LDLR. PCSK9 antibodies increase LDLR expression in mouse, allowing the liver to clear more HDL, thus lowering serum HDL-C levels.

[0388]

(Example 14)

Effect of Antibody 31H4 on LDLR Levels in a 6-Day Study

The present example demonstrates that an antigen binding protein alters LDLR levels in a subject over time, as expected. A Western blot analysis was performed in order to confirm the effect of antibody 31H4 on LDLR levels. Fifty to 100 mg of liver tissue obtained from the sacrificed mice described in Example 13 was homogenized in 0.3 mL of RIPA buffer (Santa Cruz Biotechnology Inc.) containing a complete protease inhibitor (Roche). The homogenate was incubated on ice for 30 minutes and centrifuged to sediment the cellular debris. Protein concentration in the supernatant was measured using BioRad protein assay reagent (Bio Rad laboratories). One hundred micrograms of protein was denatured at 70°C for 10 minutes and separated on 4 to 12% Bis-Tris SDS gradient gel (Invitrogen). The protein was transferred to a 0.45 μm PVDF membrane (Invitrogen) and blocked in washing buffer (50 mM Tris PH7.5, 150 mM NaCl, 2 mM $CaCl_2$, and 0.05% Tween 20) containing 5% non-fat milk at room temperature for 1 hour. Then, the blot was probed with goat anti-mouse LDLR antibody (R&D system) 1:2000 or anti-β actin (sigma) 1:2000 at room temperature for 1 hour. The blot was washed for a short time and incubated with bovine anti-goat IgG-HRP (Santa Cruz Biotechnology Inc.) 1:2000 or goat anti-mouse IgG-HRP (Upstate) 1:2000. After incubation at room temperature for 1 hour, the blot was washed thoroughly, and immunoreactive bands were detected using ECL plus kit (Amersham biosciences). The Western blot showed an increase in LDLR protein levels in the presence of antibody 31H4, as illustrated in Figure 9.

[0389]

(Example 15)

Serum Cholesterol Lowering Effect of Antibody 31H4 in a 13-Day Study

In order to assess total serum cholesterol (TC) lowering in wild type (WT) mice via antibody therapy against PCSK9 protein in a 13-day study, the following procedure was performed.

[0390]

Male WT mice (C57BL/6 strain, 9 to 10 weeks old, 17 to 27 g) obtained from Jackson Laboratory (Bar Harbor, ME) were fed a normal diet (Harland-Teklad, Diet 2918) throughout the duration of the experiment. Mice were administered either anti-PCSK9 antibody 31H4 (2 mg/mL in PBS) or control IgG (2 mg/mL in PBS) at a level of 10 mg/kg through the mouse's tail vein at t = 0. Naive mice were also set aside as a naive control group.

[0391]

Dosing groups and time of sacrifice are shown in Table 10. Animals were sacrificed, and livers were extracted and prepared as in Example 13.

[0392]

[Table 13]

Table 10

Group	Treatment	Time point after dosing	Number	Dose
1	IgG	72 hours	6	10 mg/kg
2	31H4	72 hours	6	10 mg/kg
3	31H4	72 hours	6	1 mg/kg
4	IgG	144 hours	6	10 mg/kg
5	31H4	144 hours	6	10 mg/kg
6	31H4	144 hours	6	1 mg/kg
7	IgG	192 hours	6	10 mg/kg
8	31H4	192 hours	6	10 mg/kg
9	31H4	192 hours	6	1 mg/kg
10	IgG	240 hours	6	10 mg/kg
11	31H4	240 hours	6	10 mg/kg
12	31H4	240 hours	6	1 mg/kg
13	IgG	312 hours	6	10 mg/kg
14	31H4	312 hours	6	10 mg/kg
15	31H4	312 hours	6	1 mg/kg
16	Naive	n/a	6	n/a

[0393]

When the 6-day experiment was extended to a 13-day study, the same serum

cholesterol lowering effect observed in the 6-day study was also observed in the 13-day study. More specifically, animals dosed at 10 mg/kg showed a 31% decrease in serum cholesterol on day 3, which gradually returned to pre-dosing levels by day 13. Figure 10A illustrates the results of this experiment. Figure 10C illustrates the results of repeating the above procedure using 31H4 at a dose of 10 mg/kg and another antibody 16F12 also at a dose of 10 mg/kg. Dosing groups and time of sacrifice are shown in Table 11.

[0395]

As shown in Figure 10C, both 16F12 and 31H4 resulted in significant and substantial decreases in total serum cholesterol after only a single dose, and were beneficial over a week or more (10 days or more). The results of the repeated 13-day study are consistent with those of the first 13-day study, and a decrease in serum cholesterol levels of 26% on day 3 is observed. For Figure 10A and Figure 10B, the change in % is relative to the control IgG at the same time point (*P < 0.01). For Figure 10C, the change in % is relative to the control IgG at the same time point (*P < 0.05).

[0422]

(Example 26)

Mouse Model for the Ability of PCSK9 and ABP to Lower LDL in Vivo

In order to prepare mice which over-express human PCSK9, three-week-old WT C57B1/6 mice were injected via tail vein administration with various concentrations of adeno-associated virus (AAV) recombinantly modified to express human PCSK9 so as to measure the correct titer that would give a measurable increase of LDL-cholesterol in the mice. By using this virus which expresses human PCSK9, it was determined that 4.5 × 10E12 pfu of virus resulted in LDL-cholesterol levels of about 40 mg/dL in the circulating blood (normal levels of LDL in WT mice are about 10 mg/dL). The human PCSK9 levels in these animals were found to be about 13 μg/mL. A colony of mice was prepared using this injection criteria.

[0423]

One week after injection, mice were assessed for LDL-cholesterol levels and randomly assigned to different treatment groups. Then, a single bolus injection of either 10 mg/kg or 30 mg/kg of 16F12, 21B12, or 31H4 antigen binding protein was administered to the animals via tail vein injection. IgG2 ABP was administered to a separate group of animals as a dosing control. Then, subgroups of the animals (n = 6 to 7) were euthanized at 24 and 48 hours after ABP animals. After IgG2 administration, there were no effects on LDL-cholesterol levels at either dose. Both

31H4 and 21B12 showed significant LDL-cholesterol lowering up to 48 hours (including 48 hours) after administration, as compared to IgG2 control (shown in Figures 14A and 14B at two different doses). By the time point of 48 hours, 16F12 showed an intermediary LDL-cholesterol lowering response at levels returning to baseline of about 40 mg/dL. These data are consistent with in vitro binding data (Biacore and Kinexa) that show a nearly equivalent binding affinity between 31H4 and 21B12 for human PCSK9 and a lesser affinity of 16F12 for PCSK9.

[0426]

(Example 27)

31H4 and 21B12 Bind to ProCat Region of PCSK9

The present example describes one method for determining where various antibodies bind to PCSK9.

[0427]

The ProCat (31 to 449 of SEQ ID NO: 3) or V domain (450 to 692 of SEQ ID NO: 3) of the PCSK9 protein was combined with either antibody 31H4 or 21B12. The samples were analyzed by non-denaturing PAGE for complex formation. As is apparent from Figure 16A and Figure 16B, gel shifts were present for the samples of ProCat/31H4 and ProCat/21B12, demonstrating that the antibodies bound to the ProCat domain.

[0428]

(Example 28)

LDLR EGFa Domain Binds to Catalytic Domain of PCSK9 The present example presents the solved crystal structure of PCSK9 ProCat (31 to 454 of SEQ ID NO: 3) bound to the LDLR EGFa domain (293 to 334) at 2.9 angstrom resolution (the condition described in the following examples).

[0429]

An illustrated explanation of the structure of PCSK9 bound to EGFa is shown in Figure 17. The crystal structure (and its illustrated explanation in Figure 17) reveals that the EGFa domain of LDLR binds to the catalytic domain of PCSK9. In addition, the interaction between PCSK9 and EGFa appears to occur across a surface of PCSK9 that is present between residues D374 and S153 in the structure illustrated in Figure 17.

[0430]

Specific core PCSK9 amino acid residues of the interaction interface with the LDLR EGFa domain were defined as PCSK9 residues that are present within 5 angstroms of the EGFa domain. The core residues are as follows: S153, I154, P155,

R194, D238, A239, I369, S372, D374, C375, T377, C378, F379, V380, and S381.
[0431]

Boundary PCSK9 amino acid residues of the interaction interface with the LDLR EGFa domain were defined as PCSK9 residues that are present at 5 angstroms to 8 angstroms of the EGFa domain. The boundary residues are as follows: W156, N157, L158, E159, H193, E195, H229, R237, G240, K243, D367, I368, G370, A371, S373, S376, and Q382. The underlined residues are mostly or completely buried within PCSK9.
[0432]

As will be appreciated by a person ordinarily skilled in the art, the results from the present example demonstrate that PCSK9 and EGFa interact with each other. Thus, an antibody which interacts with or blocks any of these residues can be useful as an antibody which inhibits the interaction between PCSK9 and the EGFa domain of LDLR (and/or LDLR in general). In some embodiments, when bound to PCSK9, antibodies which interact with or block any of the above residues or are present at 15 to 8, 8, 8 to 5, or 5 angstroms of the above residues are contemplated to provide useful inhibition of PCSK9 binding to LDLR.
[0438]

(Example 30)

21B12 binds to the catalytic domain of PCSK9, has a binding site different from 31H4, and can bind to PCSK9 simultaneously with 31H4.
[0439]

The present example presents the crystal structure of PCSK9 ProCat (31 to 449 of SEQ ID NO: 3) bound to the Fab fragments of 31H4 and 21B12, which was measured at 2.8 angstrom resolution (the conditions described in the following examples). This crystal structure illustrated in Figures 19A and 19B shows that 31H4 and 21B12 have different binding sites on PCSK9 and that both antigen binding proteins can bind to PCSK9 simultaneously. The structure shows that 21B12 interacts with amino acid residues derived from the catalytic domain of PCSK9. In this structure, the interaction between PCSK9 and 31H4 is similar to what was observed above.
[0440]

Specific core PCSK9 amino acid residues of the interaction interface with 21B12 were defined as PCSK9 residues that are present within 5 angstroms of the 21B12 protein. The core residues are as follows: S153, S188, I189, Q190, S191, D192, R194, E197, G198, R199, V200, D224, R237, D238, K243, S373, D374, S376,

T377, and F379.

[0443]

As will be appreciated by a person ordinarily skilled in the art, the results obtained from Example 30 demonstrate where the antigen binding protein to PCSK9 can interact with PCSK9, and that the antigen binding protein to PCSK9 can still block PCSK9 from interacting with EGFa (and thus with LDLR). Thus, an antigen binding protein which interacts with any of these PCSK9 residues or which blocks any of these residues can be useful as an antibody which inhibits the interaction between PCSK9 and EGFa (and thus LDLR). Therefore, in some embodiments, an antibody which interacts with any of the above residues or which interacts with residues that are present within 5 angstroms of the above residues is contemplated to provide useful inhibition of PCSK9 binding to LDLR. Similarly, an antigen binding protein which blocks any of the above residues (which can be determined, e.g., via a competition assay) can also be useful for inhibition of the PCSK9/LDLR interaction.

[0444]

(Example 31)

Interaction between EGFa, PCSK9, and Antibodies

The structure of the ternary complex (PCSK9/31H4/21B12) obtained from the above example was superimposed on the PCSK9/EGFa structure (determined as described in Example 28) and the result of this combination is illustrated in Figure 20A. This figure shows regions on PCSK9 which can be usefully targeted so as to inhibit PCSK9 interaction with EGFa. The figure shows that both 31H4 and 21B12 partially overlap with the position of the EGFa domain of LDLR and sterically interfere with its binding to PCSK9. In addition, as is apparent from the structures, 21B12 directly interacts with a subset of amino acid residues specifically involved in binding to the LDLR EGFa domain.

[0445]

As described above, analysis of the crystal structures identified specific amino acids involved in the interaction between PCSK9 and the counter-proteins (the core and the interface boundary regions on the PCSK9 surface) and the spatial requirements of these counter-proteins to interact with PCSK9. These structures suggest methods to inhibit the interaction between PCSK9 and LDLR. First, as described above, binding of a factor to PCSK9 that shares residues in common with the binding site of the LDLR EGFa domain inhibits the interaction between PCSK9 and LDLR. Second, a factor which binds outside of the residues in common can sterically interfere with the EGFa domain or regions of the LDLR that are either N-

terminal or C-terminal to the EGFa domain to interfere with the interaction between PCSK9 and LDLR.

[0446]

In some embodiments, the residues that are involved in EGFa binding and are close to the regions to which the above antigen binding proteins bind are especially useful for manipulating the binding of PCSK9 to LDLR. For example, amino acid residues derived from common interfaces in both the core region and boundary region for different binding partners are listed in Table 12 below. Amino acid residues completely buried within the PCSK9 protein are underlined.

[0447]

[Table 15]

Table 12

Parameter	Amino acid position
31H4/EGFa, both less than 5 angstroms	D374, V380, S381
31H4, less than 5 angstroms / EGFa, 5 to 8 angstroms	D367, Q382
31H4, 5 to 8 angstroms / EGFa, less than 5 angstroms	I369, S372, C378, F379
31H4/EGFa, both 5 to 8 angstroms	H229, S373
21B12/EGFa, both less than 5 angstroms	S153, R194, D238, D374, T377, F379
21B12, less than 5 angstroms / EGFa, 5 to 8 angstroms	R237, K243, S373, S376
21B12, 5 to 8 angstroms / EGFa, less than 5 angstroms	I154, A239, I369, S372, C375, C378
21B12/EGFa, both 5 to 8 angstroms	H193, E195

[0448]

As will be appreciated by a person ordinarily skilled in the art, in some embodiments, the antigen binding protein binds to and/or blocks at least one of the above residues.

[0489]

(Example 37)

Epitope Mapping - Binning

Another set of binning experiments was performed in addition to the set in Example 10. As in Example 10, ABPs which compete with each other can be considered to bind to the same site on the target and, in common parlance, are said to form "bin" with each other.

[0490]

A modification of the multiplexed binning method stated by Jia et al. (J.

Immunological Methods, 288 (2004) 91-98) was used. Each bead cord of streptavidin-coated Luminex beads was incubated in 100 μL of 0.5 μg/mL biotinylated monovalent mouse anti-human IgG capture antibody (BD Pharmingen, #555785) at room temperature for 1 hour in the dark, and then washed three times with PBSA (phosphate buffered saline (PBS) plus 1% bovine serum albumin (BSA)). Each bead cord was separately incubated with 100 μL of 2 μg/mL anti-PCSK9 antibody (Coating Antibody) for 1 hour, and then washed three times with PBSA. The beads were pooled and then dispensed to a 96-well filter plate (Millipore, #MSBVN1250). One hundred microliters of 2 μg/mL purified PCSK9 protein was added to half the wells. Buffer was added to the other half as control. The reaction was incubated for 1 hour and then washed. One hundred microliters of 2 μg/mL anti-PCSK9 antibody (Detection Ab) was added to all the wells, and the solution was incubated for 1 hour, and then washed. As another subject, irrelevant human IgG (Jackson, #009-000-003) was allowed to run. To each well, 20 μL of PE-conjugated monovalent mouse anti-human IgG (BD Pharmingen, #555787) was added, the solution was incubated for 1 hour, and then washed. Beads were resuspended in 100 μL of PBSA, and a minimum of 100 events/bead cord were collected on the BioPlex instrument (BioRad).

[0491]

The median fluorescent intensity (MFI) of the antibody pair without PCSK9 was subtracted from the signal of the corresponding reaction containing PCSK9. For the antibody pair to be considered to bind simultaneously (and thus in different bins), the subtracted signal had to be three times greater than the signal of the antibody competing with itself and three times greater than the signal of the antibody competing with the irrelevant antibody.

[0492]

The data obtained from the above is illustrated in Figures 23A to 23D. The ABPs belonged to five bins. The shaded boxes indicate ABPs which can bind simultaneously to PCSK9. The non-shaded boxes indicate ABPs which compete with each other for binding. A summary of the results is shown in Table 37.1.

[0493]

[Table 17]

Table 37.1

Bin 1	Bin 2	Bin 3	Bin 4	Bin 5
01A12.2	27B2.1	16F12.1	11G1.5	30A4.1
03B6.1	27B2.5	22E2.1	03C4.1	13B5.1
09C9.1	12H11.1	27A6.1		13H1.1
17C2.1		28B12.1		31A4.1
21B12.2		28D6.1		31B12.1
23G1.1		31G11.1		
25G4.1		31H4.1		
26E10.1		08A1.2		
11H4.1		08A3.1		
11H8.1		11F1.1		
19H9.2				
26H5.1				
27E7.1				
27H5.1				
30B9.1				
02B5.1				
23B5.1				
27B2.6				
09H6.1				

[0494]

Bins 1 (competing with ABP 21B12) and 3 (competing with 31H4) are exclusive of each other, bin 2 competes with bins 1 and 3, and bin 4 does not compete with bins 1 and 3. In this example, bin 5 is represented as a "catch all" bin to describe ABPs that fit into other bins. Thus, the above ABPs in each of the bins are representative of different types of epitope positions on PCSK9, some of which overlap with each other.

[0495]

As will be appreciated by a person ordinarily skilled in the art, if the reference ABP prevents the binding of the probe ABP, the antibodies are referred to be in the same bin. The order in which the ABPs are used can be important. If ABP A is used as the reference ABP and blocks the binding of ABP B, the converse is not always true. ABP B used as the reference ABP does not necessarily block ABP A. There are a number of factors that play a role here. The binding of an ABP can cause conformational changes in the target, which prevents the binding of the second ABP, or epitopes which overlap but does not completely block each other can allow the second ABP to still have enough high-affinity interaction with the target to allow binding. ABPs with a much higher affinity can have a greater ability to push out blocking ABPs. In general, if competition is observed in any order, the ABPs are

referred to as bins with each other, and if both ABPs can block each other, it is likely that the epitopes overlap more completely.

[0521]

Table 39.5 displays a summary of all of the hits for the various antibodies.

[0522]

[Table 22]

Table 39.5

EC50 shift hits					Bmax shift hits				
21B12	31H4	31A4	12H11	3C4	21B12	31H4	31A4	12H11	3C4
R207E	R185E	R439E	T132R	E582R	D162R			S123R	R519E
D208R*		E513R	S351R		R164E			E129R	H521R
		V538R	A390R		E167R			A311R	Q554R
		E539R	A413R					D313R	
								D337R	

* Decreases EC50

[0523]

To further examine how these residues form some or all of the relevant epitopes, the above positions were mapped onto various crystal structure models, of which the results are shown in Figures 27A to 27E....

[0526]

Figure 27D illustrates the 12H11 epitope hits mapped onto the crystal structure of PCSK9 with 31H4 and 21B12 antibodies. The structure identifies PCSK9 residues as follows. Light gray indicates unmutated residues (except for residues explicitly indicated on the structure) and darker gray indicates mutated residues (some of which could not express). Residues explicitly indicated (regardless of the shading indicated on the figure) were tested, and a significant change in EC50 and/or Bmax were obtained. 12H11 competes with 21B12 and 31H4 in the binning assay described above.

QEDEDGDYEELVLALRSEEDGLAEAPEHGTTATFHRCAKDPWRLPGTYVVVLKEETHL
SQSERTARRLQAQAARRGYLTKILHVFHGLLPGFLVKMSGDLLELALKLPHVDYIEEDS
SVFAQSIPWNLERITPPRYRADEYQPPDGGSLVEVYLLDTSIQSDHREIEGRVMVTDFEN
VPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAGVAKGASMRSLRVLNCQGKGTVSGT
LIGLEFIRKSQLVQPVGPLVVLLPLAGGYSRVLNAACQRLARAGVVLVTAAGNFRDDAC
LYSPASAPEVITVGATNAQDQPVTLGTLGTNFGRCVDLFAPGEDIIGASSDCSTCFVSQS
GTSQAAAHVAGIAAMMLSAEPELTLAELRQRLIHFSAKDVINEAWFPEDQRVLTPNLVA
ALPPSTHGAGWQLFCRTVWSAHSGPTRMATAIARCAPDEELLSCSSFSRSGKRRGERME
AQGGKLVCRAHNAFGGEGVYAIARCCLLPQANCSVHTAPPAEASMGTRVHCHQQGHV
LTGCSSHWEVEDLGTHKPPVLRPRGQPNQCVGHREASIHASCCHAPGLECKVKEHGIPA
PQGQVTVACEEGWTLTGCSALPGTSHVLGAYAVDNTCVVRSRDVSTTGSTSEEAVTAV
AICCRSRHLAQASQELQ

SEQ ID NO: 1

FIG. 1A

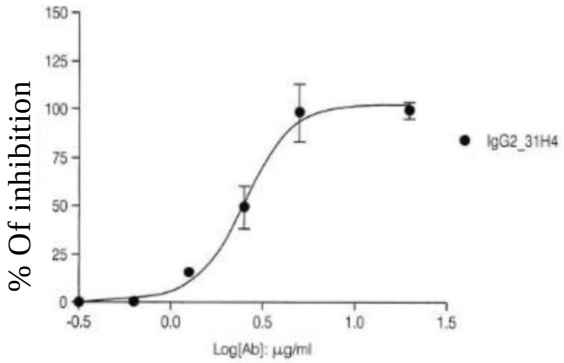


FIG. 7A

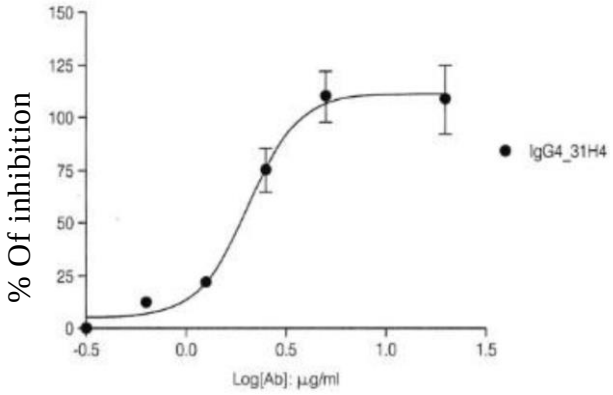


FIG. 7B

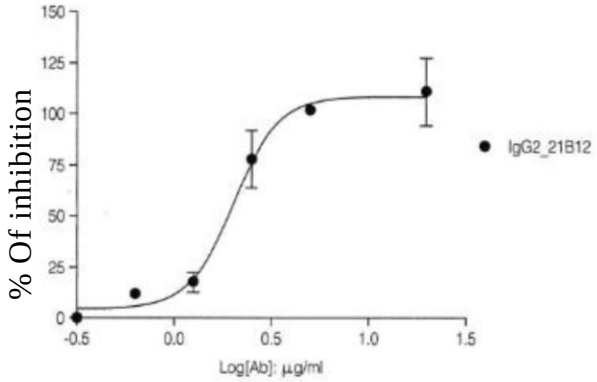


FIG. 7C

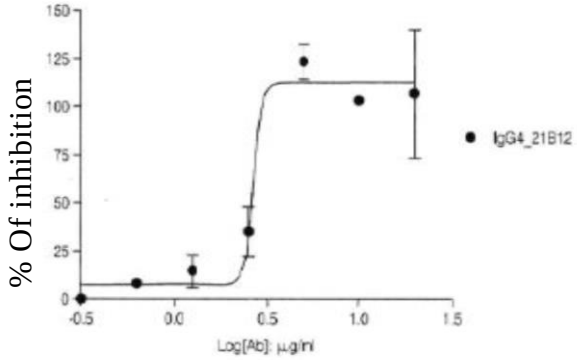


FIG. 7D

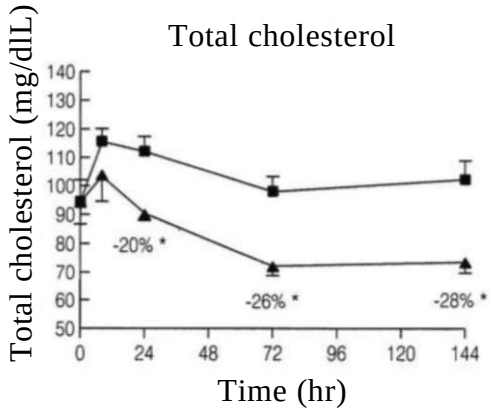


FIG. 8A

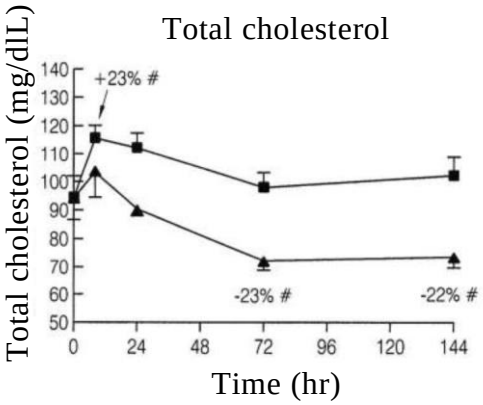


FIG. 8B

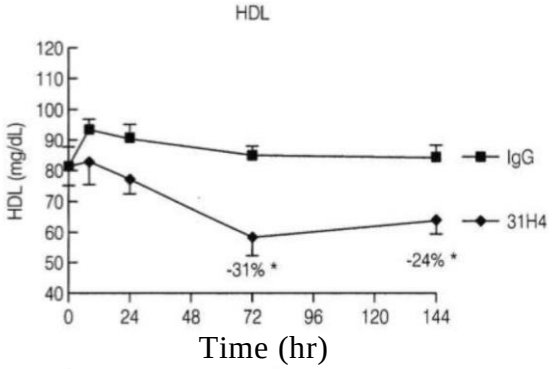


FIG. 8C

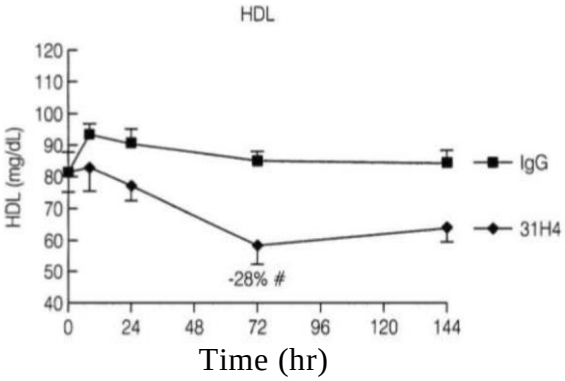


FIG. 8D

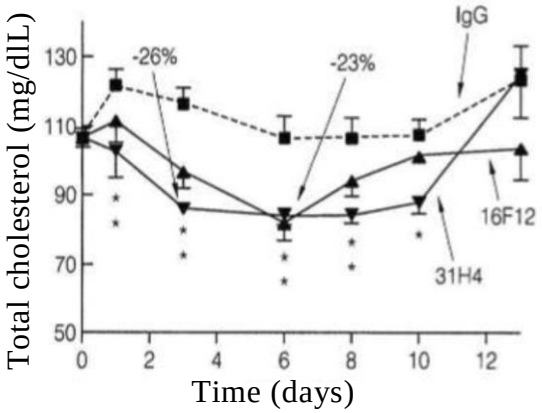


FIG. 10C

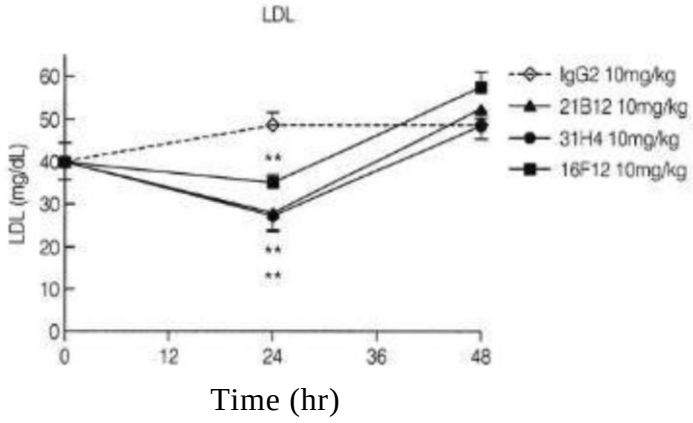


FIG. 14A

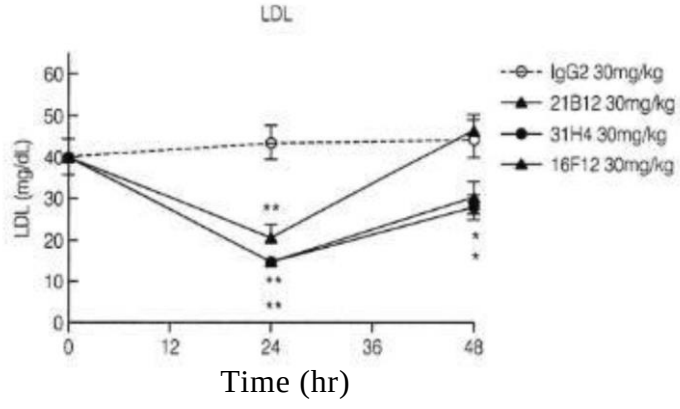


FIG. 14B

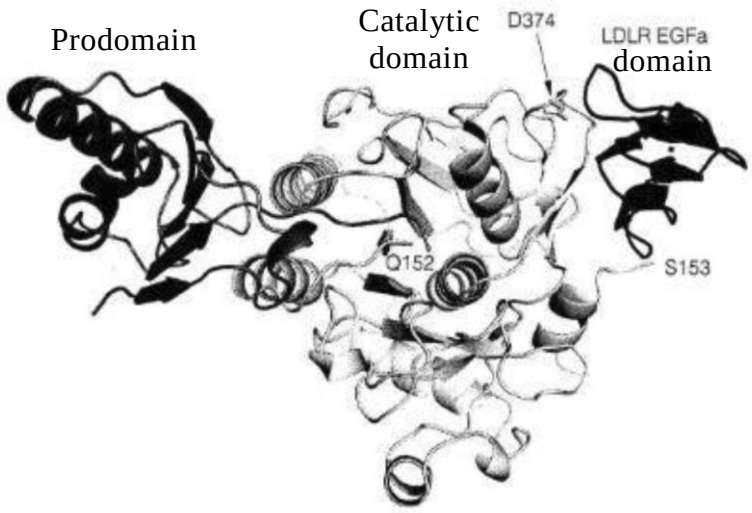


FIG. 17

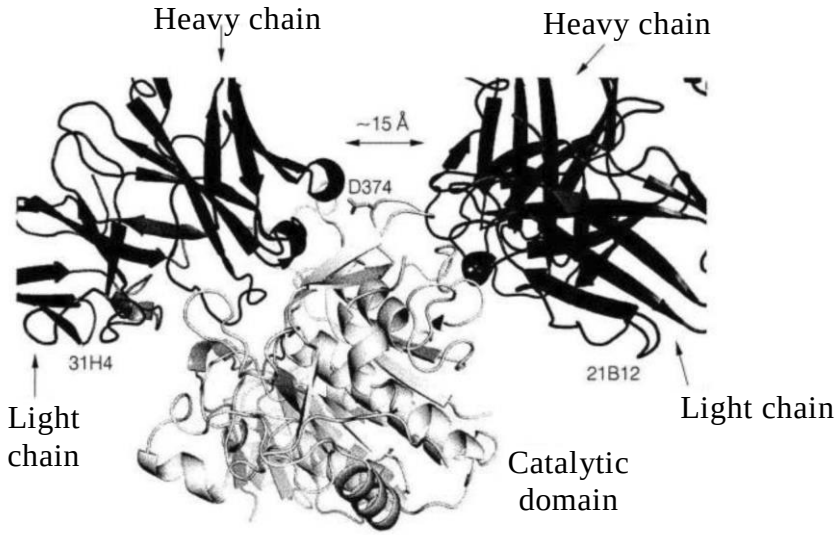


FIG. 19A

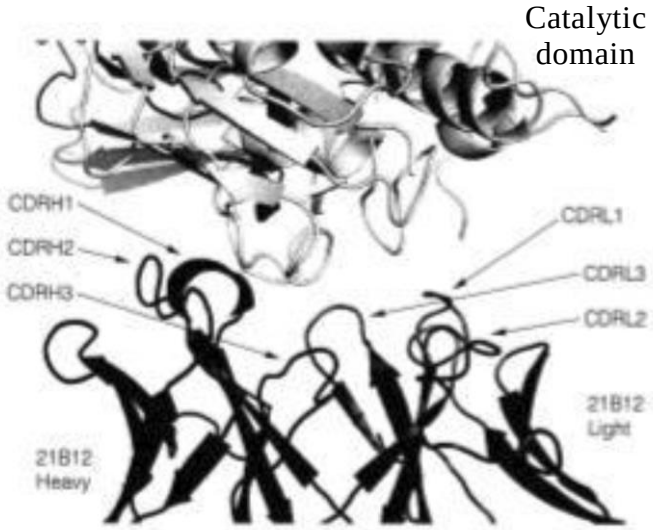


FIG. 19B

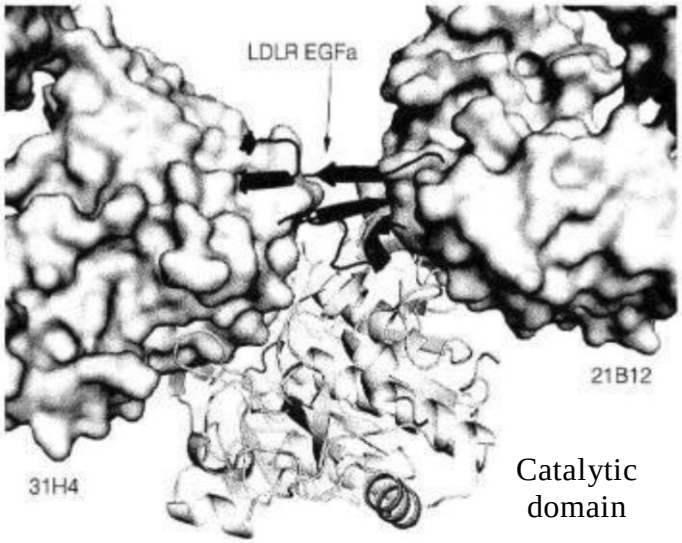


FIG. 20A

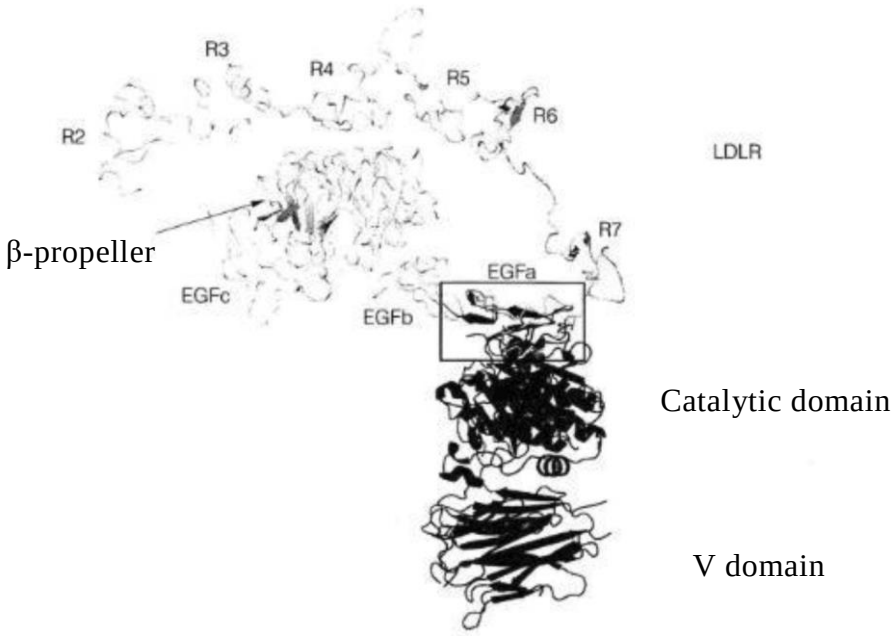


FIG. 20B

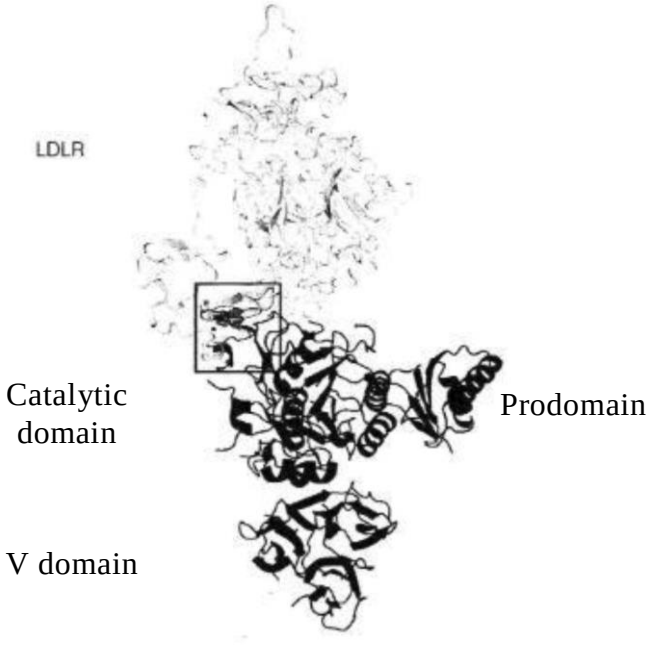


FIG. 20C

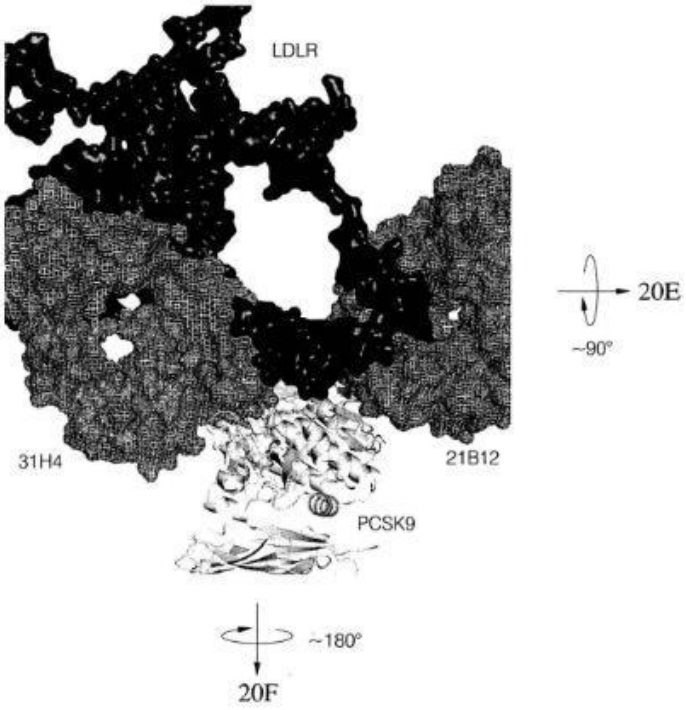
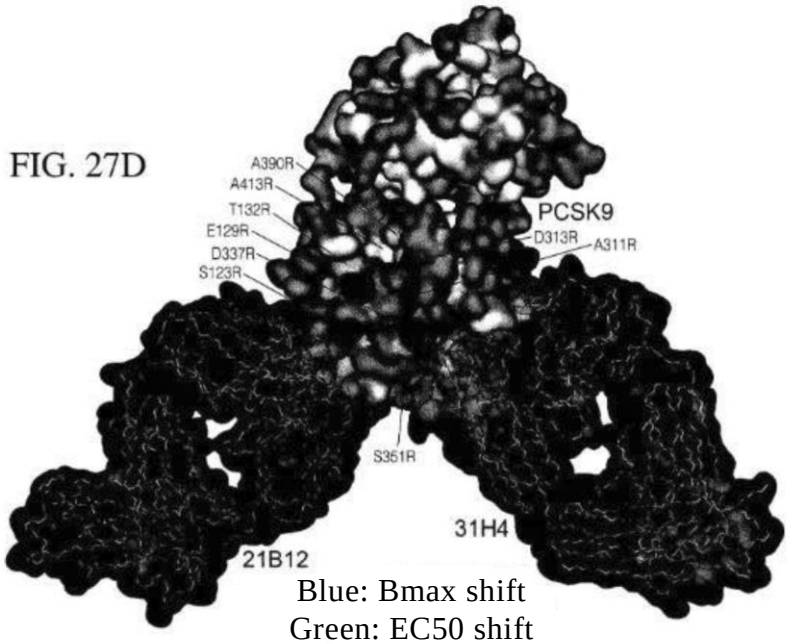


FIG. 20D

FIG. 27D



Blue: Bmax shift
Green: EC50 shift

(Attachment 2)

* When the relevant portion of Exhibit Ko 1 below (page number and line number are based on the original text) is summarily excerpted, it is abbreviated as, for example, "Exhibit Ko 1-1".

1. Page 413, Abstract

"Proprotein convertase subtilisin kexin type 9 (PCSK9) lowers the number of surface low-density lipoprotein (LDL) receptors through an undefined mechanism.... Biosensor studies show that PCSK9 binds to the extracellular domain of LDL receptor with K_d = 170 nM at the neutral pH of plasma and with a K_d as low as 1 nM at the acidic pH of endosomes. The D374Y gain-of-function mutant is associated with hypercholesterolemia and early-onset cardiovascular disease, binds to the receptor 25 times more strongly than wild-type PCSK9 at neutral pH, and remains exclusively a high-affinity complex at acidic pH. PCSK9 can decrease LDL receptors by a mechanism that requires direct binding but not necessarily receptor proteolysis."

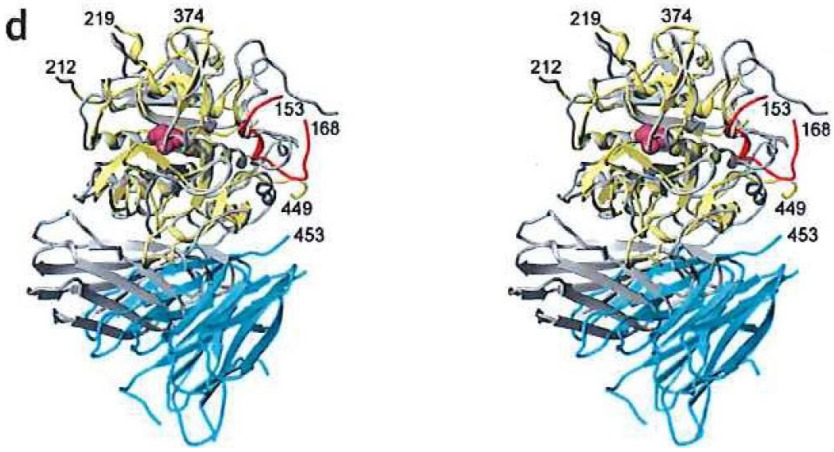
2. Page 413, Left Column, Lines 1 to 21

"Familial hypercholesterolemia produces results of mutations that cause defects in hepatic cholesterol clearance, elevated levels of plasma LDL cholesterol (LDL-C), and an early onset of cardiovascular disease.... Recently, a third locus related to familial hypercholesterolemia has been found: it was a gene encoding a serine protease PCSK9. The protein is predominantly expressed in the liver and lowers the number of cell-surface LDLR by an unknown mechanism. Gain-of-function mutations of PCSK9 are characterized through clinical studies in multiple populations and result in more severe decrease in the number of LDLR with consequent hypercholesterolemia. Loss-of-function mutations lead to increased number of receptors, increasing clearance of LDL-C from the circulating blood and reducing the risk of cardiovascular disease. Thus, PCSK9 is viewed as an attractive new target for therapeutic intervention in dyslipidemia. In addition, this attraction is further heightened by the following evidence. That is, lipid-lowering effects provided by PCSK9 have synergistic effects with those provided by statins widely used as cholesterol biosynthesis inhibitor. This suggests that a PCSK9 inhibitor has benefits that improve those existing therapies."

3. Page 413, Left Column, Lines 22 to 25

"PCSK9 (or NARC-1, Document 11) is the ninth known member of the proprotein convertase family. Convertase zymogens invariably have an N-terminal prodomain, a subtilisin-like catalytic domain, and a C-terminal domain."

4. Page 416, Figure 4



"Figure 4 Structure and orientation of the C-terminal domain.... (d) Stereo view of PCSK9 and furin superimposed with catalytic domains. Gray, furin; red, PCSK9 P' peptide; yellow, PCSK9 catalytic domain (ends at 449); cyan, PCSK9 C-terminal domain (starts at 453); magenta, space-fill, serine 386. Some other PCSK9 residues are displayed."

5. Page 417, Left Column, Lines 1 to 20

"Biophysical Analysis of PCSK9 Binding to LDLR

Secreted PCSK9 interacts with human liver cell surfaces and can be immunoprecipitated with LDLR. Ligand blotting of the recombinant extracellular domain (ECD) of LDLR subjected to SDS-PAGE under non-denaturing conditions has also indicated that the LDLR ECD directly binds to PCSK9.... Most importantly, these studies show that PCSK9 binds to LDLR with more greatly increased affinity (by as much as 170-fold) at the endosomal pH."

6. Page 417, Left Column, Lines 28 to 41

"Gain-of-function Mutations

Gain-of-function mutations in human PCSK9 are associated with familial hypercholesterolemia. In particular, the D374Y mutant is about 10 times more active than wild-type PCSK9 in lowering the number of LDLR. We overexpressed and purified D374Y and two other gain-of-function mutants and tested their binding to LDLR ECD by SPR (Table 1). At pH 7.5, the D374Y showed 25 times greater affinity (K_d = 6 nM) for LDLR extracellular domain (ECD) than that of wild-type PCSK9. Moreover, at pH 5.4, D374Y had a single binding mode with a K_d of 1.6 nM (Table 1). Because 50% of the wild-type PCSK9 had a K_d of 42 nM at acidic pH,

this portion of the binding is strengthened by 25-fold in the case of the D374Y mutant. The results strongly suggest that the increased effect of the D374Y mutant in lowering LDLR results from its enhanced binding to LDLR ECD."

7. Page 417, Table 1

PCSK9	K_d at pH 7.5 (nM)	K_d at pH 5.4 (nM)
Wild-type	169 ± 13	1.0 ± 0.47 (49% ± 9%), 42 ± 14 (51% ± 9%)[a]
F216L	207 ± 21	0.8 ± 0.03 (50% ± 5%), 50 ± 10 (50% ± 5%)
S127R	32 ± 8 (51% ± 1%), 86 ± 23 (51% ± 1%)	0.8 ± 0.10 (41% ± 3%), 56 ± 10 (59% ± 3%)
D374Y	6 ± 1	1.6 ± 0.12

"Table 1 Affinities of PCSK9 and Mutants Binding to Immobilized LDLR ECD

Each value represents the average of three to four determinations and is shown with standard deviations. [a] Two values indicate that the data fit a model with two populations of binding sites or conformations; the fractions of each population are shown in parentheses."

8. Page 418, Left Column, Lines 1 to 17

"LDLR Lowering by PCSK9

The extracellular domain of LDLR contains seven LDLR type A modules (LA1 to LA7), followed by two epidermal growth factor (EGF) repeats, a YWTD β-propeller domain, another EGF repeat, and a highly glycosylated 58-residue segment. At neutral pH, LDL particles primarily bind to the LA3 to LA5 modules. The LA modules often use three conserved, Ca^{2+}-binding acidic residues for protein-protein interactions. Binding of LDL to the cell-surface LDLR is followed by intracellular uptake of the LDL-bound receptor into the endosomal compartment, where the acidic pH promotes a conformational change, which leads to self-association of LDLR between the LA3 to LA5 modules and the β-prodomain. This rearrangement aids in the release of bound LDL and recycling of the ligand-free LDLR to the cell surface, where the LDL liberated in the endosome is degraded in the lysosome. Usually, LDLRs undergo a rapid process of intracellular uptake and recycling, but some LDLR mutants which do not release the bound ligand in endosomes are not recycled to the cell surface."

9. Page 418, Left Column, Lines 18 to 28

"Ligand-blotting experiments have shown that PCSK9 binds directly to LDLR

extracellular domain.... Thus, the increased effect of D374Y in LDLR lowering can indeed arise from enhanced binding to cell-surface LDLR."

10. Page 418, Left Column, Line 29 to Right Column, Line 6

"PCSK9 moves with LDLR into endosomes, suggesting that LDLR-bound PCSK9, like LDL, moves the receptor together into this acidic compartment; however, in contrast to the weakened binding between LDL and LDLR, the affinity between PCSK9 and LDLR can be increased in the endosome. Failure to release PCSK9 can prevent recycling and reduce the number of LDLR on the cell-surface. The D374Y mutant binds to LDLR even more strongly than wild-type PCSK9 does, which can account for its increased activity in LDLR lowering."

11. Page 418, Right column, Lines 10 to 17

"Whether PCSK9 interacts with an unidentified partner to facilitate the release of the prodomain and activate PCSK9 as a protease for LDLR lowering is an open question. The retention of the prodomain in recombinant and plasma PCSK9, together with enhanced LDLR binding of the prodomain S127R mutant, suggests that the prodomain contributes to LDLR binding. The prodomain and catalytic domain are insufficient for activity, suggesting that all three domains are involved in forming an extensive binding surface for LDLR."

12. Page 418, Right column, Lines 23 to 32

"Genetic evidence suggests that PCSK9 is an attractive target for the treatment of cardiovascular disease. In theory, PCSK9 could be targeted by a cell-permeable protease inhibitor which inhibits self-processing and secretion of PCSK9 and achieves effects similar to those of PCSK9 loss-of-function mutations. Because binding to LDLR in plasma and receptor-dependent intracellular uptake are mostly the rate-determining step for PCSK9 function, antibodies or small molecules which bind to PCSK9 in plasma and inhibit its binding to LDLR can also be effective inhibitors of PCSK9 function. Our structure reported here, particularly the structure of the PCSK9-LDLR complex, will be useful for designing novel therapies."

(Attachment 3)

1. "3. By a representative of Regeneron, I was requested to test an ability of various antibodies which bind specifically to hPCSK9 to compete with two reference antibodies (including the variable heavy chain (VH) and variable light chain (VL) regions of 21B12 antibody and 31H4 antibody, respectively)." (Page 1, lines 28 to 32)

2. "4.1 Regeneron Antibody

From Dr. [G]([G]) of Regeneron, we have received 63 different monoclonal antibodies named by the following designations....

4.2 Amgen Antibody

From Dr. [H]([H]) of InSCREENeX, I have received five monoclonal antibodies named 9C9, 3B6, 27B2, 21B12, and 31H4...." (Page 2, line 1 to page 3, line 19)

3. "5.1 Binding of MAb (translator's note: monoclonal antibody) to PCSK9

...

Antibodies from Regeneron and Amgen were diluted with PBS and coated on a 384-well ELISA plate at room temperature for 1 hour (Greiner #781061, > 5 μg/ml, 30 μl/well). Uncoated (antibody-free) wells on the plate were used as negative controls. After uncoated antibodies were removed by washing the plate with H_2O (containing 0.05% Tween 20), blocking of the ELISA plate was performed using 2% BSA solution (containing 0.05% Tween 20) at room temperature for 30 minutes (80 μl/well). At the time when the blocking solution was removed, PCSK9 (received from InSCREENeX) was added to each immobilized antibody in duplicate (30 μl/well, 5 μg/ml in 2% BSA solution containing 0.05% Tween 20). As negative controls, 2% BSA solution (containing 0.05% Tween 20) was added to each immobilized antibody in duplicate. The solution was incubated at room temperature for 45 minutes. After the plate was washed with H_2O (containing 0.05% Tween 20), the captured antigen (Myc-tagged-PCSK9) was detected with HRP-bound anti-Myc antibody (2% BSA solution (containing 0.05% Tween 20) in 1:100,000 dilution, 30 μl/well) at room temperature for 1.5 hours. After a further washing step, TMB (30 μl/well) was added and incubated at room temperature for 20 minutes before stopping the HRP reaction with 30 μl of H_2SO_4 (0.5 M). Absorbance was measured at 450 nm (reference wavelength 620 nm).

Based on the absorbance value, the arithmetic mean value and standard deviation for the same measurement value were calculated.

The results of this experiment are shown in column 2 of the table attached as Material B1. If a signal-to-noise ratio of < 10 is detected when comparing binding

signals with/without PCSK9, it is considered that the MAb (translator's note: monoclonal antibody) concerned "does not bind to soluble PCSK9", which has been highlighted in red. If a signal-to-noise ratio of > 10 is detected when comparing binding signals with/without PCSK9, it is considered that the MAb concerned "binds to soluble PCSK9", which has been highlighted by shading." (Page 4, line 8 to page 5, line 10)

4. "5.2 Competition with 31H4 or 21B12 for Binding to PCSK9"

...

Antibodies from Regeneron and Amgen were diluted with PBS and coated on a 384-well ELISA plate at room temperature for 1 hour (Greiner #781061, > 5 μg/ml, 30 μl/well). Uncoated (antibody-free) wells on the plate were used as negative controls. After uncoated antibodies were removed by washing the plate with H_2O (containing 0.05% Tween 20), blocking of the ELISA plate was performed using 2% BSA solution (containing 0.05% Tween 20) at room temperature for 30 minutes (80 μl/well). At the time when the blocking solution was removed, PCSK9 (received from InSCREENeX) was added to each immobilized antibody in duplicate (30 μl/well, 5 μg/ml in 2% BSA solution containing 0.05% Tween 20). As negative controls, 2% BSA solution (containing 0.05% Tween 20) was added to each immobilized antibody in duplicate. The solution was incubated at room temperature for 45 minutes. After the plate was washed with H_2O (containing 0.05% Tween 20), the captured antigen was detected at room temperature for 45 minutes by adding biotinylated 21B12 or biotinylated 31H4 antibody (30 μl/well, 1.25 μg/ml in 2% BSA solution containing 0.05% Tween 20). After a further washing step, a streptavidin-HRP reagent was added (30 μl/well, 1:5000 dilution in 2% BSA solution containing 0.05% Tween 20) and the solution was incubated at room temperature for 45 minutes. The plate was washed again, and TMB was added (30 μl/well) and incubated at room temperature for 20 minutes before stopping the HRP reaction with 30 μl of H_2SO_4 (0.5 M). Absorbance was measured at 450 nm (reference wavelength 620 nm).

Based on the absorbance value, the arithmetic mean value and standard deviation for the same measurement value were calculated. The compatibility of the 21B12 antibody and the immobilized antibody was quantified by standardizing each individual binding signal to the binding signal of the 21B12 antibody and the immobilized 31H4 antibody (100%). The compatibility of the 31H4 antibody and the immobilized antibody was quantified by standardizing each individual binding signal to the binding signal of the 31H4 antibody and the immobilized 21B12 antibody (100%).

The results of this experiment are shown in column 3 from the left (for 21B12 antibody) and column 4 from the left (for 31H4 antibody) in the table attached as Material B1.

I think that it is necessary to note that Claim 1 of Present Patent 1 and Claim 1 of Present Patent 2 do not indicate a specific value for inhibition of the binding; i.e., competition, between the claimed antibody and the reference antibodies to be achieved in order that other antibodies can be considered to be "competing antibodies" with one of the reference antibodies. As mentioned above, the compatibility of the 31H4 antibody and the immobilized antibody was quantified by standardizing each individual binding signal to the binding signal of the 31H4 antibody and the immobilized 21B12 antibody (100%). However, as is apparent from Material B1 attached, when BSA was immobilized instead of the PCSK9 antibody, the remaining fluorescence (background control) resulted in a signal of 3% and 4% for 21B12-binding compatibility or 31H4-binding compatibility, respectively. Thus, full competition is achieved when the signal is reduced to about 3%. On such grounds, I thought that it was reasonable to use an MAb (translator's note: monoclonal antibody) having a binding compatibility of < 50% with either 21B12 or 31H4 as a competing antibody. The competing antibodies are highlighted by shading in column 3 from the left (for 21B12 antibody) and column 4 from the left (for 31H4 antibody) in the table attached as Material B1." (Page 5, line 11 to page 7, line 2).

5. "5.3 Neutralization of LDLR Binding to PCSK9

...

LDL-R was diluted with PBS and coated on a 384-well ELISA plate at 4°C for 16 hours (Greiner #781061, 1 μg/ml, 30 μl/well). Uncoated (antibody-free) wells on the plate were used as negative controls. After uncoated antigens were removed, blocking of the ELISA plate was performed using 2% BSA solution (containing 0.05% Tween 20) at room temperature for 2 hours (80 μl/well). Then, the plate was washed with 2% BSA solution (containing 0.05% Tween 20).

In the meantime, dilution series of Regeneron and Amgen antibodies were prepared (20 μg/ml, 2 μg/ml, 0.2 μg/ml, and 0 μg/ml in 2% BSA solution (containing 0.05% Tween 20)). Each 50 μl of the antibody dilutions was added to 50 μl of PCSK9 (from Regeneron) in a 96-well plate (1 μg/ml in 2% BSA solution (containing 0.05% Tween 20)) and preincubated at room temperature for 1 hour. After preincubation, each antibody:antigen mixture was transferred to the washed ELISA plate (30 μl/well) and incubated at room temperature for 1 hour. After the plate was washed with H_2O (containing 0.05% Tween 20), PCSK9 was detected using anti-Myc

antibody (30 μl/received, diluted 1:1000 in 2% BSA solution (containing 0.05% Tween 20)) at room temperature for 1 hour. The plate was washed again, and TMB was added (30 μl/well) and incubated at room temperature for 20 minutes before stopping the HRP reaction with 30 μl of H_2SO_4 (0.5 M). Absorbance was measured at 450 nm (reference wavelength 620 nm).

The PCSK9 binding signals in the absence of antibodies were standardized to 100%. The remaining PCSK9 binding signals in the presence of the antibody were calculated accordingly. If the PCSK9 binding activity at the highest antibody concentration was ≤ 60%, the antibody was considered to neutralize.

The values for "PCSK9 activity on LDL-R" observed in these experiments are shown in columns 6 to 9 of the table in Material B1 for 10 μg/ml Mab, 1 μg/ml Mab, 0.1 μg/ml Mab, and 0 μg/ml Mab, respectively. I think that it should be noted that Claim 1 of Present Patent 1 and Claim 1 of Present Patent 2 do not indicate a specific value that needs to be observed for reduced binding of LDLR to PCSK9. However, in order to classify the results, it was necessary to select a threshold value for defining the antibody as neutralizing or non-neutralizing. I thought that it was reasonable to refer to an MAb which reduces binding of PCSK9 to LDL-R by at least 40% at a concentration of 10 μg/ml (i.e., a high MAb with the remaining PCSK9 binding to LDLR being toward 60%) as "neutralizing". Similarly, at 10 μg/ml MAb, if the remaining PCSK9 which binds to LDLR is higher than 60% of the maximum level (i.e., the reduction in PCSK9-LDLR binding is less than 40%), the MAb was referred to as non-neutralizing. In column 5 from the left of the table in Material B1, the neutralizing MAbs are highlighted by shading." (Page 7, line 3 to page 8, line 20)

(Material B1)

Material B1

Antibody	Antibody binding property	21B12 binding compatibility [%]	31H4 binding compatibility [%]	Neutralizing property	PCSK9 activity on LDL-R [%] (10 μg IgG/ml)	PCSK9 activity on LDL-R [%] (1 μg IgG/ml)	PCSK9 activity on LDL-R [%] (0.1 μg IgG/ml)	PCSK9 activity on LDL-R [%] (0 μg IgG/ml)
190515-34	Bind to soluble PCSK9	76	47	Non-neutralizing	-	-	-	-

081211 B	Not bind to soluble PCSK9	-	-	-	-	-	-	-
081203 A	Bind to soluble PCSK9	3	26	Neutralizing	29	31	101	100
081205 B	Bind to soluble PCSK9	3	6	Neutralizing	44	40	89	100
190515 -35	Not bind to soluble PCSK9	-	-	-	-	-	-	-
081212 A	Bind to soluble PCSK9	96	11	Neutralizing	46	45	108	100
190515 -36	Bind to soluble PCSK9	100	107	Non-neutralizing	-	-	-	-
190515 -37	Bind to soluble PCSK9	4	126	Non-neutralizing	-	-	-	-
190515 -48	Bind to soluble PCSK9	97	74	Non-neutralizing	-	-	-	-
190515 -49	Bind to soluble PCSK9	6	6	Non-neutralizing	-	-	-	-
190515 -38	Bind to soluble PCSK9	4	5	Neutralizing	55	43	96	100
190515 -1	Bind to soluble PCSK9	3	102	Non-neutralizing	-	-	-	-
190515 -50	Bind to soluble PCSK9	53	35	Non-neutralizing	-	-	-	-
190515 -51	Bind to soluble PCSK9	54	34	Non-neutralizing	-	-	-	-
190515 -2	Bind to soluble PCSK9	62	39	Non-neutralizing	-	-	-	-
190515 -3	Bind to soluble PCSK9	80	48	Non-neutralizing	-	-	-	-

190515-4	Bind to soluble PCSK9	94	73	Non-neutralizing	-	-	-	-
190515-5	Bind to soluble PCSK9	67	41	Non-neutralizing	-	-	-	-
190515-6	Bind to soluble PCSK9	52	31	Non-neutralizing	-	-	-	-
190515-7	Not bind to soluble PCSK9	-	-	-	-	-	-	-
190515-39	Bind to soluble PCSK9	3	116	Non-neutralizing	-	-	-	-
190515-8	Not bind to soluble PCSK9	-	-	-	-	-	-	-
190515-9	Not bind to soluble PCSK9	-	-	-	-	-	-	-
190515-10	Bind to soluble PCSK9	89	76	Non-neutralizing	-	-	-	-
190515-11	Bind to soluble PCSK9	63	42	Non-neutralizing	-	-	-	-
190515-12	Bind to soluble PCSK9	66	43	Non-neutralizing	-	-	-	-
190515-13	Bind to soluble PCSK9	64	46	Non-neutralizing	-	-	-	-
190515-14	Bind to soluble PCSK9	97	33	Non-neutralizing	-	-	-	-
190515-15	Bind to soluble PCSK9	84	57	Non-neutralizing	-	-	-	-
190515-16	Bind to soluble PCSK9	70	46	Non-neutralizing	-	-	-	-
190515-17	Bind to soluble	69	45	Non-neutralizi	-	-	-	-

	PCSK9			ng				
190515-18	Bind to soluble PCSK9	98	84	Non-neutralizing	-	-	-	-
190515-19	Bind to soluble PCSK9	59	35	Non-neutralizing	-	-	-	-
190515-20	Bind to soluble PCSK9	45	28	Non-neutralizing	-	-	-	-
190515-21	Bind to soluble PCSK9	94	76	Non-neutralizing	-	-	-	-
190515-22	Bind to soluble PCSK9	83	64	Non-neutralizing	-	-	-	-
190515-23	Bind to soluble PCSK9	98	90	Non-neutralizing	-	-	-	-
190515-24	Bind to soluble PCSK9	66	48	Non-neutralizing	-	-	-	-
190515-25	Bind to soluble PCSK9	80	68	Non-neutralizing	-	-	-	-
190515-26	Bind to soluble PCSK9	86	84	Non-neutralizing	-	-	-	-
190515-27	Bind to soluble PCSK9	50	31	Non-neutralizing	-	-	-	-
190515-28	Bind to soluble PCSK9	89	74	Non-neutralizing	-	-	-	-
190515-29	Bind to soluble PCSK9	53	35	Non-neutralizing	-	-	-	-
190515-30	Bind to soluble PCSK9	61	44	Non-neutralizing	-	-	-	-
190515-31	Bind to soluble PCSK9	101	102	Non-neutralizing	-	-	-	-
190515-33	Bind to soluble PCSK9	85	75	Non-neutralizing	-	-	-	-
190515	Bind to	101	3	Neutralizi	40	49	101	100

-46	soluble PCSK9			ng				
080924A	Bind to soluble PCSK9	82	45	Non-neutralizing	-	-	-	-
080924D	Bind to soluble PCSK9	63	38	Non-neutralizing	-	-	-	-
190515-40	Bind to soluble PCSK9	3	3	Non-neutralizing	-	-	-	-
080924C	Bind to soluble PCSK9	62	41	Non-neutralizing	-	-	-	-
080927A	Bind to soluble PCSK9	33	23	Non-neutralizing	-	-	-	-
190515-41	Bind to soluble PCSK9	53	32	Non-neutralizing	-	-	-	-
080930A	Bind to soluble PCSK9	86	63	Non-neutralizing	-	-	-	-
190515-42	Bind to soluble PCSK9	101	101	Neutralizing	42	111	110	100
081006A	Bind to soluble PCSK9	4	7	Non-neutralizing	-	-	-	-
081006B	Bind to soluble PCSK9	62	19	Non-neutralizing	-	-	-	-
081013C	Bind to soluble PCSK9	3	64	Non-neutralizing				
190515-45	Bind to soluble PCSK9	89	63	Non-neutralizing	-	-	-	-
190515-44	Bind to soluble PCSK9	99	102	Non-neutralizing	-	-	-	-
081008B	Bind to soluble PCSK9	101	127	Neutralizing	42	56	98	100
190515-43	Bind to soluble PCSK9	101	4	Neutralizing	46	55	94	100

081017C	Bind to soluble PCSK9	81	56	Non-neutralizing	-	-	-	-
9C9	Bind to soluble PCSK9	3	70	Neutralizing	37	58	84	100
3B6	Bind to soluble PCSK9	3	105	Neutralizing	43	66	96	100
27B2	Bind to soluble PCSK9	9	5	Non-neutralizing	-	-	-	-