Patent Right	Date	December 27, 2018	Court	Intellectual Property High Court, Fourth Division
	Case number	2017 (Gyo-Ke) 10225		
- A case in which, with regard to a patent titled "ANTIGEN BINDING PROTEINS TO PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 (PCSK9)", the court determined that it does not lack inventive step, nor does it violate the provision of support requirement or enablement requirement.				

Case type: Rescission of Trial Decision to Maintain

Result: Dismissed

References: Article 29, paragraph (2); Article 36, paragraph (4), item (i); and Article 36, paragraph (6), item (i) of the Patent Act

Number of related rights, etc.: Patent No. 5705288

Summary of the Judgment

The present case is a case of seeking rescission of JPO decision that dismissed claims for invalidation trial according to Claims 1 and 9 of Patent (Patent No. 5705288), titled "ANTIGEN BINDING PROTEINS TO PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 (PCSK9)". In the present case, Plaintiff argued about the errors in the determination of inventive step, nonconformance to support requirement, and nonconformance to enablement requirement as a ground for rescission.

In summary, the court decision has made the following determination and dismissed the claims by the Plaintiff.

1 Errors in the determination of inventive step

A person ordinarily skilled in the art who reads Exhibit Ko 1 would be motivated to obtain binding neutralizing antibodies of PCSK9 and LDLR, and could have obtained certain monoclonal antibodies (the constitution of Corrected Invention 1 according to different feature A) capable of neutralizing the binding of PCSK9 and LDLR, on the basis of Exhibit Ko 1 and well-known techniques.

On the other hand, in producing an antibody with an animal immunization method, it is a matter of common technical knowledge that antibodies with different reactivities against an antigen may be obtained depending on the difference in "an infusion condition" including the selection of animals, a dosage amount and a dosage form of an antigen, the use of immunization adjuvant, the infusion route and times, and an interval between infusions. It is recognized that an antibody produced from an immunized mouse that is immunized according to an immunization program described

in the description and an antibody produced from an immunized mouse that is immunized according to an immunization program with a different condition and schedule from the above have different reactivities against PCSK9.

Therefore, it is recognized that it takes trial and errors that go beyond the extent that could be usually expected to optimize a condition and a schedule of an immunization program and produce an immunized mouse suitable for obtaining a reference antibody.

Further, the use of transgenic mouse for the production of human antibody and a method of immobilizing an antigen by biotinylation for screening an antibody were well-known as of the priority date in a process of producing a monoclonal antibody; however, a certain level of originality is required in constructing a screening system suitable for obtaining a reference antibody from hybridoma produced by use of the above immunized mouse by utilizing these techniques.

However, Exhibit Ko 1 fails to describe or suggest a condition of immunization program and schedule described in the description. First of all it fails to describe a method for producing an antibody that inhibits the binding of PCSK9 and LDLR.

Therefore, a person ordinarily skilled in the art who read Exhibit Ko 1 could not have easily conceived of obtaining a reference antibody on the basis of Exhibit Ko 1 and well-known techniques. It cannot be recognized that an antibody competing with the reference antibody (the constitution of the Corrected Invention 1 according to the different feature B) was easily conceivable.

2 Error in the determination of nonconformance to the support requirement

A person ordinarily skilled in the art who read the description could recognize that various neutralizing antibodies competing with the reference antibody included in the scope of the claims of the Corrected Invention 1 (Claim 1) other than neutralizing antibodies competing with the reference antibody in the description might be obtained by repetitively implementing the production and selection of immunized mouse in accordance with a procedure and a schedule of immunization program in the description, the hybridoma generation in which a selected immunized mouse is used, and a screening and an epitope binning assay for identifying an antibody that strongly blocks a binding interaction between PCSK9 and LDLR in the description from the start.

Therefore, it is recognized that Corrected Invention 1 (Claim 1) conforms to the support requirement.

Further, in view of the description that it may be therapeutically beneficial since it may treat or prevent or reduce the risk of the diseases associated with increased

cholesterol level such as hypercholesteremia, it can be seen from the description that a person ordinarily skilled in the art can use an antibody of Corrected Invention 1 as a pharmaceutical composition.

Therefore, Corrected Invention 9 (Claim 9) conforms to the support requirement.

3 Error in the determination of nonconformance to the enablement requirement

It can be seen from the description that an antibody of Corrected Invention 1 and a pharmaceutical composition of Corrected Invention 9 may be produced and used. Therefore, the Detailed Description of the Invention of the description is clearly and sufficiently described to the extent that a person ordinarily skilled in the art can work Corrected Inventions 1 and 9.

Therefore, it is recognized that Corrected Inventions 1 and 9 conform to the enablement requirement.

Judgment rendered on December 27, 2018
2017 (Gyo-Ke) 10225 A case of seeking rescission of JPO decision
Date of conclusion of oral argument: October 10, 2018

Judgment

Plaintiff Sanofi

Defendant Amgen Incorporated

Main text

1. The Plaintiff's claim shall be dismissed.
2. The court costs shall be borne by the Plaintiff.
3. An additional period for filing a final appeal and a petition for acceptance of the final appeal against this judgment shall be 30 days.

Facts and reasons

No. 1 Claims

In connection with a trial decision which JPO made on August 2, 2017 with regard to the case of Invalidation Trial No. 2016-800004, a part corresponding to Claims 1 and 9 of Patent No. 5705288 shall be rescinded.

No. 2 Outline of the case

1 History of the procedures, etc. in Japan Patent Office

(1) Defendant divided a part of a patent application with an international filing date of August 22, 2008 (priority date: August 23, 2007, December 21, 2007, January 9, 2008, and August 4, 2008 (hereinafter referred to as 'the priority date'), claiming priority in the United States) (Japanese Patent Application No. 2010-522084) into a patent application on September 20, 2013 for an invention titled 'ANTIGEN BINDING PROTEINS TO PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 (PCSK9)' (Hereinafter referred to as 'the present application') and a patent right was registered on March 6, 2015 (Patent number: No. 5705288, number of claims: 9, hereinafter this patent is referred to as 'the Patent' Exhibits Ko 201, 211).

(2) Plaintiff claimed a trial for patent invalidation with respect to the Patent on January 18, 2016 (Invalidation Trial No. 2016-800004) (Exhibit Ko 212).

Defendant received an advance notice of a trial decision (Exhibit Ko 225) on March 9, 2017, and claimed a correction to correct Claims 1 and 9 and cancel Claims 2 to 4 from a group of claims consisting of Claims 1 to 4 and 9 of the scope of the claims, and cancel a group of claims consisting of Claims 5 to 8 on May 8, 2017 (Hereinafter referred to as 'the Correction.' Exhibit Ko 203).

Thereafter, Japan Patent Office accepted the Correction on August 2, 2017 and made a trial decision to the effect that 'a claim for trial with regard to the inventions according to Claims 1 and 9 of the Patent should be dismissed. A claim for trial according to Claims 2 to 8 should be dismissed by a ruling' (Hereinafter referred to as 'the trial') and its certified copies were served for Plaintiff on August 10.

(3) Plaintiff filed a suit to seek a rescission of a part of a trial decision with regard to Claims 1 and 9 of the Patent on December 8, 2017.

2 Recitation of the claims

The recitation of Claims 1 to 9 of the scope of the claims after the Correction is set forth as below (Hereinafter the invention according to Claim 1 is referred to as 'Corrected Invention 1', and the invention according to Claim 9 is referred to as 'the Corrected Invention 9', Exhibit Ko 203).

[Claim 1]

An isolated monoclonal antibody capable of neutralizing the binding of PCSK9 and LDLR protein, competing with the antibody, comprising: a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO. 49; and a light chain comprising a light chain variable region consisting of the amino acid sequence of SEQ ID NO. 23 for the binding with PCSK9.

[Claim 9]

A pharmaceutical composition comprising an isolated monoclonal antibody of Claim 1.

3 Abstract of reasons of trial decision

The reason for trial decision is as per the attached written trial decision (copy). Particularly, the abstract of a part according to Claims 1 and 9 is set forth below:

(1) Violation of support requirement (Reason 1 for invalidation)
[i] The Detailed Description of the Invention (of the description) describes multiple kinds of specific antibodies encompassed into Corrected Invention 1, and a person ordinarily skilled in the art could recognize from the description of a method for preparing the same and a screening method that an antibody included into Corrected Invention 1 might be further obtained. Thus Corrected Invention 1 is described in the Detailed Description of the Invention over the whole range, and [ii] it is theoretically and experimentally described that an antibody of Corrected Invention 1 may be used as a pharmaceutical, and thus Corrected Invention 9 is also described in the Detailed Description of the Invention.

Therefore, Corrected Inventions 1 and 9 conform to the requirement as specified in Article 36, paragraph (6), item (i) of the Patent Act (support requirement), and thus the Demandant's (Plaintiff's) allegation of Reason 1 for invalidation is not reasonable.
(2) Violation of Enablement requirement (Reason 2 for invalidation)

The Detailed Description of the Invention is described to the extent that it may allow a person ordinarily skilled in the art to make and use the antibody according to Corrected Invention 1 and the pharmaceutical composition according to Corrected Invention 9, and thus the Demandant's (Plaintiff's) allegation of Reason 2 for invalidation of Corrected Inventions 1 and 9 not conforming to the requirement of Article 36, paragraph (4), item (i) of the Patent Act (Enablement requirement) is not reasonable.
(3) Lack of Inventive Step over a main cited reference of Exhibit Ko 1 (Reason 4 for invalidation)

Exhibit Ko 1, publications distributed before the priority date (‘J. Clin. Invest., vol. 116(11),pp. 2995-3005 (2006)’, Translation, Exhibit Ko 1-2) provided a motivation to search a substance that inhibits the interaction between PCSK9 and LDLR for the purpose of development of pharmaceuticals for treatment of hypercholesteremia, and it was well known that antibody is a substance that inhibits interaction between biomolecules. Thus it is recognized that a person ordinarily skilled in the art could have easily conceived of preparing an antibody that inhibits the interaction between PCSK9 and LDLR.

Taking the common technical knowledge into account, however, a specific structure of ‘the antibody comprising: a heavy chain comprising a

heavy chain variable region consisting of the amino acid sequence of SEQ ID NO. 49; and a light chain comprising a light chain variable region consisting of the amino acid sequence of SEQ ID NO. 23' cannot be deduced from Exhibit Ko 1, let alone 'an antibody competing with' the antibody. Therefore, it cannot be recognized that Corrected Inventions 1 and 9 were easily conceivable by a person ordinarily skilled in the art on the basis of Exhibit Ko 1 and well-known technique. The Demandant's (Plaintiff's) allegation of Reason 4 for invalidation is not reasonable.

(omitted)

No. 4 Judgment of this Court

1 Reason 1-1 for Rescission (Errors in the determination of inventive step of Corrected Invention 1)

(1) Described matters of the description

A. The recitation of the scope of the claims of the Corrected Inventions 1 and 9 (Claims 1 and 9) is as per described in the aforesaid No. 2, item 2.

In 'the Detailed Description of the Invention' of the description (Exhibit Ko 201), there are the following descriptions:(see Attachment 1 with respect to 'Table 2', 'Table 3', 'Table 8.3', 'Table 37.1', 'FIG. 1A', 'FIGs. 7A to D', 'FIGs. 14A and B', 'FIGs. 20A to D', and 'FIG. 27D' cited in the following).

(A) Technical Field

[0002]

The present invention relates to antigen binding proteins to proprotein convertase subtilisin kexin type 9 (PCSK9) and a method for using and producing the same.

(B) Background Art

[0003]

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a serine protease involved with the modulation of low-density lipoprotein receptor (LDLR) protein level (Horton et al., 2007; Seidah and Prat, 2007). An in vitro experiment demonstrates that the addition of PCSK9 to HepG2 cell may decrease LDLR level on a cell surface (Benjannet et al., 2004; Lagace et al., 2006; Maxwell et al., 2005; Park et al., 2004). An experiment with mice has demonstrated that the increase of PCSK9 protein level results in a

decreased level of LDLR protein in the liver (Benjannet et al., 2004; Lagace et al., 2006; Maxwell et al., 2005; Park et al., 2004), whereas PCSK9 knockout mice have increased level 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 along with the LDLR, and to be co-immunofluorescent with LDLR throughout the endosomal pathway (Lagace et al., 2006). Degradation of LDLR by PCSK9 has not been observed and the mechanism through which it lowers extracellular LDLR protein levels is uncertain.

(C) Best mode for carrying out the invention

[0066]

As will be appreciated by a person ordinarily skilled in the art, in light of the present disclosure, altering the interactions between PCSK9 and LDLR can increase 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. As such, antigen binding proteins to PCSK9 can be used in various methods and compositions for treating subjects with elevated serum cholesterol levels, at who are at risk of elevated serum cholesterol levels, or who could benefit from a reduction in their serum cholesterol levels. ... 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 or 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 involving 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 that is available to bind to LDL. In some embodiments, ‘PCSK9 activity’

includes any biological activity resulting from PCSK9 signaling. Exemplary activities include, but are not limited to, PCSK9 binding to LDLR, PCSK9 enzyme activity that cleaves LDLR or other proteins, ...
[0109]

An 'antigen binding protein' ('ABP') as used herein means any protein that binds a specified target antigen. In the instant application, the specified target antigen is the PCSK9 protein or a fragment thereof. 'Antigen binding protein' includes but is not limited to antibodies and binding parts thereof, such as immunologically functional fragments. ... The term 'immunologically functional fragment' (or simply 'fragment') of an antibody or immunoglobulin chain (heavy or light chain) antigen binding protein, as used herein, is a species of antigen binding protein comprising a portion (regardless of how that 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 is still capable of specifically binding to an antigen. Such fragments are biologically active in that they bind to the target antigen and can compete with other antigen binding proteins, including intact antibodies, for binding to a given epitope. In some embodiments, the fragments are neutralizing fragments. In some embodiments, the fragments can block or reduce the likelihood of the interaction between LDLR and PCSK9. In one aspect, such a fragment will retain at least one CDR present in the full-length light or heavy chain, and in some embodiments will comprise a single heavy chain and/or light chain or portion thereof. ...
[0123]

'Antigen binding region' means a protein, or a portion of a protein, that specifically binds a specified antigen (e.g., a paratope). For example, that portion of an antigen binding protein that contains the amino acid residues that interact with an antigen and confer on the antigen binding protein its specificity and affinity for the antigen is referred to as 'antigen binding region.' An antigen binding region typically includes one or more 'complementary binding regions' ('CDRs'). Certain antigen binding regions also include one or more 'framework' regions. A 'CDR' is an amino acid sequence that contributes to antigen binding specificity and affinity. 'Framework' regions can aid in maintaining the proper conformation of the CDRs to promote binding between the antigen binding region and an antigen. Structurally, framework regions can be located in

antibodies between CDRs. Examples of framework and CDR regions are shown in FIGs. 2A-3D, 3CCC-JJJ, and 15A-15D. ...

[0127]

The variable regions typically exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair typically are aligned by the framework regions, which can enable binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The assignment of amino acids to each domain is typically in accordance with the definitions 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' encompasses a full-length light chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A 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 at the amino-terminus of the polypeptide. Light chains include κ chains and λ chains.

[0133]

The term 'heavy chain' encompasses a full-length heavy chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A 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 at the amino-terminus of the polypeptide, and the C_H domains are at the carboxyl-terminus, with the C_H3 being closest to the carboxy-terminus of the polypeptide. Heavy chains can be of any isotype, including IgG (including IgG1, IgG2, IgG3, and IgG4 subtypes), IgA (including IgA1 and IgA2 subtypes), IgM, and IgE.

(D) [0138]

The term 'neutralizing antigen binding protein' or 'neutralizing antibody' refers to an antigen binding protein or antibody, respectively, that binds to a ligand and prevents or reduces the 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 and altering the ligand' s ability to bind through indirect means (such as structural or energetic alterations in the ligand). ... In some embodiments, in the case of PCSK9 antigen binding proteins, such a neutralizing molecule can diminish the ability of PCSK9 to bind the LDLR. In some embodiments, the neutralizing ability is characterized and/or described via a competition assay. ... In some embodiments, ABPs 27B2, 13H1, 13B5, and 3C4 are non-neutralizing ABPs, 3B6, 9C9, and 31A4 are weak neutralizers, and the remaining ABPs in Table 2 are strong neutralizers. In some embodiments, the antibodies or antigen binding proteins neutralize 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 antibodies or ABPs neutralize by binding to PCSK9, and while still allowing PCSK9 to bind to LDLR, preventing or reducing the PCSK9 mediated degradation of LDLR. ...

[0140]

The term ‘compete’ when used in the context of antigen binding proteins (e.g., neutralizing antigen binding proteins or neutralizing antibodies) that compete for the same epitope means competition between antigen binding proteins as determined 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 a reference antibody) to a common antigen (e.g., PCSK9 or a fragment thereof). Numerous types of competitive binding assays can be used to determine if one antigen binding protein competes with another; for example: 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 involves the use of purified antigen bound to a solid surface or cells bearing either of an unlabelled test antigen binding protein and a labeled reference antigen binding protein. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test antigen binding

protein. Usually the test antigen binding protein is present in excess. Antigen binding proteins identified by competition assay (competing antigen binding proteins) include antigen binding proteins binding to the same epitope as the reference antigen binding proteins and antigen binding proteins binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antigen binding protein for steric hindrance to occur. ...

[0142]

The term 'epitope' includes any determinant capable being bound by an antigen binding protein, such as an antibody or a T-cell receptor. An epitope is a region of an antigen that is bound by an antigen binding protein that targets that antigen, and when the antigen is a protein, includes specific amino acids that directly contact the antigen binding protein. Most often, epitopes reside on proteins, but in some instances can reside on other kinds of molecules, such as nucleic acids. Epitope determinants can include chemically active surface groupings of molecules such as amino acids, sugar side chains, or phosphoryl or sulfonyl groups, and can have specific three dimensional structural characteristics, and/or specific charge characteristics. Generally, antibodies specific for a particular target antigen will preferentially recognize an epitope on the target antigen in a complex mixture of proteins and/or macromolecules.

(E) [0154]

Antigen binding proteins against PCSK9

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a serine protease involved in regulating the levels of the 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 includes a signal sequence, an N-terminal prodomain, a subtilisin-like catalytic domain, and a C-terminal domain.

[0155]

Antigen binding proteins (ABPs) that bind to PCSK9, including human PCSK9, are provided herein. In some embodiments, the antigen binding proteins provided are polypeptides which comprise one or more complementary determining regions (CDRs), as described herein. In some

antigen binding proteins, the CDRs are embedded into a ‘framework’ region, which orients the CDR(s) such that the proper antigen binding properties of the CDR(s) are achieved. In some embodiments, antigen binding proteins provided herein can interfere with, block, reduce, or modulate the interaction between PCSK9 and LDLR. Such antigen binding proteins are 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 PCSK9's ability to result in the degradation of LDLR, without having to prevent the binding interaction between PCSK9 and LDLR. Such ABPs can be specifically described as ‘non-competitively neutralizing’ ABPs. In some embodiments, the neutralizing ABP binds to PCSK9 in a location and/or manner that prevents PCSK9 from binding to LDLR. Such ABPs can be specifically described as ‘competitively neutralizing’ ABPs. Both of the above neutralizers can result in a greater amount of free LDLR being present in a subject, which results in more LDLR binding to LDL (thereby reducing the amount of LDL in the subject). In turn, this results in a reduction in the amount of serum cholesterol present in a subject.

(F)[0170]

Specific examples of some of the variable regions of the light and heavy chains of the antibodies that are provided and their corresponding amino acid sequences are summarized in TABLE 2.

[0172]

Again, 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 light and heavy chain pairings found in several of the antibodies disclosed herein. …

(G) [0261]

… In some embodiments, the ABP competes with ABP 21B12.

[0268]

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

[0269]

Competing antigen binding proteins

In another aspect, antigen binding proteins are provided that compete with one of the exemplified antibodies or functional fragments binding to the epitope described herein for specific binding to PCSK9. Such antigen binding proteins can also bind to the same epitope as one of the herein exemplified antigen binding proteins, or an overlapping epitope. Antigen binding proteins and fragments that compete with or bind 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, including those with the heavy and light chains, variable region domains, and CDRs included in TABLE 2 and/or FIGs. 2-3 and 15. Thus, as a specific example, the antigen binding proteins that are provided include those that compete with an antibody or antigen binding protein having:

(a) all 6 of the CDRs listed for an antibody listed in FIGs. 2-3 and 15;

(b) a VH and a VL listed for an antibody listed in Table 2; or

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

[0270]

Certain Therapeutic Uses and Pharmaceutical Compositions

In certain instances, PCSK9 activity correlates with a number of human disease states. For example, in certain instances, too much or too little PCSK9 activity correlates with certain conditions, such as hypercholesterolemia. Therefore, 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 methods can treat and/or prevent and/or reduce the risk of disorders that relate to elevated serum cholesterol levels or in which elevated cholesterol levels are relevant.

[0271]

As will be appreciated by a person ordinarily skilled in the art, in light of the present disclosure, disorders that relate to, involve, 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, a ‘cholesterol related disorder’ (which encompasses ‘serum

cholesterol related disorders') includes any one or more of the following: hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimers disease, and generally dyslipidemias, which can be manifested, for example, by an elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated VLDL, and/or low HDL. ...

[0276]

In some embodiments, antigen binding proteins to PCSK9 are used to decrease the amount of PCSK9 activity from an abnormally high level or even a normal level. In some embodiments, antigen binding proteins to PCSK9 are used to treat or prevent hypercholesterolemia and/or in the preparation of medicaments therefore and/or for other cholesterol related disorders (such as those noted herein). 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, reduction of PCSK9 activity to below normal can provide a therapeutic effect.

(H) [0312]

(Example 1)

Immunization and Titering

Generation of Anti-PCSK9 Antibodies and Hybridomas

Antibodies to the mature form of PCSK9 (depicted as the sequence in FIG. 1A, with the pro-domain underlined), were raised in XenoMouse$^{(R)}$ mice (Abgenix, Fremont, CA), which 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 the XenoMouse$^{(R)}$ strain XMG2-KL, which produces fully human IgG2$_{\kappa}$ and IgG2$_{\lambda}$ antibodies. Group 1 mice were immunized with human PCSK9. PCSK9 was prepared using standard recombinant techniques using the GenBank sequence as reference (NM_174936). Group 2 involved mice of the XenoMouse$^{(R)}$ strain XMG4-KL, which 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 antigen eleven times, according to the schedule in Table 3. In the initial immunizations, each mouse was injected with a total of 10 μg of antigen delivered

intraperitoneally into the abdomen. Subsequent boosts were 5 μg doses and injection method was staggered between intraperitoneal injections into the abdomen and subcutaneous injections at the base of the tail. For intraperitoneal injections, antigen is prepared as an emulsion with TiterMax(R) Gold (Sigma, Cat # T2684) and for subcutaneous injections, antigen is mixed with Alum (aluminum phosphate) and CpG oligos. In injections 2 through 8 and 10, each mouse was injected with a total of 5 μg of antigen in the adjuvant alum gel. A final injection of 5 μg of antigen per mouse was delivered in Phospho buffered saline and delivered into 2 sites, 50% IP into the abdomen and 50% SQ at the base of tail. The immunization programs are summarized in Table 3, shown below.

[0320]

Titers of the antibody against human PCSK9 were tested by ELISA assay for mice immunized with soluble antigen as described. Table 4 summarizes the ELISA data and indicates that there were some mice which appeared to be specific for PCSK9. See, e.g., Table 4. Therefore, at the end of the immunization program, 10 mice (in bold in Table 4) were selected for harvest, and splenocytes and lymphocytes were isolated from the spleens and lymph nodes respectively, as described herein.

[0322]

(Example 2)

Recovery of Lymphocytes, B-cell Isolations, Fusions, and Generation of Hybridomas

This example outlines how the immune cells were recovered and the hybridomas were generated. Selected immunized mice were sacrificed by cervical dislocation and the draining lymph nodes were harvested and pooled from each cohort. The B cells were dissociated from lymphoid tissue by grinding in DMEM to release the cells from the tissues, and the cells were suspended in DMEM. The cells were counted, and 0.9 ml DMEM per 100 million lymphocytes was added to the cell pellet to resuspend the cells gently but completely.

[0323]

Lymphocytes were mixed with nonsecretory myeloma P3X63Ag8.653 cells purchased from ATCC, cat.# CR11580 (...) at a ratio of 1:4. The cell mixture was gently pelleted by centrifugation at 400 × g 4 min. After decanting of the supernatant by tilting the vessel, the cells

were gently mixed using a 1 ml pipette. Preheated PEG/DMSO solution from Sigma (cat# P7306) (1 ml per one million of B-cells) was slowly added with gentle agitation over 1 min, followed by 1 min of mixing. Preheated IDMEM (2 ml per one million of B cells) (DMEM without glutamine, L-glutamine, pen/strep, MEM non-essential amino acids (all from Invitrogen), was then 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 400 × g 6 min and resuspended in 20 ml selection media (DMEM (Invitrogen), 15% FBS (Hyclone), supplemented with L-glutamine, pen/strep, MEM Non-essential amino acids, Sodium Pyruvate, 2-Mercaptoethanol (all from Invitrogen), HA-Azaserine Hypoxanthine and OPI (oxaloacetate, pyruvate, bovine insulin) (both from Sigma), and IL-6 (Boehringer Mannheim)) per million B-cells. Cells were incubated for 20-30 min at 37°C and then resuspended in 200 ml selection media and cultured for 3-4 days in a T175 flask prior to 96 well plating. Thus, hybridomas that produced 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 PCSK9 binding to LDLR and affinity. Binding to soluble PCSK9 was analyzed by ELISA, as described below. BIAcore(R) (surface plasmon resonance) was used to quantify binding affinity.

[0326]

Primary Screening

A 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 employed. The plates were coated with neutravadin 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. The plates were then washed using a Titertek plate washer (Titertek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 90 μl of 1×PBS/1% milk and incubated approximately 30 minutes at room temperature. The plates were then washed. Again, a 3-cycle wash was performed. The capture sample was biotinylated-PCSK9, without a V5 tag, and was added at 0.9 μg/ml in 1×PBS/1% milk/10 mM Ca^{2+} at a volume of 40 μl/well. The plates were then incubated for 1 hour at room temperature. Next, the plates were washed using the Titertek plate washer operated using a 3-cycle wash. 10 μl of supernatant was transferred into 40 μl of 1×PBS/1% milk/10mM Ca^{2+} and incubated 1.5 hours at room temperature. Again the plates were washed using the Titertek plate washer operated using a 3-cycle wash. 40 μl/well of Goat anti-Human IgG Fc POD at a concentration of 100 ng/ml (1:4000) in 1×PBS/1% milk/10mM Ca^{2+} was added to the plate and was incubated 1 hour at room temperature. The plates were washed once again, using a 3-cycle wash. Finally, 40 μl/well of One-step TMB (Neogen, Lexington, Kentucky) was added to the plate, and quenching with 40 μl/well of 1N hydrochloric acid was performed after 30 minutes at room temperature. ODs were read immediately at 450 nm using a Titertek plate reader.

[0328]

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

[0329]

Confirmatory Screening

The 3000 positives were then rescreened for binding to wild-type PCSK9 to confirm that stable hybridomas were established. ... A total of 2441 positives repeated in the second screening. These antibodies were then used in the subsequent screenings.

[0330]

Mouse Cross-reactivity Screening

The panel of hybridomas was then screened for cross-reactivity to mouse PCSK9 to make certain that the antibodies could bind to both human and mouse PCSK9. ... 579 antibodies were observed to cross-react with mouse PCSK9. These antibodies were then used in the subsequent screenings.

[0331]

D374Y Mutant Binding Screening

The D374Y mutation in PCSK9 has been documented in the human population (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 for 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. ... Over 96% of the positive hits on the wild-type PCSK9 also bound to mutant PCSK9.

[0332]

Large Scale Receptor Ligand Blocking Screening

To screen for the antibodies that block PCSK9 binding to LDLR, an assay was developed using the D374Y PCSK9 mutant. The mutant was used for this assay because it has a higher binding affinity to LDLR, enabling development of a more sensitive receptor ligand blocking assay. The following protocol was employed in the receptor ligand blocking screening: Costar 3702 medium binding 384 well plates (Corning Life Sciences) were employed in the screening. The plates were coated with goat anti-LDLR (R&D Cat #AF2148) at 2 µg/ml in 1×PBS/0.05%Azide at a volume of 40 µl/well. The plates were incubated at 4°C overnight. The plates were then washed using a Titertek plate washer (Titertek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 90 µl of 1×PBS/1% milk and incubated for approximately 30 minutes at room temperature. The plates were then washed using the Titertek plate washer. A 3-cycle wash was performed. The capture sample was LDLR (R&D, Cat #2148LD/CF), and was added at 0.4 µg/ml in 1×PBS/1% milk/10mM Ca^{2+} at a volume of 40 µl/well. The plates were then incubated for 1 hour and 10 minutes at room temperature. Contemporaneously, 20 ng/ml of biotinylated human D374Y PCSK9 was incubated with 15 microliters of hybridoma exhaust supernatant in Nunc polypropylene plates and the

exhaust supernatant concentration was diluted 1:5. The plates were then pre-incubated for about 1 hour and 30 minutes at room temperature. Next, the plates were washed using the Titertek plate washer operated using a 3-cycle wash. 50 µl/well of the pre-incubated mixture was transferred onto the LDLR coated ELISA plates and incubated for 1 hour at room temperature. To detect LDLR-bound b-PCSK9, 40 µl/well streptavidin HRP at 500 ng/ml in assay diluent was added to the plates. The plates were incubated for 1 hour at room temperature. The plates were again washed using a Titertek plate washer. A 3-cycle wash was performed. Finally, 40 µl/well of One-step TMB (Neogen, Lexington, Kentucky) was added to the plate, which was quenched with 40 µl/well of 1N hydrochloric acid after 30 minutes at room temperature. ODs were read immediately at 450 nm using a Titertek plate reader. The screening identified 384 antibodies that blocked the interaction between PCSK9 and the LDLR well, including 100 antibodies that blocked the interaction strongly (OD < 0.3). These antibodies inhibited the binding interaction of PCSK9 and LDLR at a rate greater than 90% (greater than 90% inhibition).

[0333]

Receptor Ligand Binding Assay on Blocker Subset

The receptor ligand assay was then repeated using the mutant enzyme on the 384 member subset of neutralizers identified in the first large scale receptor ligand inhibition assay. The same protocol was employed in the screening of the 384 member blocker subset assay as was done in the large scale receptor ligand blocking screening. This repeat screening confirmed the initial screening data.

[0334]

This screening of the 384 member subset identified 85 antibodies that blocked interaction between the PCSK9 mutant enzyme and the LDLR at a rate greater than 90%.

[0335]

Receptor Ligand Binding Assay of Blockers that Bind to the Wild Type PCSK9 but not to the D374Y Mutant

In the initial panel of 3000 sups there were 86 antibodies shown to specifically bind to the wild-type PCSK9 and not to the huPCSK9 (D374Y) mutant. These 86 sups were tested for the ability to block binding of wild-type PCSK9 to the LDLR receptor. ...

[0336]

Screening Results

Based on the results of the assays described, several hybridoma lines were identified as producing antibodies with desired interactions 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). In general, no difference among the different clones of a particular line was detected by the functional assays described herein. In a few cases, clones were identified from a particular line that behaved differently in the functional assays; for example, 25A7.1 was found not to block PCSK9/LDLR but 25A7.3 (referred to herein as 25A7) was neutralizing. The isolated clones were each expanded in 50-100 ml of hybridoma media and allowed to grow to exhaustion, (i.e., less than about 10% cell viability). The concentration and potency of the antibodies to PCSK9 in the supernatants of those cultures were determined by ELISA and by in vitro functional testing, as described herein. As a result of the screening described herein, the hybridomas with the highest titer of antibodies to PCSK9 were identified. The selected hybridomas are shown in FIGs 2A-3D and Table 2.

[0373]

(Example 10)

Epitope Binning

Competition ELISA was used for anti-PCSK9 antibody binning. Briefly, 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. The plate was then washed and blocked with 3% BSA. Meanwhile, 30 ng/ml of biotinylated hPCSK9 was incubated with the second antibody (mAb2) for 2 hours at room temperature. The mixture was applied to coated mAb1 and incubated for 1 hour at room temperature. The ELISA plate was then washed and incubated with Neutravidin-HRP (Pierce) at 1:5000 dilutions for 1 hour. After another wash, the plate was incubated with TMB substrate and signal was detected at 650 nm using a Titertek plate reader. Antibodies with the same binding profiles were grouped together into the same epitope bin. The results of the antibody binning studies are presented in Table 8.3.

(I) [0377]

(Example 11)

Efficacy of 31H4 and 21B12 for Blocking D374Y PCSK9/LDLR Binding

This example provides the IC_{50} values for two of the antibodies in blocking PCSK9 D374Y's ability 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). Plates were washed thoroughly with buffer A and then blocked for 2 hours with buffer B (1% milk in buffer A). After washing, plates were incubated for 1.5 hours with 0.4 μg/ml of LDL receptor (R&D Systems) diluted in buffer C (buffer B supplemented with 10 mM $CaCl_2$). Concurrent 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, which 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 them and incubated for 1 hour at room temperature. Binding of the biotinylated D374Y to the LDL receptor was detected by incubation with streptavidin-HRP (Biosource) at 500 ng/ml in buffer C followed by TMB substrate (KPL). The signal was quenched with 1N HCl and the absorbance read at 450 nm.

[0378]

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

[0379]

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

[0380]

(Example 12)

Cell LDL Uptake Assay

This example demonstrates the ability of various antigen binding proteins to reduce LDL uptake by cells. ...

[0381]

The results of the cellular uptake assay are shown in FIGs. 7A-7D. Summarily, IC_{50} values were determined for each antibody and found to be 16.7 nM for 31H4 IgG2 (FIG. 7A), 13.3 nM for 31H4 IgG4 (FIG. 7B), 13.3 nM for 21B12 IgG2 (FIG. 7C), and 18 nM for 21B12 IgG4 (FIG. 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.

(J) [0382]

(Example 13)

Serum cholesterol Lowering Effect of the 31H4 Antibody in 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, aged 9-10 weeks, 17-27 g) obtained from Jackson Laboratory (Bar Harbor, ME) were fed a normal chow (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.

[0385]

Mice were sacrificed with CO_2 asphyxiation at the pre-determined time points shown in Table 9. Blood was collected via the vena cava into eppendorf tubes and was allowed to clot at room temperature for 30 minutes. The samples were then spun down in a tabletop centrifuge at 12,000 × g for 10 minutes to separate the serum. 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 FIGs. 8A-8D. Summarily, mice to which antibody 31H4 was administered showed decreased serum cholesterol levels over the course of the experiment (FIG. 8A and FIG. 8B). In addition, it is noted that the mice also showed decreased HDL levels (FIG. 8C and FIG. 8D). For FIG. 8A and FIG. 8C,

the percentage change is in relation to the control IgG at the same time point (*P<0.01, # P<0.05). For FIG. 8B and FIG. 8D, the percentage change is in relation to total serum cholesterol and HDL levels measured in naive animals at t=0 hrs (*P<0.01, # P<0.05).

[0387]

In respect to the lowered HDL levels, it is noted that a person ordinarily skilled in the art will appreciate that the decrease in HDL in mice is not indicative that an HDL decrease will occur in humans and merely further reflects that the serum cholesterol level in the organism has decreased. It is noted that mice transport the majority of serum cholesterol in high density lipoprotein (HDL) particles, which is different from the case of humans, who carry most serum cholesterol on LDL particles. In mice the measurement of total serum cholesterol most closely resembles the level of serum HDL-C. Mouse HDL contains apolipoprotein E (apoE), which is a ligand for the LDL receptor (LDLR) and allows it to be cleared by the LDLR. Thus, examining HDL is an appropriate indicator for the present example, in mice (with the understanding that a decrease in HDL is not expected for humans). For example, human HDL, in contrast, does not contain apoE and is not a ligand for the LDLR. As PCSK9 antibodies increase LDLR expression in mouse, the liver can clear more HDL, and therefore lowers 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 the level of LDLR in a subject, as predicted, over time. A Western blot analysis was performed in order to ascertain the effect of antibody 31H4 on LDLR levels. 50-100 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 complete protease inhibitor (Roche). The homogenate was incubated on ice for 30 minutes and centrifuged to pellet cellular debris. Protein concentration in the supernatant was measured using BioRad protein assay reagents (BioRad laboratories). 100 µg of protein was denatured at 70°C for 10 minutes and separated on 4-12% Bis-Tris SDS gradient gel (Invitrogen). Proteins were 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 for 1 hour at room temperature. The blot was then probed with goat anti-mouse LDLR antibody (R&D system) 1:2000 or anti-β actin (sigma) 1:2000 for 1 hour at room temperature. The blot was washed briefly and incubated with bovine anti-goat IgG-HRP (Santa Cruz Biotechnology Inc.) 1:2000 or goat anti-mouse IgG-HRP (Upstate) 1:2000. After a 1-hour incubation at room temperature, 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 depicted in FIG. 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, aged 9-10 weeks, 17-27 g) obtained from Jackson Laboratory (Bar Harbor, ME) were fed a normal chow ... 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.

[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 demonstrated a 31% decrease in serum cholesterol on day 3, which gradually returned to pre-dosing levels by day 13. FIG. 10A depicts the results of this experiment. FIG. 10C depicts the results of repeating the

above procedure with the 10 mg/kg dose of 31H4, and with another antibody, 16F12, also at 10 mg/kg. Dosing groups and time of sacrifice are shown in Table 11.

[0395]

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

(K) [0422]

(Example 26)

Mouse Model for PCSK9 and ABP ability to lower LDL in vivo

To generate mice which over-expressed human PCSK9, three-week-old WT C57B1/6 mice were injected via tail vein administration with various concentrations of adenoassociated virus (AAV), recombinantly modified to express human PCSK9, to determine the correct titer which would provide a measurable increase of LDL-cholesterol in the mice. Using this particular virus that expressed human PCSK9, it was determined that 4.5×10E12 pfu of virus would result in an LDL-cholesterol level of approximately 40 mg/dL in circulating blood (normal levels of LDL in a WT mice are approximately 10 mg/dL). The human PCSK9 levels in these animals were found to be approximately 13 μg/mL. A colony of mice was generated using this injection criteria.

[0423]

One week after injection, mice were assessed for LDL-cholesterol levels, and randomized into different treatment groups. Animals were then administered, via tail vein injection, a single bolus injection of either 10 mg/kg or 30 mg/kg of 16F12, 21B12, or 31H4 antigen binding proteins. IgG2 ABP was administered in a separate group of animals as a dosing control. Subgroups of animals (n=6-7) were then euthanized at 24 and 48 hours after ABP administration. There were no effects on LDL-cholesterol levels following IgG2 administration at either dose. 31H4 and 21B12

demonstrated significant LDL-cholesterol lowering up to 48 hours (and including 48 hours) post-administration, as compared to IgG2 control (shown in FIGs. 14A and 14B at two different doses). 16F12 shows an intermediary LDL-cholesterol lowering response, with levels returning to baseline of approximately 40 mg/dL by the 48 hour time point. These data are consistent with in vitro binding data (Biacore and Kinexa), which show near equivalent binding affinity between 31H4 and 21B12, and a lesser affinity of 16F12 to human PCSK9.

(L) [0438]

(Example 30)

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

[0439]

The present example presents the crystal structure of PCSK9 ProCat (31-449 of SEQ ID NO: 3) bound to the Fab fragments of 31H4 and 21B12, determined at 2.8 Å resolution (the conditions for which are described in the below Examples). This crystal structure, depicted in FIG. 19A and FIG. 19B, shows that 31H4 and 21B12 have distinct 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 from PCSK9's catalytic domain. 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 within 5 Å 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 from Example 30 demonstrate where antigen binding proteins to PCSK9 can interact on PCSK9 and still block PCSK9 from interacting with EGFa (and thus LDLR). Thus, antigen binding proteins that interact with any of these PCSK9 residues or that block any of these residues can be useful as antibodies that inhibit the interaction of PCSK9 and EGFa (and LDLR accordingly). Thus, in some embodiments, antibodies that interact

with any of the above residues or interact with residues that are within 5 Å of the above residues are contemplated to provide useful inhibition PCSK9 binding to LDLR. Similarly, antigen binding proteins that block any of the above residues (which can be determined, for example, via a competition assay) can also be useful for inhibition of PCSK9/LDLR interaction.

(M) [0489]

(Example 37)

Epitope Mapping-Binning

An alternative set of binning experiments was conducted in addition to the set in Example 10. As in Example 10, ABPs that compete with each other can be thought of as binding to the same site on the target and in common parlance are said to 'bin' together.

[0490]

A modification of the Multiplexed Binning method described by Jia, et al (J. Immunological Methods, 288 (2004) 91-98) was used. Individual bead cords of streptavidin-coated Luminex beads were incubated in 100 µl of 0.5 µg/ml biotinylated monovalent mouse-anti-human IgG capture antibody (BD Pharmingen, #555785) for 1 hour at room temperature in the dark, then washed 3× 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 then washed 3× with PBSA. The beads were pooled and then dispensed to a 96-well filter plate (Millipore, #MSBVN1250). 100 µl 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 then washed. 100 µl of a 2 µg/ml anti-PCSK9 antibody (Detection Ab) was added to all the wells, incubated for 1 hour, then washed. An irrelevant human-IgG (Jackson, #009-000-003) was run as another control. 20 µl PE-conjugated monovalent mouse-anti-human IgG (BD Pharmingen, #555787) was added to each well and incubated for 1 hour, then washed. Beads were resuspended in 100 µl PBSA and a minimum of 100 events/bead cord were collected on the BioPlex instrument (BioRad).

[0491]

Median Fluorescent Intensity (MFI) of the antibody pair without PCSK9 was subtracted from signal of the corresponding reaction containing PCSK9. For the antibody pair to be considered bound simultaneously, and

therefore in different bins, the subtracted signal had to be greater than 3 times the signal of the antibody competing with itself and 3 times the signal of the antibody competing with the irrelevant antibody.

[0492]

The data from the above are depicted in FIGs. 23A-23D. The ABPs fell into five bins. The shaded boxes indicate ABPs that can bind simultaneously to PCSK9. The nonshaded boxes indicate those ABPs that compete with each other for binding. A summary of the results is shown in Table 37.1.

[0494]

Bins 1 (competes with ABP 21B12) and 3 (competes with 31H4) are mutually exclusive; bin 2 competes with bins 1 and 3; and bin 4 does not compete with bins 1 and 3. Bin 5, in this example, is presented as a 'catch all' bin to describe those ABPs that fit into the other bins. Thus, the above identified ABPs in each of the binds are representative of different types of epitope locations 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, then the antibodies are said to be in the same bin. The order in which the ABPs are employed can be important. If ABP A is employed as the reference ABP and blocks the binding of ABP B, the converse is not always true: ... In general, if competition is observed in either order the ABPs are said to be bin together, and if both ABPs can block each other then it is likely that the epitopes overlap more completely.

(N) [0521]

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

[0523]

To further examine how these residues form part of or all of the relevant epitopes, the above noted positions were mapped onto various crystal structure models, and the results are shown in FIGs. 27A through 27E. ...

[0526]

FIG. 27D depicts the 12H11 epitope hits, as mapped onto the crystal structure of PCSK9 with 31H4 and 21B12 antibodies. The structure

identifies PCSK9 residues as follows: light gray indicates those residues that were not mutated (with the exception of those residues that are explicitly indicated on the structure) and darker gray indicates those residues that were mutated (a minority of which failed to express). Residues that are explicitly indicated were tested (regardless of the shading indicated on the figure) and resulted in a significant change in EC50 and/or Bmax. 12H11 competes with 21B12 and 31H4 in the binning assay described above.

B. According to the described matter of the aforesaid A, it is recognized that the Detailed Description of the Invention has the following disclosure with regard to Corrected Inventions 1 and 9.

(A) PCSK9 (Proprotein convertase subtilisin kexin type 9) is a serine protease that binds to LDLR (low density lipoprotein receptor) and interacts therewith to be incorporated into liver cells together with LDLR and decrease LDLR level in liver, and further decrease an amount of LDLR available for the binding to LDL on cell surface (extracellular) to increase an amount of LDL in a subject ([0002], [0003], [0071]).

An isolated monoclonal antibody ‘competing’ with an antibody (‘21B12’) (reference antibody) comprising: a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO. 49; and a light chain variable region consisting of the amino acid sequence of SEQ ID NO. 23 that binds to PCSK9 is a neutralization antigen binding protein (neutralizing ABP) that blocks or reduces, and ‘competitively neutralizes’ the interaction (binding) between PCSK9 and LDLR in a location and/or a manner that prevents PCSK9 from binding to LDLR ([0138], [0140], [0155], [0261], [0269], [Table 2]).

(B) This neutralizing ABP against PCSK9 can neutralize the binding of PCSK9 and LDLR and increase the amount of LDLR, thereby decreasing the amount of serum LDL in a subject, resulting in a reduction in the subject's serum cholesterol level, and this effect may treat or prevent or reduce the risk of the diseases associated with increased cholesterol level such as hypercholesteremia, and thus may be therapeutically beneficial ([0155], [0270], [0271], [0276]).

(2) Disclosure of Exhibit Ko 1

A. Exhibit Ko 1 (translation, Exhibit Ko 1-2) has the following descriptions: (see the attachment 2 with respect to ‘FIG. 2A’ and ‘FIG. 3’ cited in the following description).

(A) ‘Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice’ (page 2995, article name, translation, page 1)

(B) ‘We show that purified PCSK9 added to the medium of HepG2 cells reduces the number of cell-surface LDLRs in a dose- and time-dependent manner. This activity was approximately 10-fold greater for a gain-of-function mutant, PCSK9(D374Y), that causes hypercholesterolemia. Binding and uptake of PCSK9 were largely dependent on the presence of LDLRs. Coimmunoprecipitation and ligand blotting studies indicated that PCSK9 and LDLR directly associate; ... To determine whether PCSK9 was active in plasma, transgenic PCSK9 mice were parabiosed with wild-type littermates. After parabiosis, secreted PCSK9 was transferred to the circulation of wild-type mice and reduced the number of hepatic LDLRs to nearly undetectable levels. We conclude that secreted PCSK9 associates with the LDLR and reduces hepatic LDLR protein levels.’ (page 2995, lines 3 to 14, translation, page 1)

(C) ‘The biological activity of PCSK9 was revealed through overexpression studies in mice. Overexpression of PCSK9 posttranscriptionally reduced the amount of LDLR protein in liver (3, 8-10). Confirmation that PCSK9 functions normally to regulate LDLR protein levels came from loss-of-function studies in humans and mice. Individuals who are heterozygous for a nonsense mutation in allele PCSK9 have significantly lower plasma LDL cholesterol levels, suggesting that a reduction in PCSK9 activity leads to an increase in LDLRs (11). These conclusions were supported by studies in PCSK9-knockout mice, which revealed that loss of PCSK9 resulted in increased numbers of LDLRs in hepatocytes, accelerated plasma LDL clearance, and significantly lowered plasma cholesterol levels (12). In the most recent studies, humans heterozygous for loss-of-function mutations in PCSK9 were shown to have a significant reduction in the long-term risk of developing atherosclerotic heart disease (13).

The genetic data from humans and the in vivo studies in mice demonstrate that one function of PCSK9 is to reduce the number of the

LDLRs and that this function is manifest in humans in the basal state.' (page 2995, right column, lines 6 to 25, Translation, page 1 to page 2)

(D) 'FIG. 2

Reduction of endogenous LDLRs in HepG2 cells following the addition of recombinant purified PCSK9 to the culture medium. (A) Dose response of exogenous PCSK9-mediated LDLR degradation in HepG2 cells.'

(page 2996, left column, Translation page 2)

'As shown in FIG. 2A, the number of cell-surface LDLRs declined by approximately 50% after incubation with the physiologically relevant concentration of 0.5 μg/ml PCSK9 (lane 2) and became nearly undetectable after exposure to 2.5 μg/ml PCSK9 (lane 4). Incubation of HepG2 cells for 4 hours with 5 or 10 μg/ml PCSK9 decreased whole-cell LDLR protein levels by approximately 50% (lanes 11 and 12). FLAG-tagged PCSK9 was detected in whole-cell extracts in a concentration-dependent manner (lanes 7-12), but it was not detected among the biotin-labeled cell-surface proteins (lanes 1-6), suggesting that most of the cell-associated PCSK9 had been internalized.' (page 2997, left column, lines 11 to 21, Translation, page 2 to page 3)

(E) 'As discussed in the Introduction, certain point mutations in PCSK9 cause hypercholesterolemia. To determine whether one such mutation increases the activity of PCSK9 in a cell-based assay, varying amounts of wild-type PCSK9 and the PCSK9 mutant D374Y (4) were added to HepG2 cells, after which LDLR protein levels were measured (FIG. 3). The D374Y mutation was chosen for study because individuals who harbor this mutation have been shown to manifest severe hypercholesterolemia (16). PCSK9 (D374Y) was at least 10-fold more active than wild-type PCSK9 in reducing cell surface LDLRs. Thus, PCSK9 (D374Y) at 0.25 μg/ml was at least as effective as 2.5 μg/ml of wild-type PCSK9 (compare lanes 5 and 11). After incubation with wild-type PCSK9, the number of LDLRs was significantly reduced in whole-cell extracts, and similar results were obtained with 10-fold lower concentrations of PCSK9 (D374Y) (lanes 13-24). Despite the different concentrations were employed, the amounts of wild-type and mutant PCSK9 measured in the cell extracts were similar, indicating that the mutant protein was taken up by the cell approximately 10-fold more efficiently than the wild-type protein.' (page 2997, left column, lines 30 to right column, 7, Translation, page 3)

‘FIG. 3

Increased cell association and LDLR degradation by addition of purified mutant PCSK9(D374Y) to the medium of HepG2 cells. Cells were cultured for 18 hours in medium C and then incubated for 4 hours with the indicated amounts of purified human PCSK9 or PCSK9(D374Y). Immunoblot analysis of LDLR, FLAG-tagged PCSK9, and transferrin receptor was carried out as described in the legend to FIG. 2. The asterisk indicates a nonspecific band. Similar results were obtained in 3 independent experiments.’ (page 2997, Translation pages 3 to 4)

(F) ‘Consistent with the coimmunoprecipitation, the PCSK9(D374Y) mutant appeared to bind to the LDLR protein with a greater affinity. Combined, the results of these studies indicate that PCSK9(D374Y) binds to LDLRs with higher affinity than does wild-type PCSK9, a finding that correlates with the enhanced ability of the mutant PCSK9 to destroy LDLRs.’ (page 2998, right column, lines 20 to 25, Translation, page 4)

(G) ‘In the current report, we demonstrate that endogenous PCSK9 is rapidly secreted from cells and that secreted PCSK9 destroys LDLRs when added to the medium of cultured HepG2 cells and mouse primary hepatocytes. The effective concentration of PCSK9 required to reduce the number of LDLRs in cultured cells was within the range of plasma PCSK9 concentrations measured in human plasma. The cell association and uptake of PCSK9 occurred via binding to the LDLR, and both proteins colocalized to a late endocytic/lysosomal compartment. The internalization of PCSK9 with the LDLR into an endosomal/lysosomal compartment was required for PCSK9 to reduce LDLR protein levels, since this activity was blocked in the absence of ARH. Finally, we show that PCSK9 was present in plasma of transgenic mice and that the secreted protein was active in destroying hepatic LDLRs.

Insights into the mechanism of secreted PCSK9's action were derived from studies in MEFs and mouse hepatocytes, which showed that LDLRs were required for the majority of PCSK9 to associate with the cell surface (FIG. 4A and FIG. 6B). These studies suggested that the LDLR and PCSK9 might directly interact with the LDLR and PCSK9, which was confirmed by coimmunoprecipitation and ligand blotting studies, (FIG. 5).’ (page 3001, left column, line 24 from the bottom to line 2 from the bottom, Translation, page 4)

(H) ‘Considered together, the available data now suggest that PCSK9 can function both extra- and intracellularly, but we do not know which pathway predominates under normal and/or pathologic conditions. Currently, all studies suggesting that the protein functions intracellularly have been performed using PCSK9 overexpression via a strong CMV promoter. Overexpression may permit association of PCSK9 and the LDLR in an intracellular compartment that does not occur physiologically. In the current studies, we were able to demonstrate that physiologically relevant concentrations of PCSK9 could significantly reduce the number of cell-surface LDLRs when added to HepG2 cells (FIGs. 2 and 3).’ (page 3002, left column, line 7 from the bottom to right column, line 4, Translation, pages 4 to 5).

(I) ‘The genetic data from humans with loss-of-function mutations in PCSK9 combined with the studies in knockout mice that lack PCSK9 clearly indicate that inhibitors of the protease would be of therapeutic benefit for the treatment of hypercholesterolemia. Inasmuch as overexpression of the catalytically inactive form of PCSK9 in mice did not alter LDLR protein levels (Document[9]), an inhibitor of PCSK9's protease activity in the ER should be sufficient to block its ability to reduce LDLR protein levels. If PCSK9 functions as a secreted factor as suggested by the current data, then additional approaches to neutralize its activity, including the development of antibodies to block its interaction with the LDLR or inhibitors to block its action in plasma, can be explored for the treatment of hypercholesterolemia.’ (page 3002, right column, line 13 from the bottom to last line, Translation, page 5)

(J) ‘Antibodies. For the anti-human PCSK9 polyclonal antibody, the human PCSK9 amino acid sequence was analyzed using Protean software (Lasergene; DNAStar) for immunogenic regions. Amino acids 165-180 (RYRADEYQPPDGGSLV) and 220-240 (ASKCDSHGTHLAGVVSGRDAG) were synthesized, conjugated to keyhole-limpet hemocyanin using the Imject Maleimide Activated mcKLH kit (Pierce), and rabbits were injected with a mixture of the peptides (20 μg each) as described previously (28). IgG fractions from sera were purified using the ImmunoPure (A/G) IgG purification kit (Pierce).’ (page 3003, left column, lines 26 to 33, Translation, page 5)

B. According to the described matter of the aforesaid item A, Exhibit Ko 1 discloses that [i] By an experiment adding a purified PCSK9 to a medium of cultured HepG2 cells, it was demonstrated that the purified PCSK9 reduced the number of cell-surface LDLRs (FIGs. 2 and 3) (aforesaid items A(B), (D), (E), (G), (H)), [ii] We conclude from the experimental result of [i] that secreted PCSK9 associates with the LDLR and reduces hepatic LDLR protein levels (aforesaid item A(B)), [iii] The genetic data from humans with loss-of-function mutations in PCSK9 combined with the studies in knockout mice that lack PCSK9 would be sufficient to inhibit the ability of an inhibitor of the protease activity of PCSK9 in cells to decrease LDLR level for the treatment of hypercholesterolemia, however, as is suggested by data of this study, an additional method for neutralizing the activity of PCSK9 including the development of an antibody blocking the interaction (binding) between PCSK9 and LDLR and the development of an inhibitor blocking the activity in plasma may be sought as a treatment of hypercholesteremia (aforesaid item A(I)).

C. In this regard, Plaintiff alleges that according to the described matter of Exhibit Ko 1, Exhibit Ko 1 discloses an antibody inhibiting the binding of PCSK9 and LDLR (binding neutralizing antibody).

However, Exhibit Ko 1 discloses that ‘anti-human PCSK9 polyclonal antibody’ was obtained as an antibody against PCSK9 by purifying from a serum obtained by the injection to rabbit (aforesaid item A (J)), and it fails to disclose that this polyclonal antibody neutralized the binding of PCSK9 and LDLR.

Further, as in the aforesaid item B, Exhibit Ko 1 discloses an additional method for neutralizing the activity of PCSK9 including the development of an antibody blocking the interaction (binding) between PCSK9 and LDLR may be sought as a treatment of hypercholesteremia; however, it fails to describe or suggest a specific antibody having such effect.

Therefore, the above argument presented by Plaintiff is not acceptable.

D. According to the description of the scope of the claims of the Corrected Invention 1 (Claim 1) and the aforesaid C, the Corrected Invention 1 and an antibody of Exhibit Ko 1 (‘anti-human PCSK9

polyclonal antibody') are different from each other in that [i] the Corrected Invention 1 is 'an isolated monoclonal antibody', and a binding neutralizing antibody capable of 'neutralizing the binding' of PCSK9 and LDLR, whereas an antibody of Exhibit Ko 1 is a polyclonal antibody and it is indefinite as to whether to be a binding neutralizing antibody of PCSK9 and LDLR (Hereinafter referred to as 'different feature A.'), [ii] the Corrected Invention 1 'competes' with 'the antibody comprising: a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO. 49; and a light chain comprising a light chain variable region consisting of the amino acid sequence of SEQ ID NO. 23' (reference antibody) for 'the binding with PCSK9', whereas it is indefinite as to whether an antibody of Exhibit Ko 1 competes with the reference antibody (hereinafter referred to as 'different feature B').

(3) Well-known art as of the priority date

A. Exhibits Ko 220 to 224 have the following descriptions:

(A) Exhibit Ko 220 ('Antibodies A LABORATORY MANUAL', 1988)

'Antibody laboratory manual' (book name, translation, page 1)

'For manipulating and adopting the reactivity against a certain antigen, there is only a little room left for researchers to intervene. The type of such intervention is categorized into two categories. Specifically, to modify the antigen, or change the condition of infusion. ... The second kind of intervention is the selection of animals, dose amount, and forms of antigen, the use of immunoadjuvant, infusion path and times, and an interval between infusion.' (page 92, lines 1 to 11, translation, page 1))

'For the preparation of monoclonal antibody, both mouse and rat may be used. (...).' (page 94, lines 14 to 15, translation, page 1))

(B) Exhibit Ko 221 ('Antibody Engineering Methods and Protocols', 2004)

'3.6 Primary Screening of Antigen Binding

1. An appropriate number of ELISA plates is ... into plate coating buffer (a buffer covering a plate surface) and in a case of using a plate (...) coated (a surface is covered) with a soluble antigen or streptoavidin, 100 to 300 ng/mL biotinylated antigen is coated (a surface is covered) in 50 uL/well.' (page 197, lines 12 to 17, Translation, page 1 to page 2)

'3.7 Screen of Secondary ELISA

1. Primary Screening

By use of a similar condition to (...), the number of ELISA plates twice as many as the number of cultivation plates are coated with 50 uL/well soluble or biotinylated antigen (a surface is covered).' (page 197, lines 33 to 36, translation, page 2)

(C) Exhibit Ko 222 ('Phage Display of Peptides and Proteins: A Laboratory Manual', 1996) (Book name, Translation, page 1)

'Selection using a biotinylated antigen' (page 101, line 15, translation, page 2)

'Selection of Phage-antibody library by biotin selection of Protocol 12' (page 101, line 5 from the bottom, Translation, page 2).

(D)Exhibit Ko 223 ('REVIEW 'Selecting and screening recombinant antibody libraries'', September, 2005) (title, translation, page 1)

'Phage display

Antibody display on a surface of two kinds of bacteriophage fd and M13 is the most commonly used method for the display and selection of a large number of collections of antibodies and for the engineering of selected antibodies (...).'

(page 1106, left column, lines 10 to right column, line 2, Translation, page 1)

'FIG. 3

Method for ex vivo (in vitro) selection for binding. The selection from display library is implemented with several methods (or a combination thereof).

(Omitted)

(b) Biotinylated antigen

(Biotin (red) is captured via beads (gray) coated with Streptoavidin)' (page 1111, Translation 1 to 2)

(E) Exhibit Ko 224 ('REVIEWS, 'Potent antibody therapeutics by design'', May, 2006) (title, translation, page 1)

'Table 1 Monoclonal antibody approved for the therapeutic use in the United States' (page 344, Translation, page 1)

'The use of transgenic mouse for the production of human antibody is relatively simple, and based on a widely used technique.' (page 347, left column, lines 18 to 19, Translation, page 2)

'Human antibody from phage display library' (page 347, line 9 from the bottom, Translation, page 2)

‘After isolating from phage display library, several antibody fragments have sufficiently high binding affinity and biological effect for the indication of treatment.’ (page 347, right column, lines 30 to 33, Translation, page 2)

‘The affinity nature of the current antibody and the subsequent functional screening are widely used for promoting the effectiveness of antibodies, and are strategies that achieve success at a high frequency.’ (page 350, right column, line 2 from the bottom to page 351, left column, line 2, translation, page 3)

B. Comprehensively taking the described matter of the aforesaid item A into account, [i] a method of preparing a monoclonal antibody having a specific binding to an antigen by an animal immunization method or a phage display method, [ii] a method of using a transgenic mouse for the preparation of a human antibody and immobilizing an antigen by biotinylation for screening of the antibody in the preparation process and a means for obtaining a phage display library of a recombinant antibody were recognized as well-known as of the priority date.

(4) Whether the different feature was easily conceivable

A. The scope of the claims of the Corrected Invention 1 (Claim 1) fails to specify the meaning of ‘competing with the antibody’ in the context of ‘capable of neutralizing the binding of PCSK9 and LDLR protein and competing with the antibody comprising: a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO. 49; and a light chain comprising a light chain variable region consisting of the amino acid sequence of SEQ ID NO. 23 for the binding with PCSK9’ of Corrected Invention 1. The specification discloses that ‘Antigen binding proteins identified by competition assay (competing antigen binding proteins) include antigen binding proteins binding to the same epitope as the reference antigen binding proteins and antigen binding proteins binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antigen binding protein for steric hindrance to occur.’ ([0140]), ‘the neutralizing ABP binds to PCSK9 in a location and/or manner that prevents PCSK9 from binding to LDLR. Such ABPs can be specifically described as 'competitively neutralizing' ABPs.’ ([0155]).

Comprehensively taking into account the language of the scope of the claims of the Corrected Invention 1 (Claim 1) and the above described

matter of the description, ‘competing with an antibody’ of Corrected Invention 1 is construed as meaning binding to a same site where ‘an antibody comprising: a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO. 49; and a light chain variable region consisting of the amino acid sequence of SEQ ID NO. 23’ (reference antibody) binds to PCSK9 or binding to a site on PCSK9 that may become a steric barrier for the binding of the reference antibody and PCSK9.

B. Exhibit Ko 1 discloses that ‘an inhibitor of the protease activity of PCSK9 in cells would be sufficient to inhibit the ability to decrease LDLR level for the treatment of hypercholesterolemia; however, as is suggested by data of this study, an additional method for neutralizing the activity of PCSK9 including the development of an antibody blocking the interaction (binding) between PCSK9 and LDLR and the development of an inhibitor blocking the activity in plasma may be sought as a treatment of hypercholesteremia’ (aforesaid item (2)B[iii]). This disclosure suggests the possibility of usefulness of antibody blocking the interaction (binding) between PCSK9 and LDLR and neutralizing the PCSK9 activity for the treatment of hypercholesteremia, and is thus recognized as a motivation for a person ordinarily skilled in the art who read Exhibit Ko 1 to obtain a binding neutralizing antibody of PCSK9 and LDLR.

In addition, Exhibit Ko 1 fails to describe a monoclonal antibody capable of neutralizing the binding of PCSK9 and LDLR; however, as of the priority date, a common method for the preparation of a monoclonal antibody was well-known by an animal immunization method or a phage display method (aforesaid item (3)B[i]). In view of this, it is recognized that a person ordinarily skilled in the art could have obtained any monoclonal antibody (the constitution of Corrected Invention 1 according to Different feature A) capable of neutralizing the binding of PCSK9 and LDLR, on the basis of Exhibit Ko 1 and the above well-known art.

C.(A) On the other hand, according to the described matter of Exhibit Ko 220 (aforesaid item (3)A(A)), in producing an antibody with an animal immunization method, it was a matter of common technical knowledge as of the priority date that antibodies with different reactivities against an antigen may be obtained depending on the difference in ‘an infusion condition’ (for animals) including the selection of animals, a dosage amount

and a dosage form of an antigen, the use of immunization adjuvant, the infusion route and times, and an interval between infusions.

In this regard, the description discloses in Example 1 that human PCSK9 antigen was injected 11 times to mice in two groups containing human immunization globulin gene to prepare immunized mice and select mice (10 mice) that produce an antibody specific to PCSK9 according to a schedule of an immunization program described in Table 3 to obtain a reference antibody and a binding neutralizing antibody of PCSK9 and LDLR that competes with the reference antibody ([0312], [0313], [0320], Table 3). Specifically, in a first accelerated immunization, 10 μg antigen was intraperitoneally injected into each mouse, and the subsequent accelerated immunization was implemented at each dose of 5 μg by each hypodermic injection into a base part of a tail and intraperitoneal injection alternatingly. For intraperitoneal injections antigen was prepared as an emulsion with TiterMax$^{(R)}$ Gold and for subcutaneous injections antigen was mixed with Alum (aluminum phosphate) and CpG oligos; in the second to eighth and tenth accelerated immunization, each mouse was injected with a total of 5 μg of antigen in the adjuvant alum gel, a final injection of 5 μg of antigen per mouse was delivered in Phospho buffered saline (PBS), and was delivered by 50% IP into the abdomen and by 50% SQ at the base of the tail, and titers of the antibody against human PCSK9 were tested by ELISA assay, and at the end of the immunization program, 10 mice which appeared to be specific for PCSK9 were selected.

Further, the description discloses that [i] hybridomas producing antigen binding proteins to PCSK9 were produced by use of the above selected immunized mouse (Example 2, [0322] to [0324]), [ii] total 3104 antigen specific hybridomas were obtained by ‘Primary Screening’ by ELiSA with a capture sample of biotinylated-PCSK9, without a V5 tag, binding to a plate coated with neutravadin (Example 3, [0325] to [0328]), [iii] the 3000 positives in total were rescreened and further the 2441 positives in total were then rescreened (‘Confirmatory Screening’) to confirm stable hybridomas were established, and then it was confirmed that 579 antibodies could cross-react to mouse PCSK9 by ‘mouse cross-reactivity screening’ ([0329], [0330]); [iv] to screen for the antibodies that block PCSK9 binding to LDLR, LDLR was bound to a plate coated with goat anti-LDL receptor as a capture sample, and biotinylated

human D374Y PCSK9 was transferred to the plate with hybridoma exhaust supernatant, and there was implemented a screening for detecting LDLR-bound biotinylated PCSK9 with streptoavidin HRP (‘Large scale receptor ligand blocking screening’), and of 384 identified antibodies that strongly blocked the interaction between PCSK9 and the LDLR well, 100 antibodies blocked the binding interaction of PCSK9 and LDLR by 90% or more ([0332]); [v] for the 384 member subset of neutralizers (blockers) identified in [iv], a repetitive screening (‘receptor ligand binding assay for a subset of blockers’) was implemented by use of the same protocol as for the large scale receptor ligand blocking screening, and 85 antibodies blocking the interaction between PCSK9 mutant enzyme and LDLR by 90% or more were identified ([0333], [0334]); [vi] On the basis of the results of these assays (screening), there were identified several hybridoma lines producing antibodies with desired interactions with PCSK9 included a reference hybridoma (21B12) ([0336], Table 2); and [vii] the reference antibody is a neutralizing antibody that strongly blocks the binding of PCSK9 and LDLR (Example 11, [0138], [0378]).

Comprehensively taking the above description into account, the first immunized mice producing a specific antibody against PCSK9 were prepared and selected according to an immunization program in the description, and then hybridoma (3104 in total of antigen-specific hybridoma) was prepared by use of selected immunized mice. For antibodies produced from this hybridoma, a screening with a specific protocol was implemented in combination for identifying antibodies that strongly shield the binding interaction between PCSK9 and LDLR, and on the basis of this result, a reference antibody strongly neutralizing the binding of PCSK9 and LDLR was obtained as one antibody having a desired interaction with identified PCSK9.

However, in view of the above common general knowledge as of the priority date, it is recognized that an antibody produced from an immunized mouse that is immunized according to an immunization program described in the description and an antibody produced from an immunized mouse that is immunized according to an immunization program with a different condition and schedule from the above have different reactivities against PCSK9. It is recognized that it takes trial and error that go beyond the extent that could be usually expected to optimize a condition and a schedule

of an immunization program and to produce an immunized mouse suitable for obtaining a reference antibody.

Further, the use of transgenic mouse for the production of human antibody and a method of immobilizing an antigen by biotinylation for screening an antibody were well-known as of the priority date in a process of producing a monoclonal antibody (aforesaid item (3)B[ii]); however, a certain level of originality is required in constructing a screening system suitable for obtaining a reference antibody from hybridoma produced by use of the above immunized mouse by utilizing these techniques.

However, Exhibit Ko 1 fails to describe or suggest a condition of immunization program and schedule described in the description. First of all it fails to describe a method for producing an antibody that inhibits the binding of PCSK9 and LDLR (binding neutralizing antibody).

Consequently, it cannot be recognized that a person ordinarily skilled in the art could have easily conceived of obtaining a reference antibody by an animal immunization method based on Exhibit Ko 1 and well-known art (aforesaid item (3)B).

(B) Further, it is necessary to design an amino acid sequence of CDR of an antibody and prepare a phage display library with the amino acid sequence for the preparation of monoclonal antibody by a phage display method; however, Exhibit Ko 1 fails to describe an amino acid sequence information ([0123] of the description, FIG. 3JJ) of CDR of a reference antibody, and there is no evidence sufficient to find that the above amino acid sequence information was widely known before the priority date.

Consequently, it cannot be recognized that a person ordinarily skilled in the art could have easily conceived of obtaining a reference antibody by a phage display method on Exhibit Ko 1 and well-known art (aforesaid item (3)B).

D. Comprehensively taking the aforesaid items B and C into account, it is not recognized that a person ordinarily skilled in the art who read Exhibit Ko 1 could have easily conceived of obtaining a reference antibody on the basis of Exhibit Ko 1 and the above well-known art (aforesaid item (3)B), while the person could have obtained any monoclonal antibody (the constitution of the Corrected Invention 1 according to the different feature A) capable of neutralizing the binding of PCSK9 and LDLR. Therefore, it cannot be recognized that an antibody ‘competing’ with a reference

antibody that binds to a same site where the reference antibody binds to PCSK9 or a site on PCSK9 that may become a steric barrier for the binding of the reference antibody and PCSK9 (the constitution of Corrected Invention 1 according to the different feature B) was easily conceivable.

E. In contrast, Plaintiff alleges on the grounds of: [i] FIG. 27D of the description (Figure showing the positional relationship of the binding sites of the reference antibody and LDLR on PCSK9) and Table 37.1 of Example 37 (when a plurality of binding neutralizing antibodies of PCSK9 and LDLR are prepared without any barometer as to whether or not to compete with the reference antibody, many of such antibodies (a proportion of the number of antibodies of bins 1 to 2 against the total number of antibodies of bins 1 to 4 is about 65%) competed with the reference antibody); and [ii] the declaration statement by Professor A who analyzed on the basis of data in the description, it can be said that there are many antibodies competing with a reference antibody at least in a certain proportion in a case where a binding neutralizing antibody of PCSK9 and LDLR was obtained, and thus a person ordinarily skilled in the art would have obtained a binding neutralizing antibody competing with the reference antibody only by preparing some binding neutralizing antibody of PCSK9 and LDLR, and thus a person ordinarily skilled in the art who read Exhibit Ko 1 could have easily conceived of binding neutralizing antibody competing with the reference antibody on the basis of Exhibit Ko 1 and well-known art.

However, Table 37.1 of the description has confirmed that 2441 stable hybridomas producing an antibody that binds to PCSK9 have been established by confirmatory screening ([0329]); and a part of them (39 antibodies in total) were subjected to epitope binning, and the result was summarized ([0489] to [0492]); the proportion of antibodies competing with a reference antibody to the binding neutralizing antibodies of PCSK9 and LDLR cannot be calculated from the analysis of this table.

Subsequently, the analysis based on FIG. 27D of the description in the declaration statement by Professor A stated that it is necessary for an antibody to overlap the binding site of LDLR on PCSK9 at least in two amino acid sequences for neutralizing the binding of PCSK9 and LDLR, and supposing that a size of the binding site be 20 Å x30 Å, most of anti-PCSK9 antibodies neutralizing the binding of PCSK9 and LDLR ‘obviously compete with either of 21B12 or 31H4 in view of the manner of binding of

these antibodies and the binding site of LDLR on a surface of PCSK9, as depicted in FIG. 27D.' ('4.1' of Exhibit Ko 204, translation, page 3). The above opinion does not discuss the competition of anti-PCSK9 antibody neutralizing the binding of PCSK9 and LDLR with most of the reference antibody (21B12 antibody) as shown by the language of 'competing with either of 21B12 or 31H4,' nor there is a support of demonstrative data showing such a competition with a reference antibody.

Furthermore, according to a published Japanese translation of PCT international publication for patent application according to an invention filed by Novartis (priority date: April 13, 2007. Exhibit Otsu 9), in view of the fact that a region of the binding site (the binding with amino acid residues 101 to 107 and 123 to 132 in an epitope) of 'H1-Fab' (anti-PCSK9 antibody) neutralizing the binding interaction between PCSK9 and LDLR with hPCSK9 (human PCSK9) (paragraphs [0237] to [0241]) is different from a region competing with a reference antibody (21B12 antibody) ([0440] etc. of the description), it cannot be said that every antibody neutralizing the binding of PCSK9 and LDLR competes with a reference antibody, nor does it include many antibodies competing with the reference antibody.

Therefore, the above argument presented by Plaintiff is not acceptable.

(5) Summary

For the above reason, it cannot be said that Corrected Invention 1 was easily conceivable by a person ordinarily skilled in the art on the basis of Exhibit Ko 1 and well-known technique, and thus the trial decision consistent with this is not erroneous, nor does Reason 1-1 for rescission as Plaintiff alleges have a point.

2 Reason 1-2 for Rescission (Errors in the determination of inventive step of Corrected Invention 9)

Plaintiff alleges that, Corrected Invention 9 is an invention directed to a pharmaceutical composition comprising an antibody of Corrected Invention 1, whereas Exhibit Ko 1 definitely discloses that the binding neutralizing antibody of PCSK9 and LDLR may be useful for the treatment of hypercholesteremia, a person ordinarily skilled in the art could have easily conceived of a pharmaceutical composition comprising Corrected Invention 9 based on Exhibit Ko 1 and well-known art, in a similar manner

to the case of Corrected Invention 1, and thus the judgment of the trial decision different from this is erroneous.

As in the aforesaid item 1(5), however, it cannot be said that Corrected Invention 1 was easily conceivable by a person ordinarily skilled in the art on the basis of Exhibit Ko 1 and well-known technique, and thus the above Plaintiff's allegation is based on an incorrect premise, and thus not acceptable.

Therefore, none of Reason 1-2 for rescission presented by Plaintiff is reasonable.

3 Reason 2 for Rescission (Error in the determination of the support requirement)

(1) Conformance to Support Requirement

A. Comprehensively taking the aforesaid findings 1(1) and (4)C(A) into account, it is recognized that the Detailed Description of the Invention has the following disclosure with regard to Corrected Inventions 1 and 9.

(A) PCSK9 (Proprotein convertase subtilisin kexin type 9) is a serine protease that binds to LDLR (low density lipoprotein receptor) and interacts therewith to be incorporated into liver cells together with LDLR and decrease LDLR level in the liver, and further decrease an amount of LDLR available for the binding to LDL on cell surface (extracellular) to increase an amount of LDL in a subject ([0002], [0003], [0071]).

The term ‘neutralizing antibody’ represents the antibody that binds to a ligand and prevents or reduces the biological effects of the ligand. In the anti-PCSK9 antibody, it involves the neutralization by the prevention of the binding of PCSK9 and LDLR, and the neutralization by the prevention of PCSK9-mediated decomposition of LDLR without preventing the binding of PCSK9 and LDLR ([0138]).

The term ‘competing’ means the competition between antibodies determined by various assays that measure the degree of a test antibody preventing or inhibiting a specific binding of the reference antibody to an antigen. An antibody identified by a competitive assay includes an antibody binding to an epitope identical to or overlapping with the reference antibody, and an antibody binding to an adjacent epitope sufficiently closed to interfere sterically with the binding of the reference antibody to the epitope ([0140], [0269]).

The term ‘epitope’ is a region of an antigen that is bound by an antibody, and when the antigen is a protein, includes specific amino acids that directly contact the antibody ([0142]).

(B) An isolated monoclonal antibody competing with an antibody (‘21B12’) comprising: a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO. 49; and a light chain variable region consisting of the amino acid sequence of SEQ ID NO. 23 as a neutralization antigen binding protein (neutralizing ABP) that binds to PCSK9 in a location and/or a manner that prevents PCSK9 from binding to LDLR and blocks or reduces and ‘competitively neutralizes’ the interaction (binding) between PCSK9 and LDLR ([0138], [0140], [0155], [0261], [0269], [Table 2])

This neutralizing ABP against PCSK9 can neutralize the binding of PCSK9 and LDLR and increase the amount of LDLR, thereby decreasing the amount of LDL in a subject, resulting in a reduction in the subject's serum cholesterol level, and this effect may treat or prevent or reduce the risk of the diseases associated with increased cholesterol level such as hypercholesteremia, and thus may be therapeutically beneficial ([0155], [0270], [0271], [0276]).

(C) A human PCSK9 antigen was injected 11 times to mice in two groups containing human immunization globulin gene to prepare immunized mice and select mice (10 mice) that produce an antibody specific to PCSK9 according to a procedure and schedule of an immunization program described in Table 3 to obtain a reference antibody and a binding neutralizing antibody of PCSK9 and LDLR that competes with the reference antibody (Example 1, [0312], [0313], [0320], Table 3).

Hybridomas producing antigen binding proteins to PCSK9 were produced by use of these selected immunized mice (Example 2, [0322] to [0324]), a total of 3104 antigen specific hybridomas were obtained by ‘Primary Screening’ by ELiSA with a capture sample of biotinylated-PCSK9, without a V5 tag, binding to a plate coated with neutravadin (Example 3, [0325] to [0328]).

The 3000 positives in total were rescreened from the above hybridomas obtained by ‘Primary Screening’ and further the 2441 positives in total were then rescreened (‘Confirmatory Screening’) to confirm that stable hybridomas were established, and then it was confirmed that 579 antibodies could cross-react to mouse PCSK9 by ‘mouse cross-reactivity

screening'([0329], [0330]); Furthermore, 'large scale receptor ligand blocking screening' was implemented for screening antibody that blocks the binding of PCSK9 to LDLR, and identified 384 antibodies that strongly blocked the interaction between PCSK9 and the LDLR well, of which 100 antibodies blocked the binding interaction of PCSK9 and LDLR by 90% or more ([0332]).

For the 384 member subset of neutralizers (blockers) identified in this manner, 'a receptor ligand binding assay for a subset of blockers' was implemented, and 85 antibodies blocking the interaction between PCSK9 mutant enzyme and LDLR by 90% were identified ([0333], [0334]).

On the basis of the results of these assays (screening), there were identified several hybridoma lines producing antibodies with desired interactions with PCSK9, including a reference hybridoma (21B12) ([0336], Table 2), which is a neutralizing antibody that strongly blocks the binding of PCSK9 and LDLR (Example 11, [0138], [0378]).

(D) From 32 antibodies in Table 2 (several hybridoma lines that produce an antibody having a desired interaction with PCSK9), 27B2, 13H1, 13B5, and 3C4 are non-neutralizing antibodies, and 3B6, 9C9, and 31A4 are weak neutralizing antibodies, and the others (including a reference antibody) are strong neutralizing antibodies ([0138], [0336]).

Further, according to the result of epitope binning for the above 32 antibodies, the ones competing with 21B12 antibody (reference antibody) (bin 1) total 19, the ones competing with 31H4 antibody (bin 3) total 7. They are mutually exclusive. One competes with both the reference antibody and 31H4 antibody (bin 2), and one competes with neither the reference antibody nor 31H4 antibody (bin 4) (Example 10, [0373], [0494], Table 8.3).

Further, in addition to a set of Example 10, according to the result of epitope binning implemented for the other set (39 antibodies in total), the ones not competing with 31H4 antibody but competing with 21B12 antibody (reference antibody) (bin 1) total 19, the ones competing with both 21B12 antibody and 31H4 antibody (bin 2) total 3. Those not competing with 21B12 antibody but competing with 31H4 antibody (bin 3) number 10. Further, 16 out of antibodies included in bin 1 are antibodies listed in Table 2, and according to the description of [0138], it has been confirmed that 15

out of them excluding 27B12 antibody are neutralizing antibodies (Example 37, [0489] to [0495], Table 37.1).

B. According to the finding of the aforesaid item A, it is recognized that Corrected Inventions 1 and 9 are described in the Detailed Description of the Invention.

Further, in view of the fact that Table 37.1 of the description shows that hybridomas have been prepared by use of immunized mice produced in accordance with the description, and 2441 stable hybridomas producing an antibody that binds to PCSK9 have been established by screening ([0329]); and a part of them (39 antibodies in total) were subjected to epitope binning, which resulted in 19 antibodies not competing with 31H4 antibody but competing with 21B12 antibody (reference antibody) (bin 1), out of which 15 antibodies were found to be neutralizing antibodies ([0138], Table 2), it is recognized that a person ordinarily skilled in the art who reads Exhibit Ko 1 could recognize that neutralizing antibodies competing with the reference antibody included in the scope of the claims of Corrected Invention 1 (Claim 1) were obtained when a similar epitope binning assay is implemented for the remaining antibodies obtained from the above 2441 stable hybridomas.

Furthermore, a person skilled in the art who read the description could recognize that various neutralizing antibodies competing with the reference antibody included in the scope of the claims of Corrected Invention 1 (Claim 1) other than neutralizing antibodies competing with the reference antibody in the description might be obtained by repetitively implementing the production and selection of immunized mice in accordance with a procedure and a schedule of immunization program in the description, the hybridoma generation in which a selected immunized mouse is used, a screening and an epitope binning assay (the aforesaid item A(C) and (D)) for identifying an antibody that strongly blocks a binding interaction between PCSK9 and LDLR in the description from the start.

Therefore, it is recognized that Corrected Invention 1 (Claim 1) conforms to the support requirement.

Further, as the aforesaid item A(B), in view of the description that it may be therapeutically beneficial since it may treat or prevent or reduce the risk of the diseases associated with increased cholesterol level such as hypercholesteremia, it can be seen from the description that a person skilled

in the art can use an antibody of Corrected Invention 1 as a pharmaceutical composition.

Therefore, Corrected Invention 9 (Claim 9) conforms to the support requirement.

(2) Plaintiff's allegation

A. Plaintiff alleges that the scope of the claims of Corrected Invention 1 (Claim 1) is an invention defined only by a function or properties (‘binding neutralization’ and ‘competition with reference antibody’) without specifying the structure of the antibody, and thus it encompasses an enormous number or kind of binding neutralizing antibodies with any possible structures on the principle of literal construction, whereas there are as few as three groups or three kinds of antibodies described specifically in the description, and further it cannot be said that an antibody ‘competing’ with a reference antibody may neutralize the binding of PCSK9 and LDLR, and the ‘competition’ of an antibody with the reference antibody cannot be a barometer of ‘binding neutralization,’ and thus it cannot be recognized from the description that even antibodies with any structure not described in the description may solve the problem of Corrected Invention 1 to provide the binding neutralizing antibody of PCSK9 and LDLR, and Corrected Inventions 1 and 9 do not conform to the support requirement.

However, it is a matter of common general knowledge in a production process of monoclonal antibody by an animal immunization method that an antibody specifically reacting with a specific antigen in animal body is produced, and hybridomas produced by use of the immunized animal are subjected to screening, and amino acid sequence is identified in a process of identifying an antibody having specific binding properties. Therefore, it is not recognized as essential to specify the structure of the antibody (amino acid sequence) in advance for obtaining an antibody having specific binding properties.

Further, Corrected Invention 1 (Claim 1) specifies ‘capable of neutralizing the binding of PCSK9 and LDLR protein’ and ‘for the binding with PCSK9’, ‘compete’ with a reference antibody (21B12 antibody) as matters specifying the invention. As in the above item (1)B, it is recognized that a person ordinarily skilled in the art could recognize from the description that a neutralizing antibody competing with a reference antibody included in the scope of claims of Corrected Invention 1 (Claim 1)

could be obtained under the guidance of the description without reference to amino acid sequence of antibodies.

Further, although it cannot be said that every antibody ‘competing’ with a reference antibody neutralizes the binding of PCSK9 and LDLR, Corrected Invention 1 specifies ‘capable of neutralizing the binding of PCSK9 and LDLR protein’ as a matter specifying the invention. Therefore, it does not affect the above findings.

Therefore, the above Plaintiff's allegation is not reasonable.

B. Plaintiff alleges that, as in the case of Corrected Invention 1, in the case where a product is not specified by its specific structure but is defined only functionally in the claims and specified by screening method, it does not conform to the support requirement; the functional definition and the identification of screening method do not constitute a basis for the support requirement, but it interferes with industrial progress, which is a goal of the Patent Act, and is against the spirit of the patent system to permit such format of the claims.

As in the above item A, however, it cannot be said as essential to preliminarily specify a structure of the antibody (amino acid sequence) to obtain an antibody having specific binding properties, and it is recognized that a person ordinarily skilled in the art could recognize from the description that a neutralizing antibody competing with a reference antibody included in the scope of claims of Corrected Invention 1 (Claim 1) could be obtained under the guidance of the description without reference to amino acid sequence of antibodies.

Further, it cannot be said that the format of the recitation of the claim of Corrected Invention 1 with interferes industrial progress, which is the spirit of the Patent Act, nor does it cause the circumstances where it is against the spirit of the patent system, as alleged by Plaintiff.

Therefore, the above Plaintiff's allegation is not reasonable.

(3) Summary

For the above reason, the trial decision stating that Corrected Inventions 1 and 9 conform to the support requirement is not erroneous, and thus Reason 2 for rescission as Plaintiff alleges is not reasonable.

4 Reason 3 for Rescission (Errors in the determination of conformance to the enablement requirement)

(1) Conformance to Enablement requirement

According to the aforesaid finding 3(1)A, it can be seen from the description that an antibody of Corrected Invention 1 and a pharmaceutical composition of Corrected Invention 9 may be produced and used. Therefore, the Detailed Description of the Invention of the description is clearly and sufficiently described to the extent that a person ordinarily skilled in the art can work Corrected Inventions 1 and 9.

Therefore, it is recognized that Corrected Inventions 1 and 9 conform to the enablement requirement.

(2) Plaintiff's allegation

A. Plaintiff alleges that Corrected Invention 1 is defined only functionally without specifying the antibody structure, and includes extremely various kinds of antibodies, and specific antibodies (three groups to three kinds of antibodies) described as antibodies that may be included in Corrected Invention 1 in the Detailed Description of the Invention of the description may include antibodies with various structures having totally different amino acid sequences, and of course include any antibodies that were totally unknown ever before, and excess trial and errors that can be expected by a person ordinarily skilled in the art are obviously needed to obtain every antibody encompassed into the scope of claims of Corrected Invention 1, and thus Corrected Invention 1 does not conform to the enablement requirement, which holds true for Corrected Invention 9.

In view of the findings of the above item 3(2)A, however, it cannot be said as essential to preliminarily specify a structure of the antibody (amino acid sequence) to obtain an antibody having specific binding properties, and it is recognized that a person ordinarily skilled in the art could obtain a neutralizing antibody competing with a reference antibody included in the scope of claims of Corrected Invention 1 (Claim 1) under guidance of the description without reference to amino acid sequence of antibodies.

Further, in view of the finding of the aforesaid item 3(1)B, it is recognized that a person ordinarily skilled in the art could obtain from the description various neutralizing antibodies competing with the reference antibody included in the scope of the claims of Corrected Invention 1 (Claim 1) other than neutralizing antibodies competing with the reference antibody in the description. Thus it cannot be said that it requires excess trial and error beyond the extent that might be ordinarily expected for a

person ordinarily skilled in the art to obtain antibodies encompassed into a scope of the claims of Corrected Invention 1 (Claim 1).

Therefore, the above Plaintiff's allegation is not reasonable.

B. Plaintiff alleges that Corrected Invention 1 specifies the function that antibody should have (i.e. problem to be solved) as a matter for specifying the invention, and the enablement requirement is a substantial requirement, and thus even if a function to be borne by the product should be described as a matter for specifying the invention, it cannot be directly concluded from the description that a person skilled in the art may use all the products within the scope of the invention, nor does the description conform to the enablement requirement, and should the description conform to the enablement requirement, it means that every claim having a broad scope and functionally defined may suffice the enablement requirement, which causes the determination of the enablement requirement to be reduced to a formality, and thus Corrected Invention 1 does not conform to the enablement requirement, nor does Corrected Invention 9.

As in the aforesaid item A, however, in view of the finding of the aforesaid item 3(1)B, a person ordinarily skilled in the art could recognize from the description that various neutralizing antibodies competing with the reference antibody included in the scope of the claims of Corrected Invention 1 (Claim 1) other than neutralizing antibodies competing with the reference antibody in the description might be obtained.

Therefore, the above Plaintiff's allegation is not reasonable.

(3) Summary

For the above reasons, the determination of the trial decision stating that Corrected Inventions 1 and 9 conform to the enablement requirement is not erroneous, and thus Reason 3 for rescission as Plaintiff alleges is not reasonable.

5. Conclusion

For the above reasons, none of reasons for rescission as Plaintiff argues has a point, thus the trial decision contains no illegality to be rescinded.

Therefore, the Plaintiff's claim should be dismissed.

Intellectual Property High Court, Fourth Division

Presiding Judge: OHTAKA Ichiro

Judge: FURUKAWA Kenichi

Judge: SEKINE Sumiko

Attachment 1

[Table 2]

Table 2

Typical heavy chain and light chain variable region

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

[Table 3]

Table 3

strain of mice	XMG2/kl	XMG4/kl
Number of animals	10	10
Immunogen	PCSK9-V5/His	PCSK9-V5/His
First accelerated immunization	intraperitoneal injection 10 μg for each	intraperitoneal injection 10 μg for each
	Titermax Gold	Titermax Gold
Second accelerated immunization	Tail injection 5 μg for each	Tail injection 5 μg for each
	Alum/CpG ODN	Alum/CpG ODN
Third accelerated immunization	intraperitoneal injection 5 μg for each	intraperitoneal injection 5 μg for each
	Titermax Gold	Titermax Gold
Fourth accelerated immunization	Tail injection 5 μg for each	Tail injection 5 μg for each
	Alum/CpG ODN	Alum/CpG ODN
Fifth accelerated immunization	intraperitoneal injection 5 μg for each	intraperitoneal injection 5 μg for each
	Titermax Gold	Titermax Gold
Sixth accelerated immunization	Tail injection 5 μg for each	Tail injection 5 μg for each
	Alum/CpG ODN	Alum/CpG ODN
Seventh accelerated immunization	intraperitoneal injection 5 μg for each	intraperitoneal injection 5 μg for each
	Titermax Gold	Titermax Gold
Eighth accelerated immunization	Tail injection 5 μg for each	Tail injection 5 μg for each
	Alum/CpG ODN	Alum/CpG ODN
blood drawing		
Ninth accelerated immunization	intraperitoneal injection 5 μg for each	intraperitoneal injection 5 μg for each
	Titermax Gold	Titermax Gold
Tenth accelerated immunization	Tail injection 5 μg for each	Tail injection 5 μg for each
	Alum/CpG ODN	Alum/CpG ODN
Eleventh accelerated immunization	BIP 5 μg for each	BIP 5 μg for each
	PBS	PBS
Collection		

[Table 8.3]

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

[Table 37.1]

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				

[FIG. 1A]

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

SEQ ID NO:1

FIG. 1A

[FIG. 7A]

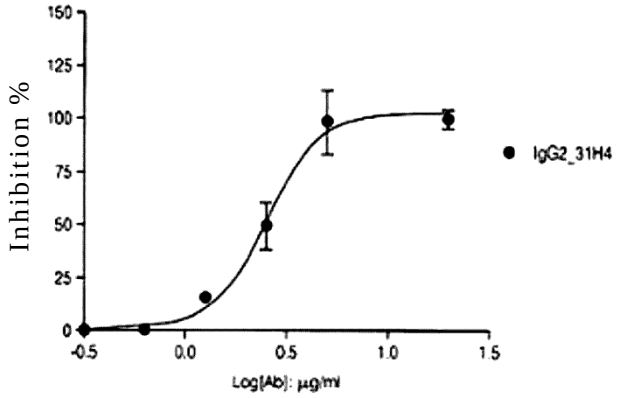


FIG. 7A

[FIG. 7B]

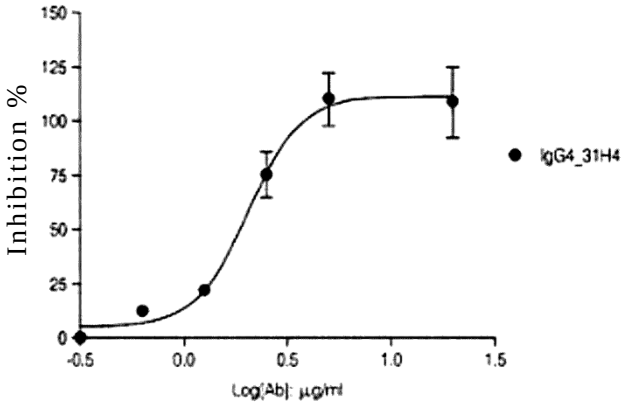


FIG. 7B

[FIG. 7C]

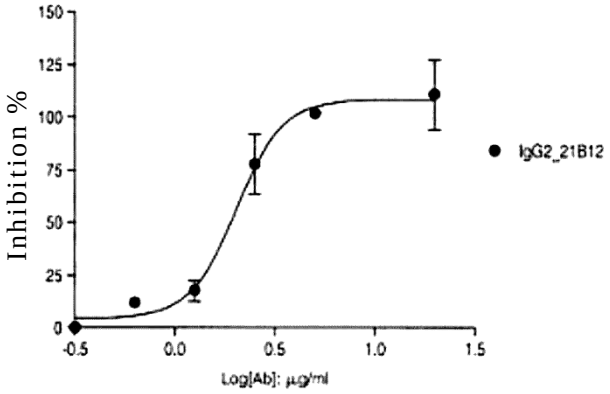


FIG. 7C

[FIG. 7D]

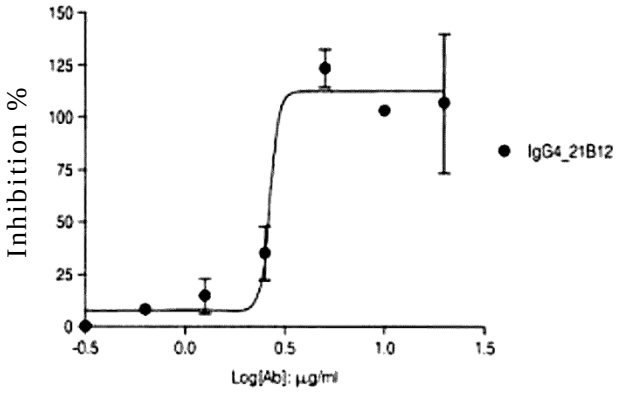


FIG. 7D

[FIG. 14A]

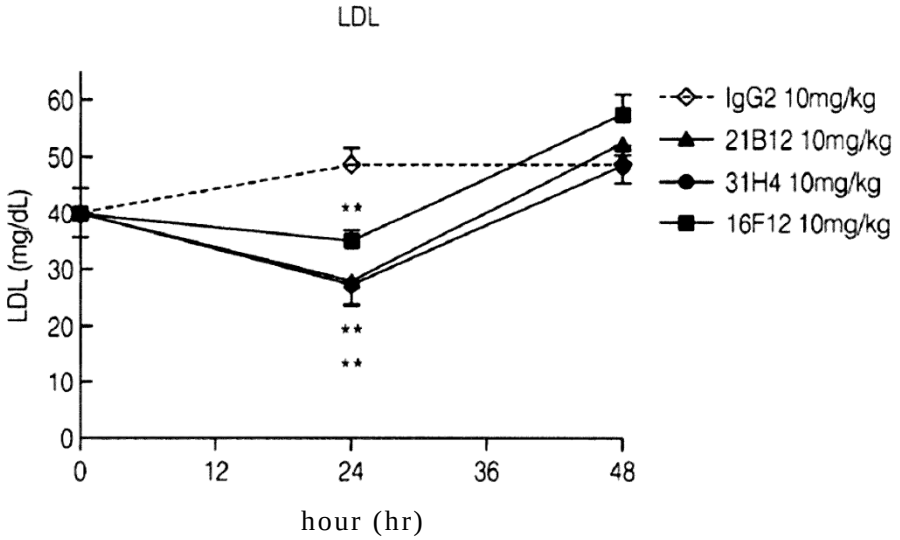


FIG. 14A

[FIG. 14B]

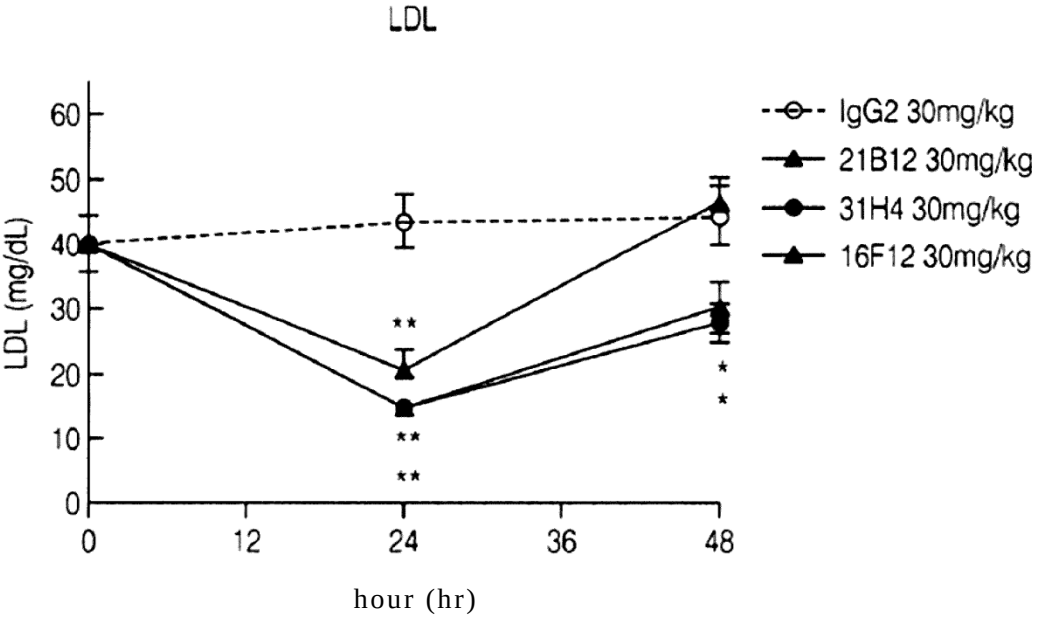


FIG. 14B

[FIG. 20A]

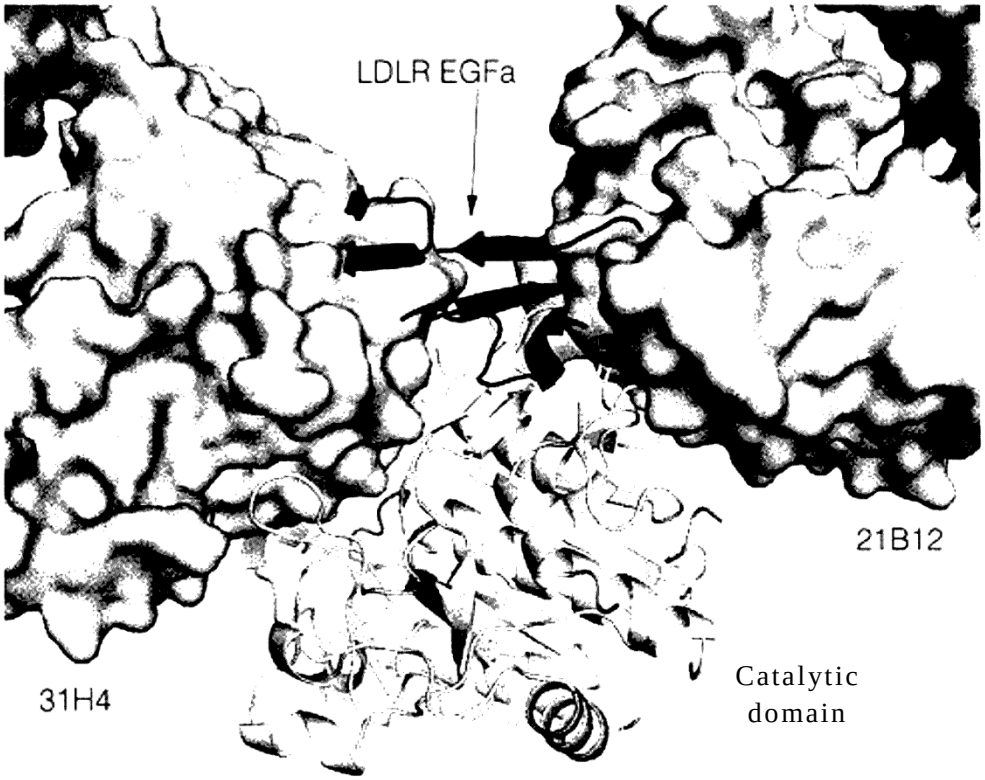


FIG. 20A

[FIG. 20B]

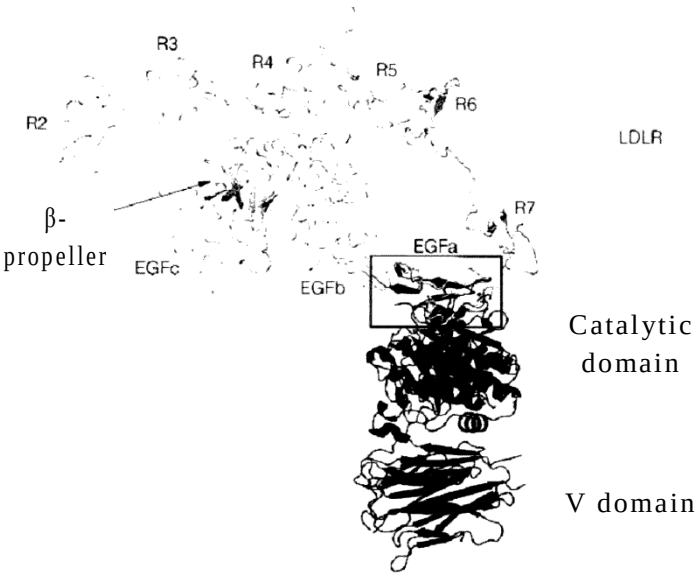


FIG. 20B

[FIG. 20C]

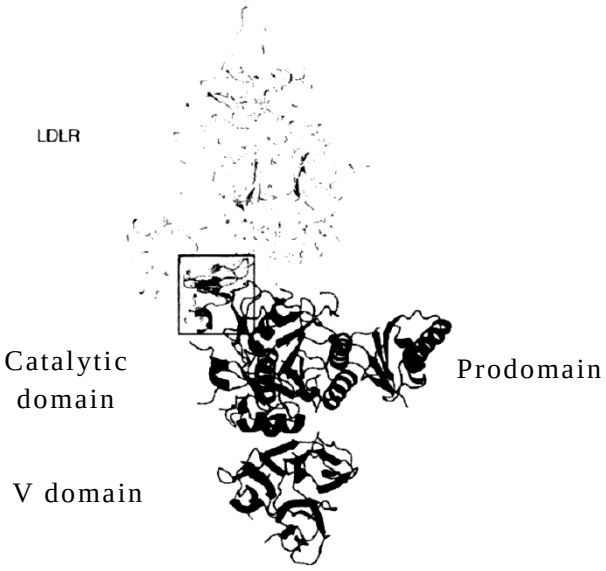


FIG. 20C

[FIG. 20D]

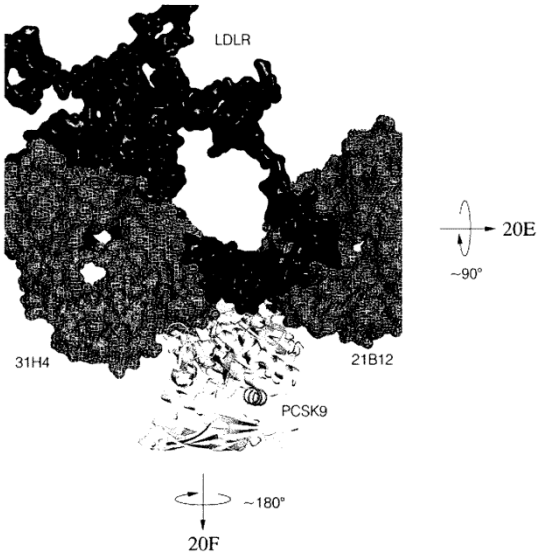
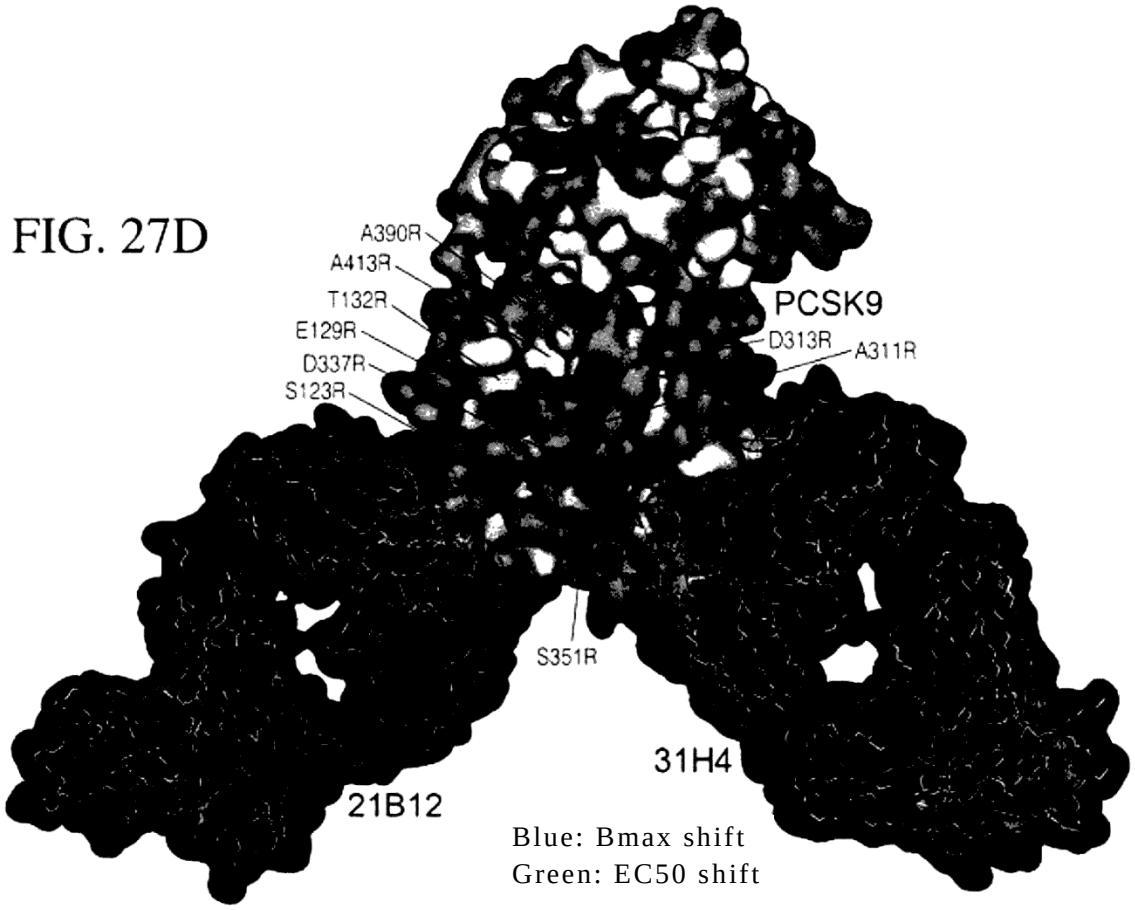


FIG. 20D

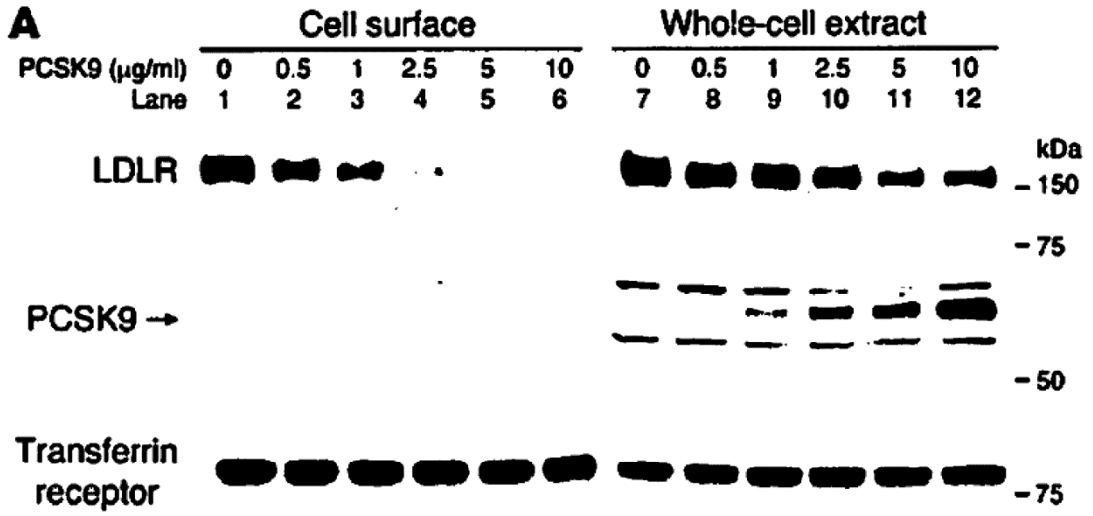
[FIG. 27D]

FIG. 27D

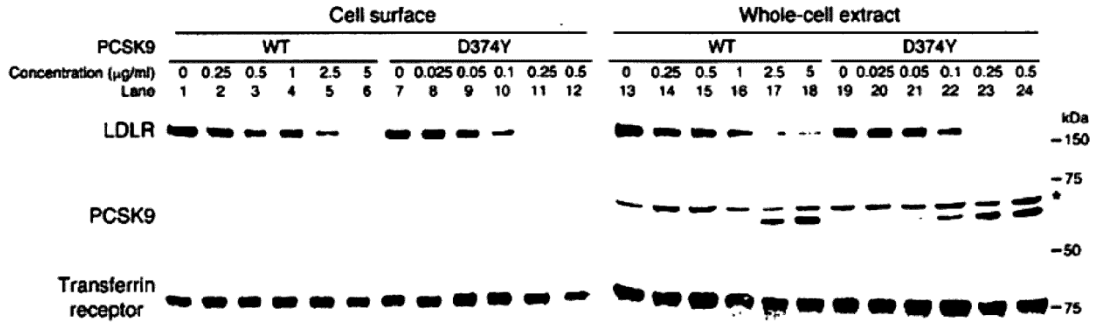


Attachment 2

[FIG. 2A]

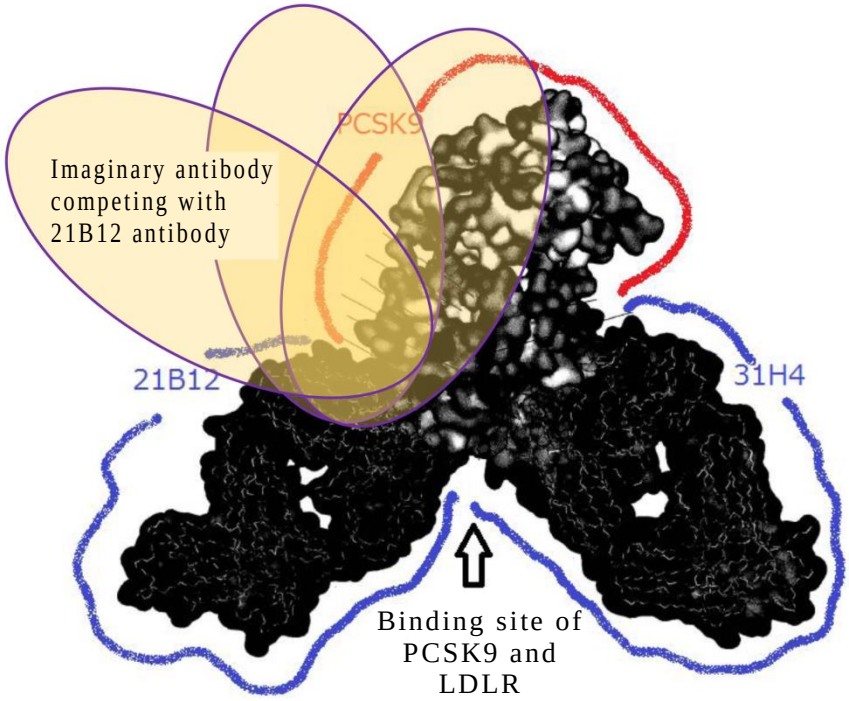


[FIG. 3]



Attachment 3

[FIG. A]



[FIG. B]