rendering them unsuitable for therapeutic administration.

Thus in light of the therapeutic potential of AXL there is a high need for monoclonal AXL antibodies, antibody fragments or derivatives thereof that effectively and specifically block AXL mediated signal transduction and which are suitable for therapeutic treatment.

Summary

In a first aspect, the present invention refers to a monoclonal antibody that binds to the extracellular domain of AXL and at least partially inhibits AXL activity, wherein the monoclonal antibody comprises a heavy chain amino acid sequence comprising (a) CDRH1 as shown in SEQ ID NO: 22, (b) CDRH2 as shown in SEQ ID NO: 23, and (c) CDRH3 as shown in SEQ ID NO: 24, and a light chain amino acid sequence comprising (d) CDRL1 as shown in SEQ ID NO: 19, (e) CDRL2 as shown in SEQ ID NO: 20, and (f) CDRL3 as shown in SEQ ID NO: 21. In a further aspect, the present invention refers to an isolated nucleic acid molecule selected from the group consisting of: (a) a nucleic acid sequence encoding a monoclonal antibody, antibody fragment or a derivative thereof of the present invention, (b) a nucleic acid sequence as shown in SEQ ID NOs: 3, 4, 34-36, and (c) a nucleic acid complementary to any of the sequences in (a) or (b). In still another aspect, the present invention refers to a vector comprising a nucleic acid sequence of the present invention. In still another aspect, the present invention refers to a host comprising the vector of the present invention. In another aspect, the present invention refers to a process of manufacturing a monoclonal antibody according to the present invention comprising the step of obtaining said polypeptide from the host of the present invention. In another aspect, the present invention refers to pharmaceutical composition comprising the monoclonal antibody of the present invention, the nucleic acid molecule of the present invention, the vector of the present invention, the host of the present invention, or a polypeptide generated by the process of the present invention. In still another aspect, the present invention refers to

the use of the monoclonal antibody according to the present invention for the manufacture of a pharmaceutical composition for the diagnosis, prevention or treatment of a hyperproliferative disease. In another aspect, the present invention refers to method for diagnosing a condition associated with the expression of AXL, comprising contacting a sample with a monoclonal antibody according to the present invention, and detecting the presence of AXL. In another aspect, the present invention refers to the use of the monoclonal antibody according to the present invention in the manufacture of a medicament for preventing or treating a condition associated with the expression of AXL in a patient. In another aspect, the present invention refers to a kit comprising a monoclonal antibody according to the present invention, a nucleic acid sequence according to the present invention or a vector according to the present invention. In still another aspect, the present invention refers to the use of a monoclonal antibody according to the present invention for the manufacture of a pharmaceutical composition for the treatment of drug resistant cancer. In still another aspect, the present invention refers to the use of a monoclonal antibody of the present invention for the manufacture of a medicament for the co-administration with an antineoplastic agent for the treatment of a hyperproliferative disease.

Accordingly a first ~~aspect of the present invention~~example relates to a monoclonal antibody including a fragment or derivative thereof that binds to the extracellular domain of AXL, particularly of human AXL, and at least partially inhibits AXL activity.

Preferably the antibody ~~of the present invention~~as described herein further possesses at least one or more of the following properties: the ability to reduce or block AXL-mediated signal transduction, the ability to reduce or block AXL phosphorylation, the ability to reduce or block cell proliferation, the ability to reduce or block angiogenesis, the ability to reduce or block cell migration, the ability to reduce or block tumor metastasis, the ability to reduce or block AXL mediated PI3K signaling and the ability to reduce or block AXL mediated anti-apoptosis, thereby increasing for example the

sensitivity of a cell against treatment with an antineoplastic agent. Moreover the antibodies ~~of the present invention~~as described herein may exhibit high specificity for AXL, particularly human AXL and do not significantly recognize other Tyro-3 family members, e.g. MER and/or SKY and/or mammalian non-primate AXL, such as murine AXL. Antibody specificity may be determined by measurements of cross-reactivity as described in the Examples.

The term "activity" refers to the biological function of AXL, which influences the phenotype of a cell, in particular but not limited to cancer phenotypes such as evasion of apoptosis, self sufficiency in growth signals, cell proliferation, tissue invasion and/or metastasis, insensitivity to anti-growth signals (anti-apoptosis) and/or sustained angiogenesis.

migration, anchorage independence, evasion of apoptosis, or secretion of angiogenic factors, that underly metastasis and are stimulated or mediated by non-catalytic or catalytic activities of AXL, preferably including AXL phosphorylation and/or AXL-mediated signal transduction.

The term "AXL mediated anti-apoptosis" refers to all AXL-involving processes that prevent human cells, preferably but not limited to human cancer cells from programmed cell death (apoptosis). In particular, it refers to processes that prevent human cells, preferably but not limited to human cancer cells from induction of apoptosis through growth factor withdrawal, hypoxia, exposure to chemotherapeutic agents or radiation, or initiation of the Fas/Apo-1 receptor-mediated signaling, and are stimulated or mediated by non-catalytic or catalytic activities of AXL, preferably including AXL phosphorylation and/or AXL-mediated signal transduction.

In addition, the present ~~invention~~disclosure includes antibodies whose binding activities to AXL are KD = 10^{-5} M or lower, preferably KD = 10^{-7} M or lower, and most preferably KD = 10^{-9} M or lower. Whether the binding activity of an antibody ~~of the present invention~~as described herein to AXL is KD = 10^{-5} M or lower can be determined by methods known to those skilled in the art. For example, the activity can be determined using surface plasmon resonance with Biacore, and/or by ELISA (enzyme-linked immunosorbent assays), EIA (enzyme immunoassays), RIA (radioimmunoassays), or fluorescent antibody techniques, e.g. FACS.

In a second ~~aspect~~example, the antibody may have at least one antigen binding site, e.g. one or two antigen binding sites. Further, the antibody preferably comprises at least one heavy immunoglobulin chain and at least one light immunoglobulin chain. An immunoglobulin chain comprises a variable domain and optionally a constant domain. A variable domain may comprise complementary determining regions (CDRs), e.g. a CDR1, CDR2 and/or CDR3 region, and framework regions. The term "complementary determining region" (CDR) is well-defined in the art (see, for example, Harlow and Lane, "Antibodies, a Laboratory Manual", CSH Press, Cold

Spring Harbour, 1988) and refers to the stretches of amino acids within the variable region of an antibody that primarily makes contact with the antigen.

A further ~~aspect of the present invention~~example relates to an antibody including a fragment or derivative thereof that binds to the extracellular domain of AXL which comprises at least one heavy chain amino acid sequence comprising at least one CDR selected from the group consisting of

(a) a CDRH1 as shown in SEQ ID NOs: 16, 22, 28, or a CDRH1 sequence differing in 1 or 2 amino acids therefrom,

(b) a CDRH2 as shown in SEQ ID NOs: 17, 23, 29, or a CDRH2 sequence differing in 1 or 2 amino acids therefrom, and

(c) a CDRH3 as shown in SEQ ID NOs: 18, 24, 30, or a CDRH3 sequence differing in 1 or 2 amino acids therefrom,

and/or at least:

one light chain amino acid sequence comprising at least one CDR selected from the group consisting of

(d) a CDRL1 as shown in SEQ ID NOs: 13, 19, 25, or a CDRL1 sequence differing in 1 or 2 amino acids therefrom,

(e) a CDRL2 as shown in SEQ ID NOs: 14, 20, 26, or a CDRL2 sequence differing in 1 or 2 amino acids therefrom, and

(f) a CDRL3 as shown in SEQ ID NOs: 15, 21, 27, or a CDRL3 sequence differing in 1 or 2 amino acids therefrom,

or a monoclonal antibody recognizing the same epitope on the extracellular domain of AXL.

In a preferred ~~embodiment~~example, the antibody comprises a heavy chain comprising at least one CDR selected from the group consisting of

(a) a CDRH1 as shown in SEQ ID NO: 16, or a CDRH1 sequence

differing in 1 or 2 amino acids therefrom,

(b) a CDRH2 as shown in SEQ ID NO: 17, or a CDRH2 sequence differing in 1 or 2 amino acids therefrom, and

(c) a CDRH3 as shown in SEQ 1D NO: 18, or a CDRH3 sequence differing in 1 or 2 amino acids therefrom,

and/or a light chain comprising at least one CDR selected from the group consisting of

(d) a CDRL1 as shown in SEQ 1D NO: 13, or a CDRL1 sequence differing in 1 or 2 amino acids therefrom,

(e) a CDRL2 as shown in SEQ 1D NO: 14, or a CDRL2 sequence differing in one or two amino acids therefrom, and

(f) a CDRL3 as shown in SEQ 1D NO: 15, or a CDRL3 sequence differing in 1 or 2 amino acids therefrom,

or an monoclonal antibody recognizing the same epitope on the extracellular domain of AXL.

In a further preferred ~~embodiment~~example, the antibody comprises a heavy chain comprising at least one CDR selected from the group consisting of

(a) a CDRH1 as shown in SEQ ID NO: 22, or a CDRH1 sequence differing in 1 or 2 amino acids therefrom,

(b) a CDRH2 as shown in SEQ ID NO: 23, or a CDRH2 sequence differing in 1 or 2 amino acids therefrom, and

(c) a CDRH3 as shown in SEQ 1D NO: 24, or a CDRH3 sequence differing in 1 or 2 amino acids therefrom,

and/or a light chain comprising at least one CDR selected from the group consisting of

(d) a CDRL1 as shown in SEQ 1D NO: 19, or a CDRL1 sequence differing in 1 or 2 amino acids therefrom,

(e) a CDRL2 as shown in SEQ 1D NO: 20, or a CDRL2 sequence differing in one or two amino acids therefrom, and

(f) a CDRL3 as shown in SEQ 1D NO: 21, or a CDRL3 sequence differing in 1 or 2 amino acids therefrom,

or an monoclonal antibody recognizing the same epitope on the extracellular domain of AXL.

In a yet further preferred ~~embodiment~~example, the antibody comprises a heavy chain comprising at least one CDR selected from the group consisting of

(a) a CDRH1 as shown in SEQ ID NO: 28, or a CDRH1 sequence differing in 1 or 2 amino acids therefrom,

(b) a CDRH2 as shown in SEQ ID NO: 29, or a CDRH2 sequence differing in 1 or 2 amino acids therefrom, and

(c) a CDRH3 as shown in SEQ 1D NO: 30, or a CDRH3 sequence differing in 1 or 2 amino acids therefrom,

and/or a light chain comprising at least one CDR selected from the group consisting of

(d) a CDRL1 as shown in SEQ 1D NO: 25, or a CDRL1 sequence differing in 1 or 2 amino acids therefrom,

(e) a CDRL2 as shown in SEQ 1D NO: 26, or a CDRL2 sequence differing in one or two amino acids therefrom, and

(f) a CDRL3 as shown in SEQ 1D NO: 27, or a CDRL3 sequence differing in 1 or 2 amino acids therefrom,

or an monoclonal antibody recognizing the same epitope on the extracellular domain of AXL.

In another ~~embodiment~~example, the present ~~invention~~disclosure refers to an antibody comprising a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, 12 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 90% thereto and/or a light chain amino acid sequence selected from the group consisting of SEQ. ID NOs: 7, 9, 11 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 90% thereto or to an antibody recognizing the same epitope on the extracellular domain of AXL.

As used herein, "sequence identity" between two polypeptide sequences, indicates the percentage of amino acids that are identical between the sequences. Preferred polypeptide sequences ~~of the invention~~as described herein have a sequence identity of at least 90%.

In a particular preferred ~~embodiment~~example, the antibody is selected from the group consisting of 11B7, 11D5, 10D12 or an antibody recognizing the same epitope on the extracellular domain of AXL.

The antibody may be any antibody of natural and/or synthetic origin, e.g. an antibody of mammalian origin. Preferably, the constant domain -if present- is a human constant domain. The variable domain is preferably a mammalian variable domain, e.g. a humanized or a human variable domain. More preferably, the antibody is a chimeric, humanized or human antibody.

The antibody ~~of the invention~~as described herein may be of the IgA-, IgD-, IgE, IgG-or IgM-type, preferably of the IgG-or IgM-type including, but not limited to, the IgG1-, IgG2-, IgG3-, IgG4-, IgM1-and IgM2-type. In most preferred ~~embodiment~~examples, the antibody is of the human IgG1-, IgG2-or IgG4-type.

As discussed, supra, there are a number of isotypes of antibodies. It will be appreciated that antibodies that are generated need not initially possess such an isotype but, rather the antibody as generated can possess any isotype and that the antibody can be isotype-switched by using the molecularly cloned V region genes or cloned constant region genes or cDNAs in appropriate expression vectors using conventional molecular biological techniques that are well known in the art and then expressing the antibodies in host cells using techniques known in the art

The term antibody includes "fragments" or "derivatives", which have at least one antigen binding site of the antibody. Antibody fragments include Fab fragments, Fab' fragments $F(ab')_2$ fragments as well as Fv fragments.

Derivatives of the antibody include single chain antibodies, nanobodies, and diabodies. Derivatives of the antibody shall also include scaffold proteins having an antibody-like binding activity that bind to AXL.

Within the context ~~of the present invention~~as described herein, the term "scaffold protein", as used herein, means a polypeptide or protein with exposed surface areas in which amino acid insertions, substitutions or deletions are highly tolerable. Examples of scaffold proteins that can be used in accordance with the present ~~invention~~disclosure are protein A from Staphylococcus aureus, the bilin binding protein from Pieris brassicae or other lipocalins, ankyrin repeat proteins, and human fibronectin (reviewed in Binz and Plückthun, Curr Opin Biotechnol,16: 459-69, 2005). Engineering of a scaffold protein can be regarded as grafting or integrating an affinity function onto or into the structural framework of a stably folded protein. Affinity function means a protein binding affinity according to the present ~~invention~~disclosure. A scaffold can be structurally separable from the amino acid sequences conferring binding specificity. In general, proteins appearing suitable for the development of such artificial affinity reagents may be obtained by rational, or most commonly, combinatorial protein engineering techniques such as panning against AXL, either purified protein or protein displayed on the cell surface, for binding agents in an artificial scaffold library displayed in vitro, skills which are known in the art (Skerra, J. Mol. Recog., Biochim Biophys Acta, 1482: 337-350, 2000; Binz and Plückthun, Curr Opin Biotechnol, 16: 459-69, 2005). In addition, a scaffold protein having an antibody like binding activity can be derived from an acceptor polypeptide containing the scaffold domain, which can be grafted with binding domains of a donor polypeptide to confer the binding specificity of the donor polypeptide onto the scaffold domain containing the acceptor polypeptide. The inserted binding domains may include, for example, at least one CDR of an anti-AXL antibody, preferably at least one selected from the group of SEQ ID NOs: 13-30. Insertion can be accomplished by various methods known to those skilled in the art including, for example, polypeptide synthesis, nucleic acid synthesis of an encoding amino acid as well by various forms of recombinant methods well known to

those skilled in the art.

As has been indicated above, the specificity of the antibody, antibody fragment, or a derivative thereof lies in the amino acid sequence of the CDR. The variable domain (the heavy chain VH and light chain VL) of an antibody preferably comprises three complementary determining regions sometimes called hypervariable regions, flanked by four relatively conserved framework regions or "FRs". Often, the specificity of an antibody is determined or largely determined by a CDR, such as a CDR of the VH chain or a plurality of CDRs. The person skilled in the art will readily appreciate that the variable domain of the antibody, antibody fragment or derivative thereof having the above-described CDRs can be used for the construction of antibodies of further improved specificity and biological function. Insofar, the present ~~invention~~disclosure encompasses antibodies, antibody fragments or derivatives thereof comprising at least one CDR of the above-described variable domains and which advantageously have substantially the same, similar or improved binding properties as the antibody described in the appended examples. Starting from an antibody that comprises at least one CDR as recited in the attached sequence listing and required by the ~~embodiment~~examples of the ~~invention~~disclosure, the skilled artisan can combine further CDRs from the originally identified monoclonal antibodies or different antibodies for an enhanced specificity and/or affinity. CDR grafting is well-known in the art and can also be used to fine-tune the specific affinity and other properties of the antibody, fragment or derivative thereof of the ~~invention~~disclosure, as long as the original specificity is retained. It is advantageous that the antibody, fragment or derivative comprises at least two, more preferred at least three, even more preferred at least four or at least five and particularly preferred all six CDRs of the original donor antibody. In further alternatives of the ~~invention~~disclosure, CDRs from different originally identified monoclonal antibodies may be combined in a new antibody entity. In these cases, it is preferred that the three CDRs of the heavy chain originate from the same antibody whereas the three CDRs of the light chain all originate from a different (but all from the same) antibody. The antibodies ~~of the present invention~~as described herein or their corresponding

immunoglobulin chain(s) can be further modified using conventional techniques known in the art, for example, by using amino acid deletion(s), insertion(s), substitution(s), addition(s), and/or recombination(s) and/or any other modification(s) known in the art either alone or in combination. Methods for introducing such modifications in the DNA sequence underlying the amino acid sequence of an immunoglobulin chain are well known to the person skilled in the art; see, e.g., Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y.

The antibodies, antibody fragments or derivative thereof are optionally deimmunized for therapeutic purposes. A deimmunized antibody is a protein devoid of or reduced for epitopes that can be recognized by T helper lymphozytes. An example how to identify said epitopes is shown in Tangri et al., (J Immunol. 174: 3187-96, 2005). The manufacture of deimmunized antibody fragments or derivative thereof may be carried out as described in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293.

In one ~~embodiment~~example the antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). The production of chimeric antibodies is described, for example, in WO 89/09622.

Preferably, the present ~~invention~~disclosure refers to a chimerized antibody comprising a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOs: 38, 39, 41, 42 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 90% thereto

and/or a light chain amino acid sequence selected from the group consisting of SEQ. ID NOs: 37, 40 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 90% thereto or to an antibody recognizing the same epitope on the extracellular domain of AXL.

In a further ~~embodiment~~example the antibodies ~~of the present invention~~as described herein are humanized or fully human antibodies. Humanized forms of the antibodies may be generated according to the methods known in the art such as chimerization or CDR grafting. Alternative methods for the production of humanized antibodies are well known in the art and are described in, e.g., EP-A1 0 239 400 and W090/07861. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be for example performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting CDRs or CDR sequences of non human origin for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in non human antibodies.

Preferably, the present ~~invention~~disclosure refers to a humanized antibody comprising a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 46 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 90% thereto and/or a light chain amino acid sequence selected from the group consisting of SEQ. ID NOs: 43, 45 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 90% thereto or to an

et al., , Cloning Stem Cells 4:91-102, 2002).

Human antibodies can also be derived by in vitro methods. Suitable examples include but are not limited to phage display (CAT, Morphosys, Dyax, Biosite/Medarex, Xoma, Symphogen, Alexion (formerly Proliferon), Affimed) ribosome display (CAT), yeast display, and the like.

For therapeutic purposes, the antibody may be conjugated with a therapeutic effector group, e.g. a radioactive group or a cytotoxic group.

For diagnostic purposes, the antibody may be labelled. Suitable labels include radioactive labels, fluorescent labels, or enzyme labels.

Further antibodies to be utilized in accordance with the present ~~invention~~disclosure are so-called xenogenic antibodies. The general principle for the production of xenogenic antibodies such as human antibodies in mice is described in, e.g., WO9110741, WO 9402602, WO 9634096 and WO 9633735.

As discussed above, the antibody ~~of the invention~~as described herein may exist in a variety of forms besides complete antibodies; including, for example, Fv, Fab´ and $F(ab')_2$ as well as in single chains; see e.g. W08809344.

If desired, the antibodies ~~of the invention~~as described herein may be mutated in the variable domains of the heavy and/or light chains to alter a binding property of the antibody. For example, a mutation may be made in one or more of the CDR regions to increase or decrease the Kd of the antibody for AXL, or to alter the binding specificity of the antibody. Techniques in site directed mutagenesis are well-known in the art. See, e.g., Sambrook et al. and Ausubel et al., supra. Furthermore, mutations may be made at an amino acid residue that is known to be changed compared to germline in a variable region of an AXL antibody. In another aspect, mutations may be introduced into one or more of the framework regions. A mutation may be made in a framework region or constant domain to increase the half-life of the AXL

antibody. See, e.g., WO0009560. A mutation in a framework region or constant domain may also be made to alter the immunogenicity of the antibody, to provide a site for covalent or non-covalent binding to another molecule, or to alter such properties as complement fixation. Mutations may be made in each of the framework regions, the constant domain and the variable regions in a single mutated antibody. Alternatively, mutations may be made in only one of the framework regions, the variable regions or the constant domain in a single mutated antibody.

In a further aspect, the antibody may have a constant domain with effector functions, whereby AXL expressing cells which have bound the antibody, antibody fragment or derivative thereof on the cell surface may be attacked by immune system functions. For example, the antibody may be capable of fixing complement and participating in complement-dependent cytotoxicity (CDC). Moreover, the antibody may be capable of binding to Fc receptors on effector cells, such as monocytes and natural killer (NK) cells, and participate in antibody-dependent cellular cytotoxicity (ADCC).

In yet a further aspect the antibodies ~~of the invention~~as described herein are applicable for therapeutic treatment, preferably for treatment of hyperproliferative diseases, cardiovascular diseases, in particular artherosclerosis and thrombosis, diabetes related diseases, in particular glomerular hypertrophy or diabetic nephropathy, and particularly of disorders associated with, accompanied by or caused by AXL expression, overexpression or hyperactivity. The hyperproliferative diseases are preferably selected from disorders associated with, accompanied by or caused by AXL expression, overexpression or hyperactivity, such as cancer, e.g. breast cancer, colon cancer, lung cancer, kidney cancer, follicular lymphoma, myeloid leukemia, skin cancer/melanoma, glioblastoma, ovarian cancer, prostate cancer, pancreatic cancer, Barrett's esophagus and esophageal cancer, stomach cancer, bladder cancer, cervical cancer, liver cancer, thyroid cancer, and head and neck cancer, or hyperplastic and neoplastic diseases or other AXL expressing or overexpressing hyperproliferative diseases.

In another aspect the antibodies ~~of the present invention~~as described herein can be used for the co-administration with an antineoplastic agent for the treatment of one of the above mentioned disorders.

Co-administration as used herein includes the administration of an antibody ~~of the present invention~~as described herein with an antineoplastic agent, preferably an apoptosis inducing antineoplastic agent. The term co-administration further includes the administration of the antibody ~~of the present invention~~as described herein and the antineoplastic agent, preferably an apoptosis inducing antineoplastic agent, in the form of a single composition or in the form of two or more distinct compositions. Co-administration includes the administration of an antibody ~~of the present invention~~as described herein with an antineoplastic agent, preferably an apoptosis inducing antineoplastic agent simultaneously (i.e. at the same time) or sequentially, (i.e. at intervals).

The ~~invention~~disclosure further relates to a nucleic acid molecule encoding the antibody, antibody fragment or derivative thereof ~~of the invention~~as described herein. The nucleic acid molecule ~~of the invention e~~as described herein encoding the above-described antibody, antibody fragment or derivative thereof may be, e.g. DNA, cDNA, RNA or synthetically produced DNA or RNA or recombinantly produced chimeric nucleic acid molecule comprising any of those nucleic acid molecules either alone or in combination. The nucleic acid molecule may also be genomic DNA corresponding to the entire gene or a substantial portion thereof or to fragments and derivatives thereof. The nucleotide sequence may correspond to the naturally occurring nucleotide sequence or may contain single or multiple nucleotide substitutions, deletions or additions. In a particular preferred ~~embodiment~~example ~~of the present invention~~as described herein, the nucleic acid molecule is a cDNA molecule.

Preferably, the ~~invention~~disclosure relates to an isolated nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid sequence encoding a polypeptide of SEQ ID NOs: 7-12, 13-30, 37-42, 43-46

(b) a nucleic acid sequence as shown in SEQ ID NOs: 1-6, 31-36

(c) a nucleic acid complementary to any of the sequences in (a) or (b); and

(d) a nucleic acid sequence capable of hybridizing to (a), (b) or (c) under stringent conditions.

The term "hybridizing under stringent conditions" means that two nucleic acid fragments hybridize with one another under standardized hybridization conditions as described for example in Sambrook et al., "Expression of cloned genes in E. coli" in Molecular Cloning: A laboratory manual (1989), Cold Spring Harbor Laboratory Press, New York, USA. Such conditions are for example hybridization in 6.0xSSC at about 45° C. followed by a washing step with 2.0xSSC at 50° C, preferably 2.0xSSC at 65°C, or 0.2xSSC at 50°C, preferably 0.2xSSC at 65°C.

The ~~invention~~disclosure also relates to a vector comprising a nucleic acid molecule ~~of the invention~~as described herein. Said vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The nucleic acid molecules ~~of the invention~~as described herein may be joined to a vector containing selectable markers for propagation in a host. Generally, a plasmid vector is introduced in a precipitate such as a calcium phosphate precipitate or rubidium chloride precipitate, or in a complex with a charged lipid or in carbon-based clusters, such as fullerens. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells.

Preferably, the vector ~~of the invention~~as described herein is an expression vector wherein the nucleic acid molecule is operatively linked to one or more control sequences allowing the transcription and optionally expression in prokaryotic and/or

eukaryotic host cells. Expression of said nucleic acid molecule comprises transcription of the nucleic acid molecule, preferably into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the lac, trp or tac promoter in E. coli, and examples for regulatory elements permitting expression in eukaryotic host cells are the AOXI or GAL1 promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen) or pSPORTI (GIBCO BRL). Preferably, said vector is an expression vector and/or a gene transfer or targeting vector. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector ~~of the invention~~as described herein into targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors; see, for example, the techniques described in Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (2001, Third Edition) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1994). Alternatively, the nucleic acid molecules ~~of the invention~~as described herein can be reconstituted into liposomes for delivery to target cells.

The ~~invention~~disclosure further relates to a host comprising the vector ~~of the invention~~as described herein.

Said host may be a prokaryotic or eukaryotlc cell or a non-human transgenic animal. The polynucleotide or vector ~~of the invention~~as described herein which is present in the host may either be integrated into the genome of the host or it may be maintained extrachromosomally. In this respect, it is also to be understood that the nucleic acid molecule ~~of the invention~~as described herein can be used for "gene targeting" and/or "gene replacement", for restoring a mutant gene or for creating a mutant gene via homologous recombination; see for example Mouellic, Proc. Natl. Acad. Sci. USA, 87 (1990), 4712-4716; Joyner, Gene Targeting, A Practical Approach, Oxford University Press.

The host can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal, mammalian or, preferably, human cell. Preferred fungal cells are, for example, those of the genus Saccharomyces, in particular those of the species S. cerevisiae. The term "prokaryotic" is meant to include all bacteria which can be transformed or transfected with a polynucleotide for the expression of a variant polypeptide ~~of the invention~~as described herein. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, E. coli, S. typhimurium, Serratia marcescens and Bacillus subtilis. A polynucleotide coding for a mutant form of variant polypeptides ~~of the invention~~as described herein can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Methods for preparing fused, operably linked genes and expressing them in bacteria or animal cells are well-known in the art (Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (2001, Third Edition). The genetic constructs and methods described therein can be utilized for expression of variant antibodies, antibody fragments or derivatives thereof ~~of the invention~~as described herein in, e.g., prokaryotic hosts. In general, expression vectors containing promoter sequences which facilitate the efficient transcription of the inserted nucleic acid molecule are used in connection with the host. The expression vector typically contains an origin of replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed cells. The transformed prokaryotic hosts can be grown in fermentors and cultured

according to techniques known in the art to achieve optimal cell growth. The antibodies, antibody fragments or derivatives thereof ~~of the invention~~as described herein can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the microbially or otherwise expressed antibodies, antibody fragments or derivatives thereof ~~of the invention~~as described herein may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies.

In a preferred ~~embodiment~~example of the ~~invention~~disclosure, the host is a bacterium, fungal, plant, amphibian or animal cell. Preferred animal cells include but are not limited to Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), 3T3 cells, NSO cells and a number of other cell lines including human cells, for example Per.C6. In another preferred ~~embodiment~~example, said animal cell is an insect cell. Preferred insect cells include but are not limited to cells of the SF9 cell lines

In a more preferred ~~embodiment~~example of the ~~invention~~disclosure, said host is a human cell or human cell line. Said human cells include, but are not limited to Human embryonic kidney cells (HEK293, 293T, 293 freestyle). Furthermore, said human cell lines include, but are not limited to HeLa cells, human hepatocellular carcinoma cells (e. g., Hep G2), A549 cells.

The ~~invention~~disclosure also provides transgenic non-human animals comprising one or more nucleic acid molecules ~~of the invention~~as described herein that may be used to produce antibodies ~~of the invention~~as described herein. Antibodies can be produced in and recovered from tissue or body fluids, such as milk, blood or urine, of goats, cows, horses, pigs, rats, mice, rabbits, hamsters or other mammals. See, e. g., U.S. Patent Nos. 5,827,690; 5,756,687; 5,750,172; and 5,741,957. As described above, non-human transgenic animals that comprise human immunoglobulin loci can be produced by immunizing with AXL or a portion thereof.

The ~~invention~~disclosure additionally relates to a method for the preparation of an antibody, comprising culturing the host ~~of the invention~~as described herein under conditions that allow synthesis of said antibody and recovering said antibody from said culture.

The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms ~~of the present invention~~as described herein, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; see, Scopes, "Protein Purification", Springer-Verlag, N.Y. (1982). The antibody or its corresponding immunoglobulin chain(s) ~~of the invention~~as described herein can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, e.g., microbially expressed antibodies or immunoglobulin chains ~~of the invention~~as described herein may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed, e.g., against the constant region of the antibody ~~of the invention~~as described herein.

It will be apparent to those skilled in the art that the antibodies ~~of the invention~~as described herein can be further coupled to other moieties for, e.g., drug targeting and imaging applications. Such coupling may be conducted chemically after expression of the antibody or antigen to site of attachment or the coupling product may be engineered into the antibody or antigen ~~of the invention~~as described herein at the DNA level. The DNAs are then expressed in a suitable host system, and. the expressed proteins are collected and renatured, if necessary.

In a preferred ~~embodiment~~example ~~of the present invention~~as described herein, the antibody is coupled to an effector, such as a radioisotope or a toxic chemotherapeutic agent. Preferably, these antibody conjugates are useful in targeting cells, e.g. cancer cells, expressing AXL, for elimination. The linking of

antibodies/antibody fragments ~~of the invention~~as described herein to radioisotopes e.g. provides advantages to tumor treatments. Unlike chemotherapy and other forms of cancer treatment, radioimmunotherapy or the administration of a radioisotope-antibody combination directly targets the cancer cells with minimal damage to surrounding normal, healthy tissue. Preferred radioisotopes include e.g. ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I.

Furthermore, the antibodies ~~of the invention~~as described herein can be used to treat cancer when being conjugated with toxic chemotherapeutic drugs such as geldanamycin (Mandler et al., J. Natl. Cancer Inst., 92(19), 1549-51 (2000» and maytansin, for example, the maytansinoid drug, DM1 (Liu et al., Proc. Natl. Acad. Sci. U.S.A. 93:8618-8623 (1996) and auristatin-E or monomethylauristatin-E (Doronina et al., Nat. Biotechnol. 21:778-784 (2003) or calicheamicit);. Different linkers that release the drugs under acidic or reducing conditions or upon exposure to specific proteases are employed with this technology. The antibodies ~~of the invention~~as described herein may be conjugated as described in the art.

The ~~invention~~disclosure further relates to a pharmaceutical composition comprising the antibody, the nucleic acid molecule, the vector, the host ~~of the invention~~as described herein or an antibody obtained by the method ~~of the invention~~as described herein.

The term "composition" as employed herein comprises at least one compound ~~of the invention~~as described herein. Preferably, such a composition is a pharmaceutical or a diagnostic composition.

It is preferred that said pharmaceutical composition comprises a pharmaceutically acceptable carrier and/or diluent. The herein disclosed pharmaceutical composition may be partially useful for the treatment of disorders associated with, accompanied by or caused by AXL expression, overexpression or[Mike Roth1] hyperactivity, e.g. hyperproliferative diseases, cardiovascular diseases, in particular artherosclerosis and thrombosis, diabetes related diseases, in particular glomerular hypertrophy or diabetic

nephropathy. Said disorders comprise, but are not limited to cancer, e.g. breast cancer, colon cancer, lung cancer, kidney cancer, follicular lymphoma, myeloid leukemia, skin cancer/melanoma, glioblastoma, ovarian cancer, prostate cancer, pancreatic cancer, Barrett's esophagus and esophageal cancer, stomach cancer, bladder cancer, cervical cancer, liver cancer, thyroid cancer, and head and neck cancer, or other hyperplastic or neoplastic diseases or other AXL expressing or overexpressing diseases.

The term "hyperactivity" herein refers to uncontrolled AXL signaling which may be caused by a lack and/or dysfunction of negative regulation. By way of example negative regulation comprises protein dephosphorylation, degradation and/or endocytosis. Moreover uncontrolled AXL signaling may be the result of genetic alterations, either somatic or germline, which result in changes of the AXL amino acid sequence.

Examples of suitable pharmaceutical carriers, excipients and/or diluents are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. The compositions ~~of the invention~~as described herein may also be administered directly to the target site, e.g., by biolistic delivery to an external or internal target site, like the brain. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Proteinaceous pharmaceutically active matter may be present in amounts between 1 µg and 100 mg/kg body weight per

dose; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it should also be in the range of 1 pg to 100 mg per kilogram of body weight per minute.

Progress can be monitored by periodic assessment. The compositions ~~of the invention~~as described herein may be administered locally or systemically. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's,

or' fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition ~~of the invention~~as described herein may comprise further agents depending on the intended use of the pharmaceutical composition. It is particularly preferred that the pharmaceutical composition comprises further active agents like, e.g. an additional antineoplastic agent, small molecule inhibitor, anti-tumor agent or chemotherapeutic agent.

The ~~invention~~disclosure also relates to a pharmaceutical composition comprising an anti-AXL-antibody, which is preferably the antibody ~~of the invention~~as described herein in combination with at least one further antineoplastic agent. Said combination is effective, for example, in inhibiting abnormal cell growth.

Many antineoplastic agents are presently known in the art. In general the term includes all agents that are capable of prevention, alleviation and/or treatment of hyperproliferative disorders. In one ~~embodiment~~example, the

antineoplastic agent is selected from the group of therapeutic proteins including but not limited to antibodies or immunomodulatory proteins. In another ~~embodiment~~example the antineoplastic agent is selected from the group of small molecule inhibitors or chemotherapeutic agents consisting of mitotic inhibitors, kinase inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, histone deacetylase inhibitors, anti-survival agents, biological response modifiers, anti-hormones, e. g. anti-androgens, and antiangiogenesis agents.

Specific examples of antineoplastic agents which can be used in combination with the antibodies provided herein include, for example, gefitinib, lapatinib, sunitinib, pemetrexed, bevacisumab, cetuximab, imatinib, trastuzumab, alemtuzumab, rituximab, erlotinib, bortezomib and the like. Other specific antineoplastic agents to be used in the compositions as described and claimed herein include for example, chemotherapeutic agents such as capecitabine, daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphor-amide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide, trimetrexate, teniposide, cisplatin and diethylstilbestrol (DES). See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed. 1987, pp. 1206-1228, Berkow et al., eds., Rahway, N.J. In particular preferred are such antineoplastic agents that induce apoptosis.

When used with the described AXL antibodies, such antineoplastic agents

may be used individually (e.g., 5-FU and an antibody), sequentially (e.g., 5-FU and an antibody for a period of time followed by MTX and an antibody), or in combination with one or more other such antineoplastic agents (e.g., 5-FU, MTX and an antibody, or 5-FU, radiotherapy and an antibody).

The term antineoplastic agent may also include therapeutic procedures, as for example irradiation or radiotherapy.

The pharmaceutical composition ~~of the invention~~as described herein can be used in human medicine and can be used also for veterinary purposes.

Additionally, the ~~invention~~disclosure relates to the use of the antibody ~~of the invention~~as described herein, the nucleic acid molecule, the vector, the host ~~of the invention~~as described herein or an antibody obtained by the method ~~of the invention~~as described herein for the preparation of a pharmaceutical composition for diagnosis, prevention or treatment of hyperproliferative diseases, cardiovascular diseases, in particular artherosclerosis and thrombosis, diabetes related diseases, in particular glomerular hypertrophy or diabetic nephropathy, and particularly of disorders associated with, accompanied by or caused by AXL expression, overexpression or hyperactivity.

A hyperproliferative disease as mentioned above includes any neoplasia, i.e. any abnormal and/or uncontrolled new growth of tissue. The term "uncontrolled new growth of tissue" as used herein may depend upon a dysfunction and/or loss of growth regulation. A hyperproliferative disease includes tumor diseases and/or cancer, such as metastatic or invasive cancers.

In a preferred ~~embodiment~~example of the use ~~of the invention~~as described herein, said hyperproliferative disease is in particular breast cancer, colon cancer, lung cancer, kidney cancer, follicular lymphoma, myeloid leukemia, skin cancer/melanoma, glioblastoma, ovarian cancer, prostate cancer, pancreatic cancer, Barrett's esophagus and esophageal cancer, stomach cancer, bladder cancer,

cervical cancer, liver cancer, thyroid cancer, and head and neck cancer, or hyperplastic or neoplastic diseases or other AXL expressing or overexpressing hyperproliferative diseases.

In yet another ~~embodiment~~example the present ~~invention~~disclosure refers to the use of an anti-AXL-antibody, preferably the antibody ~~of the present invention~~as described herein for the manufacture of a medicament for the co-administration with an antineoplastic agent for the treatment of one of the above mentioned disorders.

According to a further preferred ~~embodiment~~example the present ~~invention~~disclosure is directed to the use of an anti-AXL antibody for the manufacture of a pharmaceutical composition for the treatment of drug resistant cancer. In a particularly preferred ~~embodiment~~example, the anti-AXL antibody is a monoclonal antibody as defined in claims 1-22.

Further the present ~~invention~~disclosure relates to a diagnostic composition comprising the antibody ~~of the invention~~as described herein, the nucleic acid molecule, the vector, the host ~~of the invention~~as described herein or an antibody obtained by the method ~~of the invention~~as described herein and optionally a pharmaceutically acceptable carrier.

The diagnostic composition ~~of the invention~~as described herein is useful in the detection of an undesired expression, overexpression or hyperactivity of the mammalian AXL in different cells, tissues or another suitable sample, comprising contacting a sample with an antibody ~~of the invention~~as described herein, and detecting the presence of AXL in the sample. Accordingly, the diagnostic composition ~~of the invention~~as described herein may be used for assessing the onset or the disease status of a hyperproliferative disease.

Furthermore, malignant cells, such as cancer cells expressing AXL, can be targeted with the antibody ~~of the invention~~as described herein. The cells which have bound the antibody as described herein ~~of the invention~~ might thus be attacked by immune system functions such as the complement system or by cell-mediated cytotoxicity,

thereby reducing the number of or eradicating cancer cells. These considerations equally apply to the treatment of metastases and re-current tumors.

In another ~~aspect of the present invention~~example, the antibody as described herein~~of the invention~~ is coupled to a labelling group. Such antibodies are particularly suitable for diagnostic applications. As used herein, the term "labelling group" refers to a detectable marker, e.g. a radiolabelled amino acid or biotinyl moieties that can be detected by marked avidin. Various methods for labelling polypeptides and glycoproteins, such as antibodies, are known in the art and may be used in performing the present invention. Examples of suitable labelling groups include, but are not limited to, the following: radioisotopes or radionuclides (e.g. ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent groups (e.g. FITC, rhodamine, lanthanide phosphors), enzymatic groups (e.g. horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent groups, biotinyl groups, or predetermined polypeptide epitopes recognized by a secondary reporter (e.g. leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).

In certain ~~aspects~~examples, it may be desirable, that the labelling groups are attached by spacer arms of various lengths to reduce potential steric hindrance.

In another ~~embodiment~~example the present ~~invention~~disclosure relates to a method of assessing for the presence of AXL expressing cells comprising contacting the antibody as described herein~~of the invention~~ with cells or a tissue suspected of carrying AXL on their/its surface. Suitable methods for detection of AXL expression in a sample may be an Enzyme-Linked Immunosorbent Assay (ELISA) or Immunohistochemistry (IHC).

An ELISA assay may be carried out in a microtiter plate format, wherein e.g. wells of a microtiter plate, are adsorbed with an AXL antibody. The wells are rinsed and treated with a blocking agent such as milk protein or albumin to

prevent nonspecific adsorption of the analyte. Subsequently the wells are treated with a test sample. After rinsing away the test sample or standard, the wells are treated with a second AXL antibody that is labelled, e.g. by conjugation with biotin. After washing away excess secondary antibody, the label is detected, e.g. with avidin-conjugated horseradish peroxidase (HRP) and a suitable chromogenic substrate. The concentration of the AXL antigen in the test samples is determined by comparison with a standard curve developed from standard samples.

For IHC, paraffin-embedded tissues may be used, wherein the tissues are, e.g. first deparaffinized in xylene and then dehydrated, e.g. with ethanol and rinsed in distilled water. Antigenic epitopes masked by formalin-fixation and paraffin-embedding may be exposed by epitope unmasking, enzymatic digestion or saponin. For epitope unmasking paraffin sections may be heated in a steamer, water bath or microwave oven for 20-40 min in an epitope retrieval solution as for example 2N HCl solution (pH 1.0). In the case of an enzyme digestion, tissue sections may be incubated at 37°C for 10-30 minutes in different enzyme solutions such as proteinase K, trypsin, pronase, pepsin etc.

After rinsing away the epitope retrieval solution or excess enzyme, tissue sections are treated with a blocking buffer to prevent unspecific interactions. The primary AXL antibody is added at appropriate concentrations. Excess primary antibody is rinsed away and sections are incubated in peroxidase blocking solution for 10 min at room temperature. After another washing step, tissue sections are incubated with a secondary labelled antibody, e.g. labelled with a group that might serve as an anchor for an enzyme. Examples therefore are biotin labelled secondary antibodies that are recognized by streptavidin coupled horseradish peroxidase. Detection of the antibody/enzyme complex is achieved by incubating with a suitable chromogenic substrate.

In an additional ~~embodiment~~example the present ~~invention~~disclosure relates to a method of

blocking AXL function comprising contacting the antibody as described hereinof the invention with cells or a tissue suspected of carrying AXL on their/its surface under conditions, wherein the antibody is capable of blocking AXL function. The contacting may be in vitro or in vivo.

The inventiondisclosure also relates to a method of treating a hyperproliferative disease, cardiovascular diseases, in particular artherosclerosis and thrombosis, diabetes related diseases, in particular glomerular hypertrophy or diabetic nephropathy, comprising, administering to a patient in need thereof a suitable dose of the antibody or antibody fragment or derivative thereof of the present inventionas described herein. The hyperproliferative disease is preferably selected from disorders associated with, accompanied by or caused by AXL expression, overexpression or hyperactivity, such as cancer, e.g. breast cancer, colon cancer, lung cancer, kidney cancer, follicular lymphoma, myeloid leukemia, skin cancer/melanoma, glioblastoma, ovarian cancer, prostate cancer, pancreatic cancer, Barrett's esophagus and esophageal cancer, stomach cancer, bladder cancer, cervical cancer, liver cancer, thyroid cancer, and head and neck cancer, or hyperplastic and neoplastic diseases or other AXL expressing or overexpressing hyperproliferative diseases.

According to another preferred embodimentexample of the inventiondisclosure the cancer to be treated is a drug resistant cancer.

The inventiondisclosure further relates to a method of treating a disease wherein the antibody as described hereinof the invention is administered to a mammal and wherein said disease is correlated directly or indirectly with the abnormal level of expression or activity of AXL.

Finally, the inventiondisclosure relates to a kit comprising an anti-AXL-antibody, preferably the antibody, antibody fragment or derivative thereof as described hereinof the invention, the nucleic acid molecule encoding said components and/or the vector as described hereinof the invention.

All ~~embodiments~~ examples covering the compounds disclosed herein can be used as single compounds or in combination for the preparation of a medicament.