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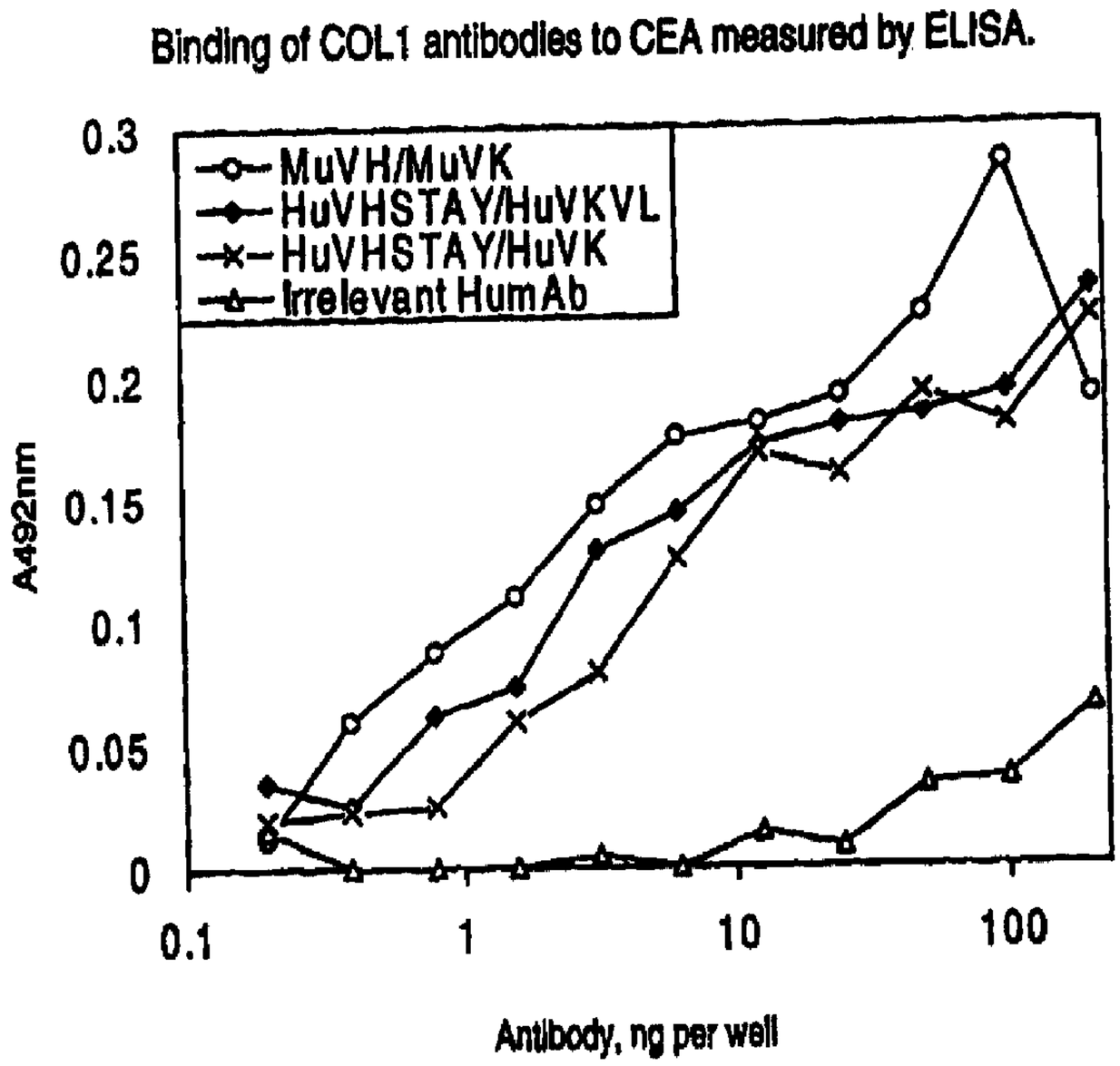
(54) **ANTICORPS MONOCLONAUX ANTI-ACE HUMANISES ET FAISANT PREUVE D'UNE GRANDE AFFINITE**

(54) **HIGH AFFINITY HUMANIZED ANTI-CEA MONOCLONAL ANTIBODIES**



(57) Cette invention concerne de nouveaux anticorps monoclonaux humanisés ainsi que des fragments ou des dérivés de ces anticorps, lesquels se lient de manière spécifique à l'antigène carcino-embryonnaire (ACE). Cette invention concerne également des procédés de production des ces anticorps et de leurs fragments ou dérivés. Ces anticorps humanisés sont utiles dans le traitement des cancers qui expriment l'ACE, ainsi qu'à des fins diagnostiques comme, par exemple, l'imagerie in vivo de tumeurs ou de cellules cancéreuses qui expriment l'ACE.

(57) Novel humanized monoclonal antibodies, fragments or derivatives thereof which specifically bind carcinoembryonic antigen (CEA) are provided as well as methods for their manufacture. These humanized antibodies are useful in the treatment of cancers which express CEA as well as for diagnostic purposes, e.g., for in vivo imaging of tumors or cancer cells which express CEA.

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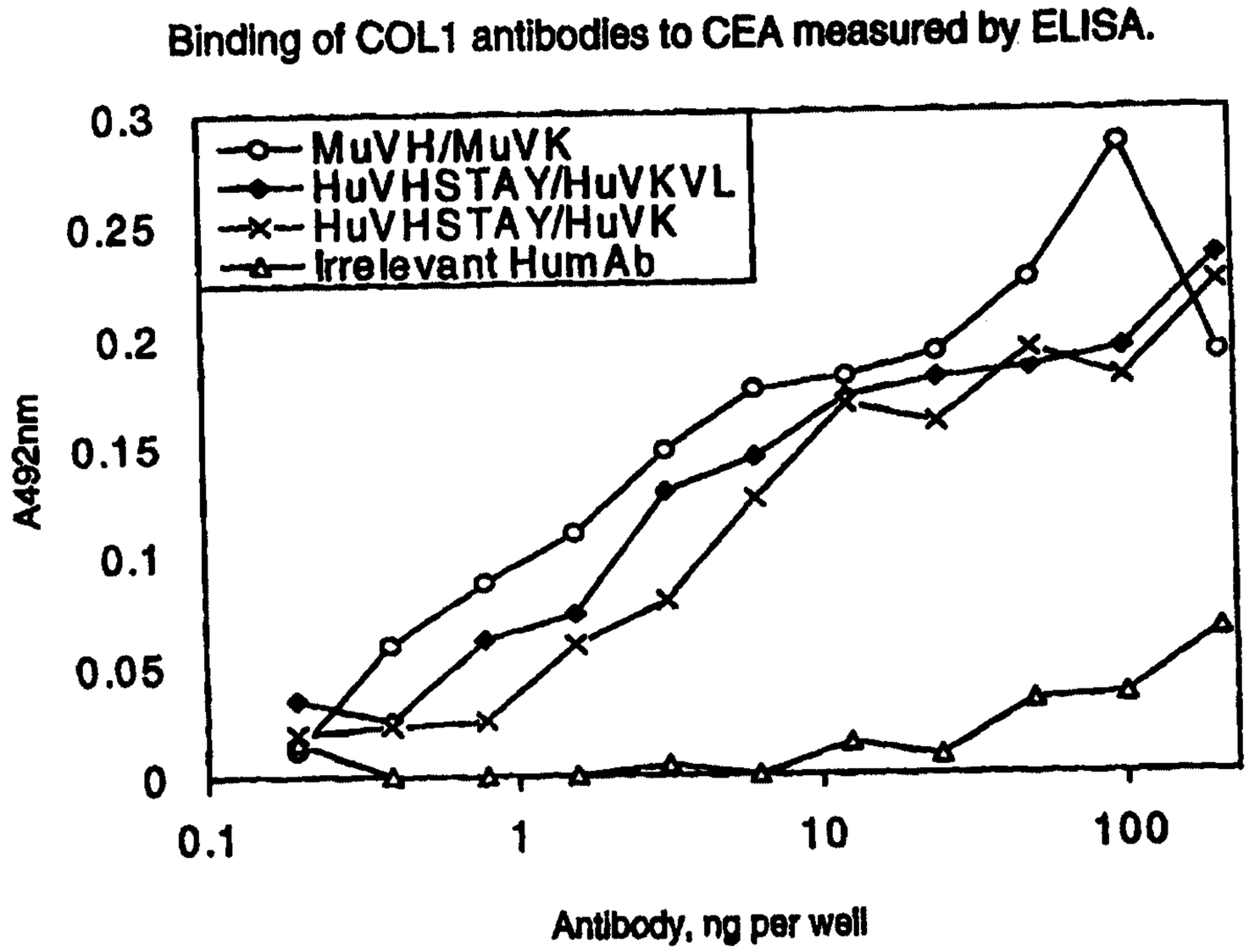

(57) Abstract

Novel humanized monoclonal antibodies, fragments or derivatives thereof which specifically bind carcinoembryonic antigen (CEA) are provided as well as methods for their manufacture. These humanized antibodies are useful in the treatment of cancers which express CEA as well as for diagnostic purposes, e.g., for *in vivo* imaging of tumors or cancer cells which express CEA.

HIGH AFFINITY HUMANIZED ANTI-CEA MONOCLONAL ANTIBODIES

FIELD OF THE INVENTION

The present invention relates to humanized monoclonal antibodies and fragments or derivatives thereof which specifically bind carcinoembryonic antigen (CEA), which is an antigen expressed by various human carcinomas including breast, lung, and gastrointestinal carcinomas such as stomach and colon cancers. More specifically, the present invention relates to humanized monoclonal antibodies and humanized antibody fragments and derivatives thereof which are derived from murine monoclonal antibody COL-1, a high affinity anti-CEA antibody. The present invention further relates to methods for producing such humanized monoclonal antibodies specific to CEA, pharmaceutical and diagnostic compositions containing such humanized monoclonal antibodies, and methods of use thereof for the treatment or diagnosis of cancer.

BACKGROUND OF THE INVENTION

The identification of antigens expressed by tumor cells and the preparation of monoclonal antibodies which specifically bind such antigens is well known in the art. Anti-tumor monoclonal antibodies exhibit potential application as both therapeutic and diagnostic agents. Such monoclonal antibodies have potential application as diagnostic agents because they specifically bind tumor antigens and thereby can detect the presence of tumor cells or tumor antigen in an analyte. For example, use of monoclonal antibodies which bind tumor antigens for *in vitro* and *in vivo* imaging of tumor cells or tumors using a labeled form of such a monoclonal antibody is conventional in the art.

Moreover, monoclonal antibodies which bind tumor antigens have well known application as therapeutic agents. The usage of monoclonal antibodies themselves as therapeutic agents, or as conjugates wherein the monoclonal antibody is directly or indirectly attached to an effector moiety, *e.g.*, a drug, cytokine, cytotoxin, etc., is well known.

Essentially, if the monoclonal antibody is attached to an effector moiety, then the monoclonal antibody functions as a targeting moiety, *i.e.* it directs the effector moiety (which typically possesses therapeutic activity) to the antibody's target, *e.g.*, a tumor which expresses the antigen bound by the monoclonal antibody. In contrast, when the monoclonal antibody itself operates as a therapeutic agent, the antibody functions both as a targeting moiety -- *i.e.* it will specifically bind a cell which expresses the antigen -- and as an effector which mediates therapeutic activity, typically tumor cell lysis. A monoclonal antibody may

possess one or more of such effector functions, which include, *e.g.*, antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC), among others; these functions are effected by the portion of the antibody molecule generally referred to in the literature as the Fc portion.

One specific tumor antigen to which various monoclonal antibodies have been produced is the carcinoembryonic antigen (CEA). CEA is an antigen complex having a molecular weight of about 180,000 D, which is expressed by numerous carcinomas including gastrointestinal carcinomas, colorectal carcinomas, breast carcinomas, ovarian carcinomas, and lung carcinomas. See, *e.g.*, Robbins *et al.*, *Int'l J. Cancer*, 53(6):892-897 (1993); Greiner *et al.*, *J. Clin. Oncol.*, 10(5):735-746 (1992); Obuchi *et al.*, *Cancer Res.*, 47(13):3565-3571 (1987); Muraro *et al.*, *Cancer Res.*, 45(11 Pt. 2):5769-5780 (1985).

The use of monoclonal antibodies to detect various, specific CEA epitopes differentially expressed on human carcinomas has been reported in the literature. See, *e.g.*, Obuchi *et al.*, *Cancer Res.*, 47(13):3565-3571 (1987); Muraro *et al.*, *Cancer Res.*, 45(11 Pt. 2):5769-5780 (1985).

In particular, Muraro *et al.* (*id.*) report generation of monoclonal antibodies designated COL-1 through COL-15, which exhibit a strong, selective reactivity for human colon carcinomas versus normal adult tissues. These antibodies react with distinct, restricted epitopes on CEA. Of these antibodies, the COL-1 antibody has been the focus of considerable attention because of its high affinity for CEA (1.4×10^9 M^{-1}) and also because it comprises no detectable reactivity for CEA-related antigens such as the nonspecific cross-treating antigen (NCA) and the normal fecal antigen (NFA). Robbins *et al.*, *Int'l J. Canc.*, 53(6):892-897 (1993).

Because of its binding properties, COL-1 is currently being evaluated for use as a therapeutic agent. For example, Siler *et al.* (*Biotech. Ther.*, 4(3-4):163-181 (1993)) report the administration of ^{131}I-labeled COL-1 to LS-M4T human colon carcinoma xenograft-containing athymic mice. They report that this treatment resulted in reduction of the rate of tumor growth, within little or no toxicity, and that their results demonstrate the potential therapeutic efficacy of radiolabeled COL-1 in clinical trials. Also, Yu *et al.* (*J. Clin. Oncol.*, 14(6):1798-1809 (1996)) report that ^{131}I-labeled COL-1 is now in phase 1 clinical trials in patients having gastrointestinal malignancies. They further indicate that the antibody conjugate is well tolerated, except for some hematologic toxicity. In addition, the use of conjugates of COL-1 and β-galactosidase has been shown to specifically kill *in vitro* tumor cells from a variety of tumor cell lines. Abraham *et al.*, *Cell Biophys.*, 24-25:127-133 (1994).

However, while murine antibodies, such as COL-1 and other anti-CEA murine antibodies, have applicability as therapeutic agents in humans, they are disadvantageous in some respects. Specifically, because murine antibodies are of foreign species origin, they may be immunogenic in humans. This may result in a neutralizing antibody response -- a human anti-murine antibody (HAMA) response -- which is particularly problematic if the antibodies are desired to be administered repeatedly, *e.g.*, for treatment of a chronic or recurrent disease condition. This is a significant drawback, as some cancer treatments are effected over a prolonged time period, *e.g.*, over several years or longer. Also, because these antibody molecules contain murine constant domains they may not exhibit human effector functions.

In an effort to eliminate or reduce such problems, chimeric antibodies have been disclosed. Chimeric antibodies contain portions of two different antibodies, typically of two different species. Generally, such antibodies contain human constant regions attached to variable regions from another species, typically murine variable regions. For example, some mouse/human chimeric antibodies have been reported which exhibit binding characteristics of the parental mouse antibody and effector functions associated with the human constant region. See, *e.g.*, U.S. Patent 4,816,567 to Cabilly *et al.*; U.S. Patent 4,978,745 to Shoemaker *et al.*; U.S. Patent 4,975,369 to Beavers *et al.*; and U.S. Patent 4,816,397 to Boss *et al.* Generally, these chimeric antibodies are constructed by preparing a genomic gene library from DNA extracted from pre-existing murine hybridomas. Nishimura *et al.*, *Cancer Res.*, 47:999 (1987). The library is then screened for variable region genes from both heavy and light chains exhibiting the correct antibody fragment rearrangement patterns. Alternatively, cDNA libraries are prepared from RNA extracted from the hybridomas and then screened, or the variable regions are obtained by polymerase chain reaction. The cloned variable region genes are then ligated into an expression vector containing cloned cassettes of the appropriate heavy or light chain human constant region gene. The chimeric genes are then expressed in a cell line of choice, usually a murine myeloma line. Such chimeric antibodies have been used in human therapy.

Moreover, the production of a chimeric mouse anti-human antibody derived from COL-1, which specifically binds CEA, has been reported. See *e.g.*, U.S. Patent 5,472,693 to Gourlie *et al.* (owned by The Dow Chemical Company).

Also, Morrison *et al.* report the preparation of several anti-tumor chimeric monoclonal antibodies, *in* Important Advances in Oncology, Recombinant Chimeric Monoclonal Antibodies, pp. 3-18 (S.A. Rosenberg, ed., 1990) (J.B. Lippincott, Philadelphia, PA). Results of

clinical trials with chimeric cMAb-17-1A in patients with metastatic colorectal carcinoma now show that this antibody has a 6-fold longer circulation time and significantly reduced immunogenicity as compared to the murine monoclonal antibody from which it was derived. LoBuglio *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:4220-4224 (1989); Meredith *et al.*, *J. Nucl. Med.*, 32:1162-1168 (1991).

However, while such chimerized monoclonal antibodies typically exhibit lesser immunogenicity, they are still potentially immunogenic in humans because they contain murine variable sequences which may elicit antibody responses. Thus, there is the possibility that these chimeric antibodies may elicit an anti-idiotypic response if administered to patients. Saleh *et al.*, *Cancer Immunol. Immunother.*, 32:185-190 (1990).

Because of the immunogenicity of chimeric antibodies, methods have been developed recently for the production of "humanized" antibodies. Ideally, "humanization" results in an antibody that is less immunogenic, with complete retention of the antigen-binding properties of the original molecule. In order to retain all the antigen-binding properties of the original antibody, the structure of its combining-site has to be faithfully reproduced in the "humanized" version. This can potentially be achieved by transplanting the combining site of the non-human antibody onto a human framework, either: (a) by grafting only the non-human complementarity determining regions (CDRs) onto human framework regions (FRs) and constant regions, with or without retention of critical framework residues (see, Jones *et al.*, Nature, 321:522 (1986) and Verhoeyen *et al.*, *Science*, 239:1539 (1988); or (b) by transplanting the entire non-human variable domains (to preserve ligand-binding properties) and also "cloaking" them with a human-like surface through judicious replacement of exposed residues (in order to reduce antigenicity) (see, Padlan, *Molec. Immunol.*, 28:489 (1991)).

Essentially, humanization by CDR-grafting involves transplanting only the CDRs onto human framework and constant regions. Theoretically, this should substantially eliminate immunogenicity (except if allotypic or idiotypic differences exist). Jones *et al.*, *Nature*, 321:522-525 (1986); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988), Riechmann *et al.*, Nature, 332:323-327 (1988). However, CDR-grafting by itself may not yield the desired result. Rather, it has been reported that some framework residues of the original antibody may also need to be preserved in order to preserve antigen binding activity. Riechmann *et al.*, *Nature*, 332:323-327 (1988); Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:10023-10029; Tempest *et al.*, *Biol. Technology*, 9:266-271 (1991); Co *et al.*, *Nature*, 351:501-502 (1991).

As discussed above, in order to preserve the antigen-binding properties of the original antibody, the structure of its combining site must be faithfully reproduced in the humanized molecule. X-ray crystallographic studies have shown that the antibody combining site is built primarily from CDR residues, although some neighboring framework residues have been found to be involved in antigen binding. Amit *et al.*, *Science*, 233:747-753 (1986); Colman *et al.*, *Nature*, 326:358-363 (1987); Sheriff *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:8075-8079 (1987); Padlan *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:5938-5942 (1989); Fischmann *et al.*, *J. Biol. Chem.*, 266:12915-12920 (1991); Tulip *et al.*, *J. Molec. Biol.*, 227:122-148 (1992). It has also been found that the structures of the CDR loops are significantly influenced by surrounding framework structures. Chothia *et al.*, *J. Molec. Biol.*, 196:901-917 (1987); Chothia *et al.*, *Nature*, 342:877-883 (1989); Tramomonteno *et al.*, *J. Molec. Biol.*, 215:175-182 (1990).

In addition to the effect of the framework residues on the CDRs, small but significant differences from the relative disposition of the variable light chain (V_L) and variable heavy (V_H) domains have been noted (Colman *et al.*, *Nature*, 326:358-363 (1987)) and those differences are ostensibly due to variations in the residues involved in the inter-domain contact (Padlan *et al.*, *Molec. Immunol.*, 31:169-217 (1994)).

Furthermore, structural studies on the effect of the mutation of interior residues, in which changes in side chain volume are involved, have shown that the resulting local deformations are accommodated by shifts in side chain positions that are propagated to distant parts of the molecular interior. This suggests that during humanization the interior residues in the variable domains and in the interface between these domains, or at least the interior volumes, should also be maintained; a humanization protocol in which an interior residue is replaced by one of different properties, such as size, charge, or hydrophobicity, could result in a significant modification of the antigen combining-site structure. One method of potentially identifying the framework residues which need to be preserved is by computer modeling. Alternatively, critical framework residues may potentially be identified by comparing known antibody combining site structures. Padlan, *Molec. Immun.*, 31(3):169-217 (1994).

The residues which potentially affect antigen binding fall into several groups. The first group comprises residues that are contiguous with the combining site surface and which could therefore make direct contact with antigens. These residues include the amino-terminal residues and those adjacent to the CDRs. The second group includes residues that could alter the structure or relative alignment of the CDRs by contacting either the CDRs or

the opposite chains. The third group comprises amino acids with buried side chains that could influence the structural integrity of the variable domains. The residues in these groups are usually found in the same positions (*id.*) according to the adopted numbering system. See Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, NIH Pub. No. 91-3242 (5th ed., 1991) (U.S. Dept. Health & Human Services, Bethesda, MD) and Genbank.

Given these effects of changes in amino acid residues, although humanized antibodies are desirable because of their potential low immunogenicity in humans, their production is unpredictable. For example, sequence modification of antibodies may result in substantial or even total loss of antigen binding affinity, or loss of binding specificity. Alternatively, "humanized antibodies" may still exhibit immunogenicity in humans, irrespective of sequence modification.

Thus, there still exists a significant need in the art for novel humanized antibodies to desired antigens. More specifically, there exists a need in the art for humanized antibodies specific to CEA, because of their potential as improved immunotherapeutic and immunodiagnostic agents for treatment and diagnosis of cancers expressing CEA, *e.g.*, gastrointestinal and colorectal cancers, breast cancers, lung cancers, and ovarian cancers, among others.

OBJECTS OF THE INVENTION

Toward this end, it is an object of the invention to provide humanized antibodies which are specific to human carcinoembryonic antigen (CEA), *i.e.* anti-CEA ("αCEA") antibodies. More specifically, it is an object of the invention to provide humanized antibodies derived from murine αCEA antibodies and in particular from COL-1, a specific murine antibody of the IgG2a isotype having high affinity for CEA.

It is also an object of the invention to provide pharmaceutical compositions containing humanized αCEA antibodies. It is a more specific object of the invention to provide pharmaceutical compositions containing humanized antibodies derived from the high affinity murine αCEA antibody, COL-1.

It is another specific object of the invention to provide methods of using humanized αCEA antibodies for treatment of cancers which express CEA, in particular breast, lung, ovarian, gastrointestinal, and colorectal cancers, among others.

It is another object of the invention to provide immunodiagnostic compositions for detecting cancer cells, the compositions containing a humanized αCEA antibody, preferably derived from COL-1, which antibody is in labeled or unlabeled form. It is another object of

the invention to provide a method of immunodiagnosis of cancer using compositions which contain a humanized αCEA antibody, preferably derived from COL-1, which is in labeled or unlabeled form.

It is still another object of the invention to provide nucleic acid sequences which encode humanized αCEA antibodies or fragments thereof. It is a more specific object of the invention to provide nucleic acid sequences which encode humanized antibodies derived from the high affinity murine αCEA antibody, COL-1. It is another object of the invention to provide vectors which provide for the expression of humanized αCEA antibodies, in particular humanized antibodies derived from the high affinity murine αCEA antibody, COL-1.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 contains an alignment of the amino acid sequences of murine COL-1 V_H (COL1MuVH), a NEWM FR template, and a humanized NEWM-based V_H (COL1NMVH or "HuVH"). The CDRs are boxed. Murine FR residues retained in the various humanized VHs exemplified herein are indicated by the symbols (↑), (A), (T), (S), (T), (A), and (Y), according to the table below.

COL1NMVH Version	Murine Residues Retained at Position						
	*	24	72	76	78	79	80
HuVH	↑						
HuVHA	↑	A					
HuVHAT	↑	A			T		
HuVHAA	↑	A				A	
HuVHAY	↑	A					Y
HuVHATAY	↑	A			T	A	Y
HuVHASTAY	↑	A		S	T	A	Y
HuVHT	↑		T				
HuVHS	↑			S			
HuVHSTAY	↑			S	T	A	Y

(* -- Retained murine residues indicated by the symbol (↑) are F-27, N-28, I-29, K-30, N-97, and T-98.) HuVHSTAY is the version of COL1NMVH expressed from the deposited cell line, ATCC CRL-12208.

Figure 2 contains an alignment of the amino acid sequences of murine COL-1 V_K (COL1MuVK or "HuVK"), an REI FR template, and a humanized REI-based V_K (COL1REVK). The CDRs are boxed. The murine FR residues retained in the humanized

sequence are indicated by the symbols: (F) for HuVKF, and (V) and (L) for HuVKVL. HuVKVL is the version of COL1REVK expressed from the deposited cell line, ATCC CRL-12208.

Figure 3 shows the IgG1 expression vectors used to express the subject humanized antibodies in NSO myeloma cells.

Figure 4 shows binding of different COL-1 antibodies to CEA, as measured by an ELISA assay.

Figure 5 contains results of ELISA assays with COL-1 antibodies including those produced according to the invention.

Figure 6 contains results of ELISA assays with COL-1 antibodies including those produced according to the invention.

Figure 7 contains results of ELISA assays with COL-1 antibodies including those produced according to the invention.

Figure 8 contains results of ELISA assays with COL-1 antibodies including those produced according to the invention.

Figure 9 contains results of ELISA assays with COL-1 antibodies including those produced according to the invention.

Figure 10 contains results of ELISA assays with COL-1 antibodies including those produced according to the invention.

Figure 11 contains results of ELISA assays with COL-1 antibodies including those produced according to the invention.

Figure 12 contains results of ELISA assays with COL-1 antibodies including those produced according to the invention.

Figure 13 contains the amino acid sequences of the humanized V_H expressed by the deposited cell line ATCC CRL-12208.

Figure 14 contains the amino acid sequences of the humanized V_K expressed by the deposited cell line ATCC CRL-12208.

Figure 15 presents the nucleotide sequence of the DNA template use to produce the initial humanized COL-1 heavy chain variable region, HuVH.

Figure 16 presents the nucleotide sequence of the DNA template used to produce a variety of HuVH derivatives.

Figure 17 presents the nucleotide sequence of the DNA template used to produce the initial humanized COL-1 light chain variable region, HuVK.

Figure 18 presents the nucleotide sequence of the DNA template used to produce a the HuVKVL derivative of HuVK.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention, definitions of certain terms which are used in this disclosure are set forth below.

Antibody - This refers to single chain, two-chain, and multi-chain proteins and glycoproteins belonging to the classes of polyclonal, monoclonal, chimeric, and hetero immunoglobulins (monoclonal antibodies being preferred); it also includes synthetic and genetically engineered variants of these immunoglobulins. "Antibody fragment" includes Fab, Fab', $F(ab')_2$, and Fv fragments, as well as any portion of an antibody having specificity toward a desired target epitope or epitopes.

Humanized antibody - This will refer to an antibody derived from a non-human antibody, typically murine, that retains or substantially retains the antigen-binding properties of the parent antibody but which is less immunogenic in humans. This may be achieved by various methods including (a) grafting only the non-human CDRs onto human framework and constant regions with or without retention of critical framework residues, or (b) transplanting the entire non-human variable domains, but "cloaking" them with a human-like

section by replacement of surface residues. Such methods as are useful in practicing the present invention include those disclosed in Jones *et al.*, Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988); Padlan, *Molec. Immun.*, 28:489-498 (1991); Padlan, *Molec. Immun.*, 31(3):169-217 (1994).

Complementarity Determining Region, or CDR - The term CDR, as used herein, refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site as delineated by Kabat et al (1991).

Framework Region - The term FR, as used herein, refers to amino acid sequences interposed between CDRs. These portions of the antibody serve to hold the CDRs in an appropriate orientation for antigen binding.

Constant Region - The portion of the antibody molecule which confers effector functions. In the present invention, murine constant regions are substituted with human constant regions. The constant regions of the subject chimeric or humanized antibodies are derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu. Further, heavy chains of various subclasses (such as the IgG subclasses of heavy chains) are responsible for different effector functions and thus, by choosing the desired heavy chain constant region chimeric antibodies with desired effector function can be produced. Preferred constant regions are gamma 1 (IgG1), gamma 3 (IgG3) and gamma 4 (IgG4). More preferred is a constant region of the gamma 1 (IgG1) isotype. The light chain constant region can be of the kappa or lambda type, preferably of the kappa type.

Chimeric antibody - This is an antibody containing sequences derived from two different antibodies, which typically are of different species. Most typically chimeric antibodies comprise human and murine antibody fragments, generally human constant and murine variable regions.

Mammals - Animals that nourish their young with milk secreted by mammary glands, preferably warm blooded mammals, more preferably humans.

Immunogenicity - A measure of the ability of a targeting protein or therapeutic moiety to elicit an immune response (humoral or cellular) when administered to a recipient. The present invention is concerned with the immunogenicity of the subject humanized antibodies or fragments thereof.

Humanized reduced immunogenicity - This refers to a humanized antibody exhibiting reduced immunogenicity relative to the parent antibody, typically a murine antibody such as COL-1.

Humanized antibody substantially retaining the binding properties of the parent antibody - This refers to a humanized antibody which retains the ability to specifically bind the antigen recognized by the parent antibody used to produce such humanized antibody. Preferably the humanized antibody will exhibit the same or substantially the same antigen-binding affinity and avidity as the parent antibody, *e.g.*, COL-1. Ideally, the affinity of the antibody will not be less than 5% of the parent antibody affinity, more preferably not less than about 30%, and most preferably the affinity will not be less than 50% of the parent antibody. Methods for assaying antigen-binding affinity are well known in the art and include half-maximal binding assays, competition assays, and Scatchard analysis. Suitable antigen binding assays are described in this application.

In its broadest embodiment, the present invention is directed to humanized antibodies which specifically bind CEA, an antigen expressed by various human cancers, in particular gastrointestinal, colorectal, breast, lung, and ovarian cancers. Preferably, such humanized antibodies will be derived from antibodies having good binding affinity to CEA, such as COL-1 through COL-15 disclosed by Muraro *et al.*, *Cancer Res.*, 45(11 Pt. 2):5769-5780 (1985).

Most preferably, such humanized antibodies will be derived from COL-1, a murine antibody of the IgG2a isotype, which has been reported to bind to CEA with high affinity ($1.4x10^9$ M^{-1}) with no detectable cross-reactivity for CEA-related antigens, such as the non-specific cross-reacting antigen (NCA) and the normal fecal antigen (NFA).

As discussed above, humanized antibodies afford potential advantages over murine and also chimeric antibodies, *e.g.*, reduced immunogenicity in humans. This is advantageous because it should reduce and potentially eliminate the eliciting of a HAMA response when such humanized antibodies are administered *in vivo*, *e.g.*, either for treatment of cancer or for diagnosis of cancer as by tumor imaging. Also, such antibodies may exhibit improved plasma clearance, pharmacokinetic, and tumor targeting properties.

However, as noted above, humanization may in some instances adversely affect antigen binding. Preferably, the humanized αCEA antibodies of the present invention will possess a binding affinity for CEA of not less than about 5% and more preferably not less than about 30%, and most preferably not less than 50% of the CEA binding antigen affinity of the parent murine antibody, preferably COL-1. Most preferably, the humanized antibodies

of the present invention will possess a binding affinity for CEA of not less than about 5% and more preferably not less than about 30% and most preferably not less than about 50% of the CEA binding affinity of COL-1, or a chimeric antibody derived therefrom.

Preferably, the humanized antibodies of the present invention will bind the same epitope as COL-1. Such antibodies can be identified based on their ability to compete with COL-1 for binding to CEA or to CEA-expressing cells.

In general, the subject humanized antibodies are produced by obtaining nucleic acid sequences encoding the variable heavy (V_H) and variable light chains (V_L, *e.g.*, V_K) of an antibody which binds CEA (preferably COL-1), identifying the CDRs in said V_H and V_L sequences, and grafting such CDR-encoding nucleic acid sequences onto selected human framework-encoding nucleic acid sequences.

Preferably, the human framework amino acid sequences are selected such that the resulting antibody is likely to be suitable for *in vivo* administration in humans. This can be determined, *e.g.*, based on previous usage of antibodies containing such human FRs. Preferably, the human FRs will not themselves be significantly immunogenic. Examples of such human frameworks include NEWM and REI.

Alternatively, the amino acid sequences of the FRs of the antibody to be humanized (*e.g.*, COL-1) will be compared to those of known human FRs, and the human FRs to be used for CDR-grafting will be selected based on their comprising sequences highly similar to those of the parent antibody, *e.g.*, a murine antibody which binds CEA. Numerous human FRs have been isolated and their sequences reported in the literature. This enhances the likelihood that the resultant CDR-grafted "humanized" antibody, which contains the CDRs of the parent (*e.g.*, murine) antibody grafted onto the selected human FRs, will substantially retain the antigen binding structure and thus retain the binding affinity of the parent antibody. As a result of such studies, the FRs of REI and NEWM antibodies have been identified as having amino acid sequences which are likely to allow the CDRs of COL-1 To retain a significant degree of antigen binding affinity. As noted, the selected human framework regions will preferably be those that are expected to be suitable for *in vivo* administration, *i.e.*, not immunogenic. Based on their amino acid sequences, REI and NEWM human framework regions are expected to be substantially non-immunogenic.

In either method, the DNA sequences encoding the V_H and V_L regions of the preferably murine αCEA antibody must be obtained. Methods for cloning nucleic acid sequences encoding immunoglobulins are well known in the art. Such methods will generally involve the amplification of the immunoglobulin-encoding sequences to be cloned

using appropriate primers by polymerase chain reaction (PCR). Primers suitable for amplifying immunoglobulin nucleic acid sequences, and specifically murine variable heavy and variable light sequences, have been reported in the literature. After such immunoglobulin-encoding sequences have been cloned, they will be sequenced by methods well known in the art. This will be effected in order to identify the V_H- and V_L-encoding sequences, and more specifically the portions thereof which encode the CDRs and FRs. This can be effected by well known methods which include, *e.g.*, those disclosed in U.S. Patent No. 4,816,397 to Boss *et al.* and U.S. Patent No. 5, 225,539 to Winter *et al.*.

Once the DNA sequences encoding the CDRs and FRs of the antibody which is to be humanized have been identified, the amino acid sequences encoding the CDRs are then identified (deduced based on the nucleic acid sequences and the genetic code and by comparison to previous antibody sequences) and the CDR-encoding nucleic acid sequences are grafted onto selected human FR-encoding sequences. This may be accomplished by use of appropriate primers and linkers. Methods for selecting suitable primers and linkers to provide for ligation of desired nucleic acid sequences is well within the purview of the ordinary artisan.

As discussed above, the selected human FRs used for humanization will preferably be those that are likely to be suitable for *in vivo* administration, *i.e.* they are not in themselves immunogenic in humans (*e.g.*, because of allotypic differences); examples thereof are REI and NEWM human FRs. Alternatively, the human FRs will be selected such that they comprise amino acid sequences which are highly similar to those of the parent antibody's FR sequences. This may be effected by comparing the amino acid sequences of the murine FRs to those of previously reported human FRs (see, *e.g.*, Kabat *et al.*, *id.*).

After the CDR-encoding sequences are grafted onto the selected human FR-encoding sequences, the resultant DNA sequences encoding the "humanized" variable heavy and variable light sequences will then be expressed to produce a humanized Fv or humanized antibody which binds CEA. Typically, the humanized V_H and V_L sequences will be expressed as part of a whole αCEA antibody molecule, *i.e.* as a fusion protein with human constant domain sequences whose encoding DNA sequences have been obtained from a commercially available library or which have been obtained using, *e.g.*, one of the above-described methods for obtaining DNA sequences. However, the V_H and V_L sequences can also be expressed in the absence of constant sequences to produce a

humanized αCEA Fv. Nevertheless, fusion of human constant sequences is potentially desirable because the resultant humanized αCEA antibody may possess human effector functions such as CDC and ADCC activity.

Methods for synthesizing DNA encoding a protein of known sequence are well known in the art. Using such methods, DNA sequences which encode the subject humanized V_L and V_H sequences (with or without constant regions) are synthesized, and then expressed in a vector system suitable for expression of recombinant antibodies. This may be effected in any vector system which provides for the subject humanized V_L and V_H sequences to be expressed as a fusion protein with human constant domain sequences and to associate to produce functional (antigen binding) antibodies or antibody fragments. Useful methods are set forth, *e.g.*, in U.S. Patent 4,816,397 to Boss *et al.* and U.S. Patent 5,225,539 to Winter *et al.*

Expression vectors and host cells suitable for expression of recombinant antibodies and humanized antibodies in particular, are well known in the art. The following references are representative of methods and vectors suitable for expression of recombinant immunoglobulins which may be utilized in carrying out the present invention: Weidle *et al.*, *Gene*, 51: 21-29 (1987); Dorai *et al.*, *J. Immunol.*, 13(12):4232-4241 (1987); De Waele *et al.*, *Eur. J. Biochem.*, 176:287-295 (1988); Colcher *et al.*, *Cancer Res.*, 49:1738-1745 (1989); Wood *et al.*, *J. Immunol.*, 145(a):3011-3016 (1990); Bulens *et al.*, *Eur. J. Biochem.*, 195:235-242 (1991); Beggington *et al.*, *Biol. Technology*, 10:169 (1992); King *et al.*, *Biochem. J.*, 281:317-323 (1992); Page *et al.*, *Biol. Technology*, 9:64 (1991); King *et al.*, *Biochem. J.*, 290:723-729 (1993); Chaudary *et al.*, *Nature*, 339:394-397 (1989); Jones *et al.*, *Nature*, 321:522-525 (1986); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988); Benhar *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:12051-12055 (1994); Singer *et al.*, *J. Immunol.*, 150:2844-2857 (1993); Cooto *et al.*, *Hybridoma*, 13(3):215-219 (1994); Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:10029-10033 (1989); Caron *et al.*, *Cancer Res.*, 32:6761-6767 (1992); Cotoma *et al.*, *J. Immunol. Meth.*, 152:89-109 (1992). Moreover, vectors suitable for expression of recombinant antibodies are commercially available. The vector may, *e.g.*, be a bare nucleic acid segment, a carrier-associated nucleic acid segment, a nucleoprotein, a plasmid, a virus, a viroid, or a transposable element.

Host cells known to be capable of expressing functional immunoglobulins include, *e.g.*: mammalian cells such as Chinese Hamster Ovary (CHO) cells; COS cells; myeloma cells, such as NSO and SP2/O cells; bacteria such as *Escherichia coli*; yeast cells such as *Saccharomyces cerevisiae*; and other host cells. Of these, CHO cells are used by many

researchers given their ability to effectively express and secrete immunoglobulins. NSO cells are one of the preferred types of host cells useful in the present invention.

Essentially, recombinant expression of humanized antibodies is obtained by one of two general methods. In the first method, the host cells are transfected with a single vector which provides for the expression of both V_H and V_L variable sequences optionally fused to selected constant regions. In the second method, host cells are transfected with two vectors, each of which provides for expression of either the V_H or V_L sequence, each optionally fused to a selected constant region.

Human constant domain sequences are well known in the art, and have been reported in the literature. Preferred human constant light chain sequences (C_L) include the kappa and lambda constant light sequences. Preferred human constant heavy chain sequences include human gamma 1, human gamma 2, human gamma 3, human gamma 4, and mutated versions thereof which provide for altered effect or function, *e.g.*, enhanced *in vivo* half-life, reduced Fc receptor binding, and the like.

After expression, the antigen binding affinity of the resultant humanized antibody will be assayed by known methods, *e.g.*, Scatchard analysis. Ideally, the antigen-binding affinity of the humanized antibody will approximate that of the parent antibody, *e.g.*, COL-1. As discussed above, ideally the affinity of the humanized antibody will not be less than 5% of the parent antibody, more preferably not less than 30%, and most preferably not less than 50% of that of the parent antibody, *e.g.*, COL-1.

In some instances, humanized antibodies produced by grafting CDRs (from an antibody which binds CEA) onto selected human FRs may provide humanized antibodies having the desired affinity to CEA. However, it may be necessary or desirable to further modify specific residues of the selected human FR in order to enhance antigen binding. This may occur because it is believed that some framework residues are essential to or at least affect antigen binding. Preferably, those framework residues of the parent (*e.g.*, murine) antibody which maintain or affect combining-site structures will be retained. These residues may be identified by X-ray crystallography of the parent antibody or Fab fragment, thereby identifying the three-dimensional structure of the antigen-binding site.

These residues may potentially be identified by X-ray crystallography of the parent Fab, thereby identifying the three-dimensional structure of the antigen-binding site. Also, framework residues which may be involved in antigen binding may be putatively selected based on previously reported humanized murine antibody sequences. Thus, it may be beneficial to retain these and other murine framework residues from the parent murine

antibody to optimize CEA binding. However, because of inherent unpredictability associated with amino acid modification of proteins, and antibodies in particular, the effects of such changes, if any, on antigen binding are unpredictable. Nevertheless, such methodology will ideally confer a "human-like" character to the resultant humanized antibody thus rendering it less immunogenic while retaining the interior and contacting residues which affect antigen-binding.

The present invention further embraces variants and equivalents which are substantially homologous to the humanized antibodies and antibody fragments set forth herein. These may contain, *e.g.*, conservative substitution mutations, *i.e.* the substitution of one or more amino acids by similar amino acids. For example, conservative substitution refers to the substitution of an amino acid with another within the same general class, *e.g.*, one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid, or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art.

The phrase "substantially homologous" is used in regard to the similarity of a subject amino acid sequence (of an oligo- or poly-peptide or protein) to a related, reference amino acid sequence. This phrase is defined as at least about 75% "correspondence" -- *i.e.* the state of identical amino acid residues being situated in parallel -- between the subject and reference sequences when those sequences are in "alignment," *i.e.* when a minimal number of "null" bases have been inserted in the subject and/or reference sequences so as to maximize the number of existing bases in correspondence between the sequences. "Null" bases are not part of the subject and reference sequences; also, the minimal number of "null" bases inserted in the subject sequence may differ from the minimal number inserted in the reference sequence. In this definition, a reference sequence is considered "related" to a subject sequence where both amino acid sequences make up proteins or portions of proteins which are either αCEA antibodies or antibody fragments with αCEA binding affinity. Each of the proteins comprising these αCEA antibodies or antibody fragments may independently be antibodies or antibody fragments or bi- or multi-functional proteins, *e.g.*, such as fusion proteins, bi- and multi-specific antibodies, single chain antibodies, and the like.

The present invention is further directed to nucleic acid sequences from which such humanized antibodies and antibody fragments may be expressed, as well as expression vectors from which these humanized antibodies and antibody fragments may be expressed in transfected host cells.

In a preferred embodiment, such humanized antibodies and corresponding nucleic acid sequences will be derived from COL-1. Most preferably, the humanized V_H sequence and the humanized V_L sequence will have the sequences substantially as depicted in Figure 1 or 13 or in Figure 2 or 14, respectively, and as discussed in the Examples set forth below. However, as discussed, the invention further contemplates other modifications of these humanized V_H and V_L sequences, *e.g.*, sequences which further comprise one or more conservative amino acid substitutions or which retain one or more additional murine framework residues which affect or do not significantly reduce antigen binding.

The subject humanized antibodies -- because they specifically bind CEA (an antigen expressed on many different cancer cell types, *e.g.*, lung carcinomas, breast carcinomas, gastrointestinal carcinomas such as stomach cancers, colorectal carcinomas such as colon cancers, ovarian carcinomas, etc.) and further because they will not be significantly immunogenic in humans -- should be suitable for use as: therapeutics for the treatment or prevention of cancers characterized by CEA expression; diagnostic agents, *e.g.*, for diagnosis and evaluating the prognosis of cancers characterized by CEA expression (based on levels of CEA expression); tumor imaging agents; or radiolabeled antibodies in the Radioimmunoguided Surgery® System (RIGS®). See Hinkle *et al.*, *Antibody, Immunoconjugates and Radiopharmaceuticals*, 4(3):339-358 (1991).

One skilled in the art would be able (by routine experimentation) to determine what amount of antibody would be effective and non-toxic for the purpose of treating cancer. Generally, however, an effective dosage will be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

The humanized antibodies or humanized antibody fragments of the invention may be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce a therapeutic or prophylactic effect. The antibodies of the subject invention can be administered to such human or other animal in a conventional dosage form prepared by combining the antibody of the invention with a conventional, pharmaceutically acceptable carrier, diluent, and/or excipient according to known techniques. It will be recognized by one of ordinary skill in the art that the form and character of the pharmaceutically acceptable carrier, diluent, and/or excipient is dictated by the amount of active ingredient with which it is to be combined, the route of administration, and other well-known variables.

Pharmaceutically acceptable formulations may include, *e.g.*, a suitable solvent, preservatives such as benzyl alcohol if desired, and a buffer. Useful solvent may include,

e.g., water, aqueous alcohols, glycols, and phosphonate and carbonate esters. Such aqueous solutions contain no more than 50% by volume of organic solvent. Suspension-type formulations may include a liquid suspending medium as a carrier, *e.g.*, aqueous polyvinylpyrrolidone, inert oils such as vegetable oils or highly refined mineral oils, or aqueous cellulose ethers such as aqueous carboxymethylcellulose. A thickener such as gelatin or an alginate may also be present, one or more natural or synthetic surfactants or antifoam agents may be used, and one or more suspending agents such as sorbitol or another sugar may be employed therein. Such formations may contain one or more adjuvants.

The route of administration of the antibodies (or fragment thereof) of the present invention may be oral, parenteral, by inhalation, or topical. The term parenteral as used herein includes intravenous, intramuscular, subcutaneous, rectal, vaginal, or intraperitoneal administration. The subcutaneous, intravenous, and intramuscular forms of parenteral administration are generally preferred.

The daily parenteral and oral dosage regimens for prophylactically or therapeutically employing humanized antibodies of the present invention will generally be in the range of about 0.005 to 100, but preferably about 0.5 to 10, milligrams per kilogram body weight per day.

The antibodies of the present invention may also be administered by inhalation. By "inhalation" is meant intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques. The preferred dosage amount of a compound of the invention to be employed is generally within the range of about 0.1 to about 100, more preferably about 10 to 100, milligrams per kg body weight.

The antibody of the invention may also be administered topically. By topical administration is meant non-systemic administration. This includes the administration of a humanized antibody (or humanized antibody fragment) formulation of the invention externally to the epidermis or to the buccal cavity, and instillation of such an antibody into the ear, eye, or nose, and wherever it does not significantly enter the bloodstream. By systemic administration is meant oral, intravenous, intraperitoneal, subcutaneous, and intramuscular administration. The amount of an antibody required for therapeutic, prophylactic, or diagnostic effect will, of course, vary with the antibody chosen, the nature and severity of the condition being treated and the animal undergoing treatment, and is ultimately at the

discretion of the physician. A suitable topical dose of an antibody of the invention will generally be within the range of about 1 to 100 milligrams per kilogram body weight daily.

Formulations

While it is possible for an antibody or fragment thereof to be administered alone, it is preferable to present it as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, *e.g.*, from 1% to 2% by weight of the formulation, although it may comprise as much as 10% w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the formulation.

The topical formulations of the present invention, comprise an active ingredient together with one or more acceptable carrier(s) therefor and optionally any other therapeutic ingredients(s). The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear, or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified and sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing

the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogels. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surface active such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

Kits according to the present invention include frozen or lyophilized humanized antibodies or humanized antibody fragments to be reconstituted, respectively, by thawing (optionally followed by further dilution) or by suspension in a (preferably buffered) liquid vehicle. The kits may also include buffer and/or excipient solutions (in liquid or frozen form) - - or buffer and/or excipient powder preparations to be reconstituted with water – for the purpose of mixing with the humanized antibodies or humanized antibody fragments to produce a formulation suitable for administration. Thus, preferably the kits containing the humanized antibodies or humanized antibody fragments are frozen, lyophilized, pre-diluted, or pre-mixed at such a concentration that the addition of a predetermined amount of heat, of water, or of a solution provided in the kit will result in a formulation of sufficient concentration and pH as to be effective for *in vivo* or *in vitro* use in the treatment or diagnosis of cancer. Preferably, such a kit will also comprise instructions for reconstituting and using the humanized antibody or humanized antibody fragment composition to treat or detect cancer. The kit may also comprise two or more component parts for the reconstituted active composition. For example, a second component part – in addition to the humanized antibodies or humanized antibody fragments – may be bifunctional chelant, bifunctional chelate, or a therapeutic agent such as a radionuclide, which when mixed with the humanized antibodies or humanized antibody fragments forms a conjugated system therewith. The above-noted buffers, excipients, and other component parts can be sold separately or together with the kit.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a humanized antibody or humanized antibody fragment of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular animal being treated, and that such optima can

be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, *i.e.*, the number of doses of an antibody or fragment thereof of the invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

The subject humanized antibodies may also be administered in combination with other anti-cancer agents, *e.g.*, other antibodies or drugs. Also, the subject humanized antibodies or fragments may be directly or indirectly attached to effector moieties having therapeutic activity. Suitable effector moieties include by way of example cytokines (IL-2, TNF, interferons, colony stimulating factors, IL-1, etc.), cytotoxins (*Pseudomonas* exotoxin, ricin, abrin, etc.), radionuclides, such as ^{90}Y, ^{131}I, ^{99m}Tc, ^{111}In, ^{125}I, among others, drugs (methotrexate, daunorubicin, doxorubicin, etc.), immunomodulators, therapeutic enzymes (*e.g.*, beta-galactosidase), anti-proliferative agents, etc. The attachment of antibodies to desired effectors is well known. See, *e.g.*, U.S. Patent No. 5,435,990 to Cheng *et al.* Moreover, bifunctional linkers for facilitating such attachment are well known and widely available. Also, chelators (chelants and chelates) providing for attachment of radionuclides are well known and available.

Alternatively, the subject humanized αCEA antibodies or fragments may be used as immunodiagnostic agents both *in vivo* and *in vitro*. A particularly preferred usage is for *in vivo* imaging of cancer cell lesions which express CEA. The subject antibodies are preferred because they should elicit no significant HAMA or allergic response. Thus, they may be used repeatedly to monitor the disease status of a patient.

As noted above, another preferred application of the subject humanized antibodies or fragments thereof is in the Radioimmunoguided System® (RIGS®). This technique involves the intravenous administration of a radiolabeled monoclonal antibody or its fragment prior to surgery. After allowing for tumor uptake and blood clearance of radioactivity, the patient is taken to the operating room where surgical exploration is effected with the aid of a hand-held gamma activity probe, *e.g.*, the Neoprobe® 1000. This helps the surgeon identify the tumor metastases and lessen the complications of excision.

The RIGS® system is advantageous because it allows for the detection of tumors not otherwise detectable by visual inspection and/or palpation. See, O'Dwyer *et al.*, *Arch. Surg.*, 121:1391-1394 (1986). This technique is described in detail in Hinkle *et al.*, *Antibody, Immunoconjugates and Radiopharmaceuticals*, 4:(3)339-358 (1991). This reference discloses the use of this technique with αCEA antibodies including the COL-1 monoclonal antibody. This technique is particularly useful for cancers of the colon, breast, pancreas,

and ovaries. Thus, this technique should be applicable to the subject humanized antibodies which react with CEA expressed by colon, breast, and ovarian cancers. Also, Hinkle *et al.* (*id.*) cite numerous references describing this technique. The subject humanized antibodies or fragments thereof may be radiolabeled with radionuclides which are suitable for *in vivo* administration, *e.g.*, iodine radionuclides such as ^{131}I and ^{125}I. Moreover, ^{111}In and ^{99m}Tc are also suitable.

The subject humanized antibodies may be used alone or in combination with other antibodies. Also, the subject humanized antibodies may be prepared in the form of a diagnostically effective composition. Generally, this will entail the incorporation of diagnostically acceptable carriers and excipients, and labels which provide for detection. Suitable labels include diagnostic radionuclides, enzymes, etc. Methods for using antibodies for tumor imaging are well known in the art.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The invention will be further clarified by a consideration of the following examples, which are intended to be purely exemplary of the present invention and thus to be construed as merely illustrative examples and not limitations of the scope of the present invention an any way.

EXAMPLES

Materials and Methods

DNA Template Preparation

All recombination work was performed upon DNA sequences in plasmid M13 vectors. The source of the NEWM framework regions for producing the initial humanized COL-1 VH was an M13 construct bearing -- between the M13 *Bam*H I and *Hin*d III sites -- a DNA segment having the nucleotide sequence shown in figure 13. The source of REI framework regions for producing the initial humanized COL-1 VL was an M13 construct bearing -- between the M13 *Bam*H I and *Hin*d III sites -- a DNA segment encoding the REI amino acid sequence of Figure 2.

When overlap-extension procedures were used to introduce mutations into a given DNA sequence, double stranded M13 DNA was utilized. In contrast, when extension-ligation procedures were used instead, the oligonucleotides were designed to anneal to only one of the two DNA strands. In this latter procedure, the M13 DNA was first treated to substitute uridine for every thymidine base in the DNA, to produce uridinylated DNA. This was

accomplished by transfecting the M13 plasmid DNA into competent cells lacking dUTPase and uracil glycosylase, normally RZ1032 cells (though CJ236 cells available from Bio-Rad of Hercules, CA, are also suitable) by combining the following ingredients.

1 µL of M13 plasmid DNA
4 mL of LB broth
40 µL of competent RZ1032 cells.

The culture was shaken for 5 hours at 37°C and the resulting single-stranded plasmid DNA (ssDNA) was isolated and dissolved in 50 µL Tris-EDTA buffer. The DNA was then treated with uracil glycosylase by mixing together:

1 µL uridinylated ssDNA
1 µL 10x glycosylase buffer
1U uracil glycosylase (Gibco BRL, Gathersburg, MD)
40 µL 25mM $MgCl_2$.

This mixture was then incubated at 37°C for one hour and then 6.6 µL 25mM $MgCl_2$ and 9.9µL 1M NaOH. The mixture was then further incubated for 5 minutes at 37°C and 16.5 µL of 0.6M HCl was then added to neutralize the mixture. The DNA was then ethanol precipitated and dissolved in water.

M13 Oligonucleotide Primers

The following oligonucleotide primers were used throughout the process of preparing the humanized COL-1 VHs and VLs exemplified below.

10. 5'-CTAAAACGACGGCCAGT-3' and
11. 5'-AACAGCTATGACCATG-3' (both for using in producing VHs); and
385. 5'-GCGGGCCTCTTCGCTATTACGC-3' and
391. 5'-CTCTCTCAGGGCCAGGCGGTGA-3' (both for use in producing VKs).

These primers are complementary to regions of the plasmid M13 which are external both to the (NEWM or REI) target framework sequences and to the *Bam*H I site -to- *Hin*d III site section of M13.

Murine Variable Regions

In order to compare the antibody binding characteristics of the antibodies produced according to the examples set forth below, antibodies having a chimeric heavy chain (*i.e.* a heavy chain having a murine COL-1 VH region and a human IgG1 constant region) and/or a chimeric light chain (*i.e.* a light chain having a murine COL-1 VL and a human κ constant region) were expressed. The source of these chimeric chains was the

ATCC-deposited cell line CRL 11217 (Budapest) which expresses a chimeric COL-1 antibody having both chains. The heavy chain of this antibody was termed "MuVH" and the light chain thereof was termed "MuVL."

Oligonucleotide Phosphorylation Protocol

Mutating oligonucleotides used in non-overlap extensions were phosphorylated according to the procedure below. In a final volume of 25µL, the following ingredients were combined:

10 pmol of each oligonucleotide,
5µL of a 5x polynucleotide kinase buffer, and
5U of T4 polynucleotide kinase (Gibco BRL).

The phosphorylation reaction was started with the addition of the enzyme and allowed to proceed for one hour at 37°C.

Annealing Protocol for Non-Overlap Extension-Ligations

The annealing step for non-overlap extension-ligations involved performing one annealing in which all mutation-carrying oligonucleotides and one primer oligonucleotide were annealed to a single stranded DNA template in which all thymidine bases had been replaced with uridine bases. The mutating oligonucleotides were first phosphorylated according to the above oligonucleotide phosphorylation protocol. In a final volume of 20 µL, the following ingredients were combined:

1 pmol of each mutation-carrying phosphorylated oligonucleotide
1 pmol of a primer oligonucleotide
4 µL 5x annealing buffer
0.2 pmol ssU-DNA template

The mixture was then heated to 90°C for 30 sec., then quickly cooled to 70°C, and finally allowed to slowly cool to 37°C.

Extension-Ligation Protocol for Non-Overlap Extension-Ligations

After completion of the annealing step in which the primer and phosphorylated mutating oligonucleotides were annealed to the ssU-DNA template, extension-ligation was performed as follows. In a final volume of 30 µL, the following ingredients were combined:

20 µL annealed ssU-DNA (*i.e.* the contents of the above annealing procedure)
2 µL 5x annealing buffer
2 µL 0.1M dithiothreitol
0.3 µL 0.1M ATP
1µL 6.25mM dNTP mixture of equimolar amounts of dATP, dTTP, dGTP, dCTP

2.5U T7 DNA polymerase (USB, now Amersham Life Sciences, Cleveland, OH)
0.5U T4 DNA ligase (Gibco BRL)
Water to 30µL

This mixture was then incubated at room temperature for 1 hour.

Standard PCR Protocols

The following procedure was used, alternately, both to amplify the non-overlap extension-ligation DNA sequences and to perform extension of each overlap DNA sequence.

In a final volume of 50µL, the following ingredients were combined:

2 µL template DNA (either annealed ssU-DNA or non-annealed ssDNA)
5 µL 10x Vent buffer (NEB, *i.e.* New England Biolabs, Beverly, MA) or
10x Thermalase buffer (IBI of New Haven, CT)
2 µL 6.25mM dNTP mixture of equimolar amounts of dATP, dTTP, dGTP, dCTP
25 pmol of one oligonucleotide primer
25 pmol of either a mutation-carrying oligonucleotide (for overlap-extension) or
a second oligonucleotide primer
1U Vent DNA polymerase (NEB) or Thermalase DNA polymerase (IBI)

Reactions were initiated with the addition of the DNA polymerase and then treated with about 15 cycles of: (1) 94°C for 30 sec., (2) 50°C for 30 sec., and (3) 30-60 seconds at either 75°C (for Vent DNA polymerase) or 72°C (for Thermalase). Reactions were brought to completion with 5 minutes at a constant temperature of either 75°C (for Vent DNA polymerase) or 72°C (for Thermalase).

PCR Overlap-Extension Amplification Protocol

After a pair of PCR reactions were performed – one for each of the two (partially complementary) overlapping DNA segments, the two resulting segments were joined according to the following PCR procedure. In a final volume of 50 µL, the following ingredients were mixed:

1 µL of each overlap DNA (from the above overlap PCR extension reactions)
5 µL 10x Vent buffer (NEB) or Thermalase buffer (IBI)
2 µL 6.25mM dNTP mixture of equimolar amounts of dATP, dTTP, dGTP, dCTP
25 pmol of each oligonucleotide primer used in the overlap PCR extensions
1U Vent DNA polymerase (NEB) or Thermalase DNA polymerase (IBI)

Reactions were initiated with the addition of the DNA polymerase and then treated with about 15 cycles of: (1) 94°C for 30 sec., (2) 50°C for 30 sec., and (3) 30-60 seconds at either 75°C (for Vent DNA polymerase) or 72°C (for Thermalase). Reactions were brought

to completion with 5 minutes at a constant temperature of either 75°C (for Vent DNA polymerase) or 72°C (for Thermalase).

Transfer of Humanized COL-1 Variable Region DNA Sequences from M13 to pSV Vectors and Subsequent Antibody Expression

Humanized antibodies were expressed in pSV vectors grown in NSO cells as follows. The humanized variable region constructs which were produced in the plasmid, M13, were obtained by ethanol precipitating the M13 plasmids (as cytosolic DNA), redissolving them in aqueous solution, and digesting them with 10U each of *Hin*d III and *Bam*H I (both from BRL, *i.e.* Gibco BRL) for 1 hour at 37°C in a final volume of 100 µL with Tris-EDTA buffer. The resulting DNA fragments were then run on a low melting point agarose gel, the band containing the humanized construct DNA was cut out, and the DNA was purified using an ELUTIP 'd' column with 20 µL Tris-EDTA buffer. 10 µL of the purified DNA preparation was then combined with 1 µL of a *Hin*d III and *Bam*H I-digested pSV preparation, 3 µL of 5x ligase buffer, and 1U of T4 DNA ligase (BRL), in order to insert the construct into a pSV plasmid. Humanized COL-1 VH constructs were inserted into pSVgpt vectors bearing a human IgG1 heavy chain constant region; the pSVgpt vector used is the "aLYS-30" shown in Figure 5. Humanized COL-1 VL constructs were inserted into pSVhyg vectors bearing a human κ light chain constant domain; the pSVhyg vector used in the "aLys-17" shown in Figure 5. Each humanized variable region construct was inserted adjacent to the respective constant region, *i.e.* so as to replace either the HuVHLYS or the HuVLLys segment illustrated in Figure 5.

The resulting vectors were transfected into NSO cells as follows. About 3 µg of the VH vector, or about 6µg of the VL vector, produced by the pSV-insertion procedures, was then linearized by digestion with 10U *Pvu*I (Gibco BRL). The digested DNA was then precipitated with ethanol and redissolved in 50 µL of water. NSO cells were collected by centrifugation and resuspended in 0.5mL Dulbecco's Modified Eagle's Medium (DMEM) and then transferred to a Gene Pulser cuvette (Bio-Rad). The DNA from both one VH and one VL construct was gently mixed with the cells by pipetting and the cuvette was left on ice for 5 minutes. Next, the cuvette was inserted between the electrodes of the Bio-Rad Gene Pulser and a single pulse of 170V at 960 µF was applied. The contents of the cuvette were then transferred to a flask containing 20mL DMEM and the cells were allowed to rest for 1-2 days at 37°C. Cells were again harvested by centrifugation and resuspended in 36mL selective DMEM. 1.5mL aliquots of this resuspension were placed in each well of a 24-well plate and

incubated at 37°C for 4 days, at which time the medium in each well was replaced with 1.5mL of fresh selective DMEM. After 6 more days of incubation at 37°C, surviving cell colonies were visible to the naked eye and the supernatants of each well were assayed for antibody production. Both whole antibody production (*i.e.* without purification) and purified antibody production were assayed. To obtain purified antibodies, the supernatants were passed through a protein A column.

ELISA Assay Protocols

Antibody concentrations and antibody binding characteristics were tested using enzyme-linked immunosorbent assay (ELISA) procedures which are set forth as follows

Measurement of IgG concentration

The concentration of IgG secreted from transfected cells was measured using an enzyme-linked immunosorbent assay (ELISA) procedure which is set forth as follows.

Polyvinyl chloride (PVC) microtiter plates (Dynatech Laboratories, Chantilly, VA, catalog # 001-010-2101) were coated with goat anti-human IgG (10mg/mL, GAHIG, Southern Biotechnology Associates, Inc., Birmingham, AL, catalog # 2010-01) diluted with Milli-Q® water and placed on the plates using 50mL/well. Plates were air-dried overnight at ambient temperature or at 37°C for 3 hours. Prior to use, non-specific binding was blocked the addition of 0.2 mL/well of 1% (w/v) bovine serum albumin (Sigma, St. Louis, MO catalog # A7888) in phosphate buffered saline (Sigma, catalog # 1000-3) (PBS/BSA). All incubations were carried out in a humidified container. Plates were incubated for 1-2 hours at 37°C and the blocking solution removed prior to sample addition. Two-fold serial dilutions of samples or a standard IgG solution set at 500 ng/mL (50 µl/well) were made in triplicate in the PBS/BSA solution. The plate was incubated at 37°C for 3 hours or overnight at 4°C. The plate was washed 3-times with 0.025% Tween-20 (v/v, Sigma) using an automatic plate washer. 50 µl/well of 1:1000 dilution of a goat anti-human IgG conjugated to Horseradish Peroxidase (Southern Biotechnology Associates Inc.) was added and incubated at 37°C for 1.5 hours. The wells were washed 3 times with 0.025% Tween-20 (v/v, Sigma) using an automatic plate washer and 50 µl/well OPD substrate buffer added. The color was developed for 4 minutes, stopped with 12.5 µl 12.5% H_2SO_4 and the absorbance at 492 nm read. The concentration of IgG in the test sample was estimated by comparison of the mean of the optical densities to a standard curve constructed from the standard IgG.

Determination of relative affinities of humanized antibodies

Antibody binding characteristics were tested in an ELISA using partially purified CEA antigen immobilized on Polyvinyl chloride (PVC) microtiter plates (Dynatech Laboratories, Chantilly, VA, catalog # 001-010-2101)

PVC plates were coated with 50 µl/well CEA (Dow Chemical, lot #040191), diluted 1:300 in Milli-Q water. Plates were air-dried overnight at ambient temperature or at 37°C for 3 hours. Prior to use, non-specific binding was blocked by the addition of 0.2 mL/well of 1% (w/v) bovine serum albumin (Sigma, St. Louis, MO catalog # A7888) in phosphate buffered saline (Sigma, catalog # 1000-3) (PBS/BSA). Plates were incubated for 1-2 hours at 37°C and the blocking solution removed prior to sample addition. All incubations were carried out in a humidified container. Two-fold serial dilutions (starting concentration range of 1.0µg/ml - 10µg/ml) of the samples to be tested in the PBS/BSA solution were added to triplicate wells of the TAG-coated plate (50 µl/well). The plate was incubated overnight at 4°C or 1-2 hours at 37°C. The plate was washed 3-times with 0.025% Tween-20 (v/v, Sigma) using an automatic plate washer. 50 µl/well of 1:1000 dilution of a goat anti-human IgG conjugated to Horseradish Peroxidase (Southern Biotechnology Associates Inc.) was added and incubated at 37°C for 1.5 hours. The wells were washed 3 times with 0.025% Tween-20 (v/v, Sigma) using an automatic plate washer and 50 µl/well OPD substrate buffer added. The color was developed for 4 minutes, stopped with 12.5 µl 12.5% H_2SO_4 and the absorbance at 492 nm read.

Determination of Affinity Constants for binding to CEA

Two-fold dilutions of purified Hu-COL-1 were prepared in PBS/BSA over a range of 1.0µg/ml - 0.003 µg/ml and samples (20 µl/well) were applied in triplicate to TAG coated PVC prepared and blocked as described *supra*. Plates were incubated overnight at 4°C. Following this incubation, samples were transferred from the plate to the corresponding wells on the GAHIG-coated trap plate. The original TAG plate was washed 3-times with 0.025% Tween-20 (v/v, Sigma, catalog # P1379) using an automatic plate washer. An ^{125}I-labeled goat anti-human IgG probe (ICN Biomedicals, Inc., catalog # 68088) was diluted to 75,000 cpm/25µl in PBS/BSA and added (25µl/well) to all wells. This TAG plate was incubated for 1 hour at 37°C.

After a 1 hour incubation at 37°C, the trap plate was washed as described above and ^{125}I-labeled GAHIG probe was added. This plate was incubated for 1 hour at 37°C. Both

plates (TAG and GAHIG-trap) containing probe were then washed with a microplate washer to remove the unbound probe. A plate cutter (D. Lee, Sunnyvale, CA, Model HWC-4) was used to separate the wells from the plate frame. The radioactivity in each well was quantified by a gamma counter. The resulting data was analyzed according to the method of Scatchard (*Ann. NY Acad.*, 51:600-672 (1946)).

EXAMPLE 1

Synthesis of Initial CDR-Grafted (Humanized) Antibody from Murine COL-1

We describe in this Example the construction of humanized COL-1 monoclonal antibodies ("Mabs") -- COL-1 HuVH/HuVK -- using the V_L and V_H frameworks of human Mabs REI and NEWM, respectively. The CDRs for murine COL-1 were grafted onto human frameworks according to known methods as discussed *supra*. In particular, human frameworks were selected from antibodies which, based on previous studies, were predicted to be suitable, *i.e.* which should not adversely affect antigen binding and not exhibit significant immunogenicity in humans. The human frameworks selected for the variable heavy and variable light chains, respectively, were NEWM and REI.

In the production of the initial version of the humanized VH, certain murine framework residues were also retained which, based on previous studies, might allow retention of antigen binding properties. Specifically, residues F27, N28, I29, K30, N97, and T98 of the murine heavy chain were initially retained.

The production of this NEWM-grafted humanized COL-1 V_H was accomplished using two rounds of a dual PCR procedure: the standard PCR protocol (for overlap-extension) followed by PCR amplification with oligonucleotide primers 10 and 11. This procedure was carried out as described above using a single-stranded M13 DNA template bearing, between the *Hin*d III and *Bam*H I sites thereof, a DNA segment having the nucleotide sequence shown in Figure 13. The mutating oligonucleotides used in the first round (with Vent DNA polymerase)were designed and synthesized with the following sequences:

836. 5'-TGAGAATGGTGATACTGAATATGCCCCGAAGT; and
837. 5'-TCGGGGCATATTCAGTATCACCATTCTCAGGATC-3'.

Those for the second round (with Thermalase) were:

838. 5'-ACTATGATTACGACGCGTTGGTTCTTCGATGTCTGGGGCCAAGGGTCCTT GGTCACCGTC-3'; and
839. 5'-ACGCGTCGTAATCATAGTAGATAGACCCCGTGTATTACAGTAATAGACCGCG GTG-3'.

The resulting humanized VH was named COL1NMVH or "HuVH."

In the production of the initial version of the humanized VL, no uniquely murine framework residues were retained. The production of the REI-grafted humanized COL-1 V_L was accomplished by using two rounds of a procedure involving performing the annealing and extension-ligation protocols described above – followed by amplification by the standard Thermalase PCR protocol -- using a ssM13 template bearing, between the *Hind* III and *Bam*H I sites thereof, a ssU-DNA segment produced by the above procedure using the REIVK sequence shown in Figure 17 (or in the second round, the mutated M13 template resulting from the first round). The mutating oligonucleotides used in round number 1 were designed and synthesized with the following sequences:

842. 5'-TATAGCCAGATGCACTGACACTTTTGCTGGCCCTACAGGTGATG-3';
844. 5'-GCTCTGGGTCATCTGGATGTCGG-3';
849. 5'-TTCTACTCACGTGTGATTTGCAGCTTGGTCCCTTGGCCGAACGTAGGAAG CTCCCTACTGTGCTGGCAGTAG-3';
850. 5'-ATGGTGAAGGTGTAGTCGGTACCGC-3'; and
851. 5'-GCCTTACCTGGCGTCTGCTGGTACC-3'.

The mutating oligonucleotide used in the second round was:

841. 5'-GCACACCAGATTGTAGGTTGGATGCAAGG-3'.

The resulting humanized VL was called "COL1REVK" or "HuVK."

Concurrently, a second humanized COL-1 VL was made by the same procedure using the following mutating oligonucleotides.

For round 1:

842. 5'-TATAGCCAGATGCACTGACACTTTTGCTGGCCCTACAGGTGATG-3';
844. 5'-GCTCTGGGTCATCTGGATGTCGG-3';
849. 5'-TTCTACTCACGTGTGATTTGCAGCTTGGTCCCTTGGCCGAACGTAGGAAG CTCCCTACTGTGCTGGCAGTAG-3'; and
851. 5'-GCCTTACCTGGCGTCTGCTGGTACC-3'.

For round 2:

841. 5'-GCACACCAGATTGTAGGTTGGATGCAAGG-3'.

The resulting VL was termed "HuVKF."

A number of amino acid substituted-versions of HuVH and HuVK were also constructed, using the above-described overlap extension technique employing oligonucleotide primers 10 and 11 or primers 385 and 391, followed by PCR amplification with Vent DNA

polymerase. The mutating oligonucleotides and DNA templates are described below under their resulting humanized variable region name:

HuVHT (using the HuVH DNA shown in Figure 16 as a template)
954. 5'-GACAATGCTGACAGACACCAGCAA-3' and
955. 5'-TGCTGGTGTCTGTCAGCATTGTCA-3';

HuVHS (using the HuVH DNA as a template)
684. 5'-CACCAGCAGCAACCAGTTCAG-3' and
683. 5'-ACTGGTTGCTCGTGGTGTCTA-3',

HuVHSTAY (using the HuVHS DNA as a template)
1026. 5'-ACCAGCAGCAACACAGCCTACCTGAGACTCAGCAG-3' and
1028. 5'-TGCTGAGTCTCAGGTAGGCTGTGTTGCTGCTGGTGT-3';

HuVHA (using the HuVH DNA as a template)
745. 5'-TGACCTGCACCGCGTCTGGCTTCAAC-3' and
746. 5'-TTGAAGCCAGACGCGGTGCAGGTCAG-3';

HuVHAA (using the HuVHA DNA as a template)
1071. 5'-GAGACTCAGCAGCGTGACAG-3' and
1072. 5'-CGCTGCTGAGTCTCAGGCTGAATGTGTTCTTGCTGGTGTC-3';

HuVHAT (using the HuVHA DNA as a template)
1071. 5'-CCTGAGACTCAGCAGCGTGACAG-3' and
1074. 5'-CGCTGCTGAGTCTCAGGCTGGCCTGGTTCTTGCTGGTG-3';

HuVHAY (using the HuVHA DNA as a template)
1071. 5'-CCTGAGACTCAGCAGCGTGACAG-3' and
1073. 5'-CGCTGCTGAGTCTCAGGTAGAACTGGTTCTTGC-3';

HuVHATAY (using the HuVHA DNA as a template)
1071. 5'-CCTGAGACTCAGCAGCGTGACAG-3' and
1075. 5'-CGCTGCTGAGTCTCAGGTAGGCTGTGTTCTTGCTGGTGTC-3';

HuVHASTAY (using the HuVHS DNA as a template)
745. 5'-TGACCTGCACCGCGTCTGGCTTCAAC-3' and
746. 5'-TTGAAGCCAGACGCGGTGCAGGTCAG-3'; and

HuVKVL (using the HuVK DNA shown in Figure 18 as a template)
1010. 5'-ACTCCGACATCGTGCTGACCCAGAG-3' and
1011. 5'-CTCTGGGTCAGCACGATGTCGGAG-3'.

The above-produced M13 DNA constructs were transferred to pSV vectors which were then transfected into NSO host cells, according to the above-described procedures. The VH construct- and VL construct-containing pSV vectors were transfected into NSO cells in the following combinations (to produce antibodies having the indicated combinations of VH and

VL regions):

MuVH/MuVK;
HuVH/HuVK;
HuVH/MuVK;
MuVH/HuVK;
HuVHA/HuVK;
HuVHT/HuVK;
HuVHT/HuVKF;
HuVH/HuVKVL;
HuVHS/HuVKVL;
HuVHSTAY/HuVK;
HuVH/HuVKF;
MuVH/HuVKF; and
HuVHSTAY/HuVKVL.

Combinations of the other VHs with the various VKs are expected to behave in an analogous manner, though having an unpredictable variation in degree of binding. Final selection of the optimum combination will be a function of obtaining an antibody having the best, selective antigen binding properties with the fewest murine amino acid substitutions.

The amino acid sequences of the initial humanized (CDR-grafted) COL-1 variable heavy (V_H) and variable light (V_K) regions, HuVH and HuVK, are show in Figures 1 and 2, respectively. NSO transfectants were screened for whole antibody expression and the antigen binding characteristics of the antibodies produced thereby were measured by an ELISA test against CEA. Results are presented in Figures 4-9. This data shows that – of the graft-humanized and the mix-and-match clones – MuVH/HuVK outperformed both HuVH/MuVK and HuVH/HuVK, but did not perform as well as the chimeric MuVH/MuVK antibodies.

These results indicate that the initial fully humanized antibody (HuVH/HuVK) exhibits a 20-fold loss in CEA antigen binding affinity. Thus, it exhibits about 5% the binding affinity of murine COL-1. Based on analysis of CEA binding of mix-and-match antibodies (HuVH/HuVK) and (MuVH/HuVK), it was determined that the reduction in CEA antigen binding was apparently largely attributable to the amino acid sequence of humanized heavy chain. However, an approximate 2-fold reduction in antigen binding also occurred apparently because of the amino acid sequence of the humanized kappa chain. This can be appreciated from the ELISA data in Figure 4.

As a result of this research, an antibody comprising the VHSTAY- and VKVL-containing heavy and light chains was deposited on October 16, 1996 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 and accorded Accession Number ATCC CRL-12208 (this is a murine plasmacytoma cell line). This deposit was made in accordance with the Budapest Treaty. This deposited cell line will be made irrevocably available, without restriction, upon issuance of a patent to this application, or any patent claiming benefit of priority to this application under 35 U.S.C. §120.

Based on the foregoing, it will be appreciated that the humanized antibodies disclosed herein exhibit antigen-binding characteristics, *i.e.*, CEA affinities comparable to the parent monoclonal antibody, nCOL-1 (murine antibody), and to chimeric antibodies derived from nCOL-1, *e.g.*, ChCOL-1γ1 (ATCC No. CRL 11217). Moreover, based on the foregoing results, these antibodies possess properties which will render them well suited for usage as *in vivo* diagnostics or therapeutics, *e.g.*, improved serum clearance, metabolic properties, and little or no immunogenicity in humans.

These properties are highly significant because they will enable the subject humanized antibodies to be administered repeatedly, in large dosages, and over a prolonged period of time without significant adverse effects, *e.g.*, a HAMA response or non-specific cytotoxicity. This is important for cancer treatment as well as for cancer diagnosis as it enables these antibodies to be used over prolonged time periods. Moreover, the clearance properties of the subject human antibodies will enable these antibodies to effectively target desired target sites, *e.g.*, CEA expressing carcinomas (because of the effects of serum clearance on targeting efficiency). Therefore, the humanized antibodies of the present invention comprise a substantial improvement in relation to previously disclosed antibodies specific to CEA.

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

SEQUENCE LISTING

GENERAL INFORMATION:
APPLICANT:
NAME: The Dow Chemical Company
STREET: 1790 Bldg. Washington Street
CITY: Midland
STATE: MI
COUNTRY: U.S.A.
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TITLE OF INVENTION: High Affinity Humanized Anti-CEA Monoclonal Antibodies

NUMBER OF SEQUENCES: 53

COMPUTER READABLE FORM:
MEDIUM TYPE: 3-1/2" diskette
COMPUTER: IBM PC Compatible
OPERATING SYSTEM: MS-DOS
SOFTWARE: PatentIn

INFORMATION FOR SEQ ID NO:1

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Murine COL-1 VH
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Ser Gly Ala
5 10 15

Ser Val Lys Met Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Lys Ala Thr Met Thr Thr Asp Tyr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
100 105 110

Val Trp Gly Ala Gly Thr Thr Val Ala Val Ser Ser
115 120

INFORMATION FOR SEQ ID NO:2

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: NEWM VH FR template
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Xaa Xaa Xaa Xaa Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Xaa Xaa
85 90 95

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
100 105 110

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Thr Val Ser Ser
115 120

INFORMATION FOR SEQ ID NO:3:

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVH
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
100 105 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
115 120

INFORMATION FOR SEQ ID NO:4:

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVHA
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
5 10 15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
100 105 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
115 120

INFORMATION FOR SEQ ID NO:5:

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVHAT
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
5 10 15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Thr Phe Ser
65 60 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
100 105 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
115 120

INFORMATION FOR SEQ ID NO:6:

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVHAA
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
5 10 15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Ala Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

```
Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
            100                 105                 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
        115                 120
```

INFORMATION FOR SEQ ID NO:7:

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVHAY
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                5                  10                  15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Tyr
65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
            100                 105                 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
        115                 120
```

INFORMATION FOR SEQ ID NO:8:

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVHATAY
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                5                  10                  15
```

```
Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
                20                  25                  30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
            35                  40                  45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
        50                  55                  60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
            100                 105                 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
        115                 120
```

INFORMATION FOR SEQ ID NO:9:

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVHASTAY
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn Thr Ala Tyr
65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
            100                 105                 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
        115                 120
```

INFORMATION FOR SEQ ID NO:10

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVHT
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Leu Thr Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
100 105 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
115 120

INFORMATION FOR SEQ ID NO:11:

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVHS
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
100 105 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
115 120

INFORMATION FOR SEQ ID NO:12:

SEQUENCE CHARACTERISTICS:
LENGTH: 124
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, HuVHSTAY
LOCATION: 1..124

SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
100 105 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
115 120

INFORMATION FOR SEQ ID NO:13:

SEQUENCE CHARACTERISTICS:
LENGTH: 110
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Murine COL-1 VK
LOCATION: 1..110

SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Thr Val Ser Leu Gly
5 10 15

Leu Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Ala Ser
20 25 30

Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Arg Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Gln Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly THr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg
85 90 95

Glu Leu Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

INFORMATION FOR SEQ ID NO:14:

SEQUENCE CHARACTERISTICS:
LENGTH: 110
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: REI VK FR template
LOCATION: 1..110

SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45

Lys Leu Leu Ile Tyr Xaa Xaa Xaa Xaa Xaa Xaa Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser
65 70 75 80

Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Xaa Xaa Xaa
85 90 95

Xaa Xaa Xaa Thr Phe Gly Gln Gly Thr Lys Xaa Xaa Xaa Xaa
100 105 110

INFORMATION FOR SEQ ID NO:15:

SEQUENCE CHARACTERISTICS:
LENGTH: 110
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VK, HuVK
LOCATION: 1..110

SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Val Ser Ala Ser
20 25 30

Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Gln Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser
65 70 75 80

Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Ser Arg
85 90 95

Glu Leu Pro Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
100 105 110

INFORMATION FOR SEQ ID NO:16:

SEQUENCE CHARACTERISTICS:
LENGTH: 110
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VK, HuVKVL
LOCATION: 1..110

SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asp Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Val Ser Ala Ser
20 25 30

Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Gln Ser Gly Val Pro Ser
50 55 60

```
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser
 65                  70                  75                  80

Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Ser Arg
                85                  90                  95

Glu Leu Pro Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
            100                 105                 110
```

INFORMATION FOR SEQ ID NO:17:

SEQUENCE CHARACTERISTICS:
LENGTH: 110
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VK, HuVKF
LOCATION: 1..110

SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
                5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Val Ser Ala Ser
            20                  25                  30

Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro
        35                  40                  45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Gln Ser Gly Val Pro Ser
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser
 65                  70                  75                  80

Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Ser Arg
                85                  90                  95

Glu Leu Pro Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
            100                 105                 110
```

INFORMATION FOR SEQ ID NO:18:

SEQUENCE CHARACTERISTICS:
LENGTH: 125
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VH, ATCC CRL-12208 VH
LOCATION: 1..125

SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                5                  10                  15
```

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Gly Phe
50 55 60

Gln Gly Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Al Val Tyr Tyr Cys
85 90 95

Asn Thr Arg Gly Leu Ser Thr Met Ile Thr Thr Arg Trp Phe Phe Asp
100 105 110

Val Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
115 120 125

INFORMATION FOR SEQ ID NO:19:

SEQUENCE CHARACTERISTICS:
LENGTH: 110
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized COL-1 VK, ATCC CRL-12208 VK
LOCATION: 1..110

SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asp Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Val Ser Ala Ser
20 25 30

Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Gln Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser
65 70 75 80

Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Ser Arg
85 90 95

Glu Leu Pro Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
100 105 110

INFORMATION FOR SEQ ID NO:20:

SEQUENCE CHARACTERISTICS:
LENGTH: 348
TYPE: DNA
STRANDEDNESS: double

TOPOLOGY: linear

FEATURE:
NAME/KEY: Template used to produce HuVH
LOCATION: 1..348

SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG 48

ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT AAA GAC TAC 96

TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT 144

GGA TGG ATT GAT CCT GAG AAT NNN NNN NNN NNN TAT GCC CCG AAG TTC 192

CAG GGC AGA GTG ACA ATG CTG GTA GAC ACC AGC AAG AAC CAG TTC AGC 240

CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT NNN NNN 288

NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN GTC 336

ACC GTC TCC TCA 348

INFORMATION FOR SEQ ID NO:21:

SEQUENCE CHARACTERISTICS:
LENGTH: 372
TYPE: DNA
STRANDEDNESS: double
TOPOLOGY: linear

FEATURE:
NAME/KEY: Template used to produce HuVH variants
LOCATION: 1..372

SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG 48

ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT AAA GAC TAC 96

TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT 144

GGA TGG ATT GAT CCT GAG AAT GGT GAT ACT GAA TAT GCC CCG AAG TTC 192

CAG GGC AGA GTG ACA ATG CTG GTA GAC ACC AGC AAG AAC CAG TTC AGC 240

CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT TAC TGT 288

AAT ACA CGG GGT CTA TCT ACT ATG ATT ACG ACG GCT TGG TTC TTC GAT 336

GTC TGG GGC CAA GGG TCC TTG GTC ACC GTC TCC TCA 372

INFORMATION FOR SEQ ID NO:22:

SEQUENCE CHARACTERISTICS:
LENGTH: 318
TYPE: DNA
STRANDEDNESS: double
TOPOLOGY: linear

FEATURE:
NAME/KEY: Template used to produce HuVK
LOCATION: 1..318

SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GAC ATC CAR CTG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT 48

GAC AGA GTG ACC ATC ACC TGT AGG NNN NNN NNN NNN NNN NNN NNN NNN 96

GGC TAT AGT TAT ATG CAC TTG TAC CAG CAG ACG CCA GGT AAG GCT CCA 144

AAG CTG CTG ATC TAC NNN NNN NNN NNN NNN NNN TCT GGT GTG CCA AGC 192

AGA TTC AGC GGT AGC GGT AGC GGT ACC GAC TTY ACC TTC ACC ATC AGC 240

AGC CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG NNN NNN NNN 288

NNN NNN NNN ACG TTC GGC CAA GGG ACC AAG 318

INFORMATION FOR SEQ ID NO:23:

SEQUENCE CHARACTERISTICS:
LENGTH: 330
TYPE: DNA
STRANDEDNESS: double
TOPOLOGY: linear

FEATURE:
NAME/KEY: Template used to produce HuVK variants
LOCATION: 1..330

SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GAC ATC CAR ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT 48

GAC AGA GTG ACC ATC ACC TGT AGG GCC AGC AAA AGT GTC AGT GCA TGT 96

GGC TAT AGT TAT ATG CAC TGG TAC CAG CAG ACG CCA GGT AAG GCT CCA 144

AAG CTG CTG ATC TAC CTT GCA TCC AAC CTA CAA TCT GGT GTG CCA AGC 192

AGA TTC AGC GGT AGC GGT AGC GGT ACC GAC TAC ACC TTC ACC ATC AGC 240

AGC CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG CAC AGT AGG 288

GAG CTT CCT ACG TTC GGC CAA GGG ACC AAG CTG CAA ATC ACA 330

INFORMATION FOR SEQ ID NO:24:

SEQUENCE CHARACTERISTICS:
LENGTH: 17
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 10
LOCATION: 1..17

SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CTAAAACGAC GGCCAGT 17

INFORMATION FOR SEQ ID NO:25:

SEQUENCE CHARACTERISTICS:
LENGTH: 16
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 11
LOCATION: 1..16

SEQUENCE DESCRIPTION: SEQ ID NO: 25:

AACAGCTATG ACCATG 16

INFORMATION FOR SEQ ID NO:26:

SEQUENCE CHARACTERISTICS:
LENGTH: 22
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 385
LOCATION: 1..22

SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GCGGGCCTCT TCGCTATTAC GC 22

INFORMATION FOR SEQ ID NO:27:

SEQUENCE CHARACTERISTICS:
LENGTH: 22
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 391
LOCATION: 1..22

SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CTCTCTCAGG GCCAGGCGGT GA 22

INFORMATION FOR SEQ ID NO:28:

SEQUENCE CHARACTERISTICS:
LENGTH: 32
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 836
LOCATION: 1..32

SEQUENCE DESCRIPTION: SEQ ID NO: 28:

TGAGAATGGT GATACTGAAT ATGCCCCGAA GT 32

INFORMATION FOR SEQ ID NO:29:

SEQUENCE CHARACTERISTICS:
LENGTH: 34
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 837
LOCATION: 1..34

SEQUENCE DESCRIPTION: SEQ ID NO: 29:

TCGGGGCATA TTCAGTATCA CCATTCTCAG GATC 34

INFORMATION FOR SEQ ID NO:30:

SEQUENCE CHARACTERISTICS:
LENGTH: 59
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 838
LOCATION: 1..59

SEQUENCE DESCRIPTION: SEQ ID NO: 30:

ACTATGATTA CGACGCGTTG GTTCTTCGAT GTCTGGGGCC AAGGGTCCTT GGTCACGTC 59

INFORMATION FOR SEQ ID NO:31:

SEQUENCE CHARACTERISTICS:
LENGTH: 55
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 839
LOCATION: 1..55

SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ACGCGTCGTA ATCATAGTAG ATAGACCCCG TGTATTACAG TAATAGACCG CGGTG 55

INFORMATION FOR SEQ ID NO:32:

SEQUENCE CHARACTERISTICS:
LENGTH: 44
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 842
LOCATION: 1..44

SEQUENCE DESCRIPTION: SEQ ID NO: 32:

TATAGCCAGA TGCACTGACA CTTTTGCTGG CCCTACAGGT GATG 44

INFORMATION FOR SEQ ID NO:33:

SEQUENCE CHARACTERISTICS:
LENGTH: 23
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 844
LOCATION: 1..23

SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GCTCTGGGTC ATCTGGATGT CGG 23

INFORMATION FOR SEQ ID NO:34:

SEQUENCE CHARACTERISTICS:
LENGTH: 72
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 849
LOCATION: 1..72

SEQUENCE DESCRIPTION: SEQ ID NO: 34:

TTCTACTCAC GTGTGATTTG CAGCTTGGTC CCTTGGCCGA ACGTAGGAAG CTCCCTACTG 60

TGCTGGCAGT AG 72

INFORMATION FOR SEQ ID NO:35:

SEQUENCE CHARACTERISTICS:
LENGTH: 25
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 850
LOCATION: 1..25

SEQUENCE DESCRIPTION: SEQ ID NO: 35:

ATGGTGAAGG TGTAGTCGGT ACCGC 25

INFORMATION FOR SEQ ID NO:36:

SEQUENCE CHARACTERISTICS:
LENGTH: 25
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 851
LOCATION: 1..25

SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GCCTTACCTG GCGTCTGCTG GTACC 25

INFORMATION FOR SEQ ID NO:37:

SEQUENCE CHARACTERISTICS:
LENGTH: 29
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 841
LOCATION: 1..29

SEQUENCE DESCRIPTION: SEQ ID NO: 37:

GCACACCAGA TTGTAGGTTG GATGCAAGG 29

INFORMATION FOR SEQ ID NO:38:

SEQUENCE CHARACTERISTICS:
LENGTH: 44
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 842
LOCATION: 1..44

SEQUENCE DESCRIPTION: SEQ ID NO: 38:

TATAGCCAGA TGCACTGACA CTTTTGCTGG CCCTACAGGT GATG 44

INFORMATION FOR SEQ ID NO:39:

SEQUENCE CHARACTERISTICS:
LENGTH: 24
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 954
LOCATION: 1..24

SEQUENCE DESCRIPTION: SEQ ID NO: 39:

GACAATGCTG ACAGACACCA GCAA 24

INFORMATION FOR SEQ ID NO:40:

SEQUENCE CHARACTERISTICS:
LENGTH: 24
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 955
LOCATION: 1..24

SEQUENCE DESCRIPTION: SEQ ID NO: 40:

TGCTGGTGTC TGTCAGCATT GTCA 24

INFORMATION FOR SEQ ID NO:41:

SEQUENCE CHARACTERISTICS:
LENGTH: 21
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 684
LOCATION: 1..21

SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CACCAGCAGC AACCAGTTCA G 21

INFORMATION FOR SEQ ID NO:42:

SEQUENCE CHARACTERISTICS:
LENGTH: 21
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 683
LOCATION: 1..21

SEQUENCE DESCRIPTION: SEQ ID NO: 42:

ACTGGTTGCT CGTGGTCTCT A 21

INFORMATION FOR SEQ ID NO:43:

SEQUENCE CHARACTERISTICS:
LENGTH: 35
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1026
LOCATION: 1..35

SEQUENCE DESCRIPTION: SEQ ID NO: 43:

ACCAGCAGCA ACACAGCCTA CCTGAGACTC AGCAG 35

INFORMATION FOR SEQ ID NO:44:

SEQUENCE CHARACTERISTICS:
LENGTH: 36
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1028
LOCATION: 1..36

SEQUENCE DESCRIPTION: SEQ ID NO: 44:

TGCTGAGTCT CAGGTAGGCT GTGTTGCTGC TGGTGT 36

INFORMATION FOR SEQ ID NO:45:

SEQUENCE CHARACTERISTICS:
LENGTH: 26
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 745
LOCATION: 1..26

SEQUENCE DESCRIPTION: SEQ ID NO: 45:

TGACCTGCAC CGCGTCTGGC TTCAAC 26

INFORMATION FOR SEQ ID NO:46:

SEQUENCE CHARACTERISTICS:
LENGTH: 26
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 746
LOCATION: 1..26

SEQUENCE DESCRIPTION: SEQ ID NO: 46:

TTGAAGCCAG ACGCGGTGCA GGTCAG 26

INFORMATION FOR SEQ ID NO:47:

SEQUENCE CHARACTERISTICS:
LENGTH: 20
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1071
LOCATION: 1..20

SEQUENCE DESCRIPTION: SEQ ID NO: 47:

GAGACTCAGC AGCGTGACAG 20

INFORMATION FOR SEQ ID NO:48:

SEQUENCE CHARACTERISTICS:
LENGTH: 40
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1072
LOCATION: 1..40

SEQUENCE DESCRIPTION: SEQ ID NO: 48:

CGCTGCTGAG TCTCAGGCTG AATGTGTTCT TGCTGGTGTC 40

INFORMATION FOR SEQ ID NO:49:

SEQUENCE CHARACTERISTICS:
LENGTH: 38
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1074
LOCATION: 1..38

SEQUENCE DESCRIPTION: SEQ ID NO: 49:

CGCTGCTGAG TCTCAGGCTG GCCTGGTTCT TGCTGGTG 38

INFORMATION FOR SEQ ID NO:50:

SEQUENCE CHARACTERISTICS:
LENGTH: 33
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1073
LOCATION: 1..33

SEQUENCE DESCRIPTION: SEQ ID NO: 50:

CGCTGCTGAG TCTCAGGTAG AACTGGTTCT TGC 33

INFORMATION FOR SEQ ID NO:51:

SEQUENCE CHARACTERISTICS:
LENGTH: 40
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1075
LOCATION: 1..40

SEQUENCE DESCRIPTION: SEQ ID NO: 51:

CGCTGCTGAG TCTCAGGTAG GCTGTGTTCT TGCTGGTGTC 40

INFORMATION FOR SEQ ID NO:52:

SEQUENCE CHARACTERISTICS:
LENGTH: 25
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1010
LOCATION: 1..25

SEQUENCE DESCRIPTION: SEQ ID NO: 52:

ACTCCGACAT CGTGCTGACC CAGAG 25

INFORMATION FOR SEQ ID NO:53:

SEQUENCE CHARACTERISTICS:
LENGTH: 24
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1011
LOCATION: 1..24

SEQUENCE DESCRIPTION: SEQ ID NO: 53:

CTCTGGGTCA GCACGATGTC GGAG 24

WHAT IS CLAIMED IS:

1. A humanized antibody or humanized antibody fragment thereof which specifically binds CEA, characterized in that said humanized antibody has: humanized heavy chain variable regions having the amino acid sequence of Figure 1 or 13 or having the amino acid sequence of the humanized heavy chain variable region of the humanized COL-1 antibody expressed by ATCC CRL-12208; and humanized light chain variable regions having the amino acid sequence of Figure 2 or 14 or having the amino acid sequence of the humanized light chain variable region of the humanized COL-1 antibody expressed by ATCC CRL-12208.

2. [Deleted].

3. [Deleted].

4. [Deleted].

5. [Deleted].

6. The humanized antibody or humanized antibody fragment of Claim 1 wherein said humanized antibody is identical to the humanized COL-1 antibody expressed by ATCC CRL-12208.

7. The humanized antibody or humanized antibody fragment of Claim 6 wherein said humanized antibody is expressed by ATCC CRL-12208.

8. The humanized antibody fragment of any one of Claims 1 and 6-7 wherein said humanized antibody fragment has a humanized variable heavy chain sequence of Figure 1 or 13 or a humanized variable light chain sequence of Figure 2 or 14, or has both said humanized variable heavy chain sequence and said humanized variable light chain sequence.

9. A nucleic acid sequence characterized in that a humanized antibody or humanized antibody fragment according to any one of Claims 1 and 6-8 may be expressed from the nucleic acid sequence.

10. A vector characterized in that a humanized antibody or humanized antibody fragment according to any one of Claims 1 and 6-8 may be expressed from the vector.

11. The vector according to Claim 10 wherein said vector is a bare nucleic acid segment, a carrier-associated nucleic acid segment, a nucleoprotein, a plasmid, a virus, a viroid, or a transposable element.

AMENDED SHEET

12. A composition suitable for the treatment or *in vivo* or *in vitro* detection of cancer characterized in that it comprises, respectively, a therapeutically effective or a diagnostically effective amount of a humanized antibody or humanized antibody fragment according to any one of Claims 1 and 6-8.

13. The composition of Claim 12 wherein said humanized antibody or humanized antibody fragment is, directly or indirectly, associated with or linked to an effector moiety having therapeutic activity, which effector moiety is a radionuclide, therapeutic enzyme, anti-cancer drug, cytokine, cytotoxin, or anti-proliferative agent, and the composition is suitable for the treatment of cancer.

14. [Deleted].

15. The composition of Claim 12 wherein said humanized antibody or humanized antibody fragment is, directly or indirectly, associated with or linked to a detectable label, and the composition is suitable for detection of cancer.

16. The composition of Claim 15 wherein the detectable label is a radionuclide or an enzyme.

17. A method for *in vivo* treatment of a mammal having a CEA-expressing cancer characterized in that the method is performed by administering to the mammal a therapeutically effective amount of a composition according to any one of Claims 12-13.

18. A method of *in vitro* immunodetection of CEA-expressing cancer cells characterized in that the method is performed by contacting the cancer cells with a composition according to any one of Claims 12, 15, and 16.

19. The method of Claim 18 wherein the humanized antibodies or humanized antibody fragments of the composition are bound to a solid support.

20. A method of immunodetection of CEA-expressing cancer cells in a mammal characterized in that the method is performed by administering to the mammal a diagnostically effective amount of a composition according to one of Claims 12, 15, and 16.

21. The method of Claim 20 wherein said immunodetection is *in vivo* tumor imaging.

22. A method of *in vivo* treatment of cancer by (i) intravenously administering a radionuclide-labeled antibody, (ii) thereafter detecting tumor cells using a radionuclide activity probe, and (iii) thereafter removing the detected tumor cells by surgical excision, characterized in that the radionuclide-labeled antibody contains a humanized antibody or humanized antibody fragment according to any one of Claims 1 and 6-8.

AMENDED SHEET

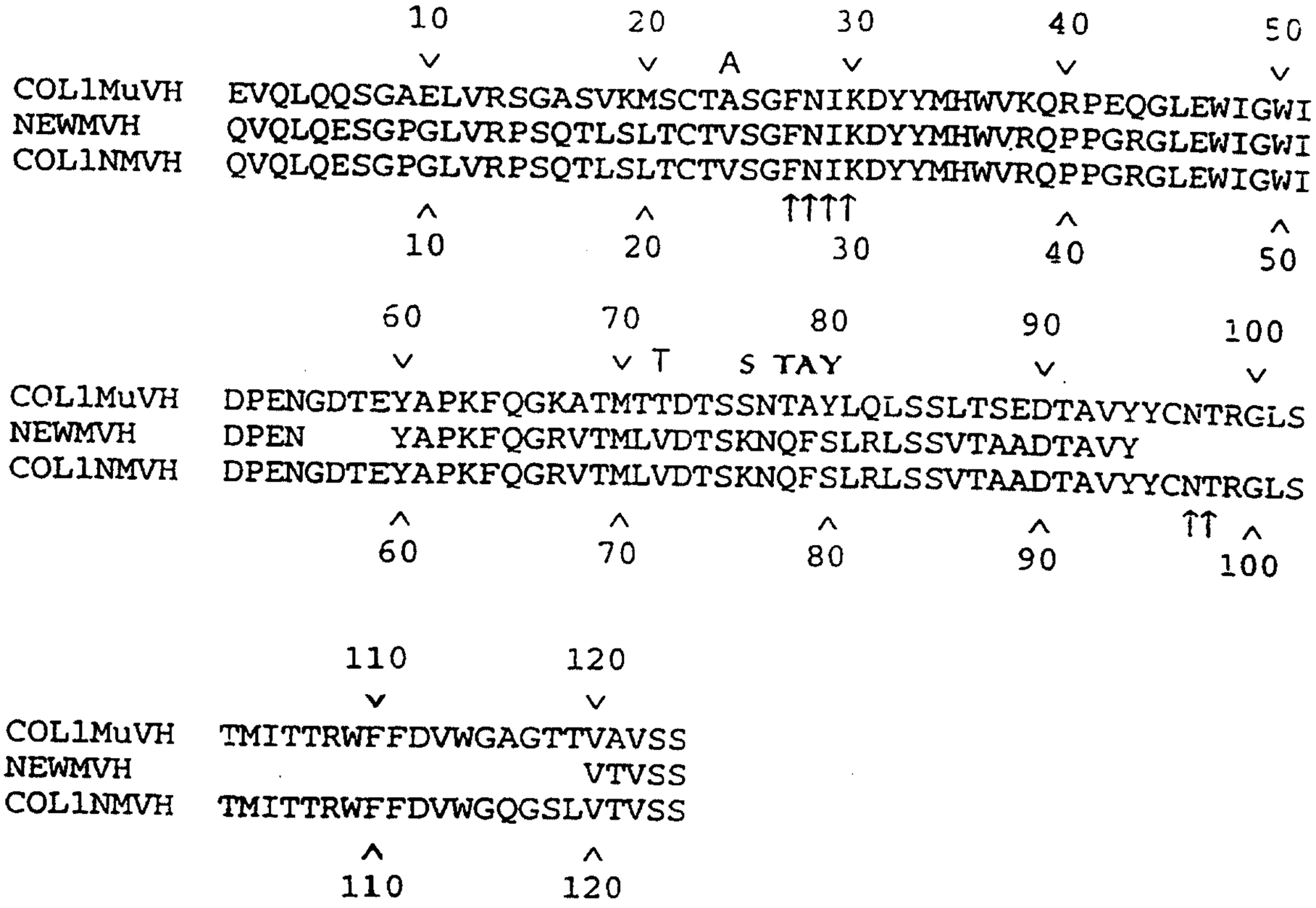
23. The method of claim 22, wherein the radionuclide is ^{125}I or ^{131}I.

24. A commercial package characterized in that it contains a composition according to any one of Claims 12-13 and 15-16 as active ingredient together with instructions for use thereof to treat or detect cancer wherein the composition is reconstituted prior to said use.

25. The use of a composition as claimed in any one of Claims 12-13 and 15-16 to treat or detect cancer.

26. The use of a humanized antibody or humanized antibody fragment according to any one of Claims 1 and 6-8 for the treatment or detection of cancer.

AMENDED SHEET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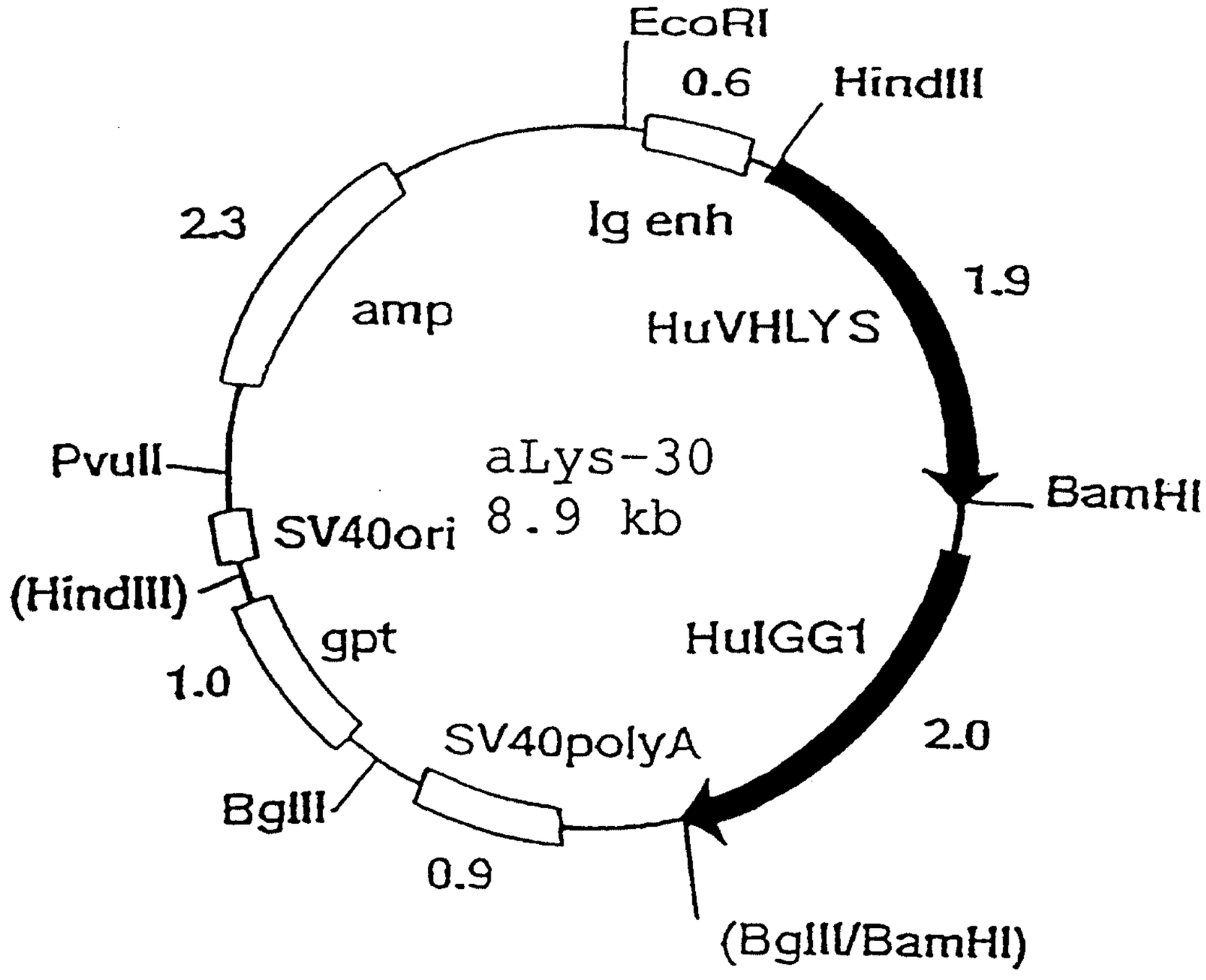
COL1MuVH EVQLQQSGAELVRSGASVKMSCTASGFNIKDYYMHWVKQRPEQGLEWIGWI
NEWMVH QVQLQESGPGLVRPSQTLSLTCTVSGFNIKDYYMHWVRQPPGRGLEWIGWI
COL1NMVH QVQLQESGPGLVRPSQTLSLTCTVSGFNIKDYYMHWVRQPPGRGLEWIGWI
10 20 30 40 50
60 70 80 90 100
T S TAY
COL1MuVH DPENGDTEYAPKFQGKATMTTDTSSNTAYLQLSSLTSEDTAVYYCNTRGLS
NEWMVH DPEN YAPKFQGRVTMLVDTSKNQFSLRLSSVTAADTAVY
COL1NMVH DPENGDTEYAPKFQGRVTMLVDTSKNQFSLRLSSVTAADTAVYYCNTRGLS
60 70 80 90 100
110 120
COL1MuVH TMITTRWFFDVWGAGTTVAVSS
NEWMVH VTVSS
COL1NMVH TMITTRWFFDVWGQGSLVTVSS
110 120

FIGURE 1

```
                  10        20        30        40        50
              VL  v         v         v         v         v
COL1MuVK  DIVLTQSPASLTVSLGLRATISCRASKSVSASGYSYMHWYQQRPGQPPKLL
REIVK     DIQLTQSPSSLSASVGDRVTITCR          GYSYMHWYQQKPGKAPKLL
COL1REVK  DIQMTQSPSSLSASVGDRVTITCRASKSVSASGYSYMHWYQQTPGKAPKLL
                  ^         ^         ^         ^         ^
                  10        20        30        40        50

                  60        70        80        90        100
                  v         v    F    v         v         v
COL1MuVK  IYLASNLQSGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPTFG
REIVK     IY      SGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQ        TFG
COL1REVK  IYLASNLQSGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQHSRELPTFG
                  ^         ^         ^         ^         ^
                  60        70        80        90        100

                  110
                  v
COL1MuVK  GGTKLEIK
REIVK     QGTK
COL1REVK  QGTKLQIT
                  ^
                  110
```

FIGURE 2



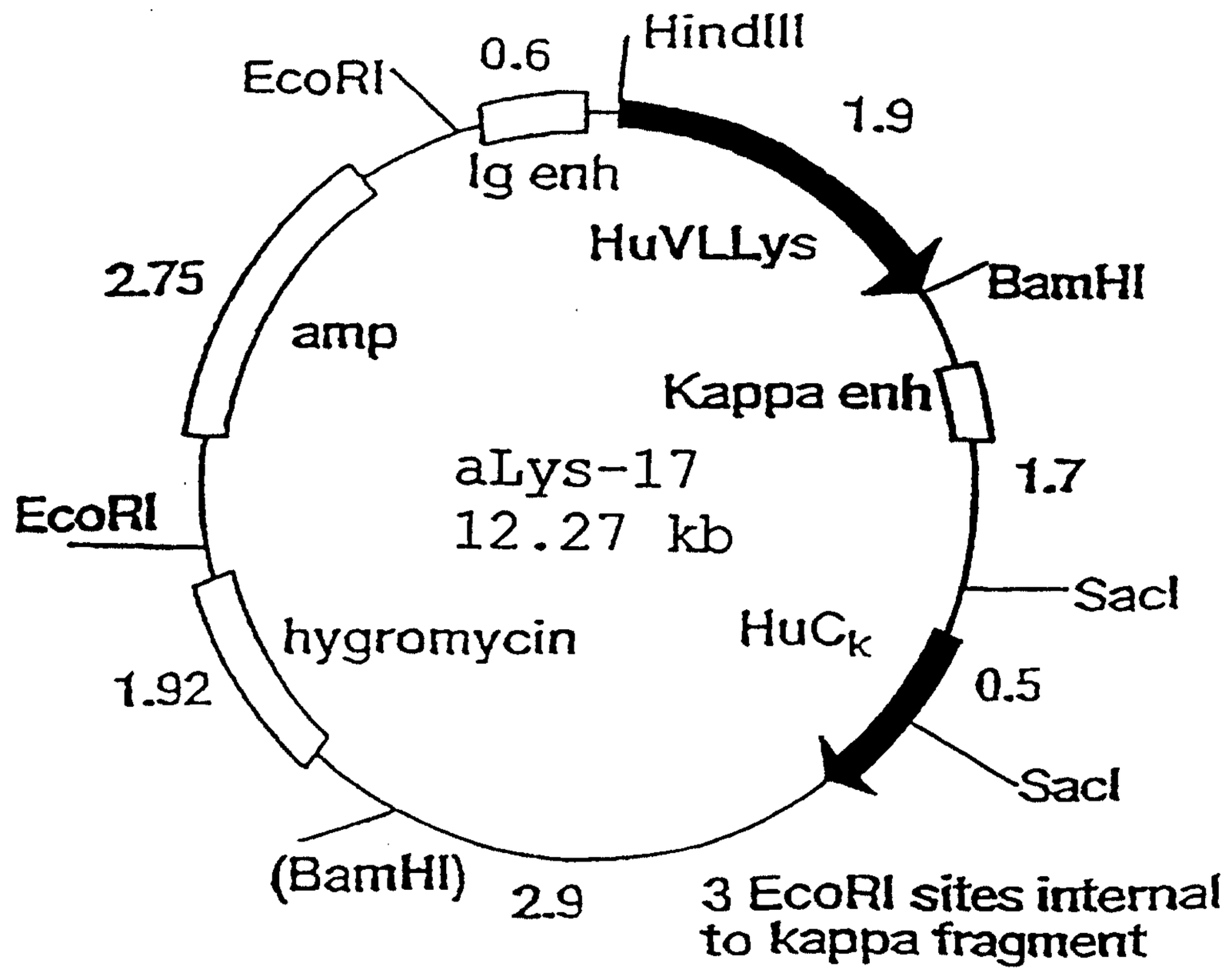
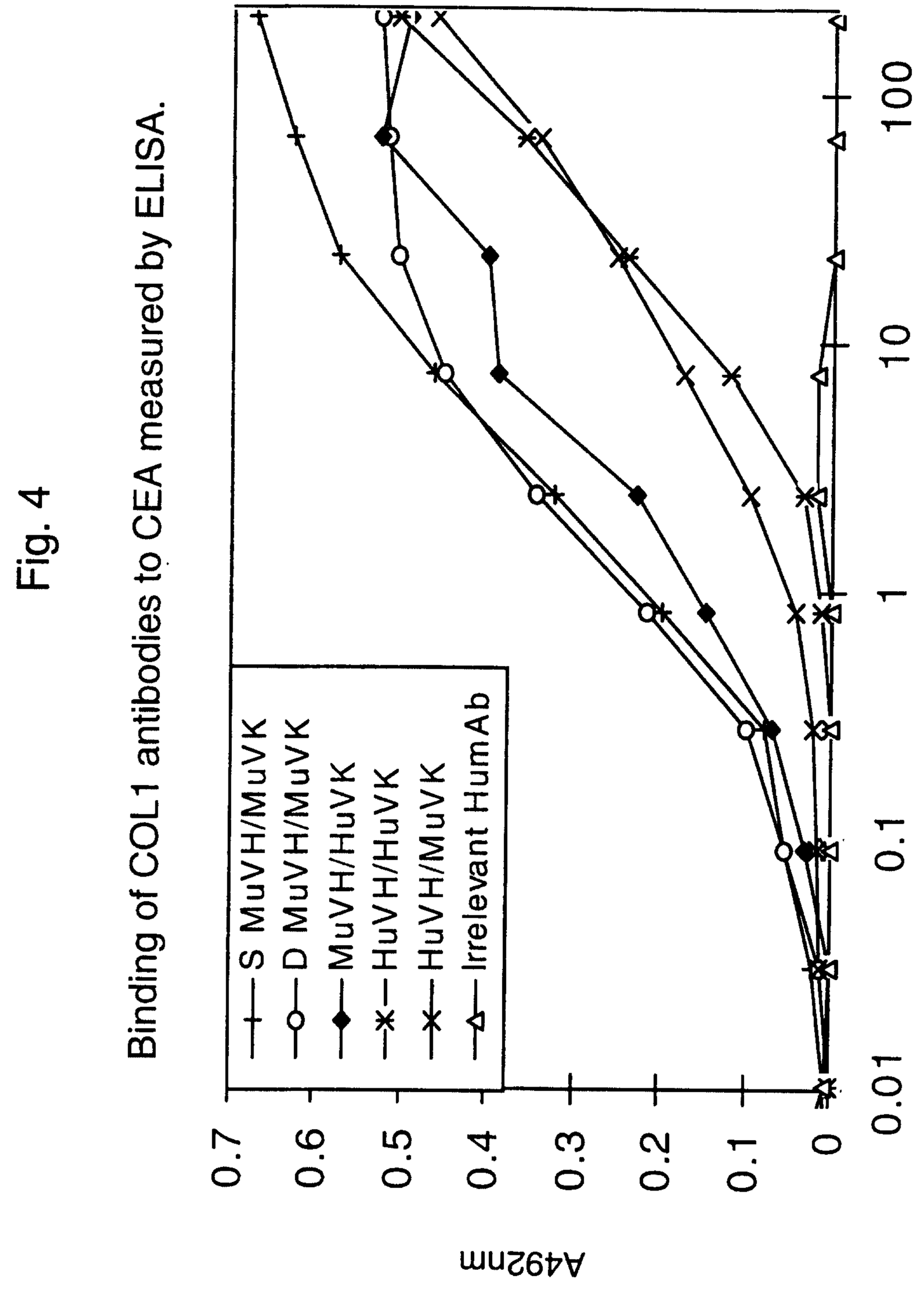


FIG. 3. Mammalian Cell Vectors for the Expression of Humanised V Regions

Fig. 4


Binding of COL1 antibodies to CEA measured by ELISA.
S MuVH/MuVK
D MuVH/MuVK
MuVH/HuVK
HuVH/HuVK
HuVH/MuVK
Irrelevant HumAb
A492nm
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
.01
0.1
1
10
100
Antibody, ng per well

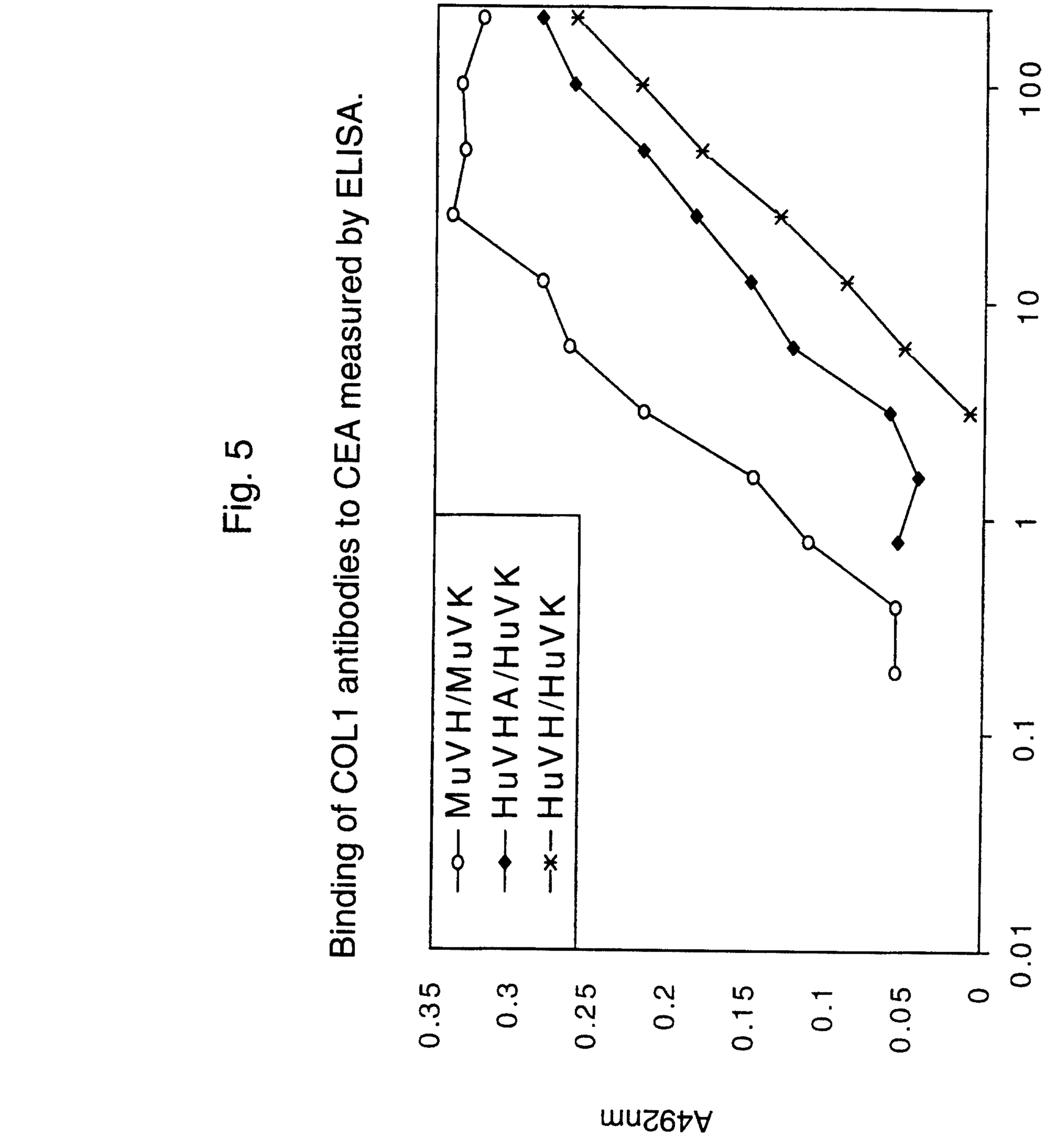

Binding of COL1 antibodies to CEA measured by ELISA.
MuVH/MuVK
HuVHA/HuVK
HuVH/HuVK
A492nm
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.01
0.1
1
10
100
Antibody, ng per well

Fig. 5

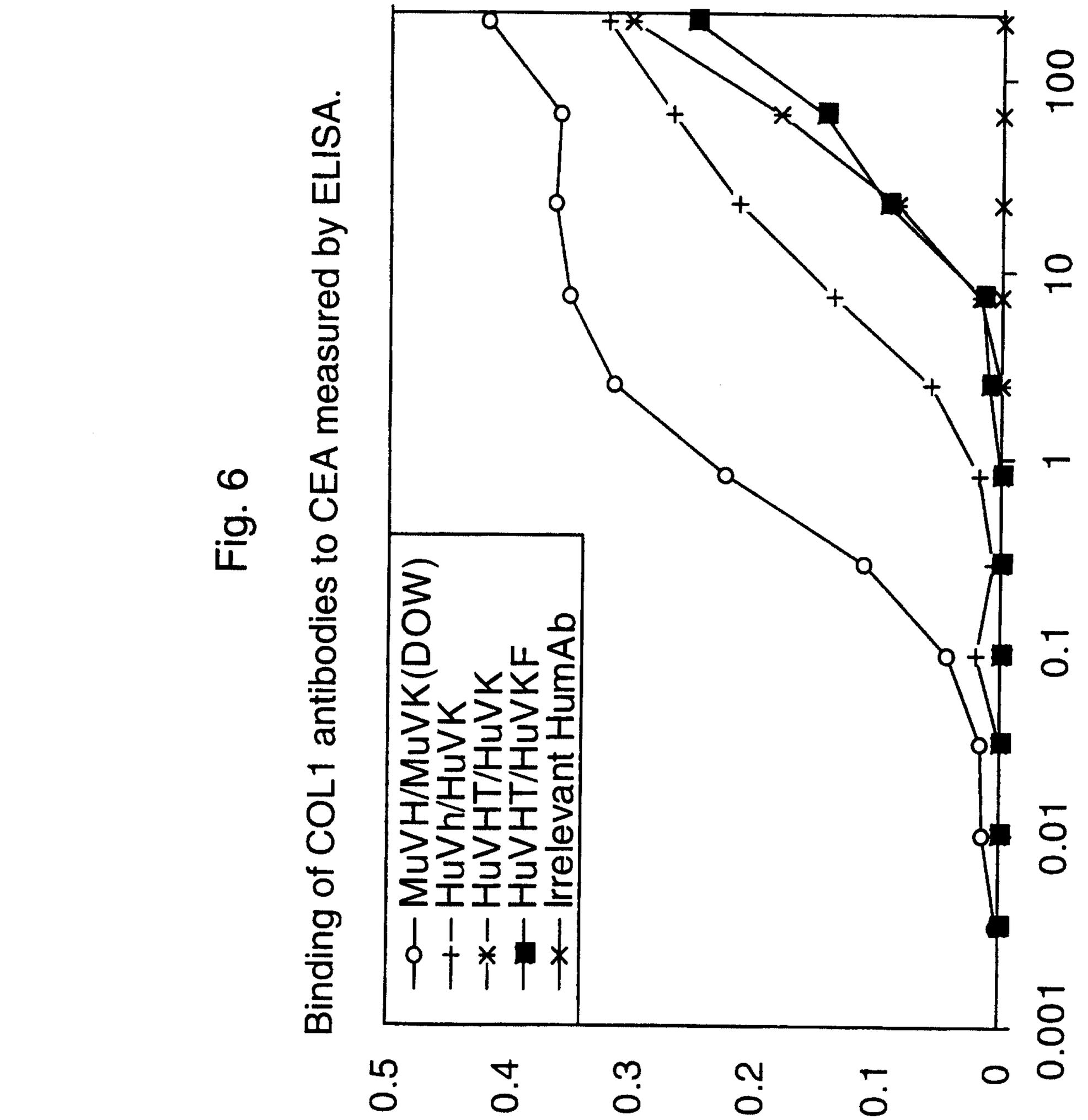


Fig. 6
Binding of COL1 antibodies to CEA measured by ELISA.
MuVH/MuVK(DOW)
HuVh/HuVK
HuVHT/HuVK
HuVHT/HuVKF
Irrelevant HumAb
A492nm
0.5
0.4
0.3
0.2
0.1
0
0.001
0.01
0.1
1
10
100
Antibody, ng per well

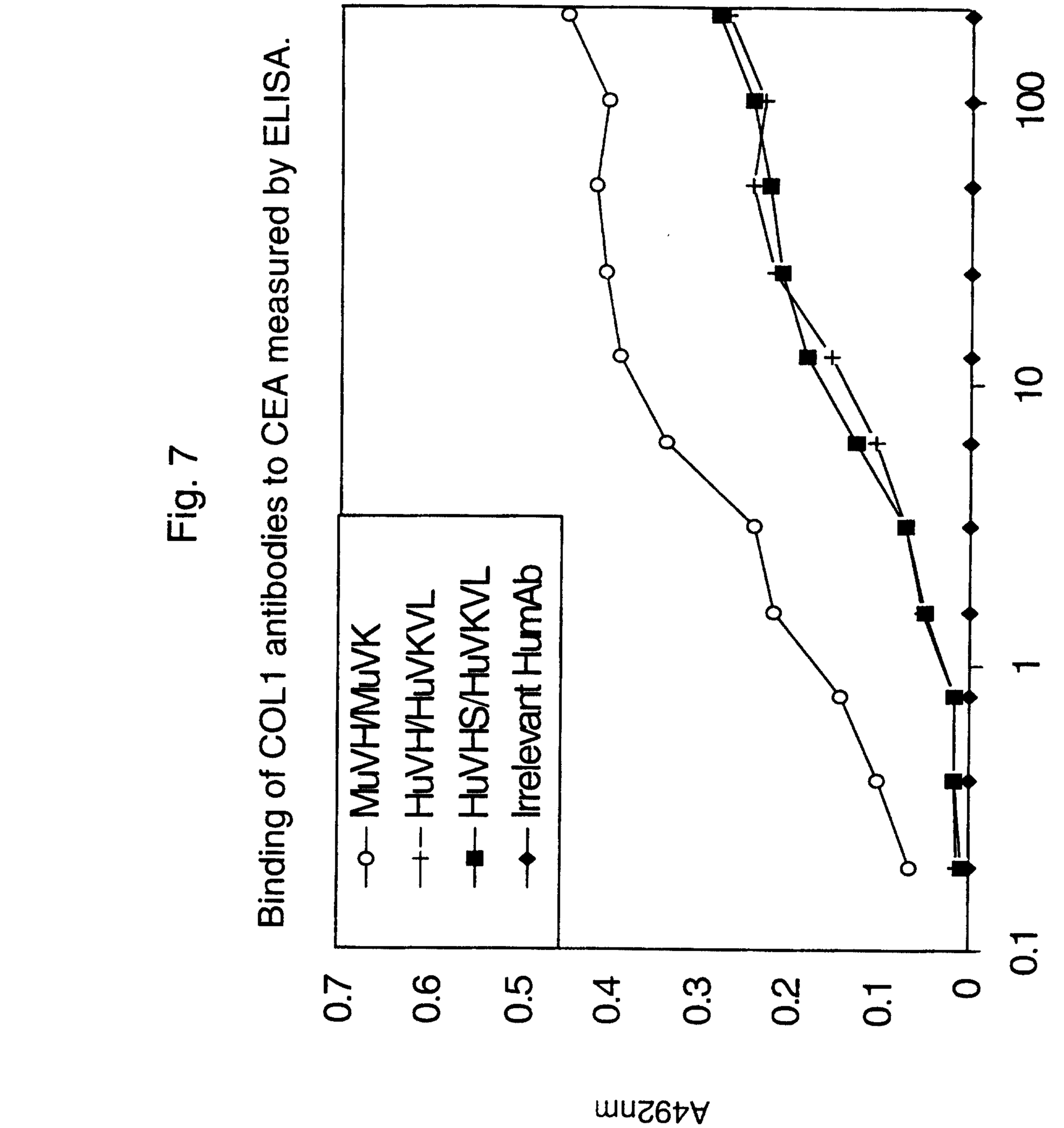

Binding of COL1 antibodies to CEA measured by ELISA.
MuVH/MuVK
HuVH/HuVKVL
HuVHS/HuVKVL
Irrelevant HumAb
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
.01
.1
1
10
100
Antibody, ng per well
A492nm

Fig. 7

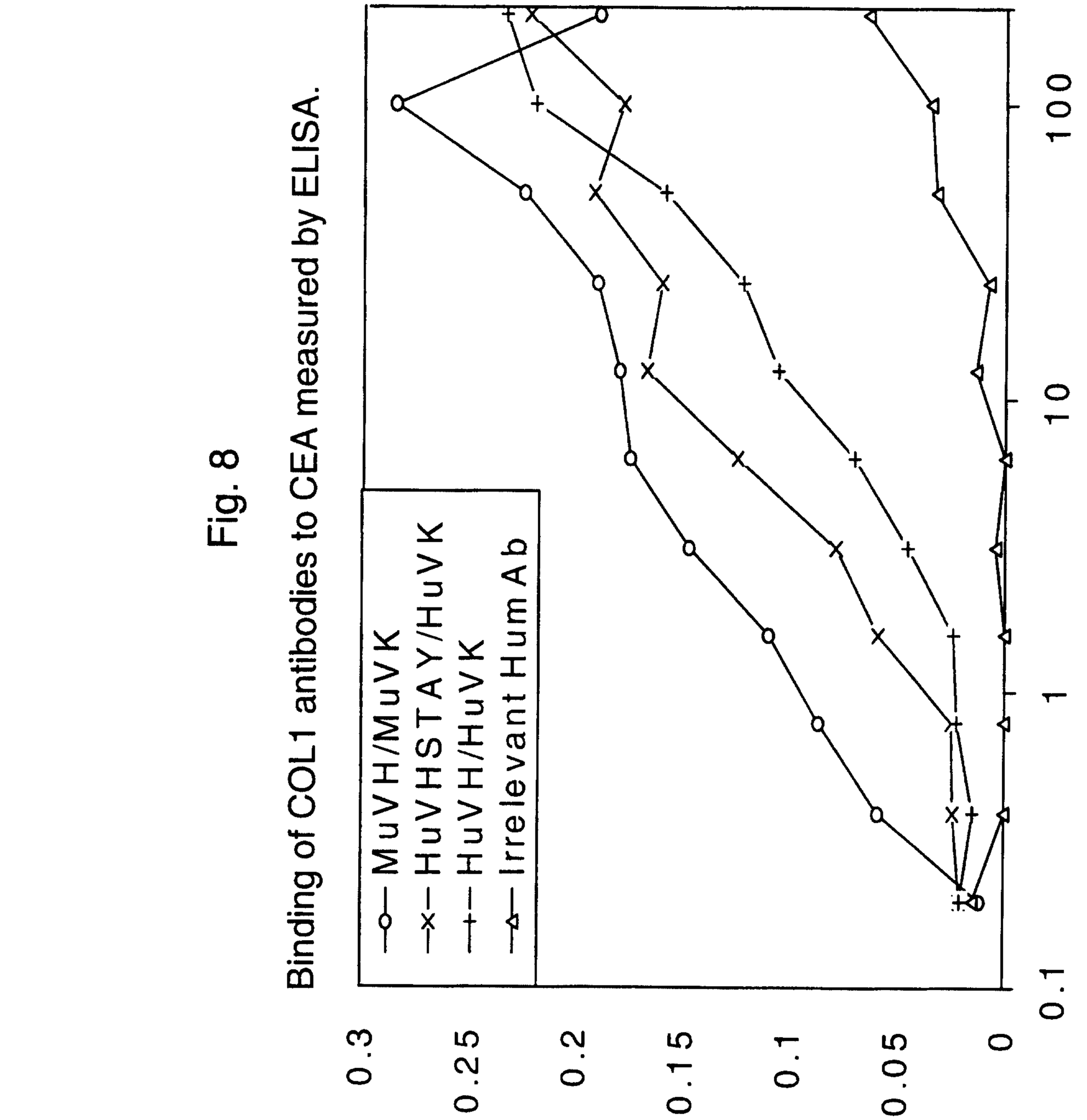
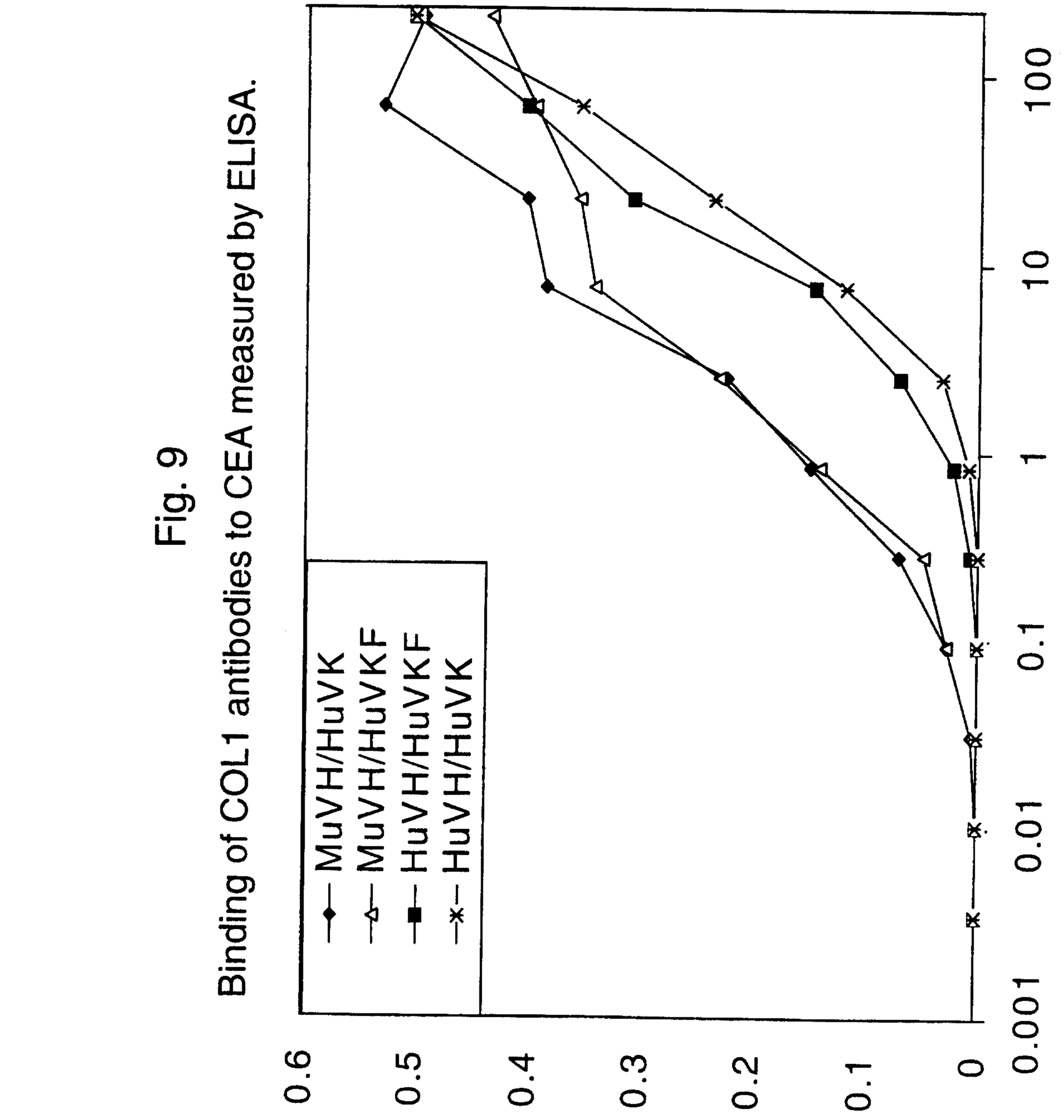

Binding of COL1 antibodies to CEA measured by ELISA.
MuVH/MuVK
HuVHSTAY/HuVK
HuVH/HuVK
Irrelevant Hum Ab
A492nm
0.3
0.25
0.2
0.15
0.1
0.05
0
.1
1
10
100
Antibody, ng per well

Fig. 8

Fig. 9


Binding of COL1 antibodies to CEA measured by ELISA.
MuVH/HuVK
MuVH/HuVKF
HuVH/HuVKF
HuVH/HuVK
A492nm
0.6
0.5
0.4
0.3
0.2
0.1
0
0.001
0.01
0.1
1
10
100
Antibody, ng per well

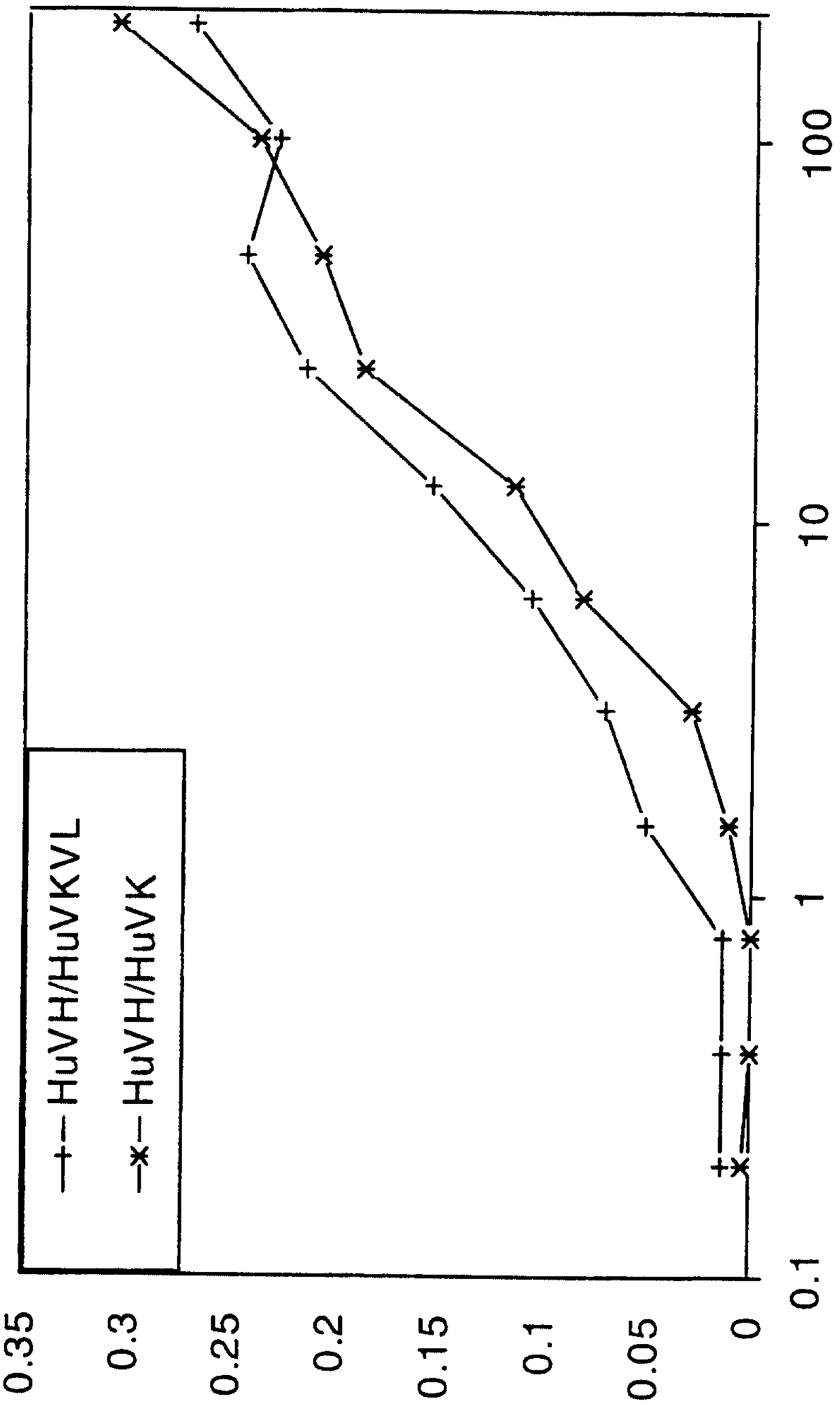


Fig. 10. Binding of COL1 antibodies to CEA measured by ELISA.

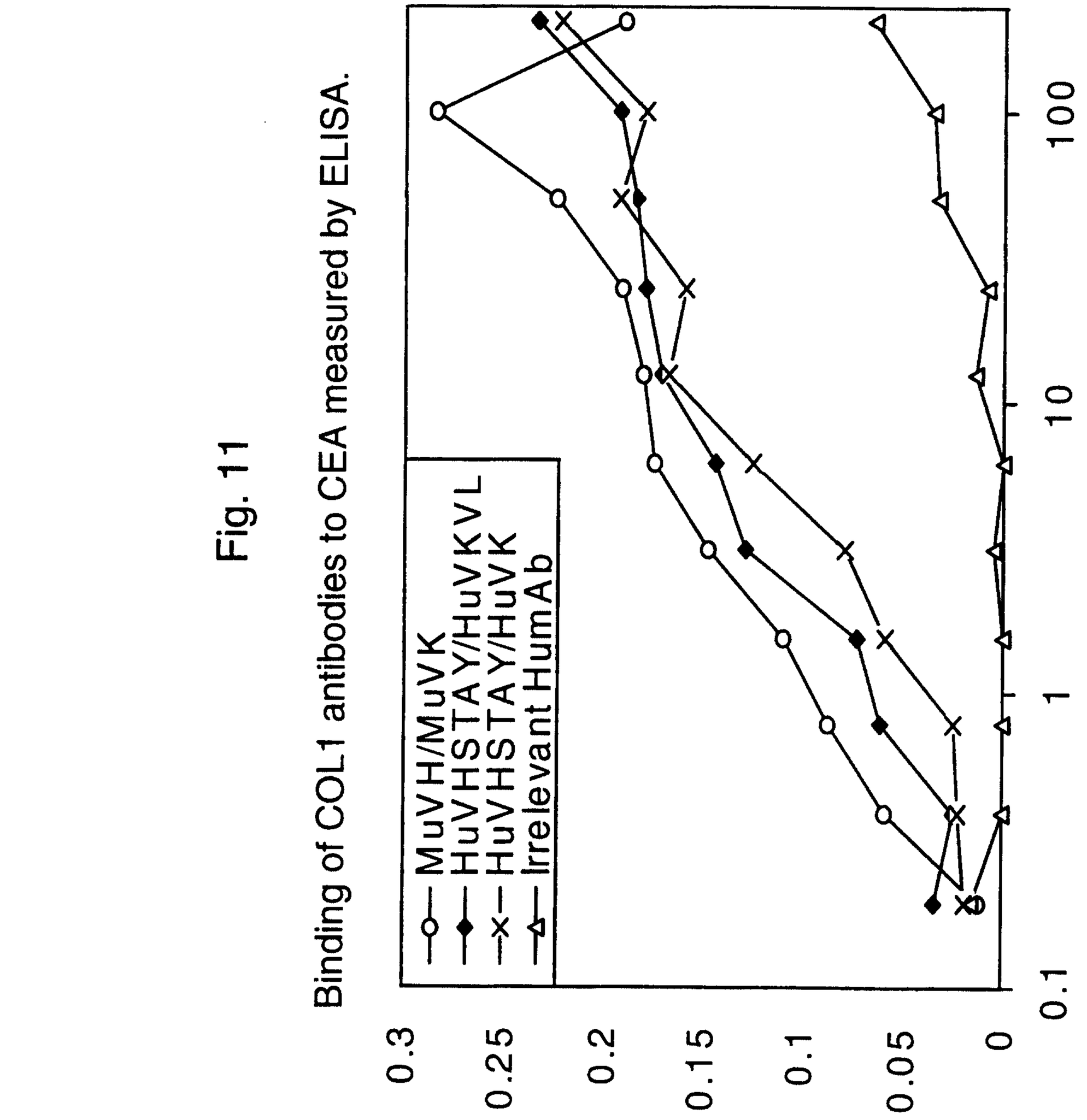


Fig. 11. Binding of COL1 antibodies to CEA measured by ELISA.

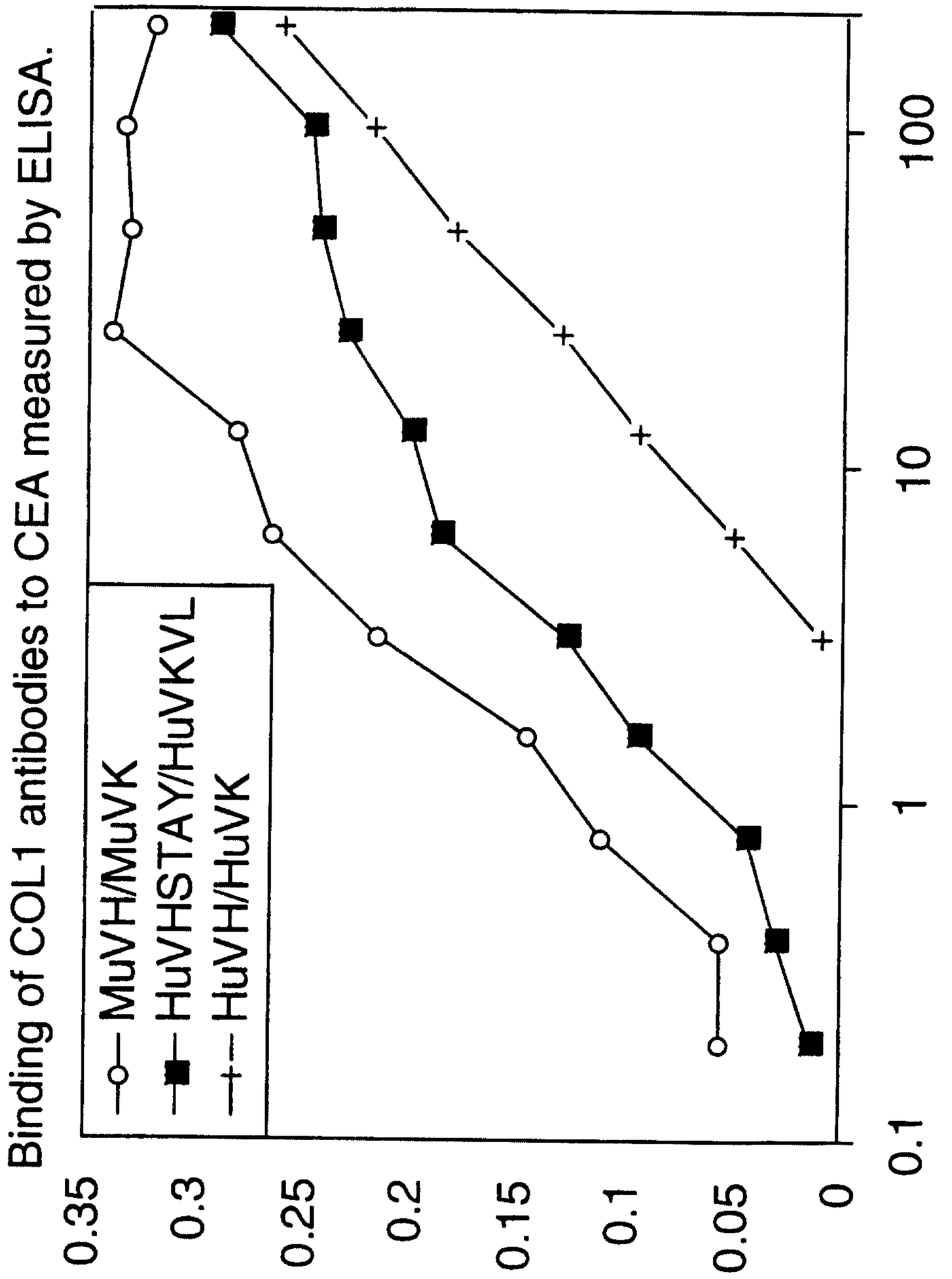

Binding of COL1 antibodies to CEA measured by ELISA.
MuVH/MuVK
HuVHSTAY/HuVKVL
HuVH/HuVK
A492nm
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.1
1
10
100
Antibody, ng per well

Fig. 12

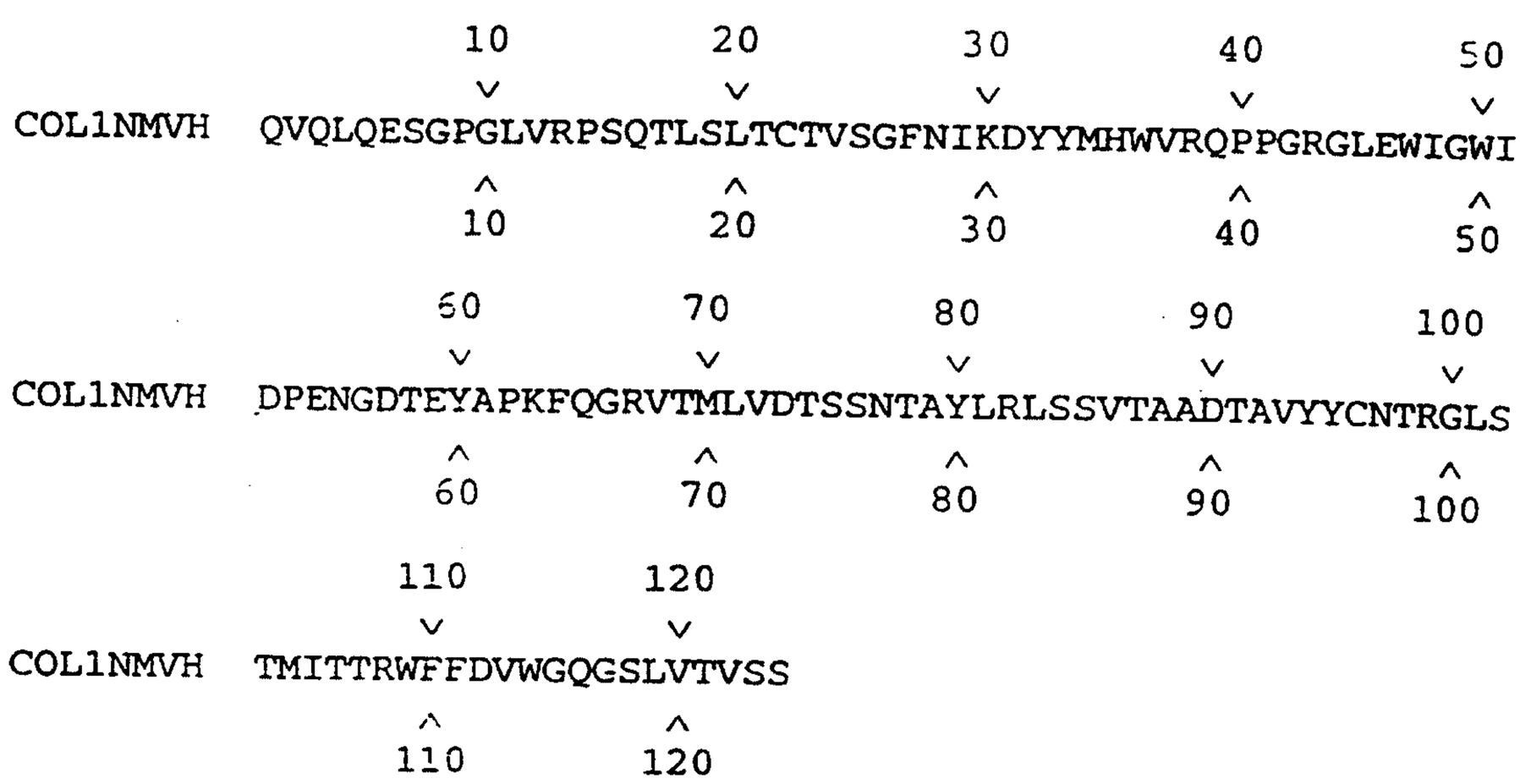

10 20 30 40 50
COL1NMVH QVQLQESGPGLVRPSQTLSLTCTVSGFNIKDYYMHWVRQPPGRGLEWIGWI
10 20 30 40 50
60 70 80 90 100
COL1NMVH DPENGDTEYAPKFQGRVTMLVDTSSNTAYLRLSSVTAADTAVYYCNTRGLS
60 70 80 90 100
110 120
COL1NMVH TMITTRWFFDVWGQGSLVTVSS
110 120

FIGURE 13

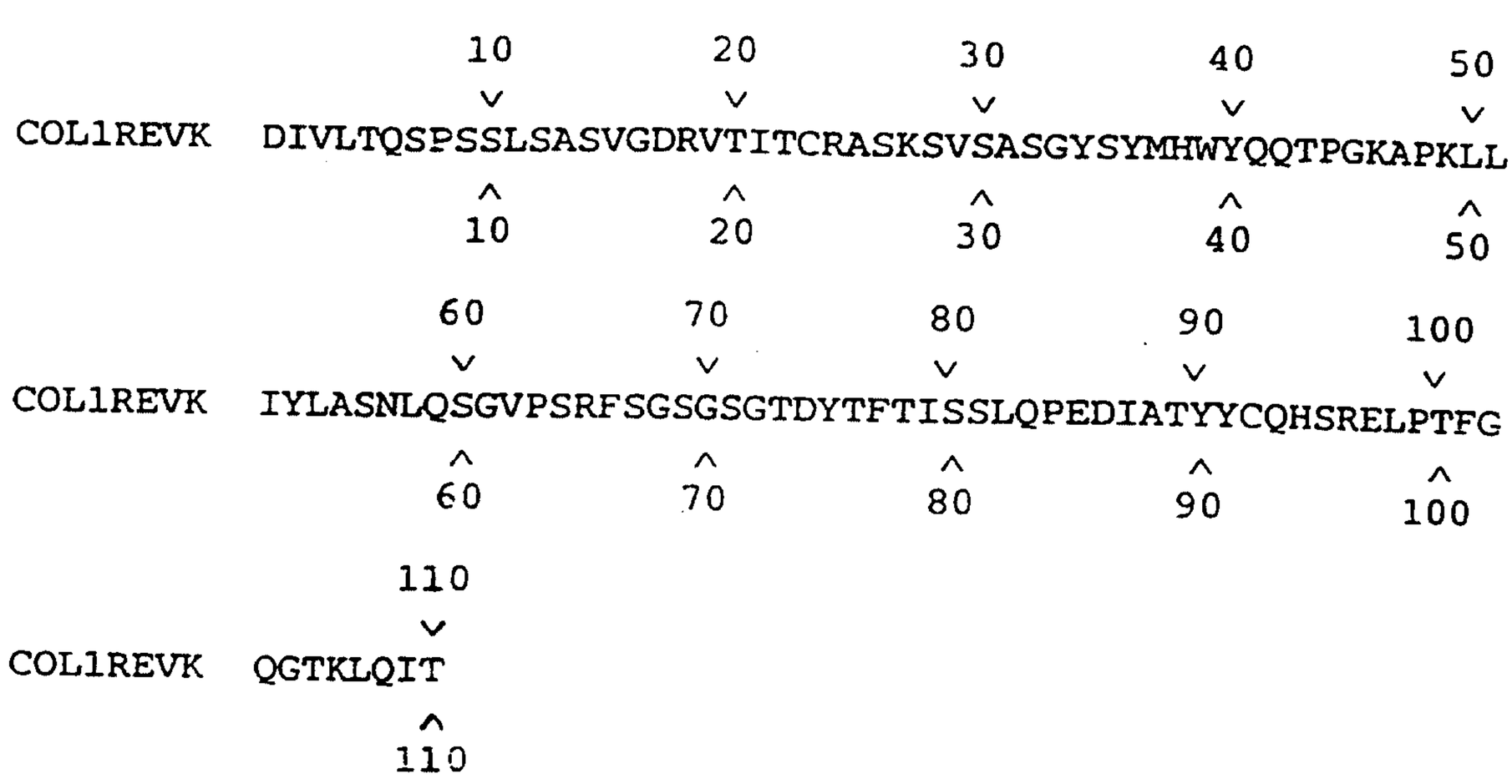

10 20 30 40 50
COL1REVK DIVLTQSPSSLSASVGDRVTITCRASKSVSASGYSYMHWYQQTPGKAPKLL
10 20 30 40 50
60 70 80 90 100
COL1REVK IYLASNLQSGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQHSRELPTFG
60 70 80 90 100
110
COL1REVK QGTKLQIT
110

FIGURE 14

```
          270        280        290        300
           V          V          V          V
CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG
GTC CAG GTT GAC GTC CTC TCG CCA GGT CCA GAA CAC TCT GGA TCG GTC

310        320        330        340        350
 V          V          V          V          V
ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT AAA GAC TAC
TGG GAC TCG GAC TGG ACG TGG CAC AGA CCG AAG TTG TAA TTT CTG ATG

   360        370        380        390        400
    V          V          V          V          V
TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT
ATA TAC GTG ACC CAC TCT GTC GGT GGA CCT GCT CCA GAA CTC ACC TAA

     410        420                   440        450
      V          V                     V          V
GGA TGG ATT GAT CCT GAG AAT           TAT GCC CCG AAG TTC CAG GGC
CCT ACC TAA CTA GGA CTC TTA...    ...ATA CGG GGC TTC AAG GTC CCG

460        470        480        490        500
 V          V          V          V          V
AGA GTG ACA ATC CTG GTA GAC ACC AGC AAG AAC CAG TTC AGC CTG AGA
TCT CAC TGT TAC GAC CAT CTG TGG TCG TTC TTG GTC AAG TCG GAC TCT

   510        520        530        540
    V          V          V          V
CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT          GTC ACC
GAG TCG TCG CAC TGT CGG CGG CTG TGG CGC CAG ATA...    ...CAG TGG

600
 V
GTC TCC TCA
CAG AGG AGT
```

FIGURE 15

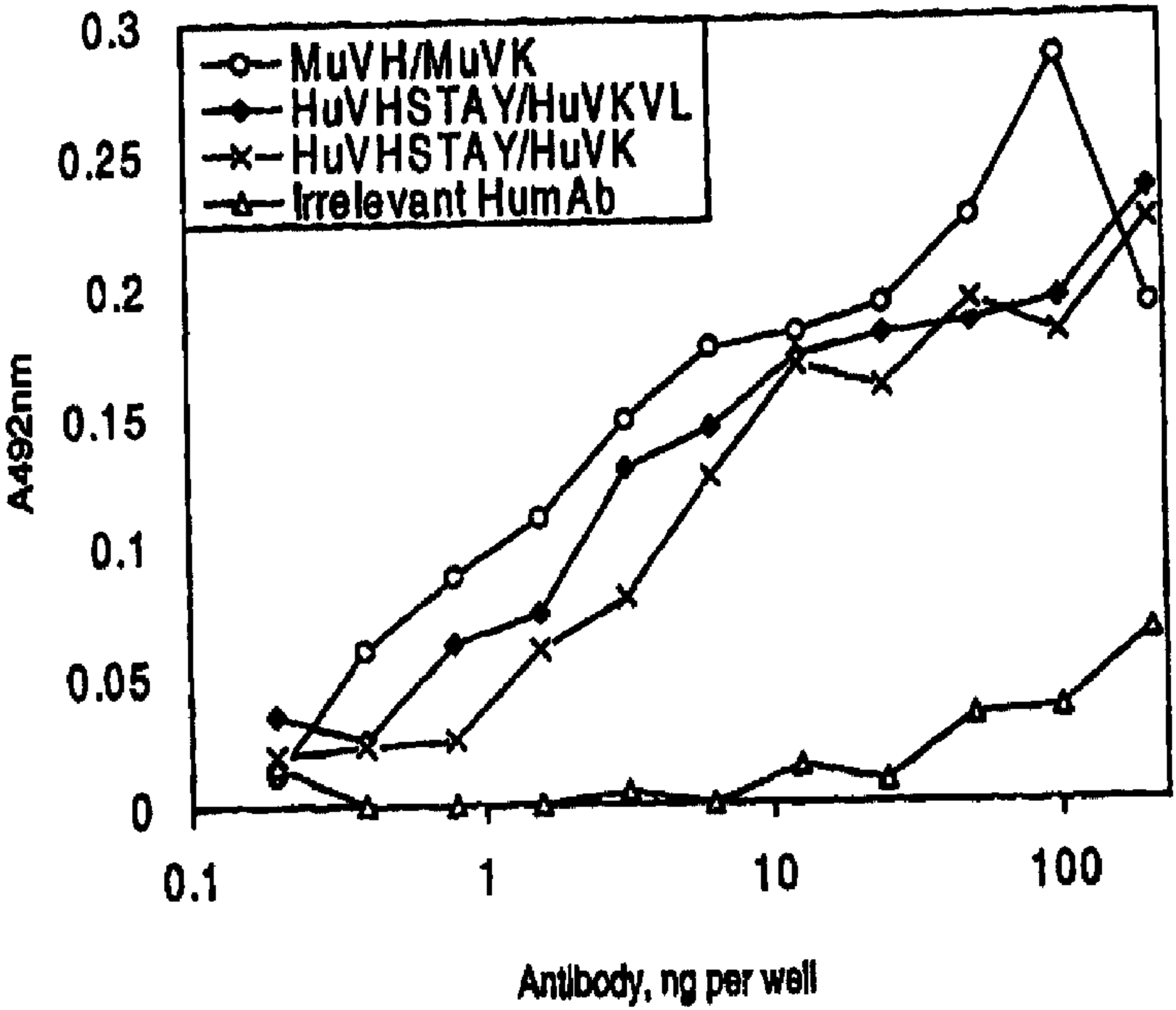
```
                270         280         290         300
                 V           V           V           V
CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG
GTC CAG GTT GAC GTC CTC TCG CCA GGT CCA GAA CAC TCT GGA TCG GTC

310         320         330         340         350
 V           V           V           V           V
ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT AAA|GAC TAC
TGG GAC TCG GAC TGG ACG TGG CAC AGA CCG AAG TTG TAA TTT|CTG ATG

   360         370         380         390         400
    V           V           V           V           V
TAT ATG CAC|TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT
ATA TAC GTG|ACC CAC TCT GTC GGT GGA CCT GCT CCA GAA CTC ACC TAA

     410         420         430         440         450
      V           V           V           V           V
GGA|TGG ATT GAT CCT GAG AAT GGT GAT ACT GAA TAT GCC CCG AAG TTC
CCT|ACC TAA CTA GGA CTC TTA CCA CTA TGA CTT ATA CGG GGC TTC AAG

          460         470         480         490         500
           V           V           V           V           V
CAG GGC|AGA GTG ACA ATG CTG GTA GAC ACC AGC AAG AAC CAG TTC AGC
GTC CCG|TCT CAC TGT TAC GAC CAT CTG TGG TCG TTC TTG GTC AAG TCG

             510         520         530         540
              V           V           V           V
CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT TAC TGT
GAC TCT GAG TCG TCG CAC TGT CGG CGG CTG TGG CGC CAG ATA.

550         560         570         580         590
 V           V           V           V           V
AAT ACA|CGG GGT CTA TCT ACT ATG ATT ACG ACG GCT TGG TTC TTC GAT
TTA TGT|GCC CCA GAT AGA TGA TAC TAA TGC TGC CGA ACC AAG AAG CTA

   600         610         620         630
    V           V           V           V
GTC|TGG GGC CAA GGG TCC TTG GTC ACC GTC TCC TCA
CAG|ACC CCG GTT CCC AGG AAC CAG TGG CAG AGG AGT
```

FIGURE 16

```
            270          280          290          300
             V            V            V            V
GAC ATC CAR CTG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT

310          320          330          360          370
 V            V            V            V            V
GAC AGA GTG ACC ATC ACC TGT AGG...    ...GGC TAT AGT TAT ATG CAC

      380          390          400          410
       V            V            V            V
TTG TAC CAG CAG ACG CCA GGT AAG GCT CCA AAG CTG CTG ATC TAC...

     440          450          460          470          480
      V            V            V            V            V
...TCT GGT GTG CCA AGC AGA TTC AGC GGT AGC GGT AGC GGT ACC GAC

          490          500          510          520          530
           V            V            V            V            V
TTY ACC TTC ACC ATC AGC AGC CTC CAG CCA GAG GAC ATC GCC ACC TAC

                       560          570
                        V            V
TAC TGC CAG...    ...ACG TTC GGC CAA GGG ACC AAG
```

FIGURE 17

```
                270                 280                 290                 300
                 V                   V                   V                   V
GAC ATC CAR ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT

310                 320                 330                 340                 350
 V                   V                   V                   V                   V
GAC AGA GTG ACC ATC ACC TGT|AGG GCC AGC AAA AGT GTC AGT GCA TGT

 360                 370                 380                 390                 400
  V                   V                   V                   V                   V
GGC TAT AGT TAT ATG CAC|TGG TAC CAG CAG ACG CCA GGT AAG GCT CCA

  410                 420                 430                 440                 450
   V                   V                   V                   V                   V
AAG CTG CTG ATC TAC|CTT GCA TCC AAC CTA CAA TCT|GGT GTG CCA AGC

      460                 470                 480                 490                 500
       V                   V                   V                   V                   V
AGA TTC AGC GGT AGC GGT AGC GGT ACC GAC TAC ACC TTC ACC ATC AGC

          510                 520                 530                 540
           V                   V                   V                   V
AGC CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC|CAG CAC AGT AGG

              560                 570                 580                 590
               V                   V                   V                   V
GAG CTT CCT ACG|TTC GGC CAA GGG ACC AAG CTG CAA ATC ACA
```

FIGURE 18

Binding of COL1 antibodies to CEA measured by ELISA.